DEVELOPMENT, MANUFACTURE AND ASSESSMENT OF CLOBETASOL 17-PROPIONATE CREAM FORMULATIONS

By

Ayeshah Fateemah Beebee Fauzee

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Faculty of Pharmacy
RHODES UNIVERSITY
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ABSTRACT

Eczema or dermatitis is the most common dermatological condition accounting for one-third of all diagnoses in the total population surveyed in South Africa. The prevalence of seborrhoeic dermatitis, extreme photodermatitis and severe psoriasis has increased markedly over the last decade and this increase may be ascribed to the HIV epidemic, first diagnosed in South Africa in 1982. Potent innovator corticosteroids, such as clobetasol 17-propionate (CP) that are used to treat skin disorders, are expensive and there is therefore a need for the production of generic topical corticosteroid products. Formulation and manufacturing processes can be challenging aspects for formulation scientists to produce a robust product that will elicit an appropriate and desirable pharmacokinetic-pharmacodynamic profile.

Laboratory scale CP creams were manufactured using different concentrations of Gelot® 64 and propylene glycol in order to establish a composition that would produce a formulation, with similar physical and chemical characteristics and *in vitro* release profile as an innovator product, Dermovate®. These formulations were assessed in terms of their viscosity, spreadability, pH, content uniformity and *in vitro* release characteristics using a Franz diffusion cell apparatus. A formulation containing 3% w/w Gelot® 64 and 46% v/v propylene glycol (CPLS-02) was found to exhibit similar viscosity and spreadability characteristics and released CP in a manner similar to Dermovate®. The mechanism of drug release was evaluated using mathematical models such as zero order, first order and Higuchi models. In addition, the *in vitro* release profiles were characterised by use of difference (f_1) and similarity (f_2 and S_d) factors.

A scale-up formulation with the same % w/w composition as the laboratory scale was also investigated following manufacture using a Wintech® cream/ointment mixer. A Central Composite Design approach was used to investigate the effect of process variables on the performance of the scale-up cream formulations. The homogenisation speed, anchor speed, homogenisation time and cooling time were the process variables investigated. Thirty scale-up batches were manufactured and analysed in terms of their viscosity, spreadability, pH, % drug content and cumulative % drug released per unit area over 72 hours. Model fitting using Design-Expert® software was undertaken and

revealed that a correlation between the process variables and the cream responses was most suitably described by quadratic polynomial relationships. The homogenisation speed had the most significant effect on the quality of the scale-up formulations, whereas the anchor speed had a secondary effect on the measured responses, for the formulations investigated. The qualitative interpretation and statistical analysis of the *in vitro* release data from the scale-up formulations using ANOVA and the f_1, f_2 and S_d factors revealed that one scale-up batch (CPSU-04), for which the process variables were a homogenisation speed of 1900 rpm, an anchor speed of 35 rpm, a homogenisation time of 100 minutes and a cooling time of 100 minutes, released CP at a similar rate and extent to Dermovate®. A diffusion-controlled mechanism appeared to be predominant in these formulations.

A human skin blanching study, using both visual and chromameter assessments, was performed to establish whether batch CPSU-04 was bioequivalent to Dermovate®. The bioequivalence of the selected scale-up formulation to Dermovate® was confirmed, following the calculation of a 90% CI.

To the Fauzee and Sohawon Families,

My late paternal grandparents, maternal grandparents and parents,

For inspiration,

For motivation &

For everlasting support.

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STUDY OBJECTIVES

Clobetasol 17-propionate (CP) is a class 1 corticosteroid used for the treatment of inflammatory conditions of the skin such as psoriasis, seborrheaic and atopic dermatitis, extreme photodermatitis and eczema [1-3]. Potent corticosteroids are expensive and the prevalence of dermatological conditions in South Africa has increased since the identification of the HIV epidemic in 1982, requiring access to affordable medicines [4]. CP is the drug of choice for the effective treatment of these dermatological conditions since it has a short lag time before an *in vivo* pharmacological effect is observed, following topical application [5,6]. Although Dovate® is a readily available generic CP cream product on the South African market, there was a need to produce a laboratory and scale-up formulation and to define a manufacturing process that would be suitable for the production of a robust product, prior to consideration of licensing such topical pharmaceutical product.

The objectives of this study were:

i. To develop and validate a Reversed-Phase High Performance Liquid Chromatographic (RP-HPLC) method that had the necessary sensitivity and selectivity to accurately and precisely quantitate CP, in methanolic solutions and semi-solid dosage forms.

ii. To design and develop a 500 g laboratory scale CP cream formulation that would have similar physical and chemical characteristics to an innovator product *viz.,* Dermovate®.

iii. To design, develop and optimise a 5000 g batch of CP cream formulation, using a minimum number of trials using experimental design techniques and to evaluate the performance characteristics of the dosage form and compare the results to those obtained for Dermovate®.

iv. To assess the rate and extent of CP release from laboratory and scale-up CP cream formulations using a Franz cell diffusion apparatus.

v. To study the *in vitro* release kinetics and release mechanisms for the laboratory and scale-up CP cream formulations.

vi. To evaluate the *in vivo* bioequivalence of a scale-up CP cream formulation, with respect to Dermovate® in healthy human volunteers using the human skin blanching assay.

TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

CLOBETASOL 17-PROPIONATE (CP)

1.1 INTRODUCTION

Clobetasol 17-Propionate (CP) is a super-high potency di-halogenated corticosteroid (Figure 1.1) that has been commercially available since 1973 [7,8]. CP has been classified as a class 1 corticosteroid [1,2,9] and is currently one of the most potent topical corticosteroids on the South African market [10-13]. CP possesses potent anti-inflammatory [14,15], anti-pruritic, vasoconstrictive [10,12,15-17], immunosuppressive [13,18] and anti-proliferative [13,15] properties.

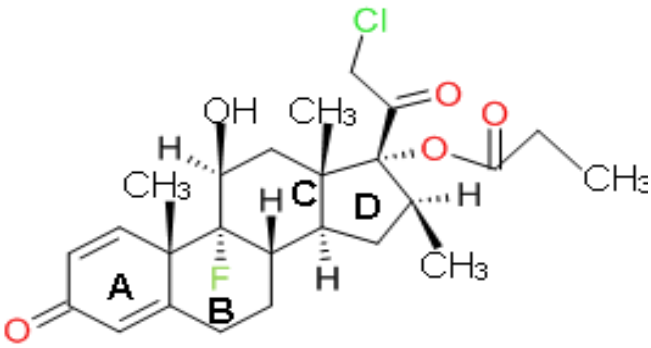


Figure 1.1. *The chemical structure of CP*

CP is the drug of choice in most corticosteroid studies since it has a short lag time before an *in vivo* pharmacological effect is observed, following topical application and it is a high potency compound [5,6].

The potency or strength of topical corticosteroids is usually measured by the Human Skin Blanching Assay (HSBA) that is also known as the Vasoconstrictor Assay (VCA) [1,19-23]. The HSBA is one of the most reliable *in vivo* methods for assessing the efficacy of topical corticosteroids [24]. It has been shown that the intensity of induced blanching is directly proportional to the clinical efficacy of the corticosteroid. This method of assessment is convenient as it can provide an indication of the potency of new corticosteroid molecules or the success of the topical vehicle as a delivery system in the assessment of bioequivalence [25]. Following evaluation of formulations using the HSBA, CP was found to be 1800 times more potent than hydrocortisone [7,10,26,27], which is classified as the least potent corticosteroid and is listed as a class 7 agent [9,28].

CP is a lipophilic drug molecule that penetrates and permeates into and through the stratum corneum via the tortuous intercellular route between corneocytes [29,30] and follicular pathways of the hair

[30]. However the cutaneous absorption of corticosteroids varies according to the physicochemical nature of the compound in addition to the site of application [30].

CP has been found to be useful for the treatment of chronic skin inflammation where the skin is thickened or lichenified, pigmented and/or scaled. CP is also effective when used for the treatment of short duration for the relief of inflammatory and pruritic manifestations of moderate-to-severe corticosteroid-responsive dermatoses [7,16,31]. Typical indications for CP include plaque psoriasis of the body, palmoplantar psoriasis, lichen planus, lichen simplex chronicus, lupus erythematosus and acute exacerbations of atopic dermatitis in adults [31,32].

CP is commercially available as cream, ointment, scalp solution [7,18,31,33], gel [34] and more recently as foam [29,35-37] or emollient cream [7] formulations, that contain 0.05% w/w of the steroid. Commercially available CP products on the South African market are sold as Dermovate®, Dovate® and Xenovate® [33,38] .

1.2 PHYSICO-CHEMICAL PROPERTIES

1.2.1. Description

CP is 21-chloro-9α-fluoro-11β, 17α-dihydroxy-16β-methyl-17α-pregna-1,4-diene-3,20-dione 17 propionate [18,32]. CP occurs as a white to creamy-white crystalline powder [1,33,39]. It is odourless [11] and tasteless [18].

CP is the di-halogenated analogue of prednisolone [7,16,40] with high glucocorticoid activity and low mineralocorticoid activity [41,42]. CP contains a fluorine (-F) atom attached on ring B at position C-9α and a chlorine (-Cl) atom on the 5-membered ring D at position C-21. The lipophilicity of this corticosteroid and its duration of action are increased as a consequence of the fluorination of ring B at position C-9 [13].

The empirical formula of CP is $C_{25}H_{32}ClFO_5$ and it has a molecular weight of 467.0 g/mol [18,33,39,43].

CP contains not less than 97.0 percent and not more than 102.0 percent of $C_{25}H_{32}ClFO_5$, calculated on an anhydrous basis [44,45].

1.2.2. Dissociation Constant (pK_a)

CP is a neutral molecule and does not possess any ionisable functional groups [18] and therefore it has no dissociation constant. Formulation and analytical issues normally associated with pH changes are therefore unlikely to occur.

1.2.3. Solubility Studies

The solubility of CP in a variety of solvents is summarised in Table 1.1.

Table 1.1. *Solubility of CP in a variety of solvents*

Solvent	Solubility	Description	References
Water	<1 in 10000	Insoluble	[18]
Alcohol	1 in 1000	Sparingly soluble	[18]
Ethanol	1 in 100	Soluble	[39]
Acetone	1 in 10	Soluble	[8]
Chloroform	1 in 10	Soluble	[8]
Dichloromethane	1 in 10	Soluble	[8]

The solubility of CP in methanol (MeOH) and propylene glycol (PG) has not been reported. As MeOH was used in the preparation of the mobile phase for RP-HPLC analysis and PG was used in *in vitro* release experiments, the solubility of CP in MeOH and PG were established. CP was added to volumetric flasks to form saturated solutions in MeOH and PG at an ambient temperature of 22°C. The solutions were shaken for 24 hours using a Junior Orbit® Shaker (Lab-line Instruments Inc., Melrose Park, ILL, USA). The saturated solutions were then filtered through a 0.45 μm Millipore® HVLP membrane filter (Millipore®, Bedford, MA, USA) and were analysed using a validated HPLC technique, as mentioned *vide infra* (§ 2.5 and § 2.6, Chapter 2). The solubility of CP was found to be 52.69 ± 1.07 mg/ml in MeOH and 9.90 ± 0.03 mg/ml in PG (n=5).

1.2.4. Partition Coefficient

During the early stages of formulation development and drug delivery investigations, the addition of different ester functional groups to the corticosteroid backbone are essential in order to produce a lipophilic molecule that would be able to pass through the stratum corneum readily [24]. In order to be well absorbed through the skin, a substance should have a molecular mass of less than 0.6 kDa, an adequate solubility in oil and water, in addition to a partition coefficient that favours equilibration in a lipophilic environment [30].

The partition coefficient is defined as an equilibrium constant for the partitioning of a molecule between a hydrophobic phase (oil) and a hydrophilic phase (water). It thus provides a measure of the affinity of a molecule for a hydrophobic environment such as the lipid bilayers of skin cells [46-48]. Therefore the partition coefficient is a good indication of the potential for absorption, permeation and distribution of a drug molecule into and through the skin. CP has an octanol/water partition coefficient (log $P_{o/w}$) of 3.5 [39,41]. Since the log $P_{o/w}$ of CP is high, it has a potential for partitioning into lipophilic media and aqueous environment during *in vitro* testing and *in vivo* studies.

1.2.5. Optical Rotation

The specific optical rotation of a 10 mg/ml solution of CP in dioxane is between +98° and +104° (t=20°C) [45].

1.2.6. Melting Range

CP melts within the range of 195.5°C to 197.0°C [39,41].

1.2.7. Ultraviolet Absorption Spectrum

The ultraviolet absorption spectrum of CP in MeOH and water, in a ratio of 68:32% v/v, over a wavelength range of 210-300 nm was generated, using a scan speed of 600 nm/min (Figure 1.2). The spectrum was generated using a double beam UV-VIS Model GBC 916 spectrophotometer (GBC Scientific Equipment Pty Ltd, Melbourne, Australia). The observed wavelength of maximum absorption (λ_{max}) for CP of 239 nm was similar to the values quoted in the literature [39,40]. The λ_{max} was used for the analysis of CP in RP-HPLC for the quantitation of CP in topical pharmaceutical dosage forms, as mentioned *vide infra* (§ 2.4.3, Chapter 2).

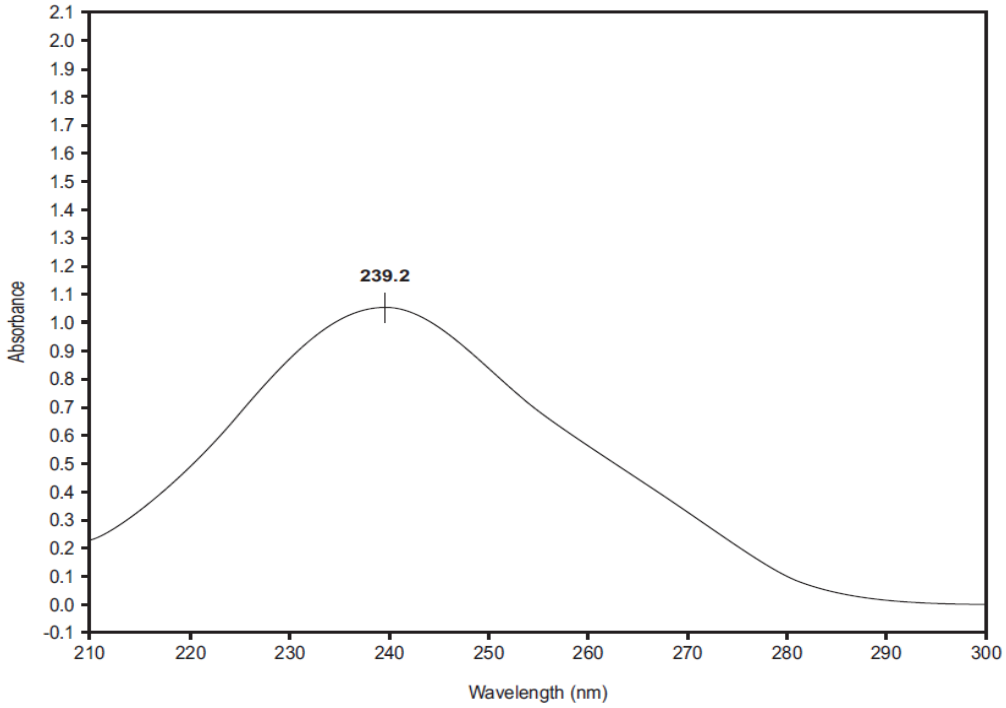


Figure 1.2. *Ultraviolet absorption spectrum of CP in MeOH and water (68:32% v/v)*

1.2.8. Infrared Spectrum

Three main methods *viz.*, Nujol® mull [49,50], potassium bromide (KBr) [51,52] and glassy film deposits [53] can be used for the preparation of solids for infrared (IR) scanning. A KBr method was used to obtain the IR spectrum of CP in the range of 2000-800 cm^{-1}, using a Perkin-Elmer® Precisely FT-IR spectrometer Spectrum 100 (Perkin-Elmer® Pty Ltd, Beaconsfield, England). The IR spectrum of CP is shown in Figure 1.3 and the relevant bands assignments are shown in Table 1.2. The principal peaks, found at wavenumbers 1733, 1661, 1606, 1065, 1009 and 888 cm^{-1} are similar to those reported in the literature [39].

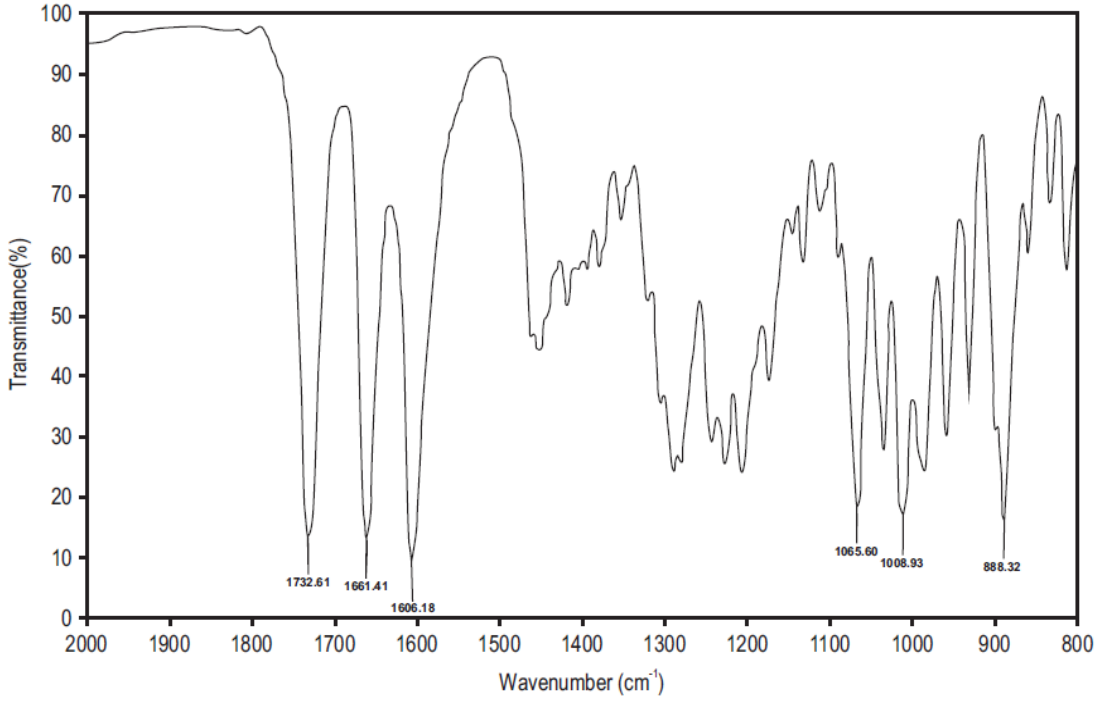


Figure 1.3. *Infrared spectrum of CP in KBr [39]*

Table 1.2. *Infrared frequencies for CP*

Major bands frequencies (cm^{-1})	**Assignments**
1733	C=O-O at position C-17
1661	C=O at position C-20
1606	C=C at positions C-1 to C-2 and C-4 and C-5
1065	C-F at position C-9
1009	C-O stretching(> C-OH) at position C-11
888	C-Cl at position C-21

1.2.9. Photochemistry of CP

Many commonly used steroidal compounds have been reported to exhibit phototoxic effects [40,54,55]. Competition between chemical reactions of separate excited moieties incorporated into a rigid structure such as in steroid structures has been investigated by Iqbal *et al.* [40]. The photochemical reactions may in part, explain the mechanism of phototoxicity of the steroid class of drugs. Therefore the photochemistry of CP was evaluated in aerobic as well as anaerobic conditions irradiated at wavelengths of 254 nm and 310 nm, in acetonitrile and 2-propanol [40]. The photoproducts were analysed by IR, ^{1}H Nuclear Magnetic Resonance (NMR), ^{13}C NMR spectroscopy and elemental analysis. CP was found to be an interesting compound since it bears two spatially separated chromophores, a cyclohexadienone moiety in ring A and a carbonyl group at position C-20 [40].

When CP was irradiated at 254 nm in argon-flushed acetonitrile or in an oxygen-saturated solution, 21-chloro-9-fluoro-11-hydroxy-16-methyl-17(1-oxopropoxy)-1,5-cyclopregn-3-ene-2, 20-dione

(***compound 1***) was produced (Figure 1.4). The presence of a cross-conjugated ketone that absorbs light at 254 nm causes the well-known lumiketone rearrangement of the chromophore and leads to the formation of a photoproduct, unaffected by the solvent vehicle [40].

However, irradiation of CP at 310 nm, either in argon-saturated acetonitrile or 2-propanol resulted in the formation of compound 1 in addition to a new compound (***compound 2***), 21-chloro-9-fluoro-11-hydroxy-16-methyl-17(1-oxopropoxy)-18, 20-cyclopregn-1,4-diene-3-one. At 310 nm, the isolated ketone at position C-20 absorbs a large fraction of light and compound 2 is formed via hydrogen atom abstraction from the close-lying 18-methyl group followed by cyclization (Figure 1.4) [40].

In oxygen-saturated solutions, the entrapment of alkyl radicals by oxygen results in the formation of peroxy radicals. The peroxy radical abstracts hydrogen from the hydrogen donating solvent (2-propanol) and produces an isolated hydroperoxy derivative compound, 9-fluoro-17-hydroxyperoxy-16-methyl-17(1-oxoperoxy) androsta-1,4-diene-3-one (***compound 3***) (Figure 1.4) [40].

The cyclohexadienone moiety in ring A and the keto group at position C-17 were found to be extensively modified following ultraviolet irradiation, resulting in a loss of biological activity following storage [40].

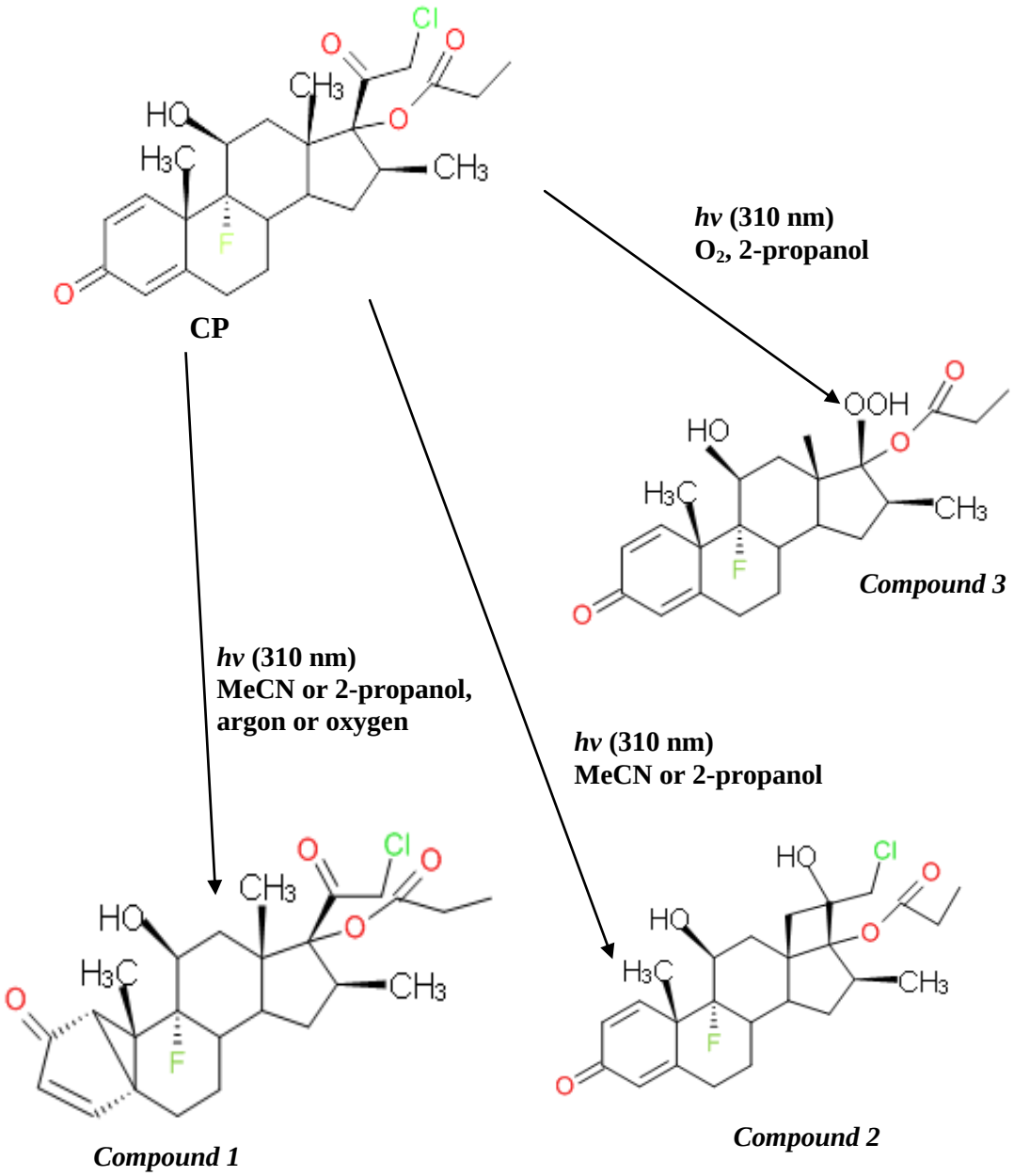


Figure 1.4. *Schematic diagram of the reaction products viz., compounds* ***1****,* ***2*** *and* ***3****, following photochemical reaction of CP under different reaction conditions*

1.3 SYNTHETIC PATHWAY

1.3.1. Synthetic route

CP is manufactured by chemical synthesis using the procedures outlined in Figure 1.5 [56]. The initial molecule, betamethasone undergoes esterification at position C-17 to form a crude product of betamethasone 17-propionate (***BEP Crude***) in the presence of para-toluene sulphonic acid, dimethyl formamide and sulphuric acid. The crude product then undergoes a purification process in methanol, methylene chloride and petroleum ether to remove most unwanted side products and any unreacted starting material to form the pure product BEP (***BEP Pure***). The next step involves the formation of

an intermediate methane sulphonate from the alcohol group on position C-21 and methane sulphonyl chloride in the presence of a non-nucleophillic base, pyridine to form 21-methanesulphonoxy betamethasone propionate. Methane sulphonates are usually used as intermediates in substitution reactions [57,58]. A substitution reaction of the sulphonyl group with a halogen atom (-Cl) takes place to give the desired product, clobetasol 17-propionate (***CP crude***) in the presence of dimethyl formamide and lithium chloride. The final step involves a purification process in methylene dichloride, activated charcoal and methanol to form the product, clobetasol 17-propionate pure product (***CP Pure***) (Figure 1.5).

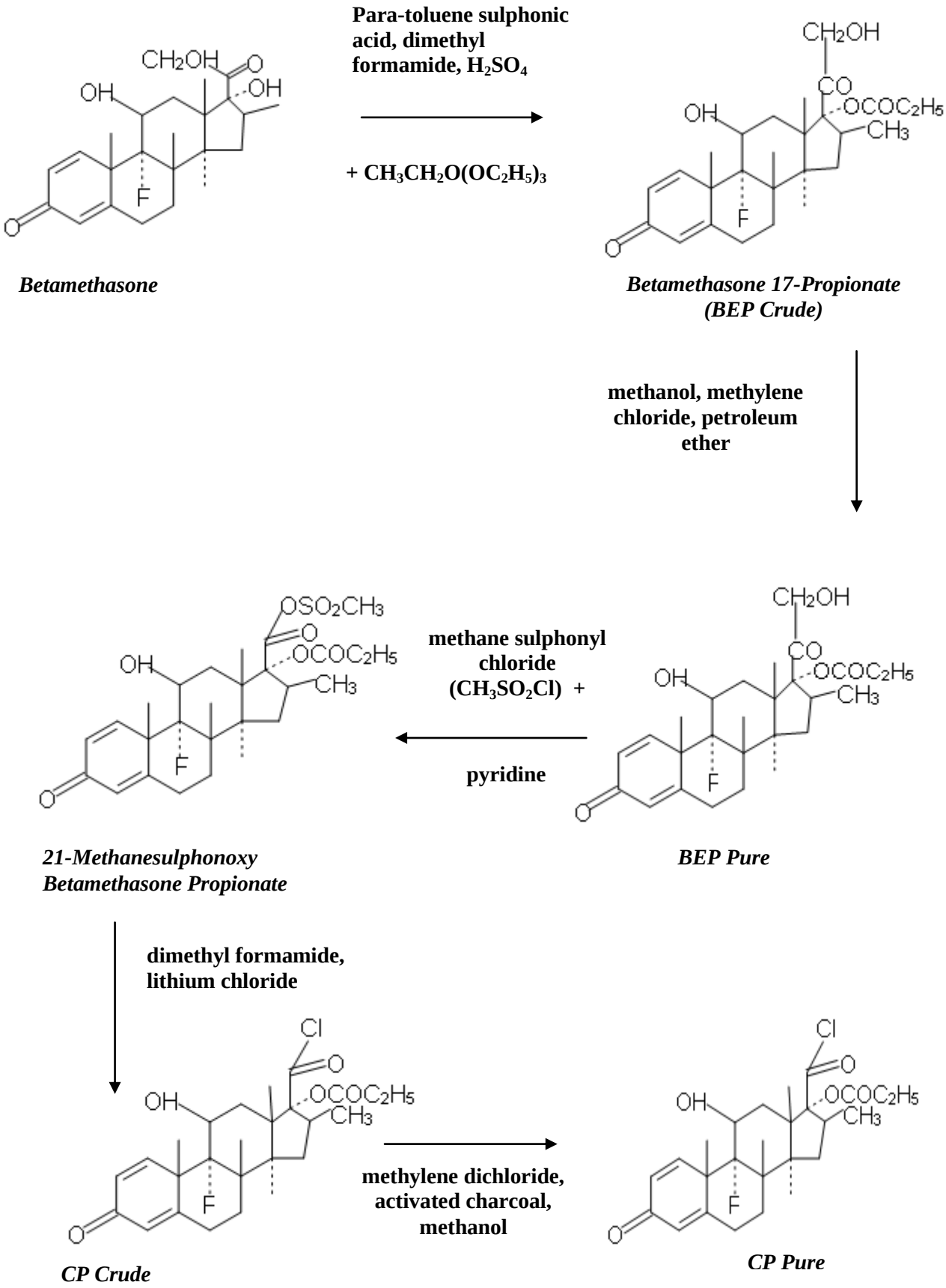


Figure 1.5. *Pathway of synthesis of CP [56]*

1.3.2. Stereochemistry and Structure Activity Relationship

Corticosteroids from natural or synthetic sources occur as a 21-carbon steroid structure, characterised by the presence of four fused rings (Figure 1.1). Generally, the chemical structure of steroids consists of three 6-membered rings (***A, B, C***) and one 5-membered ring (***D***) (Figure 1.6). The term 'steroid' refers to hydrocarbon molecules containing a hydrogenated cyclopentanoperhydrophenanthrene ring system. The steroid contains a 5-membered ring D referred to as cyclopentane and three 6-membered rings of phenanthrene [59]. Rings A, B and C invariably adopt planar, chair and chair conformations while ring D forms an envelope at position C-14 [16]. The rigid geometry of steroid compounds prevents the molecule from undergoing cyclohexane ring-flips [60]. The numbering of the components of the CP molecule commences from ring A at position C-1 and continues around each of rings A, B to position C-10. The sequence continues from position C-11 through ring D to position C-17. The angular methyl groups are attached to position C-10 to give position C-19 and position C-13 to give position C-18. Position C-17 is then attached to position C-20 to give a ketone functional group and a chlorine atom. On the opposite side of position C-17 is a propionate group as shown in Figure 1.6.

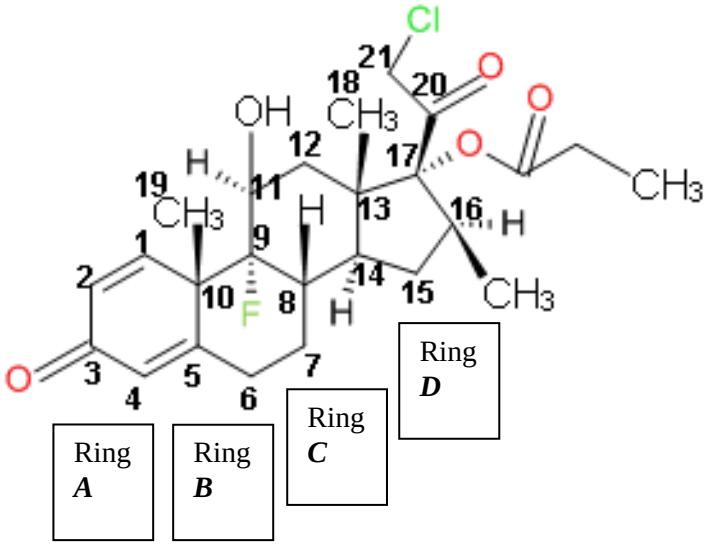


Figure 1.6. *The numbering of the carbons of clobetasol 17-propionate on rings* ***A***, ***B***, ***C*** *and* ***D***

Although all steroids are similar in structure and stereochemistry, each specific group of compounds displays different biological activity. A slight modification to a steroid structure, for example the addition of functional groups at certain positions, can result in major changes in the physiological activity of such molecules [59]. By way of example when a *trans* skeleton is adopted for steroids, the presence of the 3-keto functionality on ring A and the 11β-hydroxyl functionality on ring C are critical for anti-inflammatory activity [61]. The presence of a hydroxyl functional group at position C-11 and fluorination at position C-9 appears to be essential for glucocorticoid activity. The presence of chlorine and fluorine atoms at the positions C-21 and C-9 respectively increases the potency of the

molecule significantly. The esterification of clobetasol at position C-17 gives rise to clobetasol 17-propionate and increases the topical activity of the compound [8,62,63].

1.4 STABILITY

CP was found to be incompatible with substances such as alkali [39]. CP is reactive with oxidising agents, acidic [17] and alkaline [17,39] materials. CP should be stored in light-resistant tightly sealed containers [45,64] since it is sensitive to light [17,39,43].

CP cream, emollient cream and ointments were relatively stable and may be stored between temperatures of 15°C and 30°C (59°F to 86°F) [65]. Semi-solid formulations should not be refrigerated except for CP gel products that can be stored between 2°C and 30°C (36°F to 86°F). CP foam may be stored at room temperature but should not be exposed to temperatures > 49°C (120°F). CP lotion may be stored at temperatures between 20°C and 25°C [65].

1.5 METHODS FOR MODULATION OF CUTANEOUS PERMEABILITY OF CLOBETASOL 17-PROPIONATE

The use of traditional formulations for topical application such as creams, ointments or gels may result in unsatisfactory cutaneous penetration due to insufficient formulation being applied to an application area [66]. In recent years, research in the field of pharmaceutical dermatological technology has been undertaken and strategies have been used to increase the efficacy of dermatological vehicles using chemical, biochemical or physical approaches [30,66].

The inclusion of a penetration enhancer into topical formulation bases is important to facilitate the diffusion of an Active Pharmaceutical Ingredient (API) into and through the stratum corneum [24,67-72]. Enhancers may interact with intercellular lipids to increase the diffusibility of an API into the horny layer of the skin. Furthermore, enhancers may increase the solubility of an API in a formulation vehicle and/or improve its partition coefficient. Propylene glycol, ethanol, dimethylsulfoxide (DMSO) are examples of penetration enhancers [66,69,71].

Studies in which CP was the API of choice were performed using micelles, liposomes, sub-micron emulsions, solid lipid and polymeric nanoparticles in order to increase the percutaneous absorption of the compound [37]. Liposomal formulations can be effective in transdermal drug delivery since it facilitates penetration via the transappendageal route, which consists of hair follicles, sebaceous and sweat glands [66]. Lipid nanodispersions appear to be absorbed faster than other dermal formulations due their occlusive properties and an increase in the hydration state of the stratum corneum. This

hydration state may be increased by reducing the corneocyte packing and widening the inter-corneocyte gap permits the drug to penetrate deeper into the skin [30].

For an adequate and immediate effect, physical methods of enhancement such as sonophoresis have increased the penetration of CP into target areas [12]. The effects of different frequencies of ultra-sound in either continuous or discontinuous modes on the *in vitro* percutaneous absorption of CP have been investigated. Low frequency ultra-sound at a frequency of 20 kHz was found to increase the permeability of CP via the hair follicles and sweat glands of hairless mouse skin [12]. Ionophoresis is another physical approach for delivery enhancement and API accumulation in the skin. However, since CP is a non-ionisable molecule, it can only be transported by electro-osmosis through the dermal layers of the skin [13]. In electro-osmosis, a massive flow of ionised solvent is effected which transports ionised and neutral species and facilitates dermal penetration of the molecule [13].

1.6 CLINICAL PHARMACOLOGY

1.6.1. Mode of action

CP is a potent steroid which has high glucocorticoid and low mineralocorticoid activity [41,73]. Since CP has high glucocorticoid activity, it can bind with high affinity to glucocorticoid receptors, following intravenous administration. CP has both catabolic and anti-anabolic effects on proteins in the peripheral tissues and may also cause insulin resistance and may impair peripheral glucose utilisation [18].

The use of CP as the propionate salt results in both anti-inflammatory and immunosuppressive responses following topical administration [18]. CP seems to bind effectively to the glucocorticoid receptors located in the skin and has other therapeutic activities that are not clearly defined [7]. CP passes readily through cellular membranes and causes immediate vasoconstriction in the vascular tissues, but this mechanism of action of this effect is not fully known. CP also inhibits the adherence of neutrophils and monocyte-macrophages to endothelial cells of capillaries at the site of inflammation and blocks the effect of macrophage migration inhibitory factor whilst decreasing the conversion of inactive plasminogen to active plasmin [7].

Corticosteroids such as cortisol can act by inducing phospholipase A_2 inhibitory proteins called lipocortins. It has been reported that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibition of arachidonic acid release from membrane phospholipids facilitated by phospholipase A_2 (Figure 1.7) [59].

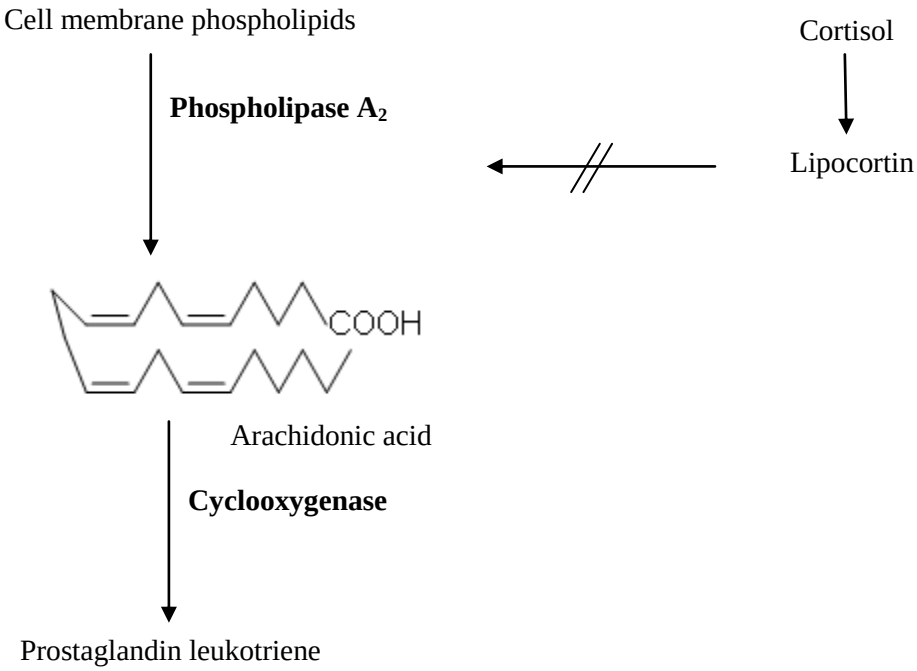


Figure 1.7. *Schematic representation of the anti-inflammatory activity of glucocorticoids [59]*

The mediators reduce inflammatory responses, thereby reducing erythema, oedema and pruritus. However these effects can only be suppressive and relapse may occur if drug therapy is withdrawn. In addition, if the pathophysiology is a consequence of an infection, the natural defence mechanisms of the biological system may exist and the resultant condition may be aggravated, despite some initial relief of the symptoms being achieved [74].

1.6.2. Indications

Topical corticosteroid-containing formulations including creams, ointments, lotions, gels and foams affect the clinical activity, potency and acceptability of a corticosteroid by patients. Although ointments have traditionally been thought to be more potent than creams, it was established that some vehicles are better suited for application to specific areas of the body [9]. Ointments are preferred for application to the glabrous areas, such as palms and soles and body sites that are susceptible to drying such as the extremities. However, ointments are not well tolerated when applied to hirsute areas of the body. Lotions, creams, foams and gels that are cosmetically elegant, are easier to use on the scalp and oily areas of the body such as the face [9,74].

Several CP-containing dosage forms are used to treat different skin conditions according to the severity of the disease state. Topical formulations of CP are mainly used for the initial control of all forms of hyperacute eczema in all age groups. However CP products can only be used for a few days in paediatric patients. CP formulations are used to treat chronic hyperkeratotic eczema of the hands and feet, and patches of chronic lichen simplex, chronic hyperkeratotic psoriasis of any area of the

body, acute contact dermatitis, hypertrophic lichen planus, localised bullous planus, keloid scarring, pretibial myxoedema, vitiligo and for the suppression of a reaction following cryotherapy [18].
A 0.05% w/w clobetasol dipropionate topical application was used to treat severe vulvar lichen sclerosis in women [75]. A scalp application of 0.05% w/v CP is effective for the treatment of moderate-to-severe scalp psoriasis [76]. Foam formulations of 0.05% w/v CP are indicated for use in the short-term topical treatment of inflammatory and pruritic manifestations of moderate-to-severe corticosteroid-responsive dermatoses of the scalp, and for short-term topical treatment of mild-to-moderate plaque-type psoriasis of non-scalp regions, excluding the face and other areas of the body such as the axilla and groin [77,78].

1.6.3. Dose

The action of corticosteroids usually lasts for only six to eight hours and therefore formulations are best applied three times a day [74]. Since CP is a highly potent topical corticosteroid, 0.05% w/w CP cream, ointment or gel should be applied in a thin layer to the affected areas of the skin twice daily, preferably in the morning and evening with gentle and thorough rubbing [79,80].

The frequency of application is usually dependent on the severity of the condition undergoing therapy. The use of more than a thin layer of formulation provides no additional beneficial therapeutic effect. If excess formulation is applied, the potential for local and systemic side effects may be enhanced [74]. Foams and solutions for application to the scalp should be applied to the affected areas twice daily, preferably morning and evening [8,81,82].

Treatment with corticosteroid formulation beyond two consecutive weeks is not recommended due to the potent nature of many of the compounds, particularly CP. Due to the potential for CP to suppress the hypothalamic-pituitary-adrenal (HPA) axis, the total dose should not exceed 50 g per week [39]. CP should be used with care in paediatric patients but should not be used in neonates due to the potential for severe local and systemic adverse effects [18].

Furthermore, CP formulations should not be applied under occlusive dressings [83]. Patients should be warned of dangerous effects following systemic absorption of significant quantities of CP, if large skin areas are covered with the formulation or if the area under treatment is wrapped so as to be an occlusive dressing [84].

1.6.4. Contraindications

Steroids, in particular highly potent corticosteroid agents, may cause several topical and systemic side effects. The use of topical corticosteroids is absolutely contraindicated in patients with primary bacterial infections and may mask bacterial infections due to their vasoconstrictive and anti-inflammatory properties, thus making diagnosis and treatment extremely difficult. Impetigo, furuncles

and carbuncles, paronychia, ecthyma, erysipelas, cellulitis and lymphagitis are all conditions in which the use of topical steroids must be avoided [85].

Patients suffering from cutaneous infections caused by viruses, bacteria or fungi should not be treated with topical corticosteroids. Such infections may be exacerbated or, in the case of fungal infections, may mask the inflammatory response. In addition, infestations such as scabies should not be treated with topical corticosteroids as the condition may be exacerbated or disguised. The use of topical CP in patients with acne vulgaris can result in suppression of the inflammatory response but may be followed by serious rebound if the therapy is stopped. In the majority of patients, rosacea responds dramatically to topical CP preparations but breakthrough occurs rapidly [18].

If potent topical corticosteroids are applied to gravitational ulcers, the resulting vasoconstriction will delay the healing process and may exacerbate infections already present in the ulcer. As noted, the use of potent topical corticosteroids is completely contraindicated in neonates. Neonatal skin is extremely thin and is thus more permeable to these molecules than that of adults or even infants. This can cause potential local and serious systemic side effects [86].

Additional contraindications include to the use of these compounds in candida and dermatophytic infections, as the organisms thrive on macerated skin. The use of topical corticosteroids in conjunction with an appropriate anti-infective agent may occasionally be appropriate. In order to treat herpes simplex and zoster-customary, the virus itself must be subjected to aggressive treatment. Potent corticosteroids have a tendency to cause topical side effects such as atrophy, stria, purpura or even ulceration, particularly when used on skin areas that are naturally thin such as the periorbital and groin regions, under occlusion or in paediatric patients. Special precautions must be taken when applying topical steroids around the eye as their use may precipitate serious glaucoma [85,87]. CP gel, cream and ointment formulations are contraindicated in patients, with a history of hypersensitivity to any of the components of topical formulations [8].

1.6.5. Drug Interactions

Some effects that have been reported with other high potency topical steroids are acneiform eruptions, allergic contact dermatitis, hypertrichosis, hypopigmentation, maceration of the skin, perioral dermatitis and secondary infection. Other drug interactions may reduce the anti-neoplastic effect of aldesleukin. It is therefore undesirable to combine the two drugs [88]. Corticosteroids may also diminish the therapeutic effect of corticorelin. Specifically, the plasma adrenocorticotropin (ACTH) response to corticorelin may be blunted by recent or current corticosteroid therapy. Monitoring of therapy is essential to avoid undesirable effects [88].

1.6.6. Adverse Reactions and Toxicology

1.6.6.1. Local Side Effects

Local cutaneous adverse reactions of topical corticosteroids are far more prevalent than systemic reactions [89,90]. The prevalence of local side effects is largely due to the anti-proliferative effects of the steroidal agents. Local corticosteroid side effects usually occur when used with excessive frequency and duration, or on areas particularly sensitive to steroids [9].

The most commonly observed local side effects of CP are burning, itching, dryness, irritation, especially if applied to bare skin, purpura, acne and telangiectasis [27,91]. Less commonly observed localised side effects include hypertrichosis, hypopigmentation, glaucoma and allergic contact dermatitis. However, reports of contact dermatitis in response to topical corticosteroids are increasing [91]. Skin atrophy and thinning of the skin, the development of stria, telangiectasis, subcutaneous haemorrhage and easy bruising and bleeding may occur as a consequence of the long-term use of topical corticosteroids [92].

Topical corticosteroids may worsen pre-existing or co-existent dermatoses such as rosacea, perioral dermatitis and tinea infections. Patients presenting with psoriasis may become prone to papulopustular flare, following withdrawal of topical corticosteroid therapy if large areas are treated with high potency agents for prolonged periods of time [9].

1.6.6.2. Systemic Side Effects

Although systemic adverse effects are uncommon following topical corticosteroid use, some locally applied corticosteroids can be absorbed through the skin and may enter the general circulatory system. However when this occurs, side effects are often clinically insignificant [9]. However, the greatest risk of systemic reactions or potentially life-threatening effects can occur when ultra-potent or high potency agents such as CP are used in large quantities over large areas of the body, leading to sufficient systemic absorption to produce adrenal suppression, Cushing's syndrome, diabetes and hypertension [9,93,94].

Infants and small children are prone to the development of systemic reactions to any potent corticosteroid medication due to their increased skin surface area-to-body mass ratio [9,89]. The major systemic effects that are observed are Hypothalamic-Pituitary-Adrenal (HPA) axis suppression, growth retardation in children, cataract formation and the development of glaucoma [9]. Several studies have reported that the potential for HPA axis suppression is greater following use of high potency topical corticosteroids. The application of 2 mg per day of a 0.05% w/w CP formulation can cause a decrease in morning cortisol levels after only a few days therapy [26,91]. It has also been

reported that the misuse of super high-potency topical corticosteroids has resulted in death due to Addisonian-type crises [91].

Most potent topical corticosteroids such as CP carry some type of prescription limit and warning in the package inserts due to serious potential side effects following prolonged use [91]. The proper use of highly potent topical steroids is fundamental for every patient and patients presenting with a history of hypertension, liver failure, glaucoma, diabetes or positive tuberculin test results, should use such products with caution [9,87].

1.6.7. Guidelines for Use

For the management of skin conditions such as eczema, patients should be educated as to how topical preparations should be applied and also of the importance of good hygiene. Daily showers, using appropriate bland cleansing detergents, are fundamental to the success of therapy. Soothing emollient preparations especially for geriatric patients are required to hydrate dry skin. Different body sites should also be treated differently due to variation in the thickness of the stratum corneum, which will result in variable skin permeability. The long-term use of topical corticosteroids should be avoided as it can lead to an increase in the systemic side effects of the molecule and may cause tachyphylaxis [95].

1.6.8. Precautions

There are a number of high risk groups in which CP must be administered with caution.

1.6.8.1. Paediatric patients

CP is not recommended for use in children under the age of 12 years as these patients have a high risk of developing HPA axis suppression and Cushing's syndrome when treated chronically with topical corticosteroid-containing products [93,94]. Furthermore, these patients are prone to the development of adrenal insufficiency during or after withdrawal of steroid therapy. In order to avoid side effects, formulations with weak potency, for example class 7 agents, may be used under the supervision of a specialist paediatrician [95].

1.6.8.2. Pregnancy

The use of CP in pregnancy should be avoided [18,96]. The least potent topical steroid-containing formulations must be chosen to ensure an absolute therapeutic response [95]. When relatively low doses of CP were systemically administered to laboratory animals, teratogenicity was evident [8,97]. The teratogenicity of CP following topical application has not been evaluated. However when CP is percutaneously and subcutaneously absorbed, it was found to be teratogenic and birth abnormalities were observed in both rabbits and mice [8,97]. Rabbits were administered doses of 3 μg/kg and 10 μg/kg that are approximately 0.02 and 0.05 times greater than the topical dose of CP in humans.

Teratogenicity in mice occurred at a dose of 0.03 μg/kg and fetotoxicity was observed at a high dose of 1 μg/kg. These doses are approximately 1.4 and 0.04 times the respective topical dose used in humans. In both cases, cleft palate, cranioschisis, and other skeletal abnormalities were observed [97].

1.6.8.3. Geriatrics

As the use of CP formulations in elderly patients carries a higher risk of local and systemic effects than in younger patients, these products should be used with caution in these individuals [95]. Clinical studies with CP formulations undertaken in the United States of America (USA) did not include a sufficient number of subjects ≥ 65 years of age and therefore no evaluation on the efficacy and safety of the drug in this age population can be made. However the adverse effects in geriatric patients were similar to those observed in younger patients. Geriatric patients usually have decreased hepatic, renal and/or cardiac function and concomitant diseases, therefore dose selection is important and must be carefully considered. Consequently corticosteroid therapy must be initiated with a low dose and gradually titrated upwards until an appropriate therapeutic response is achieved [8].

1.6.8.4. HIV/AIDS

HIV/AIDS is currently the most common cause of death in Africa and the fourth leading cause of death globally [98,99]. Skin diseases such as seborrheaic dermatitis, extreme photodermatitis, severe psoriasis and eczema are often common symptoms of HIV infection in patients with CD4 counts < 200. Since the immune system of the patient is compromised, there is a greater risk of developing serious skin lesions, thereby making therapy less effective [3]. An added complication is that most of the skin conditions may occur due to side effects of antiretroviral (ARV) therapy. HIV/AIDS patients usually suffer from oral conditions with lesions such as candidiasis with or without oropharyngeal involvement, hairy leukoplasia, recurrent aphthous-like ulcerations, oral Kaposi's sarcoma, orolabial herpes simplex infection, herpes zoster infection, intraoral or perioral warts and other HIV-associated periodontal diseases [100].

Since these infections may be bacterial, viral or fungal in origin, different therapeutic approaches are used depending on the specific skin condition to be treated. In the case of fungal infection, anti-fungal preparations are mixed with CP topical ointment or cream and can be useful for the treatment of many oral vesiculobullous diseases [100]. Patients suffering from eczema and psoriasis are usually treated with 0.05% w/w CP ointment twice daily. Ointment vehicles are usually preferred to creams since they contain a lubricant which makes the skin softer and smoother. However since CP is a highly potent agent, it should be used cautiously in patients with HIV, because it has been known to cause systemic effects [79,100].

Photosensitivity may be a major cause of skin disorders in HIV/AIDS patients [101]. Individuals of darker skin colours exhibit greater photosensitivity than individuals with lighter skin colours, and

infection with HIV itself is photosensitizing. The use of photosensitizing drugs such as trimethoprim/sulphamethoxazole when treating patients with low CD4 counts, adds to the challenges. Therefore, it is important to treat these conditions with sunscreens, topical corticosteroid therapy with compounds such as CP, lubricants and anti-histamines. The use of a steroid, even in topical formulations must be carefully considered in patients with low CD4 counts < 50, since their immune systems are already compromised and they can develop drug related reactions including hypersensitivity, interactions and idiosyncratic reactions [101,102]. Consequently it is best for patients to be referred to dermatologists that can evaluate the ARV regimen thoroughly and prescribe appropriate topical steroid therapy for these patients.

1.6.8.5. Hypertension

Hypertension is one of the most common non-communicable diseases in South Africa. It is estimated that currently 1 in 4 South Africans between the ages of 15 and 64 years suffers from high blood pressure [103]. It has been noticed that patients using topical corticosteroids develop steroid-induced ocular hypertension which is due to increased resistance to aqueous humour outflow. An elevated ocular hypertension continues as long as the steroid is in use. In these cases it is best to replace potent steroids with less potent compounds that do not cause elevated ocular pressure [104].

Glaucoma and cataracts are ophthalmic conditions that may occur when topical corticosteroid-containing ointments are applied to areas close to the eyes. In 40% of healthy people, topical ocular or periocular steroid use usually increases the intraocular pressure (glaucoma) after a few weeks [104,105]. The British National Formulary (BNF) states that topical corticosteroid formulations should be given to patients only under expert medical supervision and should not be prescribed for undiagnosed “red eye” conditions. Patients treated with long-term topical steroids must be referred to an ophthalmologist who must monitor the intraocular pressure in these patients [105].

Topically applied CP can be absorbed systemically and can cause serious adverse reactions if they are applied to large areas of the body. Hypertension is one of the systemic effects [87]. Hypertensive patients should consult their physicians prior to embarking on CP therapy. In cases where CP is used in children, CP can lead to intracranial hypertension resulting in bulging fontanelles, headaches, and bilateral papilledema [8], therefore the use of potent topical corticosteroids in children must be avoided.

1.6.8.6. Diabetes

Diabetes is another prevalent non-communicable disease in South Africa. It occurs in 10% to 16% of South Africans [106] and diabetes is one of the metabolic side effects that may occur, following the use of topical CP formulations. Diabetes may occur when highly potent topical corticosteroids are applied over large areas of the skin, thus causing significant percutaneous absorption into the systemic

circulation [87]. The consequence of excessive absorption is hyperglycaemia that results in unmasking of latent diabetes mellitus due to increased transport of alanine. Alanine is an important substrate for gluconeogenesis in the liver and increases the activity of rate-limiting enzymes, thereby causing insulin resistance. Consequently CP use in patients with pre-existing liver disease and diabetic patients, must be undertaken with caution [89]. Such patients must inform their physician prior to the administration of any topical corticosteroid-containing formulations.

1.7 CLINICAL PHARMACOKINETICS OF CLOBETASOL 17-PROPIONATE

1.7.1. Absorption

The rate and extent of percutaneous absorption of CP are dependent on many factors, such as the vehicle, severity of disease state, thickness of the stratum corneum and anatomical site, amongst others [18,66]. The absorption of an API can be assessed using either *in vitro* or *in vivo* techniques. *In vitro* studies are usually performed using human, animal or synthetic membranes and more often use the Franz diffusion cell [107,108]. The use of *in vitro* systems is an effective means of studying API release from topical formulations, however *in vivo* studies must be performed to establish API levels in the stratum corneum [109].

The Human Skin Blanching Assay (HSBA) and Tape Stripping (TS) have been used to assess the levels of CP in the stratum corneum [20-22,110,111]. The HSBA is mostly used for bioequivalence studies whereas TS is used for bioavailability and bioequivalence assessment of other topical products [111]. The HSBA has been used to assess the availability of topical corticosteroids for over 40 years, using both visual and chromameter methods of evaluation. The basis of HSBA is the measurement of blanching caused by topical application of a corticosteroid formulation [20,22,23]. TS makes use of adhesive tape strips which consecutively remove layers of corneocytes, after which the drug content is measured in each stripped layer of the skin. Since the vehicle of a topical formulation influences the amount of stratum corneum removed with each strip of tape, the amount of drug absorbed is related to the amount of stratum corneum removed. This technique permits quantification of the pseudo-absorption of the corneocytes at 430 nm, which can be correlated with the number of cell layers that have been removed [111,112].

The stratum corneum acts as a barrier as well as a reservoir for topically administered corticosteroid formulations. These two properties of the stratum corneum influence the penetrating ability of an API. The effect of CP on the horny layer by measuring the absorption of the corneocytes at a wavelength of 430 nm was established [112]. A tape stripping method was used to determine the concentration of CP in the skin following application of two different topical formulations *viz.,* Temovate® cream and Temovate® emollient cream. The results revealed that the different formulations produced different

degrees of reservoir in the stratum corneum. This correlates with the biological or blanching effect observed for these formulations [112].

The absorption of CP may only be 5% of the dose applied to a healthy skin, as compared to greater absorption following application to inflamed or diseased skin state [11,18]. Better absorption of CP is likely to occur from an ointment than a cream vehicle. A peak flux of 32.3 ng/ml/hr versus 23.1 ng/ml/hr was observed from the occlusive ointment. *In vitro* skin penetration studies have also revealed that gel formulations are absorbed more rapidly than cream formulations [113]. However, creams containing-emollient compounds may act as moisturisers that soften and soothe dry skin conditions such as moderate-to-severe dermatoses. The degree of hydration thus increases the penetration of API into the skin [7].

The bioavailability of CP in formulations manufactured using different vehicles was conducted by Franz *et al.* [108]. The bioavailability studies were assessed on scalp skin using Skin Cap® formulation and two FDA approved CP formulations *viz.,* Olux® foam and Temovate® scalp application. There was no significant difference in the percutaneous absorption of CP between Skin cap® and Olux® foam or between Olux® foam and Temovate® scalp application when applied under unoccluded conditions. However a greater absorption from Olux® foam under occluded conditions was observed [108].

1.7.2. Distribution

No human data are available regarding the systemic distribution of corticosteroids following topical application [8]. A study was conducted, in which five formulations *viz.,* foam, cream, emollient cream, lotion and solution in which CP was radio labelled, prior to application to the skin. During testing, samples were withdrawn every four hours and analysed for CP content. Following exposure for 24 hours, the skin surface was stripped twice and the CP content analysed using liquid scintillation. The results revealed that CP foam produced a quicker initial permeation of the API than the other formulations. The distribution profile showed that the foam vehicle also produced a greater percentage of accumulated CP in the collection fluid (5.9%) at 12 hours. The CP foam thus delivers the drug more efficiently [29].

1.7.3. Metabolism and Elimination

Once percutaneous absorption has occurred, a systemically absorbed topical corticosteroid undergoes elimination via pharmacokinetic pathways similar to those involving systemically administered corticosteroids [114-116]. Since circulating levels of topically administered compounds are usually below the level of detection of many analytical methods, no data relating to the metabolism of CP following topical administration, are available as yet. The small amount of CP that may be absorbed,

is metabolized in the liver. Clobetasol and its metabolites are excreted in bile and urine of animals [18].

1.8 CONCLUSION

Major structural modifications of the original glucocorticoids have led to the development of synthetic corticosteroids that have been highly effective for the treatment of chronic skin conditions. CP is one of the most potent topical corticosteroids, commercially available as a concentration of 0.05% w/w of the steroid [10-12]. Due to its potency, CP formulations are usually applied in a thin layer to the surface of the skin and there are a number of high risk groups in which CP must be administered with caution. CP has potent anti-inflammatory, anti-pruritic, vasoconstrictive and anti-proliferative properties, and this molecule is used for the treatment of chronic dermatitis, eczema and lupus erythromatosus [7,10,12,13,16]. CP has a high partition coefficient of 3.5, and therefore the compound will distribute into the lipid bilayers of skin cells from aqueous-based formulations [39]. This drug is highly insoluble in water but soluble in organic solvents such as alcohol, dichloromethane and methanol. Methanol was therefore used as the organic modifier in HPLC method development and validation. Since CP formulations are sensitive to light, they must be packed in tightly closed containers and stored away from light and heat [17]. CP is however regarded as a stable compound but a deeper understanding relating to the stability of CP is crucial for the development of topical dosage forms. Dermovate® is the innovator CP-containing cream that is commercially available in South Africa. This product is relatively expensive and therefore there is a need for the development and manufacture of a generic product for this compound. However many challenges regarding industrial manufacturing of generic cream formulations exist, including batch-to-batch and within-batch variations, formulation performance, stability and scale-up challenges. Overcoming some of these challenges is one of the important objectives of the research undertaken in this project and is reported in this thesis.

CHAPTER TWO

DEVELOPMENT AND VALIDATION OF AN HPLC METHOD FOR THE ANALYSIS OF CLOBETASOL 17-PROPIONATE IN SEMI-SOLID DOSAGE FORMS

2.1 INTRODUCTION

2.1.1. Overview

Clobetasol 17-propionate (CP) is one of the most commercially available potent corticosteroids and it is usually used at a concentration of 0.05% w/w in topical formulations, for the treatment of serious dermatological conditions [10-13]. Therefore the analysis of CP is most often undertaken to assess the content of creams, gels, ointments, lotions and solutions [7,18,31,34]. In order to optimise drug penetration through the skin while minimising the side effects of the molecule, novel particulate carrier systems such as nanoparticles, may be appropriate delivery vehicles [37]. Since these formulations are new, they are still undergoing clinical testing to establish toxicological issues, relating to such delivery technologies [30]. Therefore patients continue to rely on traditional topical products to alleviate the symptoms of their skin conditions. Reported methods have suggested techniques for the analysis of CP in pharmaceutical formulations based on spectrophotometric, Thin Layer Chromatography (TLC) coupled with densitometric evaluation, High Performance Liquid Chromatography (HPLC) with UV diode array or particle-beam-mass detectors, Normal and Reversed-Phase High Performance Liquid Chromatography (NP-HPLC, RP-HPLC), Liquid Chromatography–Mass Spectrometry (LC–MS) and LC–MS–MS [87,117]. The 2002 British Pharmacopoeia (BP) [118] recommended the use of a TLC assay for the quantification of CP. However more recent compendial methods, in the 2008 and 2010 BP [43,119] and 2005 and 2009 United States Pharmacopoeia-National Formularies (USP-NF) [45,64,120,121] recommend HPLC as the preferred analytical method as the technique is specific, accurate and precise.

The aim of these studies was to develop, optimise and validate a RP-HPLC method using UV detection for the quantitation of CP in laboratory scale and scale-up topical formulations containing CP. The method was to be simple, rapid, sensitive, reproducible and stability-indicating to generate appropriate results.

2.1.2. Principles of RP-HPLC

Liquid Chromatography (LC) is a procedure in which a mobile liquid phase facilitates the separation. Thin layer and paper chromatography are older examples of LC, whereas High Performance or High Pressure Liquid Chromatography (HPLC), Ultra-Performance Liquid Chromatography (UPLC), high speed LC and simple liquid chromatography are more modern techniques [122,123]. Modern LC has several advantages over the older LC methods in terms of convenience, accuracy, speed and the ability to develop and achieve difficult separations [123,124]. Liquid chromatographic separations occur as a result of specific interactions between sample molecules and the stationary and liquid

phases used to achieve a separation. To achieve a good separation, the stationary and mobile phases can be modified according to the properties of the analyte of interest. Furthermore a variety of detectors is available for use in LC [123,124]. HPLC methods of analysis can be divided into four types *viz.*, liquid-liquid, liquid-solid, ion-exchange and size-exclusion chromatography [124,125].

HPLC is a chromatographic technique of choice, with many applications in pharmaceutical processes in addition to research and development [126]. However it is also a practical analytical tool that has been applied in the cosmetic [117,127-129], biotechnology [130,131], biomedical [132-134], environmental [135-137], food [138-140] and biochemical [141,142] industries. In the pharmaceutical industry, HPLC is more widely used than other analytical techniques since sample preparation is rapid and records of qualitative and quantitative data, for samples of widely varying polarity, can be generated in a single run. Furthermore various forms of detection can be applied to increase the specificity and information generated from an assay, thus regulatory agencies consider HPLC a mature and reliable procedure of analysis [126]. Over the past 15 years, HPLC has had a dramatic impact on the development of drug candidates, from drug discovery to commercialisation [126].
HPLC has been diversified according to the type of interaction that occurs between an analyte and stationary phase and the differences in polarity between a stationary phase and a mobile phase. Consequently, in Normal-Phase HPLC (NP-HPLC), a polar stationary phase and a non-polar solvent are used to separate moderate-to-strongly polar compounds. However it is usually a second choice technique for most analyses when Reversed-Phase HPLC (RP-HPLC) is ineffective. NP-HPLC is a first choice technique when lipophilic samples that do not dissolve well in water and/or organic mixtures are to be analysed [143]. However RP-HPLC is more commonly used than NP-HPLC, because it can be easily implemented for routine analysis and eliminates the need to use deleterious organic solvents [144]. RP-HPLC usually makes use of a non-polar stationary phase and a polar solvent to separate low polarity compounds. RP-HPLC is the method of choice for the analysis of neutral or non-ionised compounds that dissolve in water and/or organic mixtures [143]. A third LC technique is termed ion-exchange chromatography, and relies on ionic interactions between an analyte and stationary phase to achieve a separation. Molecular interactions are involved in all three types of HPLC methods and include polar forces in NP-HPLC, dispersive forces in RP-HPLC and ionic forces in ion-exchange separations [145].

The separation of pharmaceutical analytes usually takes place on surface reacted or chemically bonded organic stationary phases or Bonded-Phase Chromatographic (BPC) columns [123]. In NP-HPLC, the stationary phase plays a major role in the retention of an analyte. Polar BPC column packing materials usually found in NP-HPLC columns include porous oxides such as silica (SiO_2) or alumina (Al_2O_3). The surface has a dense population of hydroxyl (–OH) groups, thereby making it polar. The stronger the analyte-stationary phase interaction, the longer the analyte retention [145]. The analyte molecules compete with mobile phase molecules for the adsorption sites on the surface(s) of the stationary phase. Mobile phases used in NP-HPLC are usually non-polar and the addition of a

polar solvent or modifier may therefore have an effect on the retention characteristics of an analyte [145]. The most commonly used stationary phase in RP-HPLC is a non-polar BPC with porous silica packing materials. The use of such stationary phases results in longer retention times for non-polar molecules, whereas polar molecules elute relatively rapidly. However the addition of more water to a mobile phase increases the affinity of hydrophobic analytes for the hydrophobic stationary phase, resulting in longer retention times [145]. The spreading of molecules of a specific compound along a column occurs where sample molecules follow different paths along the packed bed. Such movement is dependent on the flowstreams followed due to eddy diffusion. If the solvent velocity is high, the molecules will move rapidly or further down the column in a specific time and this will determine the degree of separation of compounds [123].

The successful separation and retention of compounds is usually achieved by partitioning or adsorption and/or a combination of both, depending on the type of stationary phase and polarity of the mobile phase. Partitioning indicates that a solute becomes fully embedded in the stationary phase, whereas in adsorption the solute interacts only at the surface of the stationary phase [123,146]. The amount of an analyte and the surface tension of a mobile phase are important factors affecting the retention time of an analyte. The interaction of an analyte and a stationary phase is proportional to the contact surface area around the non-polar part of the analyte molecule associated with a ligand in an aqueous solvent. The solvophobic theory describes the importance of the aqueous solvent, in reducing the surface area of non-polar solutes that forms cavities around the analyte and the C_{18} chains in order to accommodate the solute of interest. Thus the energy released in the separation process is proportional to the surface tension of the water in the mobile phase and the hydrophobic surface area of the analyte. The retention time can therefore be decreased by adding a small volume of polar solvent to the mobile phase in order to reduce the surface tension of the liquid [147,148].

In addition, the molecular structure and associated properties of an analyte play an important role in the retention characteristics of a solute in RP-HPLC. Sample molecules with a large number of hydrophobic functional groups will be retained longer than hydrophilic molecules since the non-polar surface area of the molecule is larger and this part of the molecule does not interact with the aqueous component of the mobile phase. The addition of an alkyl carbon such as a methyl group to an analyte can increase the retention time by 1.5- to 2.5-fold. However the addition of a polar functional group, such as a hydroxyl group to a molecule, may decrease its retention time by 0.2- to 0.3-fold since the polar compound is well integrated with the aqueous compartment of the mobile phase and will therefore elute rapidly [143].

2.2 PUBLISHED METHODS FOR THE ANALYSIS OF CLOBETASOL-17 PROPIONATE

A review of the literature for manuscripts, in which the analysis of CP in formulations was reported, was conducted prior to the development and subsequent validation of an HPLC method. A summary of all conditions reported for the analysis of CP is listed in Table 2.1.

Most reported methods of analysis for the determination of CP were performed on topical or cosmetic formulations, more specifically creams, ointments, sprays and shampoos [8,11,87,117,149-151]. However in two recent reports, analyses were performed on novel nanoparticulate delivery systems [37,152].

The analysis of CP has been achieved using silica matrix support columns, mainly C_{18} and the most common column reported was a Nova-Pak® $C_{18,}$ 150 mm x 3.9 mm [8,87,150,151]. Acetonitrile (ACN) was the solvent of choice for most separations [8,37,87,117,149-151]. Due to world-wide shortage and high cost of ACN, methanol (MeOH) was considered for use as a preferred solvent for the RP-HPLC method developed in these studies [10,11,150,152].

HPLC with UV detection was the preferred method of analysis for CP, and it is well-defined in the literature [8,10,11,30,37,87,149,150,152] and the USP-NF [45,64,120,121]. A flow rate of 1.0 ml/min was commonly used, with a detection wavelength of 240 nm for most procedures [8,37,45,87,150]. Since CP is neutral and does not dissociate in solution [18], the use of buffers and pH modification were considered unnecessary. Therefore only appropriate changes in the organic modifier content could be manipulated to produce a desirable peak shape and appropriate retention times for analysis.

Table 2.1. *Summary of published analytical methods used for the determination of CP*

Samples	Column	Technique	Mobile Phase	Flow rate (ml/min)	λ (nm)	RT (min)	Ref.
Cream, spray, shampoo	Nova-Pak® C_{18}, 150 mm x 3.9 mm, with Inertisil® ODS 2 guard column, 5 µm, Waters®	RP-HPLC	Acetonitrile:Water (50:50% v/v)	1.0	240	7.0	[87]
Nanoparticles	Luna® C_{18}, 150 mm x 3 mm, Phenomenex®	HPLC	Acetonitrile:Water (55:45% v/v)	1.0	240	-	[37]
Nanoparticles	Hypersil® C_{18}, 150 mm x 3.9 mm, Thermo Specific®	HPLC	Methanol:Water (74:26% v/v)	-	240	-	[152]
Cosmetic formulations (cream, lotion, ointment, shampoo)	Purospher-Lichrocart®, 250 mm x 4.0 mm, 5 µm, Merck®	HPLC	Acetonitrile:Water (40:60% v/v)	1.0	237	-	[117]
Cream	Lichrospher® C_{18}, 125 mm x 4.0 mm, Merck®	HPLC	Methanol:Water (65:35% v/v), pH 3.5, 0.05% acetic acid	1.1	240	9.0	[10]
Cosmetic formulations	Encapped Purospher® RP-18, 250 mm x 4.65 µm, Merck®	HPLC	Acetonitrile:Water (60:40% v/v)	1.0	239	3.42	[149]
Topical formulations	i)Symmetry® C_{18} , 75 mm x 4.6 mm, 3.5 µm , Waters® ii)Nova-Pak® C_{18} , 150 mm x 3.9 mm, 4 µm, Waters®	RP-HPLC	i)Acetonitrile:Water (18:72% v/v) ii) Methanol:Water (38:62% v/v)	1.0 -	240 -	11.40 11.06	[150]
Cream, ointment	Ultra aqueous C_{18} (USP L1), 150 mm x 4.6 mm, 3 µm, Restek®	HPLC	Acetonitrile, 0.05M monobasic sodium phosphate (adjusted with 85% phosphoric acid to a pH of 2.5) and methanol	1.0	240	1.1	[45]
Cream, ointment	Nova-Pak® $C_{18,}$ 150 mm x 3.9 mm, 5 µm, Waters®	RP-HPLC	Methanol:Water (70:30% v/v)	1.0	254	4.7	[11]
Cream	Nova-Pak® $C_{18,}$ 150 mm x 3.9 mm, 4 µm, Waters®	RP-HPLC	Acetonitrile:Water (50:50% v/v)	1.0	240	8.2	[8]

2.3 EXPERIMENTAL

2.3.1. Chemicals and Reagents

All chemicals used were at least of analytical reagent grade. Micronised CP USP standard was purchased from Symbiotec® Pharmalab P. Ltd (Indore, India). The bulk drug was used for manufacture of laboratory and scale-up cream formulations. The internal standard, Betamethasone 17-Valerate (BV) was purchased from Sigma® Chemical Co. (St Louis, USA). Dermovate® 0.05% w/w cream (Batch number 274665, Sekpharma® Pty Ltd, Sandton, Gauteng, South Africa), the innovator product that is available on the South African Market, was purchased from a local pharmacy.

HPLC grade water was prepared by reverse osmosis, using a Milli-RO® 15 water purification system (Millipore®, Bedford, MA, USA), consisting of a Super-C carbon cartridge, two Ion-X® ion-exchange cartridges and an Organex-Q® cartridge. The water was filtered through a 0.22 μm Millipak® stack filter (Millipore®, Bedford, MA, USA). HPLC grade MeOH-215 far UV Romil-SpS® Super Purity Solvent (Romil® Ltd, Waterbeach, Cambridge) was used as the organic modifier and was filtered through a 0.45 μm Millipore® HVLP membrane filter (Millipore®, Bedford, MA, USA) prior to use.

2.3.2. Preparation of Stock Solutions

Approximately 10 mg of CP was accurately weighed using a Mettler® Model AE 163 analytical balance (Mettler® Inc., Zurich, Switzerland), and transferred into a 100 ml A-grade volumetric flask. Sufficient volume of MeOH was added to dissolve the CP, and the solution was sonicated using a Branson® B12 sonicator (Branson® Inc., Shelton, Conn, USA) for two minutes to ensure complete dissolution. The solution was then covered with foil since CP solutions are sensitive to light. The solution was made to volume with MeOH, to produce a stock solution of 100 μg/ml of CP. The standard stock solution was diluted in a serial manner with MeOH to produce CP solutions of concentrations of 0.15, 0.8, 2, 4, 8, 12, 15 μg/ml respectively.

A stock solution of the internal standard was prepared by accurately weighing approximately 5 mg of BV on a Mettler® Model AE 163 analytical balance (Mettler® Inc., Zurich, Switzerland) and subsequently dissolving the material in 20 ml MeOH in a 20 ml A-grade volumetric flask, to produce a concentration of 250 μg/ml. An 800 μl aliquot volume of the BV stock solution was pipetted into a 20 ml A-grade volumetric flask and made to volume with MeOH to produce a solution with a final concentration of 10 μg/ml.

2.3.3. Preparation of Mobile Phase

A mobile phase comprised of MeOH:Water (68:32% v/v) was prepared as indicated below. Appropriate volumes of MeOH and water were measured separately, using 1000 ml and 500 ml A-grade measuring cylinders and were then mixed in a 1000 ml Schott® Duran bottle (Schott® Duran GmbH, Wertheim, Germany). The mobile phase was prepared on a daily basis and was filtered through a 0.45 μm Millipore® membrane HVLP filter (Millipore®, Bedford, MA, USA) and degassed under vacuum using an Eyela® Aspirator A-2S vacuum pump (Rikakikai® Co. Ltd, Tokyo, Japan) prior to use. HPLC can be a sensitive stability-indicating technique, particularly at low UV wavelengths during analysis. Consequently dissolved gases such as oxygen and nitrogen can affect the flow stability and/or UV absorption, which in turn can cause baseline drift and random noise in the detector output. Vacuum degassing provides an effective way of removing these gases, in order to prevent formation of bubbles at the pump inlet and to promote effective mobile phase absorbance, in particular with MeOH [153,154].

2.3.4. HPLC Apparatus

HPLC System A

The method of analysis for CP in topical pharmaceutical dosage forms was developed and validated on a modular HPLC system. The HPLC system consisted of a Spectra-Physics® SP 8810 precision isocratic pump (Spectra-Physics® Ltd ,San Jose, California, USA), an automated sample injection system Model 710B (WISP, Waters® Associates, Milford, MA, USA) and a variable UVIS 200 detector (Linear Instruments Corporation, Reno, NV). Chromatograms were recorded on a Perkin-Elmer® Model 561-3002 chart recorder (Perkin-Elmer® Ltd, Hitachi, Japan) during method development. The stationary phase was a Nova-Pak® C_{18} column, 3.9 mm x 150 mm, 4 μm (Waters® Corporation, Milford, MA, USA).

HPLC System B

For the development of an *in vitro* release method of CP and the *in vitro* assessment of laboratory and scale-up CP topical formulations, a Spectra-Physics® SP 4290 Integrator (Spectra-Physics® Ltd, San Jose, CA, USA) was used for all subsequent data capture.

2.4 METHOD DEVELOPMENT AND OPTIMISATION

2.4.1. Column Selection

The analytical column is the heart of an HPLC system and is essential for the separation of compounds that would otherwise be difficult and tedious [143]. The stationary phase is usually located in a column or tube with appropriate fittings, to perform the separation procedure. Columns must be able to withstand the back-pressure generated during use, otherwise the stationary phase

materials will be easily damaged, thereby reducing the life of the column. Most columns are made of stainless steel to withstand the high back-pressures encountered with HPLC [143,155].

The selection of an appropriate column is critical for the development of a rugged, stability-indicating and reproducible analytical method. There are a variety of commercially available columns that vary in plate number, band symmetry, retention, band spacing and lifetime characteristics [143]. In most laboratories, the most commonly applied HPLC technique is RP-HPLC and this approach usually makes the use of the C_{18} columns a popular choice. The stationary phases are readily accessible and are packed into inexpensive columns. Stationary phases are readily available in a wide range of particle size, carbon loading and degree of end capping, ensuring versatility of use [156].

Separation is usually achieved by two mechanisms *viz.*, i) mechanical separation achieved through column length, column packing, packing uniformity and particle size, and ii) chemical separation generated by physicochemical competition of the analyte(s) of interest with components of the packing material and mobile phase. Mechanical separation power is also known as column efficiency and is usually measured by the theoretical plate number (N). As the length of an HPLC column increases, the mechanical separation power also increases. This may result in long analytical run times and greater solvent consumption when using the method. A reduction in the particle size of a stationary phase may cause a reduction in the plate height of the column and an increase in the resolution power of the column [123]. Therefore columns packed with stationary phases will operate more by mechanical separation but will have higher back-pressures than larger particle-size columns [155]. In addition dense, tightly compacted and uniform column beds may also result in high column efficiencies [123]. HPLC columns of 3.9 mm i.d x 150 mm length with particle size of 4 μm were selected for analysis of CP in topical dosage forms.

The most modern HPLC column packing materials are produced using silica-based matrices. One advantage of silica particle packings is that these packing materials are able to withstand the relatively high pressures generated during manufacture and use. Silica particle packings provide high mechanical strength, thus ensuring long column life. The silica-based packings are usually found in stable columns, permitting high column efficiencies [123,143]. These packings are compatible with water and most organic solvents. Therefore no swelling of the particles occurs if a change is made in organic solvent in the mobile phase. This resistance to solvent effects enhances column stability and prolongs column use as the packing bed is stable during use. However silica is not an ideal packing material for all HPLC columns since it is soluble at low and high pH [143]. At pH values ≥ 9, soluble silica precipitation may occur and some silica supports dissolve rapidly in the mobile phase, which may result in collapse of the packing bed. Silica-based matrices are not desirable for the separation of basic compounds since they contain silanol groups that are highly acidic in nature. This may result in binding of basic solutes to the stationary phase, with a resultant increase in the retention time and

broad peak tailing. In many cases, low-purity acidic or type A silicas are used to separate neutral and non-ionisable compounds [143].

Since CP is a neutral molecule and does not require the manipulation of pH to effect a separation, a reversed-phase silica-based column was selected for this separation. A Nova-Pak® cartridge packed with dimethyl octadecylsilyl amorphous silica was used for the analysis of CP of cream formulations manufactured in these studies. The Nova-Pak® column was successfully used to evaluate CP in topical formulations such as creams, ointments, sprays and even shampoos [11,87].

2.4.2. Column Evaluation and Specifications

Prior to developing an HPLC method, chromatographic performance tests were performed to ensure column quality. Column quality or performance is measured by the number of theoretical plates, retention time, peak symmetry and column pressure drop [123].

2.4.2.1. Column Efficiency

Column efficiency can be assessed by calculating of the theoretical plate number (N) of a column using Equations 2.1 and/or 2.2.

$$N = 16\left(\frac{t_R}{W}\right)^2 \qquad \textit{Equation 2.1}$$

$$N = 5.54\left(\frac{t_R}{W_{1/2}}\right)^2 \qquad \textit{Equation 2.2}$$

Where

N=Number of theoretical plates in a chromatographic column
t_R=Retention time of the peak
W=Peak width at baseline determined by the tangent
$W_{1/2}$=Peak width at half of peak height

As N increases, the resolution for all bands increases. This results in an appropriate separation. The plate number N can be increased by using densely packed columns, longer columns, low flow rates, small diameter column-packing particles, low molecular sample molecules and mobile phases of low viscosity [143].

Prior to HPLC method development, a column test was conducted at ambient temperature (22°C). The test solution was comprised of uracil, acetophenone, benzene, toluene and naphthalene. Separation of these compounds was achieved with a Nova-Pak® C_{18}, 150 mm x 3.9 mm i.d., 4 µm, at a flow rate of 1.5 ml/min. A mobile phase of ACN and water in a ratio of 50:50% v/v was used as reported in Waters® HPLC column literature [155]. The column was found to have a plate count number of approximately 8000 and was therefore considered appropriate for use in the HPLC method

development and validation. Ideally, according to FDA guidelines [157], N depends on the elution time but should generally be greater than 2000.

2.4.2.2. Peak Asymmetry Factor

The Peak Asymmetry Factor (A_s), also known as the Peak Tailing Factor (PTF), is an important parameter for assessing band shape. A_s or PTF can be assessed using Equation 2.3 or 2.4. Symmetrical peaks should have a value for A_s close to 1.0 (range: 0.95–1.1) and columns producing A_s values of > 1.2 should preferably not be used [123].

The peak asymmetry factor [123] is defined by

$$A_s = \frac{B}{A} \qquad \textit{Equation 2.3}$$

The peak tailing factor [123] is defined by

$$PTF = \frac{(A+B)}{2A} \qquad \textit{Equation 2.4}$$

Where

A_s=Peak asymmetry factor
PTF=Peak tailing factor
B=Distance between the middle point and the right side of the peak
A=Distance between the middle point and the left side of the peak

Peak asymmetry is usually measured at 10% of the full peak height, whereas peak tailing is calculated at 5% of the full peak height (Figure 2.1). The A_s factor calculated for CP peak was found to be 1.13, with a % RSD = 1.67. The PTF measured for CP peak was 0.83, with a % RSD = 0.84. Consequently the Nova-Pak® C_{18}, 150 mm x 3.9 mm i.d., 4 µm column used was satisfactory, with A_s values close to 1.0. Since the PTF for CP peak was low, less peak tailing was observed. The FDA Guidance [157] recommends that PTF should be less than or equal to 2.0 for a column to be considered appropriate.

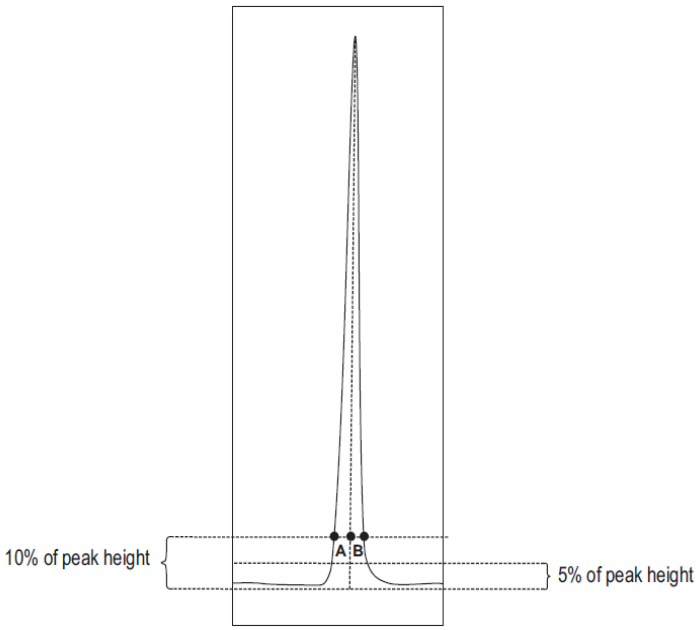


Figure 2.1. *Calculation of peak asymmetry and peak tailing [123]*

2.4.2.3. Resolution Factor

The Resolution Factor (R_s) is a useful indicator of the degree and quality of a separation between components of a mixture. The resolution is normally a measure of the extent of separation between two or more peaks of interest and can be calculated using Equation 2.5 [157].

$$R_s = \frac{(Rt_2 - Rt_1)}{1/2(t_{w1} - t_{w2})} \qquad \textit{Equation 2.5}$$

Where

R_s=Resolution factor
Rt_1=Retention time for first eluting peak
Rt_2=Retention time for second eluting peak
t_{w1}=Width of first eluting peak at the base
t_{w2}=Width of second eluting peak at the base

A resolution factor of > 2.0 indicates that an appropriate separation has been achieved between peaks and that desirable resolution has been attained [157]. Values below 1.5 are indicative of poor resolution between peaks and are therefore unacceptable [118,157]. The values for R_s generated for CP and BV were found to be 3.96, with a % RSD of 0.79, indicating that appropriate resolution between the two peaks has been achieved.

2.4.2.4. Capacity Factor

The Capacity Factor, k^1 is described as the migration rate of an analyte on a column [118] and is a measure of the retention time of the peak of interest relative to the peaks of the void volume [157]. The value for k^1 usually varies according to the mobile phase composition and the age of a column. k^1

is also affected by changes in the operating temperature of a separation [158]. The capacity factor can be calculated using Equation 2.6.

$$k^1 = \frac{V_1 - V_o}{V_o}$$ ***Equation 2.6***

Where

k^1= Capacity factor of the column
V_o= Void volume of the column
V_1= Retention volume of the peak of analyte (V_1= Retention time x Flow rate)

The parameter V_o is measured using the retention time of a molecule that is not retained on the column. Uracil was used as a non-retained molecule, giving a k^1 value of 10.6. Substances producing a capacity factor $\geq$ 2.0 are deemed acceptable. Therefore the peaks for uracil and CP were deemed to be well resolved from each other [157].

2.4.3. Method of Detection

The method of detection to be used is heavily dependent on the physicochemical properties of the analyte of interest, the sensitivity of the system, the concentration of drug to be analysed and the composition of the formulation to be assessed [151,159]. Moreover when choosing a detection system, it is important to ensure that the method is stability-indicating and selective for a particular Active Pharmaceutical Ingredient (API) [159]. Most reported methods for the HPLC analysis of CP use UV detection, since most steroid API absorb energy in the presence of UV irradiation at a wavelength of 254 nm [159]. The linear range offered by a UV detector is between 0.0001 and 2 absorbance units full scale. Therefore solutions to be analysed should preferably be at low concentrations within this range, to avoid any deviation from the Beer Lambert Law [160].

Prior to HPLC method development, a solution of CP in MeOH:Water (68:32% v/v) was scanned over a wavelength of 210-300 nm using a dual beam UV-VIS Model GBC 916 spectrophotometer (GBC Scientific Equipment Pty Ltd, Melbourne, Victoria, Australia) (§ 1.2.7, Chapter 1). A maximum absorption wavelength of 239 nm was observed and was found similar to reported values [39]. Therefore a wavelength of 239 nm was selected for use during HPLC method development and *in vitro* analyses of the formulations manufactured in these studies.

2.4.4. Choice of Internal Standard

An Internal Standard (IS) is often applied in HPLC-UV spectrophotometric analyses in order to improve the performance of the analytical method. The addition of an IS is useful in quantitative analysis as it minimises analytical errors that may easily be introduced during sample preparation or due to the analytical equipment and the technique itself [123,143,161,162]. The use of an IS usually compensates for the loss or gain in sample size or any change in sample concentration, which can occur during sample manipulation, filtration and/or extraction processes [143,162]. An IS should have

some similarity to the analyte of interest [123,143,162,163] and should mimic the behaviour in any procedure [143]. In this way, a suitable and viable IS can improve accuracy, precision (inter-assay and intra-day) and robustness during quantitation for any chromatographic analysis [164-166].

A number of drugs with structural characteristics similar to CP were selected for evaluation as potential internal standards. Betamethasone 17-valerate, mometasone furoate, betamethasone disodium phosphate, norethisterone acetate, ethinyl oestradiol, fluperolone acetate and desoxycorticosterone were tested. The chromatographic behaviour of these compounds was assessed using a mobile phase of MeOH:Water in a ratio of 68:32% v/v at a flow rate of 0.9 ml/min and the results are summarised in Table 2.2. Only betamethasone 17-valerate was found to provide adequate separation and was selected as the IS, based on resolution, retention time and peak shape parameters.

Table 2.2. *Choice of IS for CP in mobile phase*

Internal Standard	**Retention time (minutes)**	**Peak shape**	**Comments**
Betamethasone 17-valerate	8.0	Peak was well resolved, sharp, symmetrical with no tailing	Both retention time and peak shape were ideal for CP
Mometasone furoate	6.5	Peak was sharp with slight tailing	Retention time was too close to CP, peaks overlapped
Betamethasone disodium phosphate	-	No peak	No peak was observed after 20 minutes
Beclomethasone dipropionate	13.4	Peak was sharp with slight tailing	Retention time was quite far from CP
Norethisterone acetate	7.0	Peak was sharp with distinct tailing	Reasonable retention time but distinct tailing
Ethinyl oestradiol	3.6	Peak was sharp with no tailing	Retention time was too close to solvent front
Fluperolone acetate	2.9	Peak was sharp with slight shouldering	Retention time was too close to solvent front
Desoxycorticosterone	6.5	Peak was sharp, symmetrical with no tailing	Retention time was too close to CP

2.4.5. Mobile Phase and Flow Rate Selection

The composition of the mobile phase is a critical factor in achieving a suitable separation and retention time [167]. The choice of mobile phase components and composition have a profound effect on the ease of analysis and sensitivity of an HPLC detection system [143]. It is a fundamental consideration that the mobile phase does not absorb UV light at the wavelength to be used for analysis. Therefore it is critical that the cut-off wavelength of the solvents of choice is known. If an organic solvent absorbs UV radiation at the wavelength of detection, the detection of the analyte of choice will be compromised [143]. The UV cut-off of the MeOH used in the mobile phase in these studies was 215 nm.

RP-HPLC usually requires the use of organic solvents such as MeOH, ethanol, ACN and tetrahydrofuran in preparation of mobile phase [143,168]. These four solvents are used to control the selectivity of a separation of the components of a matrix to be analysed [143]. In general, the mobile phases used in an HPLC separation consist of binary mixtures of an aqueous phase *viz.*, water and buffer or water and an organic phase such as MeOH [167]. The solvents used in RP-HPLC should be of high quality and free from impurities, dust, particles and dissolved gases [169] so that the chromatographic instrumentation and the analytical results are not compromised. Vacuum degassing is therefore performed during HPLC method development, validation and all subsequent analyses. This vacuum degassing procedure alone, or in combination with filtration, is an inexpensive and effective method and it is performed to ensure that a separation is successful.

Organic solvents are used in HPLC separations as they are able to modify the polarity and selectivity of the mobile phase [143]. The polarity of an organic solvent for a specific sample must be considered when selecting a solvent for use. It is not advisable to choose a mobile phase of similar polarity to the analyte of interest since the compound will be poorly retained, resulting in rapid elution of the API. Therefore the retention time and peak shape may be adjusted using different concentrations of the organic modifier [143]. Since CP is a hydrophobic molecule, and a mobile phase of MeOH and water will be polar, there will be little or no affinity between the molecule and the mobile phase. CP will be retained on the stationary phase and separation will be better compared to that if non-polar solvents were used.

Since CP and BV are neutral molecules, pH and molarity of a mobile phase are unlikely to have any effect on the retention times of these compounds. Most HPLC methods for the analysis and quantitation of CP use ACN in the mobile phase using gradient chromatography (Table 2.1). This may be due to its low viscosity, resulting in low back-pressures on the analytical column. Furthermore ACN-Water mixtures can be used at low UV wavelengths as ACN has a UV cut-off of 205 nm [143]. However, in late 2008, an acute shortage of ACN worldwide occurred [170-172]. The primary reason behind this is due to the downturn in the global economy, which led to a reduced demand for acrylonitrile products [171,172]. Therefore it was critical to find alternative non-ACN eluants such as MeOH, ethanol and n-propanol that could be used for analytical and purification purposes [172]. MeOH is a relatively cheap solvent and is readily available. This organic solvent is an affordable renewable source that is less toxic and harmful to human health [173-176].

The flow rate of a mobile phase is another important factor in RP-HPLC. It is fundamental that flow rate be kept constant throughout all experimental procedures to ensure that precise retention time and appropriate peak shape are obtained. Variations in flow rate can however occur as a result of partial failure of the valve systems, blockage of the column and/or changes in the viscosity of the mobile phase [123]. Therefore changes in MeOH content and flow rate were investigated to establish their

effect on the retention times and resolution of CP and BV. A wavelength of 239 nm was selected for these studies. The sensitivity of the detector was set at an Absorbance Unit Full Scale (AUFS) setting of 0.05.

2.4.5.1. Effect of Methanol (MeOH) Content

Since CP is a neutral analyte, there is no need to use a buffer to modify pH or molarity of the mobile phase. CP is a lipophilic compound and the use of an organic modifier may affect the retention time of this compound. Therefore the effects of different concentrations of organic modifier in the mobile phase on the retention times and the peak shapes of CP and BV were investigated. The organic modifier contents evaluated were 66% v/v, 68% v/v, 70% v/v, 72% v/v and 74% v/v.

An increase in the proportion of MeOH resulted in a reduction in the retention time of CP and BV as shown in Figure 2.2. At a MeOH concentration of 70% v/v, slight tailing of the BV peak occurred. As the concentration of MeOH increases from 72% v/v to 74% v/v, the tailing of the BV peak became distinct and at 74% v/v, peak resolution was poor as shown in Figure 2.3. A summary of the effects of mobile phase composition on CP and BV retention times, peak shapes and resolution is presented in Table 2.3. A mobile phase of MeOH:Water in the ratio of 68:32% v/v was further evaluated during HPLC method development and validation.

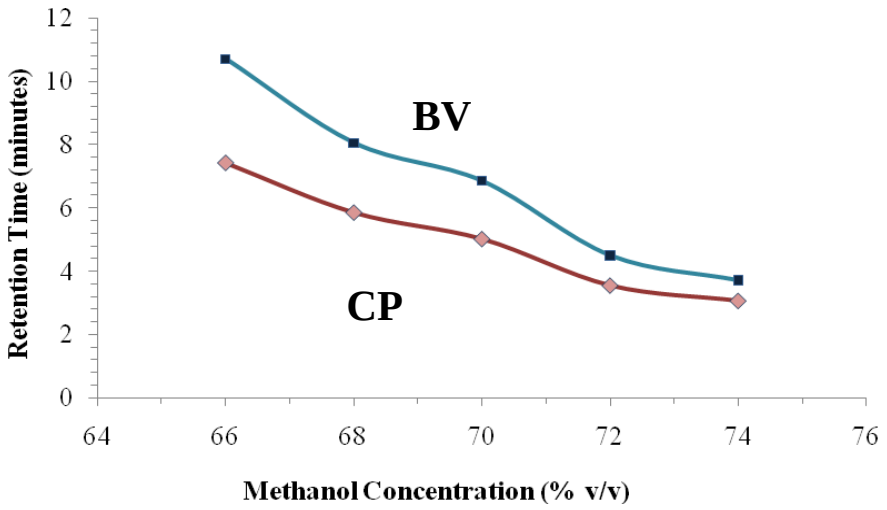


Figure 2.2. *The effect of methanol concentration on the retention times of CP and BV*

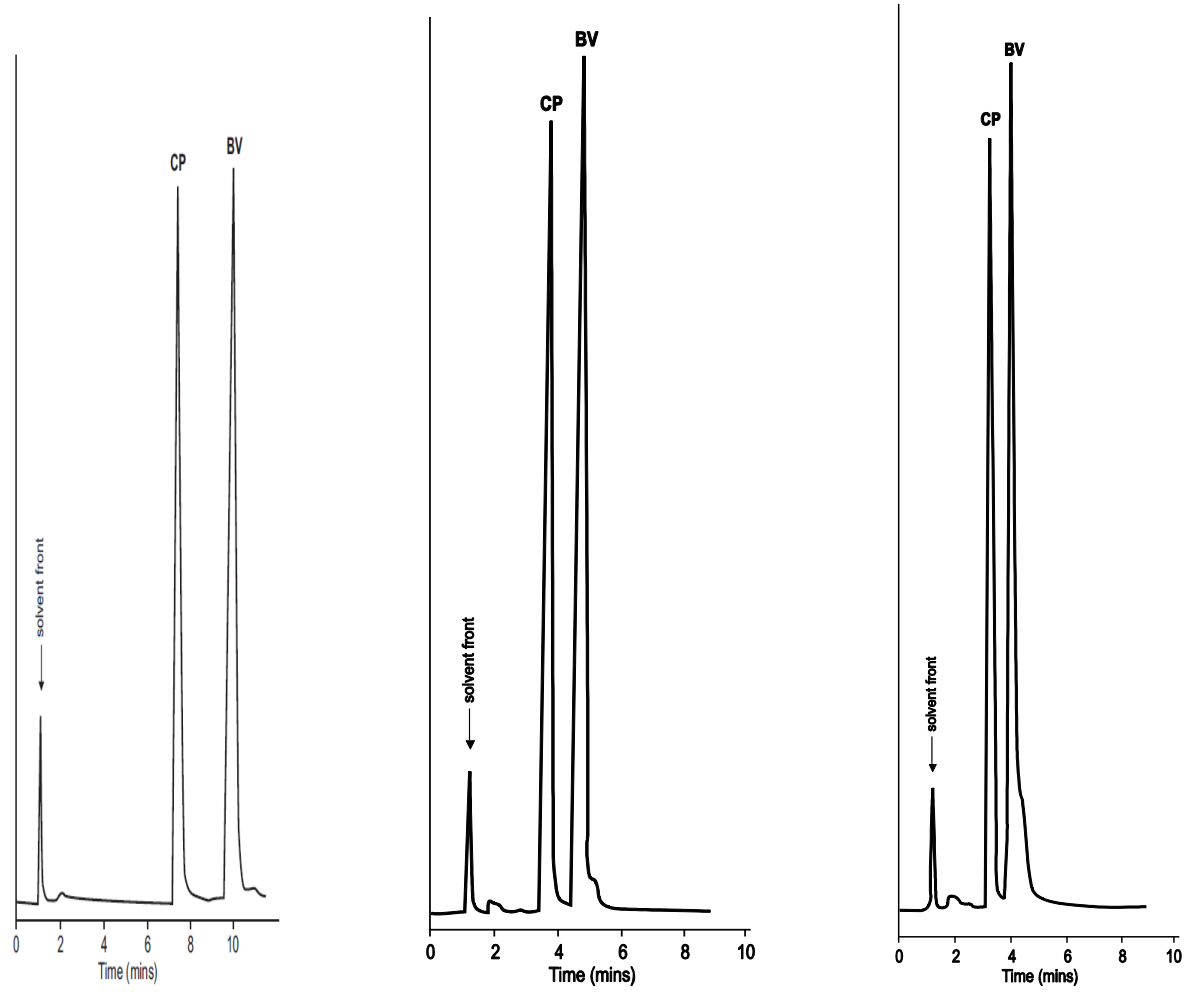


Figure 2.3. *The effect of the organic modifier on the retention times and peak shapes of CP and BV*

Table 2.3. *Effect of changing organic modifier concentration on CP and BV retention times*

	Retention time (minutes)			
% MeOH	**CP**	**BV**	**Peak shape**	**Comments**
66	7.4	10.7	Sharp, well resolved peaks with no tailing	Reasonable peak shape but quite long run time
68	6.0	8.0	Sharp, well resolved peaks with no tailing	Ideal CP and BV peak shape and appropriate run time of less than 10 minutes
70	3.8	5.1	Sharp, well resolved peak but BV showed slight tailing	BV peak tailing and run time decreased
72	3.6	4.5	Sharp CP peak but BV peak showed tailing	BV peak tailing and CP peak close to solvent front
74	3.4	4.0	Sharp CP peak but BV peak tailing increased. Peaks were not well resolved	BV peak tailing, poor peak resolution

2.4.5.2. Effect of Flow Rate

The data listed in Table 2.1 indicate that most HPLC methods used a flow rate of 1.0 ml/min for the analysis of CP. However, it was deemed necessary to investigate the effect of flow rate in this study. The flow rate was investigated over the range of 0.7-1.2 ml/min, specifically at flow rates of 0.7, 0.8,

0.9, 1.0, 1.1 and 1.2 ml/mins. Variations of flow rate may permit reduce the analysis time. However, an optimal flow rate may achieve appropriate separation of components in a matrix and may produce a stable baseline [177]. When the flow rate of a mobile phase increases, peak area, number of theoretical plates and retention time of compounds of interest will decrease [178]. Relatively low flow rates such as 0.7-0.9 ml/min consume less mobile phase and cause minimal damage to the solvent delivery module and the stationary phase. Relatively high flow rates of 1.0 ml/min to 1.2 ml/min will increase the back-pressure on equipment, which may reduce the efficacy of the solvent delivery module and stationary phase significantly.

As expected, an increase in flow rate resulted in a decrease in the retention time for CP and BV (Figure 2.4). At a flow rate of 0.7 ml/min in a mobile phase of MeOH:Water (68:32% v/v), the retention times of CP and BV were 8.1 minutes and 11.6 minutes respectively. At a flow rate of 0.9 ml/min, the retention times of CP and BV were 6.0 minutes and 8.4 minutes respectively and at the highest flow rate of 1.2 ml/min, the retention times of CP and BV were 4.8 minutes and 7.0 minutes respectively. Therefore a flow rate of 0.9 ml/min resulted in convenient retention times, adequate resolution and with little or no deleterious effects on the HPLC system and was appropriately selected for use.

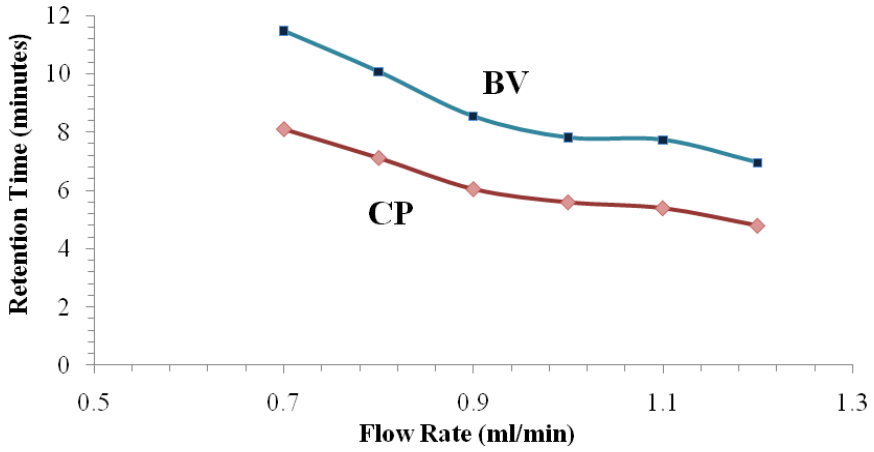


Figure 2.4. *The effect of flow rate on the retention times of CP and BV*

2.4.5.3 Volume of Injection

The volume of injection of a sample may influence the outcome of a HPLC separation. An increase in the volume of injection will increase the amount of an analyte that is loaded onto a column [143]. If a small injection volume is used, it may affect the retention characteristics, plate number and/or resolution of the peaks of interest. With too large sample volume, the resultant peak will be broad and the plate number will increase. However the retention time will decrease, resulting in poor resolution due to a volume overload [123,143]. Therefore to avoid undesirable effects on peak shapes and retention times, it was necessary to investigate the effect of different injection volumes on the

separation *viz.*, 5 µl, 10 µl, 15 µl and 20 µl. Results are shown in Figure 2.5. An injection volume of 15 µl gave an appropriate retention time and peak shape. The maximum concentration of CP that is expected to be observed during *in vitro* testing of CP cream formulations was calculated to be 12 µg/ml, with a corresponding maximum of column load of 0.18 µg of CP.

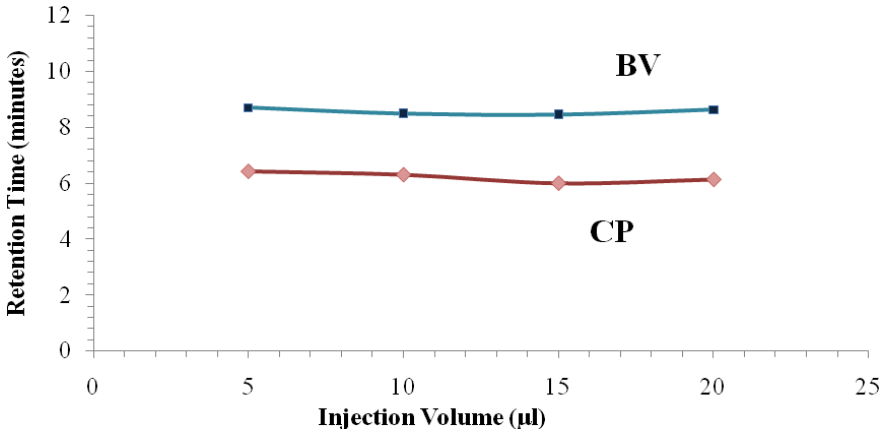


Figure 2.5. *The effect of injection volume on the retention times of CP and BV*

2.5 SELECTED CHROMATOGRAPHIC CONDITIONS

Following the evaluation of the effects of different concentrations of organic modifier and flow rates on the retention times, peak shapes and resolution of CP and BV, the optimal chromatographic conditions were selected and used throughout the HPLC method development and validation. A summary of these conditions is listed in Table 2.4, and a typical chromatogram of the separation between CP and BV is shown in Figure 2.6.

Table 2.4*. Optimal HPLC conditions for analysis of CP*

Column	Nova-Pak® Å C_{18} 4 µm (3.9 mm i.d. x 150 mm)
Flow rate	0.9 ml/min
Injection volume	15 µl
Detection wavelength	239 nm
Temperature	22°C (Ambient)
Recorder	Perkin-Elmer® Model 561-3002 strip chart recorder (Hitachi, Japan)
Recorder speed	5 mm/min
Recorder input	5 mV
Detection Sensitivity	0.05 AUFS
Mobile phase composition	MeOH:Water (68:32% v/v)
Retention time	CP 6.0 minutes BV 8.0 minutes

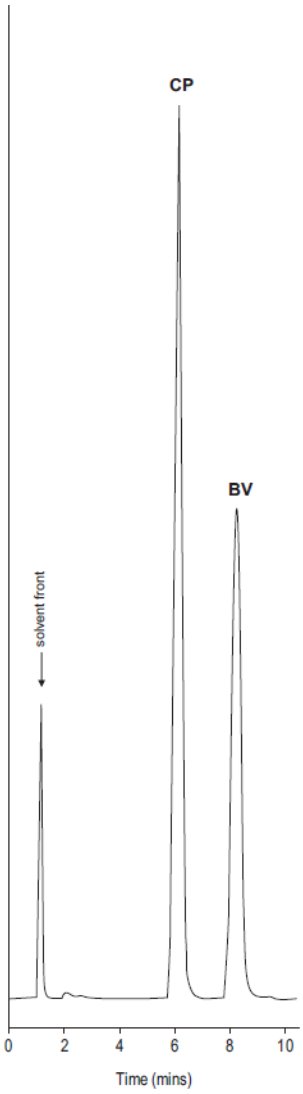


Figure 2.6. *Typical chromatogram of the separation of CP (Rt = 6.0 minutes) and the IS, BV (Rt = 8.0 minutes)*

2.6 METHOD VALIDATION

2.6.1. Introduction

Following the development of an analytical method for a compound of interest, it is important that the analytical method is validated. Method validation is usually performed to ensure that the results of all subsequent analyses are accurate, reliable, reproducible and valid when used by different analysts using the same equipment in the same and/or different laboratories. This is a process whereby an analytical procedure is demonstrated to be suitable for its intended use [179,180]. Chromatographic method validation is therefore an essential tool for quality control and assurance management in the pharmaceutical industry [169,181]. HPLC analytical methods must be validated according to standard international guidelines. Different organisations have put in place various guidelines for the purposes of validation. This includes the International Conference on Harmonization (ICH) [180,182,183],

Food and Drug Administration (FDA) [157] and the United States Pharmacopoeial convention (USP) [125,183].

The ICH guidelines Q2A [182] and Q2B [184] on validation of analytical procedures and methodology summarises the approach to validation for typical parameters such as accuracy, precision (repeatability and intermediate precision) and specificity. The limit of quantification (LOQ), detection limit (LOD), linearity, range of the method, robustness and system suitability should also be evaluated [179,183]. This may require statistical analysis of parameters, including linear regression analysis, standard deviation and relative standard deviation. The data generated therefore explain the validity of the method and determine the acceptability of an analytical procedure [179].

2.6.2. Linearity and Range

Tests of linearity ascertain whether quantitative the results are directly proportional to the concentration of analyte within a specified range [183]. The ICH guidelines [180] define linearity as the ability of a method within a given range to produce test results that are directly proportional to the concentration/amount of the analyte in a given sample. Linearity is assessed over a range of concentrations, usually between the upper and lower levels of an analyte that would be expected to be observed in experiments using a standard calibration curve [183]. When using UV spectrophotometry, it is crucial that the absorptive (UV) response to the analyte concentration follows the Beer-Lambert law in order to show that linearity for the results will be observed [157,160].

The range is used to confirm that an analytical method provides a certain degree of linearity, accuracy and precision when applied to samples of unknown concentration. For assay purposes, the range of a method should normally be 80% to 120% of the target concentration [180]. The ICH guidelines [180] recommend that a minimum of five concentration levels containing an API and an internal standard be used to assess linearity. Linearity was performed by repeated measurements (n=5) of seven calibration standard solutions containing CP and BV. The calibration standard solutions were injected in ascending order of concentration *viz.,* 0.15, 0.8, 2, 4, 8, 12, 15 µg/ml of CP. The Peak Height Ratios (PHR) of CP to BV were calculated and a calibration curve of the PHR versus concentrations was plotted and is shown in Figure 2.7. Least squares linear regression analysis was performed on these data to establish whether a correlation could be established between the absorptive response and the different analyte concentrations.

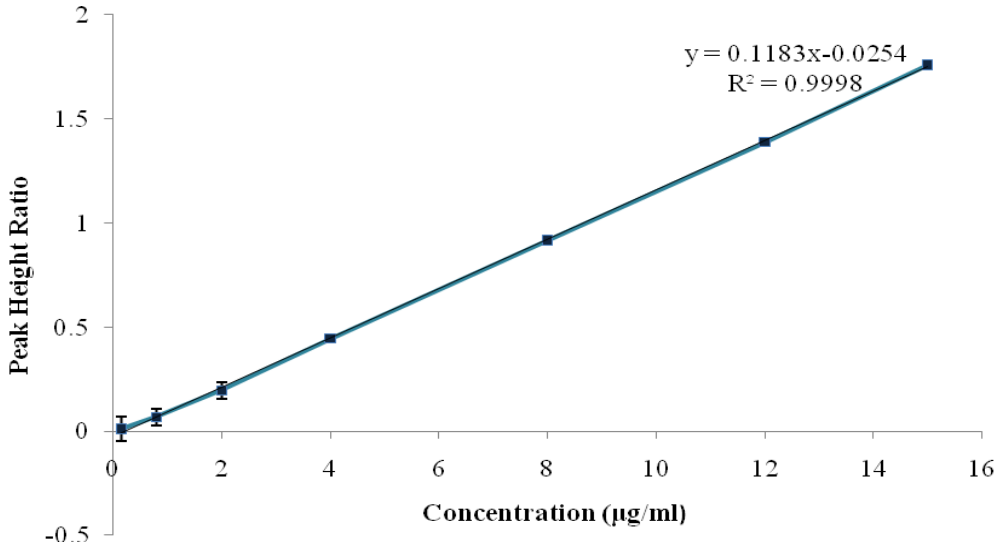


Figure 2.7. *Typical calibration curve for CP in the concentration range 0.15-15 µg/ml*

The equation for the best-fit least squares regression line is y = 0.1183x–0.0254 with a correlation coefficient (R^2) of 0.9998. R^2 should be ≥ 0.999 to establish linearity between a response and sample concentrations. The y-intercept should be < 2% of the response or near zero to establish the accuracy and linearity [185]. Since R^2 (0.9998) and the y-intercept (0.0254) were found to satisfy the specific criteria for linearity, a direct response-concentration relationship was found and the calibration curve is therefore linear.

The Response Factor (RF) is essentially the response of an API or related substance per unit weight [186] and can be calculated using Equation 2.7.

$$\text{Response factor} = \frac{\text{Response (in response units)}}{\text{Concentration } \left(\frac{\mu g}{ml}\right)} \qquad \textbf{Equation 2.7}$$

The response factor provides a better indication of linearity over the concentration range studied than least squares regression analysis [143,183,187]. This approach gives an idea of how significant the standard deviations are and how close the values of interest are to one another. The RF also known as the sensitivity factor is calculated by dividing the PHR for each concentration by a concentration of the IS producing that response [183,187]. If the responses of the concentrations are similar, there is insignificant deviation and thus the points should form a straight line with a gradient of zero or close to zero [143]. The region of interest in Figure 2.8 permits visualisation of the similarity in the response for samples of different concentration. The similarity is indicated by the limits of the values and the standard deviation between the lower limit and the upper limit in that region. A second plot of response factor versus concentration was plotted and is shown in Figure 2.8.

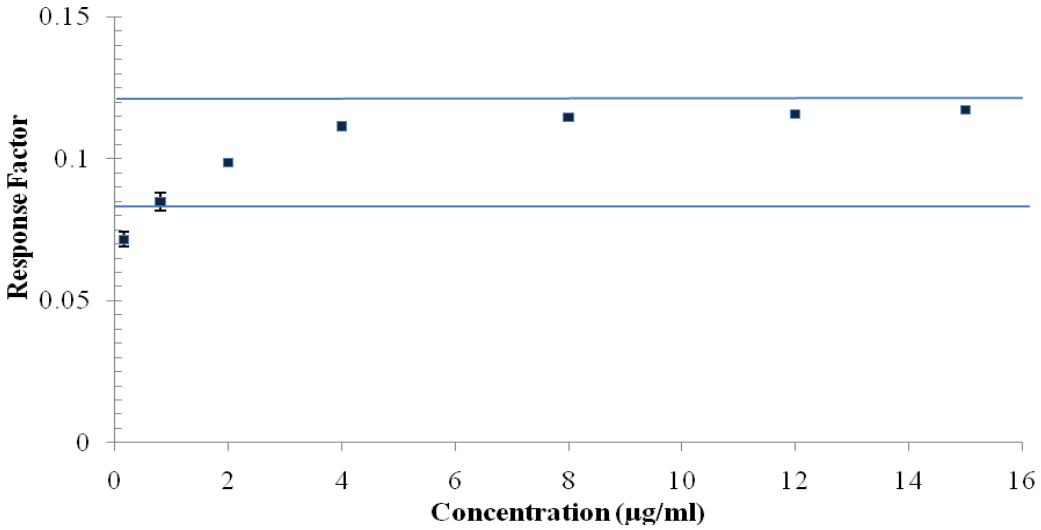


Figure 2.8. *Response factor plot for CP in the concentration range of 0.15-15 µg/ml with the region of interest showing a lower limit and an upper limit of the standard deviation for these samples*

The average response factor (n=7) is 0.1019±0.0178 and the equation for the line is y = 0.0025x+0.087. Since the gradient is close to zero, this indicates that there is a high degree of linearity in the results. All data points except the response factor at concentration 0.15 µg/ml fell within the region of interest showing that there was no significant standard deviation in the observed results.

2.6.3. Precision

Precision is a measure of the extent of reproducibility and repeatability of an analytical method, when used under normal operating conditions or when performing a series of measurements on the same analytical sample [183]. In other words it is the measure of how close the generated data values are to each other for a number of measurements taken under the same analytical conditions [157]. According to the ICH guidelines [180], precision tests should be performed at three different levels *viz.*, repeatability (intra-assay precision), intermediate precision (inter-day precision) and reproducibility (between laboratory precision).

2.6.3.1. Intra-assay Precision (Repeatability)

Repeatability is also known as intra-assay precision of an analytical method. Intra-assay precision is evaluated by the same analyst using a method under the same operating conditions, over a short interval of time [123,157,183]. This assessment is performed to evaluate the variability in experimental conditions over a short period of time [188]. This measure is established in one laboratory by one analyst, using the same analytical method on the same day. The ICH guidelines [180] suggest that repeatability can be assessed using a minimum of nine determinations covering the specific range of the analytical procedure, for example at three concentration levels with replicates of three of each concentration analysed [180].

Repeatability is established by choosing three different concentrations *viz.*, low, medium and high, falling within the calibration curve and analysing a minimum of five replicates (n=5) of each selected concentration. Three concentrations of CP *viz.*, 0.33 µg/ml, 5.7 µg/ml and 13.3 µg/ml were injected together with BV (n=5). The peak height ratio (PHR) of CP to BV was determined at each concentration. The percent relative standard deviation for each sample was calculated to assess the intra-assay precision of the method and provides an indication of the repeatability of the analytical method. For an assay method, the precision criteria for instrument precision (% RSD) can be ≤ 1%. For an intra-assay precision, % RSD can be ≤ 2%, whereas for impurity assay, the intra-assay precision can be ≤ 10% [189]. The results for repeatability of this method and the precision (n=5) are summarised in Tables 2.5 and 2.6 respectively.

Table 2.5. *Intra-assay precision for CP in the concentration range of 0.15-15 µg/ml*

Theoretical Concentration (µg/ml)	**Actual Concentration (µg/ml)**	**PHR (CP/BV) n=5**	**Standard Deviation**	**% RSD**
0.33	0.337	0.0256	0.0081	0.37
5.70	5.783	0.7306	0.0328	0.57
13.3	13.405	1.7282	0.0439	0.33

Table 2.6 *Intra-assay precision for calibration curve of CP (0.15-15 µg/ml)*

Theoretical Concentration (µg/ml)	**PHR (CP/BV) n=5**	**Standard Deviation**	**% RSD**
0.15	0.0716	0.0030	1.74
0.8	0.0847	0.0030	0.40
2.0	0.0984	0.0005	0.02
4.0	0.1115	0.0006	0.02
8.0	0.1146	0.0000	0.00
12.0	0.1157	0.0005	0.02
15.0	0.1173	0.0004	0.00

These data clearly show that all % RSD values were < 2% for all concentrations of CP investigated, and therefore the method is precise.

2.6.3.2. Inter-day Precision (Intermediate Precision)

The ICH guidelines [180] explain the extent to which intermediate precision should be established depending on the circumstances under which the analytical procedure is intended to be used. Intermediate precision, known as inter-day variability, is the reliability of the method. In other words, the method will produce the same results when similar samples are analysed in a different environment from that used during the development of the method [157]. However, intermediate precision can also refer to results from within-lab variation due to random events such as differences in experimental periods, analysts and equipment [183]. Inter-day variability can be observed despite the use of the same analytical equipment by the same analyst over a period of days to weeks.

Three different concentration solutions of CP *viz.*, 0.33 µg/ml, 5.7 µg/ml and 13.3 µg/ml were injected with BV in triplicate (n=3) on three consecutive days, using the same analytical equipment. The results for intermediate precision are recorded in Table 2.7.

Table 2.7. *Inter-day precision for CP in the concentration range of 0.15-15 µg/ml*

	Theoretical Concentration (µg/ml)	**Actual Concentration (µg/ml)**	**Standard Deviation**	**Precision (% RSD) n=3**
Day 1	0.33	0.342	0.0025	0.71
	5.70	5.786	0.0347	0.60
	13.3	13.403	0.0286	0.21
Day 2	0.33	0.344	0.0033	0.96
	5.70	5.662	0.0318	0.56
	13.3	13.312	0.1341	1.01
Day 3	0.33	0.337	0.0017	0.49
	5.70	5.752	0.0187	0.32
	13.3	13.307	0.0218	0.16

The results of these studies showed that there were no significant deviations for the three concentrations analysed in triplicate. All % RSD values for the three concentrations assessed were < 2. This indicates that the analytical method is able to produce precise results on a day-to-day basis.

2.6.3.3. Reproducibility

The reproducibility of an analytical method refers to the ability of an analytical method to produce the same precise results in more than one laboratory. In other words it is a measure of the precision between laboratories [183]. Reproducibility was not assessed as all analyses were performed by the same analyst, using the same equipment in the same laboratory for the duration of these studies.

2.6.4. Accuracy

Accuracy is defined as the measure of exactness of an analytical method or more precisely it reflects the closeness of agreement between a measured value and an acceptable true value [183]. Accuracy is usually assessed once the precision, linearity and specificity of an analytical method have been established [180]. Accuracy is a tool that measures how close experimental values are to theoretical values and is dependent on the error that may be associated with an analytical method. Accuracy must be established for methods that are used for the assay of drug substances and products and for quantitation of impurities. Accuracy studies for drug substances and products are recommended to be performed at the 80%, 100% and 120% levels of label claim as stated in the guidelines for submitting samples and analytical data for analytical method validation [157]. However for assay, accuracy may be measured as the percent recovery of a known amount of analyte added to a sample or the difference between the mean and true values calculated with confidence intervals [180,183].

According to the ICH guidelines [180], the establishment of accuracy requires a minimum of nine determinations over a minimum of three concentration levels covering the specific range of the total

analytical procedure. Statistical analysis can be performed using % RSD and % bias to determine the accuracy of the analytical method used. A % RSD limit of ≤ 2% is set for establishing acceptable accuracy, which complies with the limits set by a number of pharmaceutical industries [189]. However a % bias of ≤ 5% was set in our laboratory as the test limit since the % bias is the extent of deviation of a sample result from the sample true value. Percentage bias is calculated by Equation 2.8 and a low % bias may result in a high recovery. The analytical method thus becomes accurate.

$$\% \text{ Bias} = \frac{\text{True value} - \text{Measured value}}{\text{True value}} \times 100 \qquad \textit{Equation 2.8}$$

The accuracy of our studies was determined by using three different concentrations of CP solutions *viz.*, 0.33 µg/ml, 5.7 µg/ml and 13.3 µg/ml respectively in replicates of five (n=5) and the results are summarised in Table 2.8.

Table 2.8. *Accuracy for CP in concentration range of 0.15-15 µg/ml*

Theoretical Concentration (µg/ml)	**Actual Concentration (µg/ml)**	**Standard Deviation (n=5)**	**% Recovery**	**% RSD**	**% Bias**
0.33	0.338	0.0008	102.4	0.22	2.39
5.70	5.749	0.0265	100.8	0.46	0.86
13.3	13.415	0.0147	103.2	0.10	3.19

All % RSD and Bias values were below the limits of 2% and 5% respectively, indicating that the method of analysis for CP is accurate.

2.6.5. Limits of Quantitation (LOQ) and Detection (LOD)

The Limits of Quantitation (LOQ) and Detection (LOD) are normally applied to the analysis of related substances in an API or drug product to indicate the sensitivity of an analytical method [157]. The LOQ is defined as the lowest concentration of an analyte of interest in a specific sample that can be determined with acceptable precision and accuracy, under the required operational conditions of an analytical method [157,183]. The LOD is defined as the lowest concentration of an analyte of interest in a specific sample that can be detected but not necessarily quantitated under the required experimental conditions [157,183]. The LOD is a limit test that specifies whether or not an analyte is above or below a certain indicated value. Both the LOQ and LOD are expressed as concentrations with an acceptable precision and accuracy of measurement [183].

The LOQ and LOD of an analytical method can be determined in a number of ways according to the different international scientific standard guidelines [157,180,183]. The ICH guidelines [180] recommend three different techniques to determine the LOQ and LOD of an analyte in a sample matrix. Visual evaluation of the analytical data may be used for both non-instrumental and instrumental methods of analysis. However the LOQ and LOD are more often determined statistically, as shown in Table 2.9.

Table 2.9. *Different statistical techniques to determine LOQ and LOD in analytical method*

Methods	LOQ	LOD	References
Signal-to-Noise Ratio	10:1	3:1 or 2:1	[180,183]
Standard Deviation (SD) of Response and the Slope	$= \frac{10\sigma}{S}$ Where σ = SD of response of slope S= Slope of calibration curve	$= \frac{3.3\sigma}{S}$ Where σ = SD of response of slope S= Slope of calibration curve	[180,183]
% RSD	≤ 5%	0.3 x LOQ	[145,190,191]

The LOQ and LOD based on signal-to-noise ratio can be applied only to analytical procedures, exhibiting sufficient baseline noise [180]. This approach is not practical since the noise level observed during method development may differ when samples are analysed using different detectors [157]. A statistical method *viz.,* the % RSD approach was used to determine LOQ and LOD of this method since data was collected on a strip chart recorder.

2.6.5.1. LOQ

The lowest concentration of analyte that will produce a response with a % RSD of < 5% after multiple injections may be accepted as the LOQ [191]. Therefore, different concentrations of CP between 0.05 µg/ml and 0.30 µg/ml were analysed in replicates of five (n=5) and their PHR and % RSD were calculated. A solution of 0.05 µg/ml CP produced a peak for CP that was detectable but not quantifiable. A concentration of CP (0.10 µg/ml) produced a % RSD of 11. The LOQ was found to be approximately 0.15 µg/ml, with an average PHR of 0.02 and a % RSD of 4.55.

2.6.5.2. LOD

An estimate of the LOD for CP was obtained, by using the experimentally established value for the LOQ [191]. The LOD was taken as 0.3 times that of the LOQ value, resulting in an approximate concentration of 0.045 µg/ml for the LOD.

2.6.6. Forced Degradation Studies (Stress tests)

Forced degradation studies are also known as stress tests. Stress testing is recommended by the International Conference on Harmonization (ICH) [192] to be undertaken during the pharmaceutical development of a drug candidate. This may be performed to understand the intrinsic stability of that drug and the long-term stability of that material under different storage conditions [192-194]. Forced degradation studies are therefore conducted under experimental conditions harsher than those used to conduct short-term or long-term stability tests. The information obtained in these studies may help to establish degradation patterns during drug product development and validation of analytical procedures [193]. It is important to ensure that any degradation product formed does not interfere with the quantitation of the analyte of interest in an analytical procedure since specificity is crucial in developing a stability-indicating assay [182].

To our knowledge, no information relating to forced degradation studies when analysing CP have been published. Therefore it was essential to investigate the effect of elevated temperature, acid, alkali, oxidative, sunlight, neutral hydrolysis and dry heat-induced degradation on the stability of CP. All degradation studies were performed in solutions, except for those conducted under dry heat-induced conditions. Stock solutions containing 10 mg CP in 100 ml MeOH were prepared as previously described in § 2.3.2 (Chapter 3) and protected from light using aluminium foil. Solutions containing 100 µg/ml CP were exposed to different stress conditions and then analysed using HPLC with UV detection.

2.6.6.1. Temperature

The ICH guidelines [192] suggest that the effect of temperature on a drug substance must be evaluated and conducted over 10°C increments above the temperatures that are usually used for accelerated stability studies. Therefore to investigate whether CP degrades at elevated temperatures, five 100 µg/ml CP samples (n=3) were prepared separately as previously described in § 2.6.6 (Chapter 2). The CP samples were subjected to different temperature conditions that were maintained using a Colora® Model NB-34980 Ultra-Thermostat water bath (Colora®, Lorch, Germany) for four hours. Each sample was analysed using an HPLC method and the results are summarised in Table 2.10.

Table 2.10. *Summary of the effect of different temperatures on CP*

Temperature (°C)	**Time (hrs)**	**Observations (n=3)**	**Comments**
50	4	CP peak was resolved at 6.0 min. No extra peak	No form of degradation
60	4	CP peak was resolved at 5.9 min. No extra peak	No form of degradation
70	4	CP peak was resolved at 6.2 min. No extra peak	No form of degradation
80	4	CP peak was resolved at 6.0 min. Extra peak at 8.1min	Possible form of degradation
100	4	CP peak was resolved at 6.2 min. Extra peak at 8.1 min	Possible form of degradation

All samples of CP stored below 80°C appeared to be stable. However at temperatures 80°C and 100°C, one peak was fully resolved at 8.1 minutes from each CP sample, which may indicate the degradants of CP (A and B) as shown in Figure 2.9.

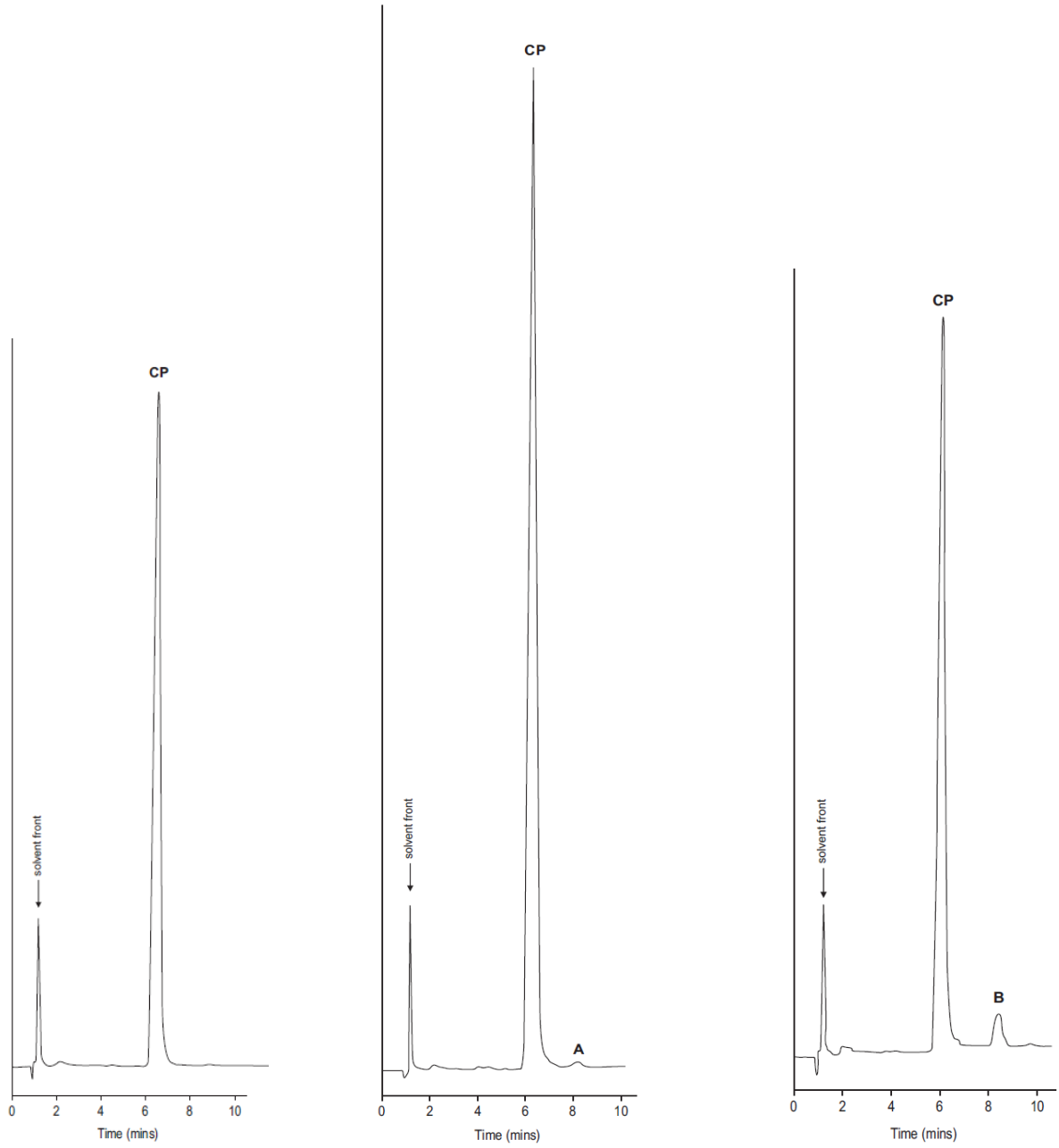


Figure 2.9. *Typical chromatogram showing degradation of CP after exposure to 60°C, 80°C and 100°C for four hours*

2.6.6.2. Acid Degradation

The hydrolytic acid degradation studies for a new chemical entity can be performed by refluxing the compound in 0.1 N HCl for eight hours [195]. If at that point, degradation had occurred, testing can be stopped. If no degradation has occurred, then more severe conditions such as longer exposure to acid or more concentrated acidic conditions may be used. Fifty ml of 0.1 M HCL was added to 50 ml of a stock solution of CP in methanol and the mixture was refluxed in a water bath at 70°C for four hours. Chromatographic analysis revealed that the peak for CP was observed at 6.0 minutes. No extra peak(s) were observed and it was therefore assumed that no degradation of CP occurred. More acidic

conditions (0.5 M HCl) were used to evaluate the effects of acid exposure on CP. The chromatographic analysis revealed that the CP peak was retained to 6.4 minutes but no other peak was observed.

2.6.6.3. Alkali Degradation

The alkali hydrolytic degradation testing of a new chemical entity can be performed by refluxing the compound in 0.1 N NaOH for eight hours [195]. Fifty ml of 0.1 M NaOH was added to 50 ml of a methanolic stock solution of CP and the mixture was refluxed in a water bath at 70°C for four hours. Chromatographic analysis showed that the peak for CP was observed at 6.0 minutes. Extra peaks *viz.*, C, D, E and F were observed at 1.8, 4.0, 5.0 and 8.0 minutes and therefore alkali degradation of CP was assumed to have occurred (Figure 2.10).

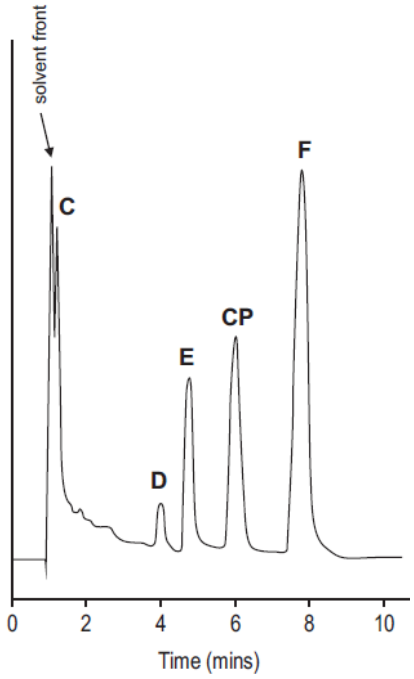
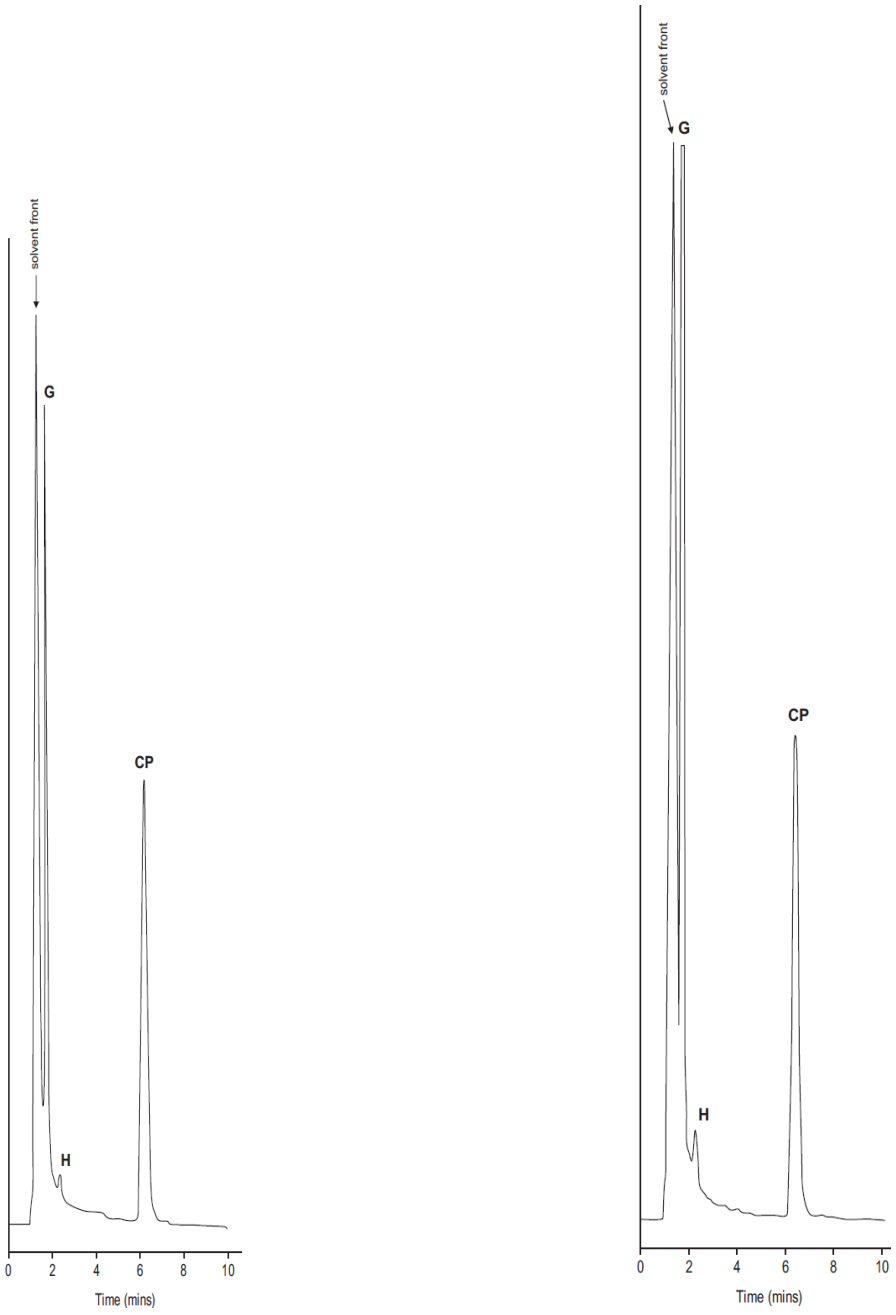


Figure 2.10. *Typical chromatogram showing degradation of CP after exposure to 0.1M NaOH at a temperature of 70°C for four hours*

Glucocorticoids, in particular the C-17 and C-21 esterified steroids, undergo degradation in aqueous and in biological fluids. Usually the ester functional groups at the C-17 and C-21 positions are hydrolysed, resulting in the steroid base remnant undergoing further degradation, via C-17 side chain alteration or alteration of ring A. The degradation in ring A has been observed with steroids possessing a 1-ene-3-keto or 1,4-diene-3-keto structure. The degradation reaction at the C-17 side chain is catalysed by the presence of hydrogen and hydroxyl ions. Since CP has an ester functional group at position C-17 on the D ring and a 1,4-diene-3-keto structure on the A ring, it is susceptible to hydrolysis [196]. However as with most other steroids, CP is found to be stable in acid but degrades rapidly when exposed to alkaline solutions.

2.6.6.4. Oxidative Degradation

In order to conduct oxidative degradation studies, it has suggested the use of 3%-30% v/v hydrogen peroxide (H_2O_2) solutions [195]. Fifty ml of a 6% v/v H_2O_2 solution was mixed with 50 ml of a 100 µg/ml of stock solution of CP and was refluxed at a temperature of 70°C for four hours. More oxidative conditions (10% v/v H_2O_2) were used to evaluate the effects of oxidation on CP. Chromatographic analysis showed that the peak for CP was observed at 6.2 minutes. Extra peaks *viz.*, G and H were observed for both concentrations of H_2O_2 on CP samples and therefore degradation of CP was assumed to have occurred (Figure 2.11).



6% v/v ***10% v/v***

Figure 2.11. *Typical chromatogram showing degradation of CP after exposure to 6% v/v and 10% v/v hydrogen peroxide at a temperature of 70°C for four hours*

2.6.6.5. Neutral Hydrolysis

Fifty ml of the stock solution of CP (100 µg/ml) was added to 50 ml of distilled water and was refluxed in the water bath at 70°C for four hours to test for degradation under neutral conditions. The chromatographic analysis revealed that the CP peak was retained to 6.0 minutes. No extra peak(s) were observed and it was therefore assumed that no degradation of CP had occurred.

2.6.6.6. Photostability

Photostability is an important component of stress testing since a number of drugs degrade in the presence of natural light [192]. Therefore the photostability of CP was tested by exposing a stock solution of CP (100 µg/ml) to direct sunlight for 24 hours. The solutions were then analysed using HPLC. The CP peak was observed at 5.8 minutes and one extra peak (I) was observed at 4.8 minutes on the chromatogram. This peak was adequately resolved from CP with no interference (Figure 2.12).

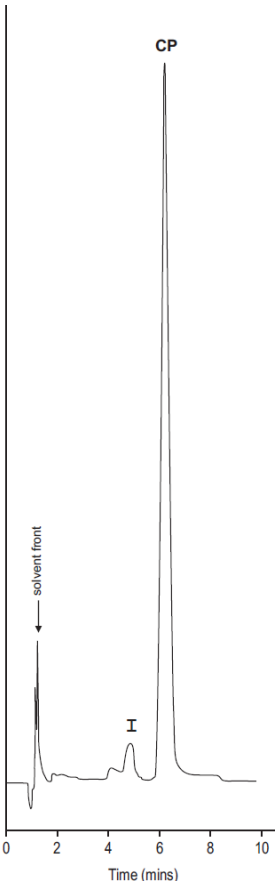


Figure 2.12. *Typical chromatogram showing degradation of CP after exposure to light for 24 hours*

Literature has reported that steroids having two double bonds on ring A are more susceptible to UV light than those with one double bond on ring A [196,197]. CP has a 1,4-diene-3-keto structure on ring A and therefore will easily undergo degradation process in the presence of light.

2.6.6.7. Dry Heat Degradation

CP powder was stored in an oven at 100°C for four hours. A stock solution then was prepared by mixing 10 mg of the heated CP in 100 ml of MeOH and the solution was analysed by HPLC. A CP peak was observed at a retention time of 6.0 minutes. CP powder appears to be unaffected by a temperature of 100°C.

2.6.7. Stability of Analyte

During validation of an analytical procedure, it is important to demonstrate the stability of an analyte in all solvents in which an API is likely to be exposed during a sample work-up process. The conditions to which the study samples are subjected should also be evaluated [198]. An analyte is said to be chemically stable under certain conditions over a certain period of time when its response compared to that of freshly prepared samples does not change significantly over time [188]. However some API might undergo degradation caused by hydrolysis or photolysis, and may be adsorbed onto the glassware used [157]. It is important to evaluate the stability of reference standard stock solutions and dilutions of these solutions over the maximum period for which the solutions will be stored prior to use. The solutions should be exposed to the same temperature as the stock solution, in the same solvent and container type used throughout the study [198]. The stability of CP and BV in MeOH was investigated under different conditions. Solutions of CP only, BV only, and CP and BV together were prepared, as described in § 2.3.2 9 (Chapter 2). Each solution was stored under refrigerated condition (4°C), on the bench (22°C) and in the autosampler (26°C). Since CP degrades in the presence of light, the solutions were protected from light using aluminium foil. Each solution was injected in triplicate (n=3) daily after preparation over a period of one week. The samples from these studies were analysed using HPLC. None of the CP solutions showed any significant changes in response for a week long period, suggesting that CP is stable in MeOH when stored in the refrigerator, on the bench or in the autosampler as shown in Figure 2.13. The results of this study are summarised in Table 2.11.

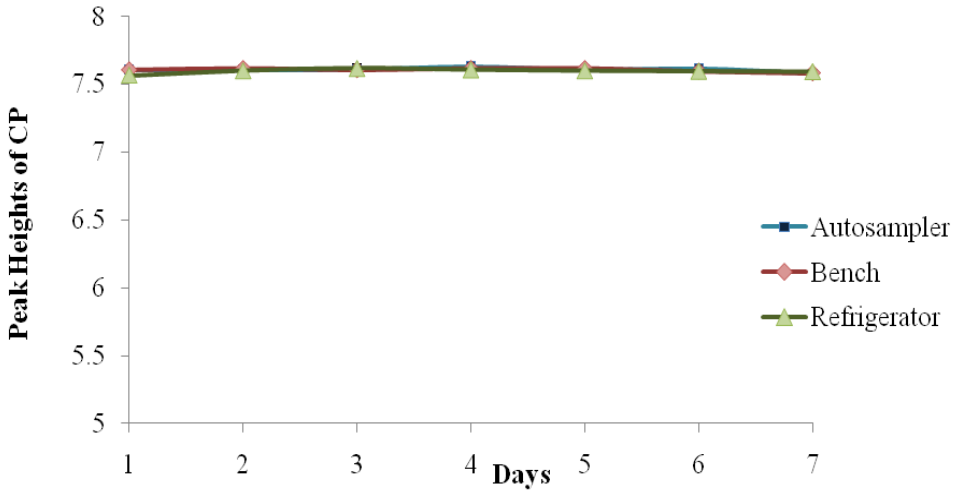


Figure 2.13. *Peak heights of CP stored at different conditions (autosampler, bench and refrigerator) protected from light for a period of 7 days*

In contrast, BV solutions stored on the bench and in the autosampler revealed major changes in peak height three days after preparation, indicating that BV degradation had occurred (Figure 2.14). However the BV solution stored in the refrigerator did not show any significant changes in response for a period of seven days. Therefore BV solution was stable in the refrigerator during that period, as shown in Figure 2.14.

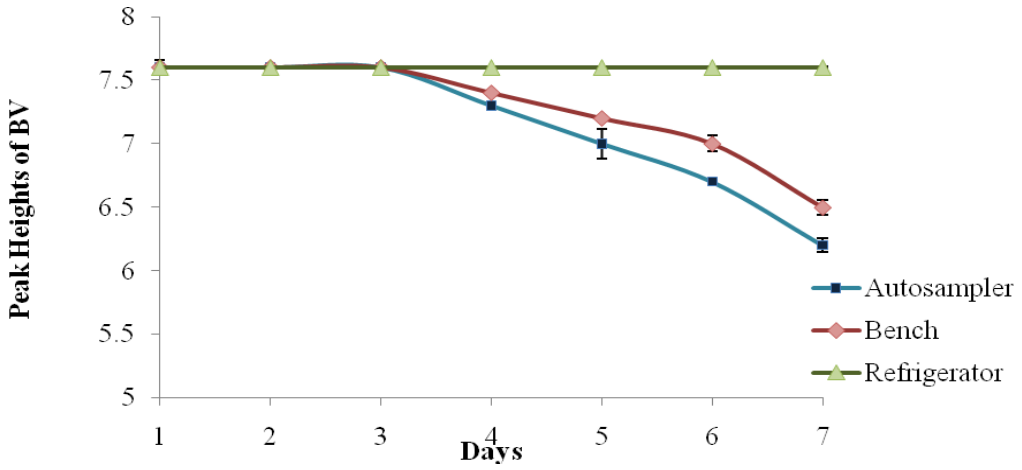


Figure 2.14. *Peak heights of BV solutions stored at different conditions (autosampler, bench and refrigerator) protected from light for a period of 7 days*

Table 2.11. *Responses of CP, BV and CP and BV stock solutions under different storage conditions for one week*

		DAYS						
	Conditions	**1**	**2**	**3**	**4**	**5**	**6**	**7**
CP	Bench(22°C)	*	*	*	*	*	*	*
	Autosampler(26°C)	*	*	*	*	*	*	*
	Refrigerator(4°C)	*	*	*	*	*	*	*
BV	Bench(22°C)	*	*	*	2.63 ± 0.15% of BV had degraded	6.57 ± 0.22% of BV had degraded	11.84 ± 0.11% of BV had degraded	15.79 ± 0.12% of BV had degraded
	Autosampler(26°C)	*	*	*	3.95 ± 0.12% of BV had degraded	6.59 ± 0.35% of BV had degraded	12.01 ± 0.11% of BV had degraded	16.02 ± 0.15% of BV had degraded
	Refrigerator(4°C)	*	*	*	*	*	*	*
CP and BV stock	Bench(22°C)	*	*	*	2.73 ± 0.32% of BV had degraded	6.10 ± 0.25% of BV had degraded	11.55 ± 0.45% of BV had degraded	16.12 ± 0.14% of BV had degraded
	Auto sampler(26°C)	*	*	*	2.77 ± 0.16% of BV had degraded	5.26 ± 0.56% of BV had degraded	12.55 ± 0.55% of BV had degraded	16.55 ± 0.35% of BV had degraded
	Refrigerator (4°C)	*	*	*	*	*	*	*

**** No significant change in response***

It was thus concluded that the stock solutions of CP and BV must be stored at 4°C and be protected from light in order to extend the shelf life of the solution to seven days. In this way daily preparation of CP and BV stock solutions is not required.

2.6.8. Specificity and Selectivity

Specificity is one of the most important investigations to be undertaken when developing and validating an analytical method. A method is said to be specific if the response produced for a single analyte is unadulterated [198]. The specificity of a method is defined as the ability of an analytical procedure to accurately and quantitatively measure the analyte of interest in the presence of other components that may be expected to be present in a sample matrix. Specificity is a measure of the degree of interference in a separation by extraneous components such as other active ingredients or excipients, impurities and degradation products that may be present. This is performed to ensure that co-elution with the analyte of interest does not occur [157,180,183,198].

The selectivity of an analytical method is different from the specificity of a method in that a method is considered selective if it produces a response that is distinguishable for the analyte of interest in the presence of any other extraneous components that may also be detectable [198]. Since most chromatographic methods produce responses not only for the analyte of interest, but for extraneous components, the term "selectivity" is more appropriate than specificity [198]. The selectivity of the analytical method must ensure that the analyte of interest is resolved from other components that may be present in a formulation.

2.6.8.1. Selectivity

The commercially available CP cream in South Africa, Dermovate® (Sekpharma® Pty Ltd, Sandton, Gauteng, South Africa) contains chlorocresol (CH) at a concentration of 0.075% w/w [199]. A solution of CH alone and a combination solution of CP and CH at a concentration of 12 µg/ml were prepared and analysed by HPLC. The results revealed that the CH and CP peaks eluted at 3.0 minutes and 6.6 minutes respectively (Figure 2.15). It can be concluded that there is no interference between CP and CH. Therefore the method can be considered selective for the analysis of CP in topical dosage forms.

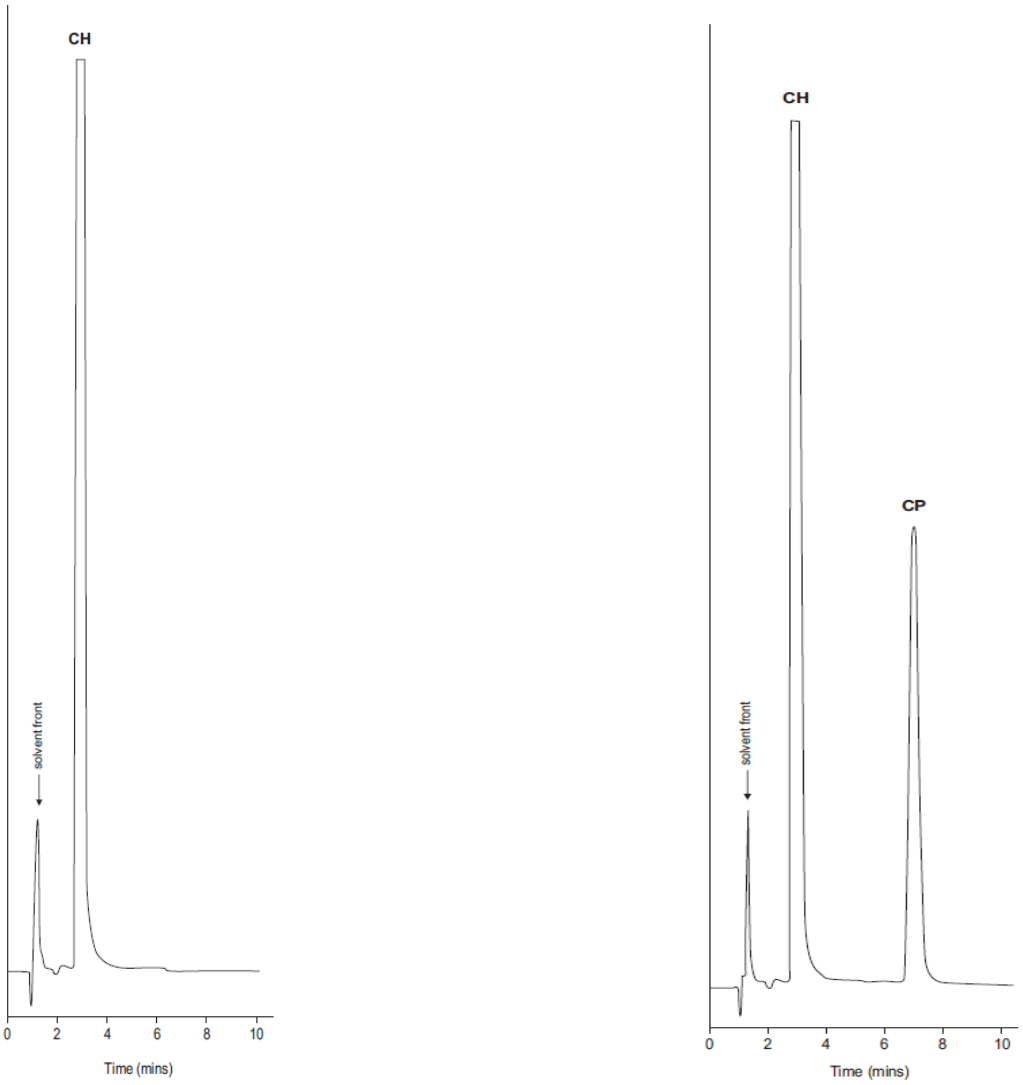


Figure 2.15. *Typical chromatogram showing CH alone (3.0 minutes) and CH and CP solution (3.0 minutes, 6.6 minutes)*

2.6.8.2. Assay of Dermovate® Cream

The assay of the commercially available, Dermovate® cream was performed using the analytical procedure developed in these studies.

2.6.8.2.1. Cream Extraction Procedure

Approximately 1200 mg aliquot of the cream, equivalent to 0.60 mg of CP was accurately weighed and transferred into a 100 ml Schott® Duran round neck Erlenmeyer flask (Schott® Duran GmbH, 51 Hattenbergstrasse, Germany). A stock solution of BV in MeOH was prepared to produce a final concentration of 10 µg/ml. A 50 ml aliquot of the BV solution was added to the stoppered flask containing the cream. The flask was placed in a Colora® Model NB-34980 Ultra-Thermostat water bath (Colora®, Lorch, Germany) and agitated at 70°C until all the cream components had melted.

After the cream had melted, the flask was removed from the bath and stirred vigorously at 1000 rpm for one minute using a magnetic stirrer plate at room temperature (22°C) until the sample had

solidified. The heating, shaking and cooling sequence was repeated twice to ensure the complete solubilisation and extraction of CP from the components of the matrix. The solution was placed in 10 ml centrifuge tubes, sealed and then placed in a freezer (-20°C) for 20 minutes, after which the sample was centrifuged at 3000 rpm using an Eppendorf® Model 5415 centrifuge (Geratebau Netheler+ Hinz GmbH 2000, Hamburg 63, West Germany) for 15 minutes. This extraction procedure ensures the complete precipitation of the lipophilic components of the cream, whereas the CP remains soluble in MeOH [200].

An 4.0 ml aliquot of the supernatant was harvested at room temperature (22°C) was filtered through a 0.45 µm HVLP Millipore® filter membrane (Millipore® Co., Bedford, MA, USA) and an aliquot of the filtered sample was injected onto the chromatographic system in replicates of five (n=5). A schematic diagram of the extraction procedure is shown in Figure 2.16. A calibration curve was constructed on the same day that the samples were prepared for analysis using the same extraction procedure and the concentration of CP was interpolated from the curve. A typical chromatogram obtained during the analysis of the commercial product is depicted in Figure 2.17.

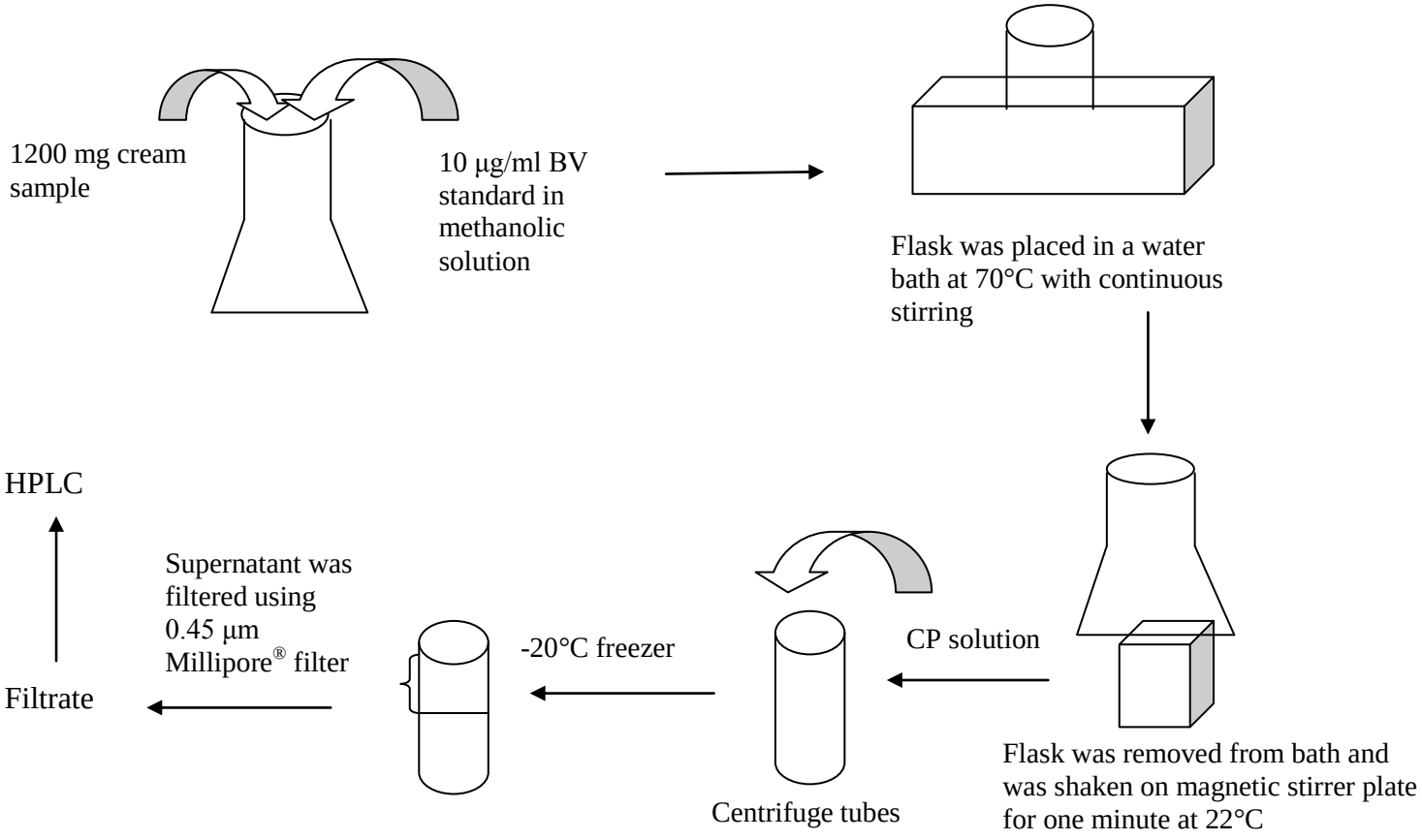


Figure 2.16. *Schematic diagram of extraction procedure of CP from cream formulations*

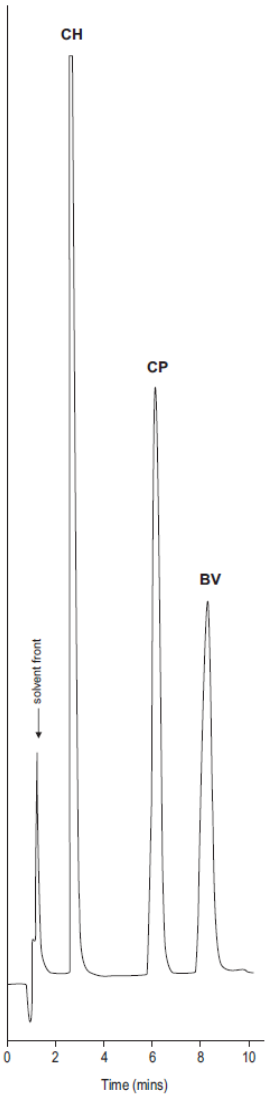


Figure 2.17. *Typical chromatogram of the extraction products (CH and CP) of Dermovate® together with the IS*

2.6.8.2.2. Validation of Cream Extraction Procedure

The validation of the extraction procedure is necessary to ensure that the extraction procedure is reliable, reproducible and accurate. Samples of CP cream were spiked with an appropriate amount of CP powder to reach a concentration of 120% of the expected amount of the analyte that would be expected in the cream samples. Samples were prepared in triplicate (n=3) and the resulting samples were then processed in the same way, as described in § 2.6.8.2 (Chapter 2). The results of validation of the extraction procedure are summarised in Table 2.12.

Table 2.12. *Results of the validation procedure to establish extraction efficiency from Dermovate® cream*

Cream sample Weight (mg)	Expected Concentration (µg/ml)	PHR (CP/BV)	%RSD (n=3)	% Recovery	Actual Concentration (µg/ml)
1200.0	14.40	1.8906	0.20	118.25	14.19
1201.0	14.41	1.8006	0.15	118.25	14.19
1200.0	14.40	1.8615	0.03	116.45	13.97

The validation data reveal that the values obtained for the % recovery were close to 120%, which is the expected amount of analyte in the cream samples. This extraction method can therefore be deemed suitable for the analysis of CP-containing creams. The results of the analysis of Dermovate® are summarised in Table 2.13.

Table 2.13. *Results of the analysis of a commercially available topical formulation of CP, Dermovate®*

Cream Sample Weight (mg)	Expected Concentration (µg/ml)	PHR (CP/BV)	%RSD (n=5)	% Recovery	Actual Concentration (µg/ml)
1200.5	12.00	1.5632	0.03	97.99	11.75
1201.0	12.01	1.5814	0.02	99.00	11.89
1200.0	12.00	1.5581	0.03	97.68	11.72
1201.5	12.02	1.5747	0.10	98.50	11.84
1200.0	12.00	1.5813	0.05	99.08	11.89

The USP [45] specifies that CP-containing cream formulations should not contain less than 90.0% or more than 115.0% of the labelled amount of CP. Since the percent recovery for all samples fell in that range, it is evident that the extraction is efficient, accurate and precise and the CP-containing cream that was tested complies with the USP standard for assays [45].

2.7 CONCLUSION

A RP-HPLC method with UV detection at 239 nm has been developed, optimised and validated for the *in vitro* quantitation of CP in cream dosage forms, according to internationally accepted and established guidelines [45,157]. The method was optimised by manipulation of the mobile phase composition, type and quantity of organic modifier in addition to evaluation of an appropriate detection wavelength, analytical column, injection volume and flow rate. Betamethasone 17-valerate was selected as the internal standard and was used throughout the application of the analytical method. Since components of mobile phase have an effect on the retention time and resolution of CP and BV, methanol can be used as a substitute for acetonitrile. The use of methanol resulted in chromatographic behaviour with the desired retention time, sharp and symmetrical peaks of CP and BV with a high degree of resolution. The peak for CP and BV were well separated with retention times of approximately 6.0 minutes and 8.0 minutes respectively. The analytical method was validated in terms of linearity, accuracy, precision, selectivity, LOQ and LOD. In addition the stability of the

analyte in methanol was evaluated. Furthermore these parameters were established in accordance with internationally recognized guidelines such as ICH, FDA and USP [157,169,180,184,192]. The method that was developed is simple, linear, accurate, precise, sensitive, selective and stability-indicating and therefore was deemed appropriate for the assessment of *in vitro* release characteristics and analyses of laboratory scale (500 g) and scale-up batches (5000 g) of CP cream formulations.

CHAPTER THREE

PREFORMULATION STUDIES AND TOPICAL DOSAGE FORM DESIGN

3.1 INTRODUCTION

3.1.1. Topical Drug Delivery Systems

Over the last few decades, the treatment of various diseases has been achieved by the administration of drugs to the human body via several different routes *viz.*, oral, sublingual, topical, rectal, parenteral, inhalation and others [201,202]. However, topical formulations are regarded as being among the most challenging products to develop when it comes to the delivery of a drug to a specific site of activity [203]. Topical drug delivery systems can be described as self-contained, discrete pharmaceutical semi-solid dosage forms designed to deliver an Active Pharmaceutical Ingredient (API) via the intact skin or body surface, for the purpose of generating a local or regional effect [204]. Topical preparations for cutaneous application are usually comprised of one or more API dissolved or dispersed in a suitable semi-solid base which may be hydrophobic or hydrophilic [205]. Semi-solid dosage forms constitute a significant portion of pharmaceutical dosage forms [206]. When the delivery system is applied topically, the API diffuses from the vehicle onto the surface tissues of the skin or through the follicular regions of the sweat ducts and unbroken stratum corneum into the underlying tissues [203,207].

Semi-solid preparations were primarily used to treat minor topical ailments. Nowadays a number of dermatological preparations are used for acute or chronic skin conditions [208]. “Topicals” or “dermatologicals” refer to a myriad of products applied to the skin or readily accessible membranes. More specifically, a “topical” is described as a formulation applied directly to external body surfaces and epithelial membranes by mechanical means, either by spreading or rubbing. The term “dermatological” is however limited to products applied to the skin or scalp for external use only [209]. Semi-solids include creams, emulsions, ointments, gels, pastes and most recently rigid foams as shown in Figure 3.1 [207,210].

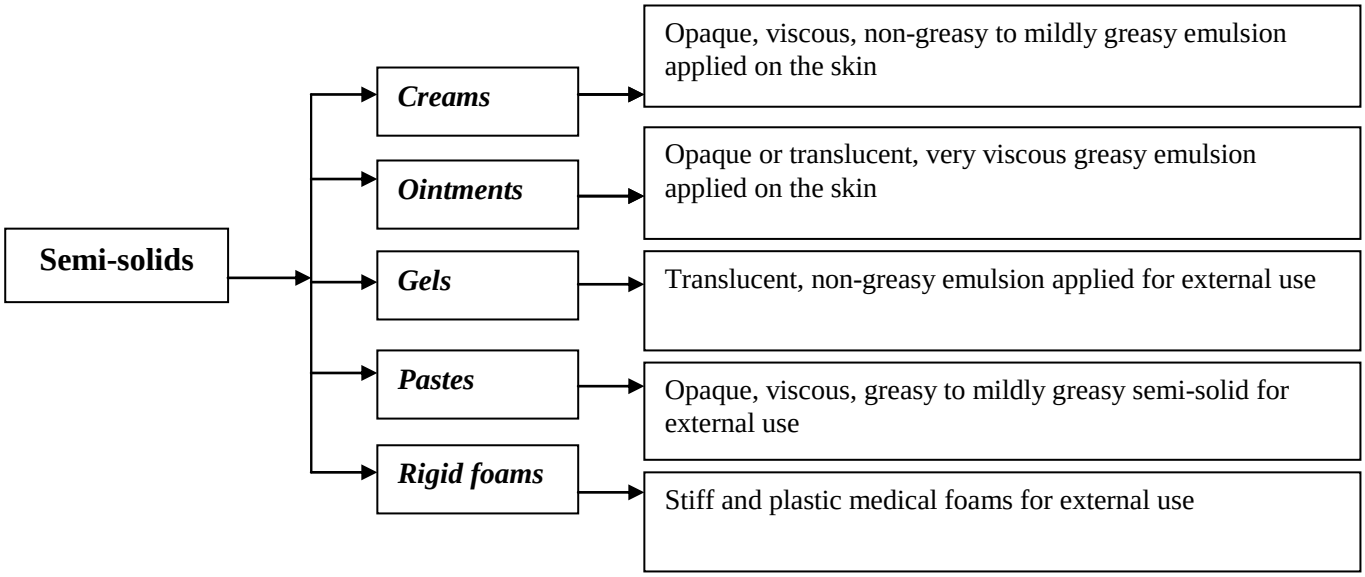


Figure 3.1. *Topical dosage forms and their physical properties*

Drug classes applied to the skin to exert a local effect include antiseptics, antifungals, anti-inflammatory agents as well as emollients for their protective effects. The application of other semi-solids products to mucous membranes, for example for ophthalmic, nasal, buccal, rectal and vaginal mucosal conditions also falls into this category [205]. These products augment or restore the primary function of the membrane, in cases of injury or biochemical malfunction, in order to pharmacologically stimulate the surface of the skin to obtain a specific therapeutic outcome [207,208,211].

Pharmaceutical semi-solid preparations have a three-dimensional (3D) structure that imparts a solid-like character to an undistributed system. These products stick to the site of application for a sufficient period of time before they are washed off or are removed by natural processes. The ability to adhere to the skin is usually due to the plastic rheological behaviour of topical formulations. This behaviour enables the topical formulations to retain their shape unless an external force is applied to the system, thereby allowing them to flow. This property also enables semi-solids to be uniformly spread over the skin to form a film that clings to the surface on application [207,209] and helps to prolong API delivery at the site of action [206].

A semi-solid dosage form is advantageous compared to other available formulations as its application to the surface of the skin or the body is fairly rapid and it has the ability to topically deliver a wide range of drug molecules to the desired site of action [206]. An ideal topical dosage form should normally have important physical, physiological and application characteristics such as elegance, non-grittiness, smooth, non-dehydrating, non-irritating and must be miscible with skin secretions. Furthermore, it should not alter skin function and must have a low sensitisation effect. It should be easily applied to the skin and must ensure efficient drug release in order to obtain an effective therapeutic outcome [212]. Formulations should optimally be thixotropic with viscous characteristics at low strain rates, and be elastic at high strain rates [213,214].

3.1.1.1. Creams

Creams are “creamy white” viscous semi-solid emulsion systems, for external application. Their consistency and rheological behaviour depend on the properties and the nature of the materials used to produce the internal or dispersed phase [207,209]. There are two types of creams *viz.*, aqueous and oily. Creams are traditionally oil-in-water (o/w) emulsions and also known as aqueous/hydrophilic creams, while oily creams, known as lipophilic creams, are water-in-oil (w/o) emulsions. Creams are relatively non-greasy and they exert emollient and moisturising effects on the skin or can be used to deliver drugs for the purpose of achieving percutaneous absorption [211].

3.1.1.2. Ointments

Ointments are hydrocarbon-based semi-solid greasy preparations, intended for application to the skin, rectum, eyes and nasal mucosa. The base is usually anhydrous and immiscible with skin secretions. Ointments are used to provide protective and emollient effects to the skin or to carry medicaments for treating certain topical ailments [208,209,211]. Ointments are also regarded as good vehicles to be applied to dry lesions. However, their main constituents, the hydrocarbons, are usually viscous in nature and they are not good vehicles for most drugs. They set a low limit for the drug delivery capabilities of the system [209].

3.1.1.3. Gels

Gels are translucent or transparent non-greasy semi-solid preparations, mainly used for external application. Gels are usually semi-solid systems in which a liquid phase is constrained within a 3D polymeric matrix, wherein a high degree of cross-linking is introduced [207,209]. The gelling agent used may be gelatin or a carbohydrate such as starch, tragacanth, sodium alginate or a cellulose derivative [207,211]. Gels may be hydrophilic or lipophilic in nature. Hydrophilic gels are used pharmaceutically as lubricants or as carriers for API. Oily gels are preparations in which the bases consist of liquid paraffin with polyethylene glycol (PEG) or fatty oils and are generally used where occlusion is required [209,210,215].

3.1.1.4. Pastes

Pastes may be manufactured using aqueous or oily vehicles, in which a high concentration of insoluble solid is dispersed. The high proportion of solid allows the formulation to thicken, thereby preventing spreading and making localisation of drug delivery simpler than with other topical formulations [210]. Pastes generally are stiffer but less greasy than ointments. Excipients such as starch, zinc oxide, calcium carbonate and talc are usually used as the dispersed solid phase. Pastes are typically semi-solid preparations for external application and therefore are used as protective barriers for the skin [209,211].

3.1.1.5. Rigid Foams

Rigid foams are systems in which air or some other gas mixture is emulsified in a liquid phase. Such foams may be relatively stiff and/or plastic. Aerosol shaving creams and certain medicated quick-breaking foams fall into this category and can be used topically [209].

A flow diagram of different dosage forms used for external application is shown in Figure 3.2.

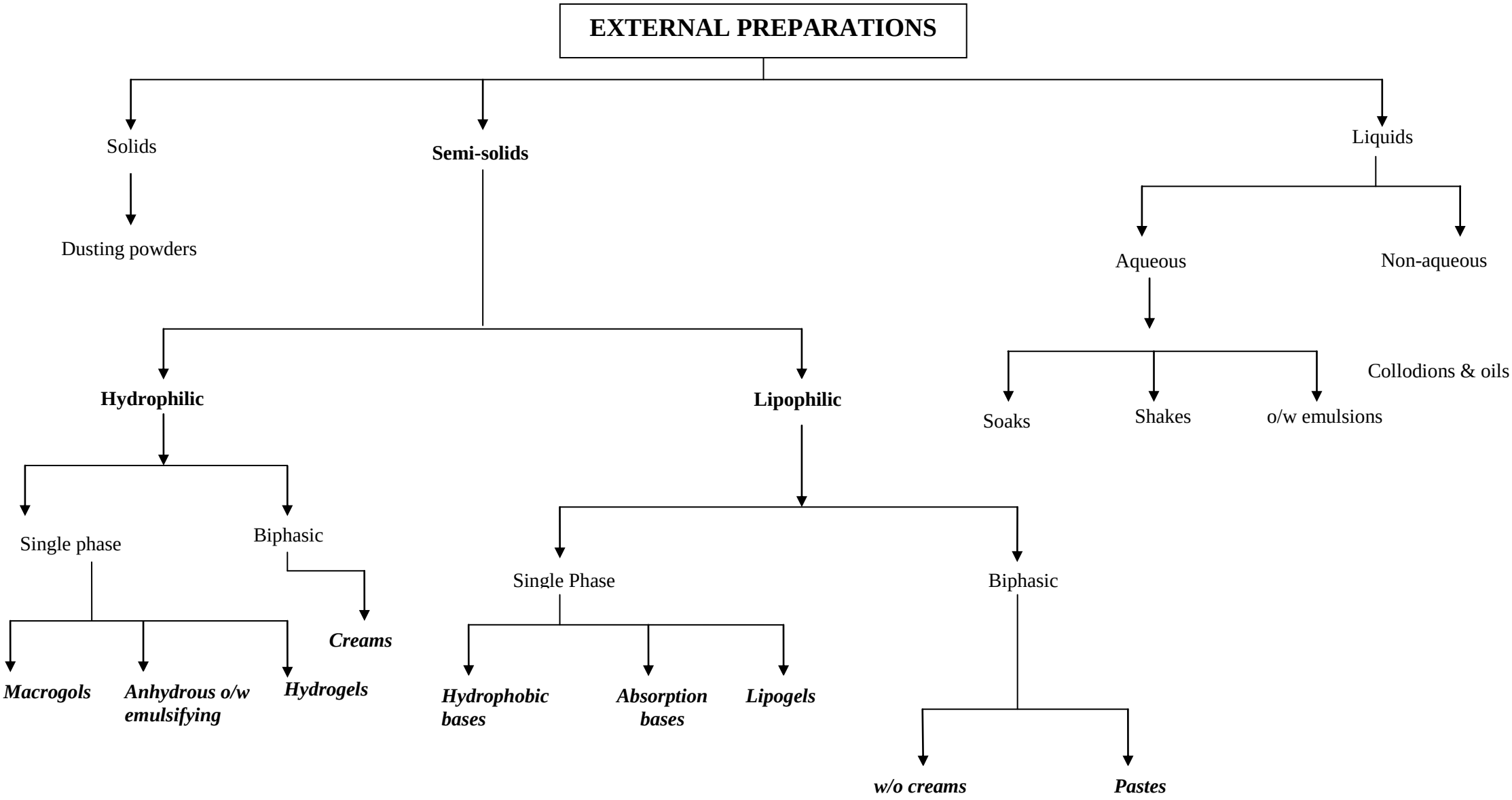


Figure 3.2. *Summary of different dosage forms available for external use [210]*

3.1.2. Topical Drug Absorption

3.1.2.1. Skin

The skin is multilayered and is the largest organ of the body. It serves as a barrier against physical and chemical attack for the underlying tissues and cells [207]. It has three distinct regions *viz.*, the epidermis, dermis and hypodermis, as shown in Figure 3.3. The hypodermis is the innermost layer and is often referred to as the subcutaneous fat. The dermis forms the bulk of the thickness of the skin and contains blood vessels, nerve fibres, openings of the eccrine or sweat glands and hair follicles [216]. The outermost region is the epidermis and it consists of several layers. The outermost layer of the epidermis is called the stratum corneum that is also known as the horny layer. This structure forms the bulk of the skin barrier and is comprised of up to 20 layers of dead keratinised cells. The stratum corneum is a rate-limiting barrier that restricts the movement of substances into and out of the skin [205,207,209,210]. However the skin can be easily damaged mechanically, chemically, biologically and by radiation. Topically-applied or systemically-active drugs may also harm the skin surface [205].

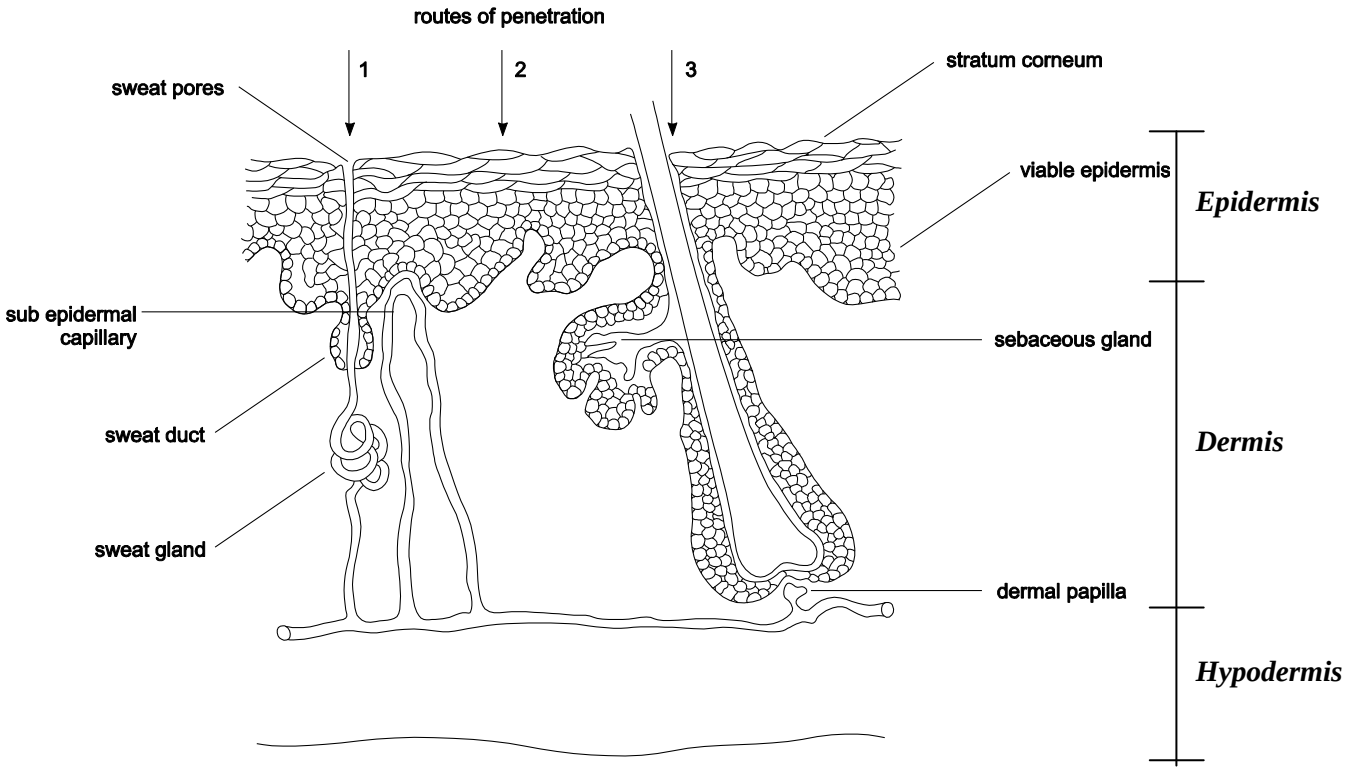


Figure 3.3. *Structure of the skin and the routes of drug penetration: (1) through the sweat ducts, (2) across the stratum corneum and (3) via the hair follicles with their associated sebaceous glands [217]*

3.1.2.2. Percutaneous Absorption

A large number of diseases may affect different regions of the skin, and the usual objective of dermatological drug therapy is to produce a desired therapeutic action at specific sites on or in the skin tissues. This requires diffusive penetration of the applied drug through the skin, or in other

words, percutaneous absorption. Percutaneous absorption is the key to topical drug delivery. When a medicated product is applied topically, the API partitions from the vehicle onto the surface of the skin and then diffuses through the multiple layers of this barrier [70]. The API comes into contact with cellular debris, microorganisms, sebum and other materials and has three potential portals of entry *viz.*, through sweat ducts, directly across the stratum corneum and via the hair follicles, to reach the site of action (Figure 3.3) [205,207,209,217].

Once the API is in the skin, lipid-soluble drugs tend to accumulate in the lipid regions whereas water-soluble drugs tend to enter the blood capillaries. A substance may take a particular route depending entirely on the physicochemical properties of that material and pre-existing skin conditions [207]. Diffusion through the horny layer of the skin occurs as a passive process since this layer has a high degree of diffusional resistance. This membrane does not permit API to pass readily, but low molecular weight molecules are able to penetrate the barrier to some extent. In cases where diseases such as eczema disrupt the barrier, easier access of the API is permitted than if the barrier was intact [205].

3.1.2.3. Factors Affecting Percutaneous Absorption through the Skin

Effective systemic therapy is generally affected by three factors *viz.*, the properties of the API, vehicle and barrier properties of the skin. Semi-solids are multi-component complex dosage forms and factors such as biological, physicochemical, API and formulation parameters may affect the flux properties of the molecule and therefore percutaneous absorption [205,207].

One of the main factors influencing skin penetration is the condition of the skin. This includes whether the skin is intact or injured, age, area to be treated, thickness of the skin, species variation and skin moisture content [218,219]. The rate of hydration and the hydration state of the stratum corneum are also important parameters that can induce or inhibit skin penetration. They are the parameters that permit the passage of all molecules to penetrate the skin. Under occlusive conditions, the stratum corneum retains water and minimal water loss occurs, which allows skin penetration to occur relatively easily. The clinical importance of skin hydration for the enhancement of percutaneous absorption has been shown by use of occlusive plastic films in topical steroid therapy [220,221]. Temperature and pH of the skin also play significant roles in the permeation of the API through the skin [217,222]. The aqueous solubility of an API will determine the concentration of that API at the absorption site(s) and water/lipid partition coefficient will highly influence the rate of API transport through that barrier. Furthermore, smaller API molecules are able to penetrate more rapidly than larger molecular size compounds [205,207,218].

The vehicle used to manufacture in a semi-solid formulation may facilitate the penetration of an API. This occurs by altering the activity of the water present in the stratum corneum, which in turn may

influence the stratum corneum/vehicle partition coefficient for that molecule [223,224]. Greases and oils are considered occlusive vehicles and they induce hydration of the barrier through sweat accumulation at the skin-vehicle interface [225-227]. Humectants that may be used in formulations usually have a high affinity for water. At times they may dehydrate the horny layer and therefore decrease penetration of the compound of interest [228]. Consequently a variety of vehicles and ingredients is required for the successful formulation of different products based on the solubility characteristics of the API in order to achieve an optimal release rate [207]. Factors affecting percutaneous absorption of drugs incorporated into semi-solid dosage forms are summarised in Table 3.1.

Table 3.1. *Factors affecting drug permeation through the skin [205,207,229-233]*

Biological Factors	Physicochemical Factors	Drug substance	Formulation Parameters
Skin condition	Skin hydration	Drug concentration	Composition
Skin age	Temperature	Partition coefficient	Vehicle
Blood flow		Molecular size and shape	Rheological behaviour
pH		Diffusion coefficient	Occlusive/Non-occlusive
Regional skin sites		Solubility	
Skin metabolism			
Species differences			

3.1.2.4. Maximising Drug Bioavailability in the Skin

A long-standing approach to improve bioavailability and to promote the permeation and penetration of drugs through the intrinsic diffusional barrier of the stratum corneum is the use of a suitable delivery vehicle, and/or the incorporation of a chemical enhancer in the formulation of a semi-solid dosage form [71,234]. Penetration enhancers are also known as sorption promoters or accelerants. They are used as "harmless" chemical or physical approaches to enhance the solubility of an API in the stratum corneum, thereby facilitating diffusion of the API through the barrier layer to the more vascular regions of the skin [69,70]. The accelerants work by reversibly disturbing the packing pattern of the intercellular lipid matrix, intracellular keratin domains or by increasing drug partitioning into the tissue, by acting as a solvent for the permeant within the membrane. The enhancers may also act on the desmosomal links between the corneocytes or by modification of metabolic activity within the skin or influencing the thermodynamic activity/solubility of an API in the vehicle of a formulation [69].

The attributes of an ideal penetration enhancer are i) it should be pharmacologically inert, non-toxic and non-irritating, ii) it should promote the penetration of compounds across the skin barrier, without exhibiting irreversible effects on that barrier, iii) it should ideally work rapidly and should have an immediate effect, iv) it should be cosmetically acceptable, v) it should not cause loss of body fluids, electrolytes or other endogenous materials, vi) it should be compatible with the API and excipients and vii) it should be a good solvent for the API [67,70,71,205,235]

3.1.2.5. Skin Penetration Enhancers

Skin penetration enhancers include chemical and physical enhancers [70-72,207,234,235].

3.1.3.5.1. Chemical Enhancers

Chemical penetration enhancers are present in topical, transdermal, dermatological and cosmetic products, to aid the dermal absorption of curatives and aesthetic agents. The selection of chemical enhancers in topical formulations requires an understanding of the mode of action of the penetration enhancers [68,236]. Chemical enhancers usually exert their effect by disrupting the highly ordered structure of the stratum corneum and/or by interacting with the intercellular proteins. They may also improve the partitioning of an API, co-enhancer or solvent into the stratum corneum, thereby facilitating drug transport [67]. Examples of chemical enhancers that have been used successfully include dimethyl sulfoxide, various alcohols, polyols, alkanes, fatty acids and esters [67,71].

3.1.3.5.2. Physical Penetration Enhancers

The penetration and permeation of drug molecules into and through the skin may require physical mechanisms of enhancement if chemical means are not successful. This may be a consequence of the different molecular sizes of molecules and therefore specific physical enhancers may achieve most desirable penetration effects [71]. Physical penetration enhancement techniques involve ways to circumvent the normal barrier function of the stratum corneum in a transient fashion in order to allow for the passage of macromolecules. Two of the best-known physical enhancement methods are iontophoresis and sonophoresis [237-239]. These two techniques create “holes” of nanometer dimensions in the stratum corneum that permit the transport of small molecules into the underlying layers of the skin structure [70,214]. Other examples include electroporation, and microneedles, needleless injection, suction ablation, radio frequency, thermophoresis and magnetophoresis [71,205,214,217]

3.1.3. Topical Formulation Bases

A topical base is usually selected and classified according to the composition of the base and its physical characteristics. The selection of an appropriate base for a formulation depends mainly on the desired site of activity of the API (topical or percutaneous absorption). However, a topical formulation base must also be compatible with other formulation components, exhibit and impart physicochemical and microbial stability to the product and facilitate the manufacturing process. Pourability and spreadability of the formulation, duration of contact with the skin, hypersensitivity reactions and ease of removal of the dosage form by washing also play a significant part in decisions relating to the selection of materials when formulating a topical base [208].

3.1.3.1. Cream Bases

An aqueous base is the vehicle for an oil-in-water semi-solid emulsion whereas an oily base is that for a water-in-oil semi-solid emulsion [208,240]. Aqueous bases are also known as water-removable bases and a large proportion of the aqueous phase is incorporated into the base, with the aid of suitable emulsifying agents. The base is easily removed from the skin due to its hydrophilic nature. Oily bases are generally hydrocarbon bases, made up of oleaginous materials and a continuous phase containing oily substances. The w/o bases provide emollient and protective properties and remain on the skin for prolonged periods of time [205,207,208,240]. The formulation approach in these studies was to use an aqueous base for the development of both laboratory scale and scale-up cream formulations containing CP and is more specifically explained in § 3.1.3.2 (Chapter 3).

3.1.3.2. Aqueous Creams

In order to formulate a stable aqueous cream, the formulation must contain at least three components *viz.*, a dispersed phase, a dispersion medium and a suitable emulsifying agent. An aqueous cream is an o/w emulsion in which water is the dispersion medium and oil is the dispersed phase. When o/w creams are rubbed into the skin, the dispersion medium or continuous phase and volatile solvents evaporate and the application shrinks to a thin film. This process results in an increase in the concentration of API in the adhering film on the surface of the skin. The concentration gradient of drug across the stratum corneum therefore increases, promoting percutaneous absorption [205,207,209]. However, formulators may add additional non-volatile, water-miscible co-solvents such as propylene glycol in the cream formulation to further enhance this process. This promotes evaporation, thus leaving a film with a high concentration of API in the non-volatile compartment, further increasing the concentration gradient. The cream therefore deposits lipids and other moisturisers onto and in the horny layer of the skin, increasing and/or restoring hydration of the tissues [205,209].

3.1.3.3. Oily Creams

Dermatological creams can also be formulated as oily creams. These are water-in-oil (w/o) emulsions containing long-chain alcohol and/or esters, acids, vegetable oil, animal oil and assorted waxes [209]. Since the continuous phase is oily in nature, these creams tend to be more occlusive than the o/w systems. Therefore oily creams leave a protective oily layer on the surface of the skin as the water of the dispersed phase evaporates [241]. Furthermore these products facilitate hydration of the stratum corneum and often increase the penetration of API, incorporated into the carrier systems. The main disadvantage of oily creams is that they can be greasy and may cause folliculitis due to the occlusion of the pilosebaceous pores of the skin. These types of creams should therefore be applied with gentle rubbing several times daily to ensure that a thin film is maintained on the surface of the skin [242].

3.1.4. Excipients used in Topical Preparations

All formulations contain excipients that form the vehicle that is necessary for delivering an API into and/or through the skin. In general, the vehicle is comprised of ingredients used to adjust the pH of the formulation, promote the delivery and prolong the effect of an API on the skin surface or in the stratum corneum in addition to making the topical product cosmetically appealing. The selection of components of a topical formulation depends on a number of factors. The surface area to be treated, the need for residual activity, presence of hair in the area to be treated and the nature of the lesion are critical [242-245]. Often an API is available in a number of different formulations and/or delivery systems and maintains a basic activity, regardless of the type of system. However their ease of use, cost and efficacy for the desired purpose are affected by the formulation selected as ideal, in addition to the method of application [242,246].

3.1.4.1. Topical Vehicles

When developing formulations for topical delivery, the choices of vehicle and excipients for a specific API influence the velocity and magnitude of absorption and consequently the bioavailability and efficacy of the API. The excipients that comprise the vehicle modulate the effects of partitioning and diffusion of the API into and through the stratum corneum [66,109]. Furthermore, topical vehicles play a major role in the appearance, feel and successful application of an API [247]. It is therefore critical to choose the most appropriate vehicle for a topical API in order to achieve a desired therapeutic outcome.

3.1.4.2. Emulsifying Agents

Both o/w and w/o creams are multi-component emulsions used for external application and they are thermodynamically unstable [248-250]. The instability is largely due to the surface energy that results from a combination of the interfacial tension between the continuous and dispersed phases, the large surface area of the dispersed phase and the difference in densities of the two phases. Therefore the inclusion of an emulsifying agent is vital to impart stability to the system [251,252]. The main purpose of an emulsifying agent is to form a condensed film around each of the droplets of the dispersed phase. A low concentration of emulsifier will have little/no effect on the stability of a cream. However an increase in the concentration of the emulsifying agent will increase the stability of a topical formulation dramatically [253,254]. The selection of a suitable emulsifier is usually the first step in the development of an emulsion or a cream. The emulsifier must be compatible with all other formulation excipients and the API to be included in the formulation. It should be non-toxic, stable and promote emulsification to maintain the stability of the topical formulation for the intended shelf-life of a product. The presence of an emulsifier may also increase the skin penetration of any API contained in that formulation [210,251,255].

Since the choice of an emulsifying agent is critical in developing a successful semi-solid formulation, formulation scientists must know the desirable properties of emulsifying agents. It is important to understand the mechanisms by which different emulsifying agents optimise the stability of a formulation. Some of the desirable properties of an emulsifying agent are that the emulsifier must be surface-active in order to reduce the interfacial surface tension to below 10 dynes/cm [256,257]. Furthermore it should be spread rapidly around the droplets of the dispersed phase in a condensed non-adherent film that prevents coalescence of the droplets, and should increase the viscosity of topical formulation when used in a reasonably low concentration [251,258].

Emulsifying agents often contain both hydrophilic and lipophilic moieties in their structure of which one is a more dominant in functionality than the other [259]. Emulsifying agents can therefore be classified on the basis of the Hydrophilic-Lipophilic Balance (HLB) value for the molecule. The HLB value is an indicator of the emulsifying characteristics of an emulsifying agent. Oil-in-water emulsions usually have weighted HLB values ranging between 8 and 16, whereas w/o emulsions have weighted HLB values ranging between 3 and 8. Emulsifying agents are more effective when used in combination, and the selection of a suitable emulsifying agent depends on the type of cream to be produced *viz.*, o/w or w/o, and the inherent charge on the other excipients *viz.*, whether they are anionic, cationic or non-ionic [210,251,256,260].

3.2 PREFORMULATION STUDIES

Topical formulations contain several excipients and preservatives in addition to low concentrations of the API of interest, making the development of a stable topical formulation rather challenging. The choice of the excipients to be used to ensure that the API is stable and is released to the biological system under the best conditions is one of the most important factors to be assessed during preformulation studies. Preformulation studies may be described as the stage of drug product development during which a formulation scientist characterises all physicochemical properties of an API that are important for the formulation of a stable, safe and effective dosage form of suitable quality [261-263]. The physicochemical properties of the API both in the solid state and in solution must be assessed at an early stage of formulation development [263-266]. Other relevant physicochemical properties include information relating to pH-solubility and stability profiles, drug-excipient compatibility, melting point of excipients and degradation studies of the API [262,267].

3.2.1. CP Topical Cream Formulation Cream Bases

The aqueous cream bases used for the formulation of CP in these studies were manufactured using the materials listed in Table 3.2.

Table 3.2. *Excipients used for formulation studies of CP cream*

Excipient name	Abbreviation	Composition	Purpose	Manufacturer
Clobetasol 17-Propionate	CP	-	API	Symbiotec Pharmalab P. Limited, Indore, India
Propylene Glycol	PG	A diol or a double alcohol	Vehicle for insoluble API and penetration enhancer	Aspen Pharmacare, Port-Elizabeth, South Africa
Sodium Citrate	SC	A weak base	pH adjusting agent	Aspen Pharmacare, Port- Elizabeth, South Africa
Citric Acid	CA	A weak acid	Buffering agent	Aspen Pharmacare, Port- Elizabeth, South Africa
Gelot® 64	G-64	Glyceryl stearate and PEG-75 stearate	o/w emulsifier	Comhan Trading Co. Limited, Sandton, South Africa
Glyceryl Monostearate	GMS	40 to 55% of monoacylglycerols, 30 to 45% of diacylglycerols and 5 to 15% of triacylglycerols	Emollient, stabilising and emulsifying agents	Croda (SA) Limited, Johannesburg, South Africa
Cetostearyl Alcohol	Ceto-A	Solid aliphatic alcohols (stearyl alcohol and cetyl alcohol)	Stiffening agent, viscosity modifier	Croda (SA) Limited, Johannesburg, South Africa
White Beeswax	WB	-	Viscosity modifier	Aspen Pharmacare, Port Elizabeth, South Africa
Chlorocresol	CH	-	Preservative	Aspen Pharmacare, Port Elizabeth, South Africa

3.2.1.1. Clobetasol 17-Propionate (CP)

CP is a lipophilic corticosteroid used in topical formulations for the treatment of dermatological conditions

3.2.1.2. Propylene Glycol (PG)

Propylene glycol (PG) is also known as propane-1-2 diol and is a clear, viscous, hygroscopic liquid that is miscible with water and alcohol. PG is widely used in pharmaceutical manufacturing as a solvent or vehicle in order to dissolve insoluble API or those that are unstable in water [33,268]. PG is commonly used as a penetration enhancer for topical dosage forms [71].

3.2.1.3. Sodium Citrate (SC)

SC is a weak base. It is a white or almost white crystalline powder which is freely soluble in water. It is commonly used as a pH-adjusting excipient in topical formulations [33,268].

3.2.1.4. Citric Acid (CA)

CA is a weak acid. It is a colourless crystalline or granular freely soluble water compound and is used in combination with SC to create a buffer system in semi-solid formulations [268].

3.2.1.5. Gelot® 64 (G-64)

G-64 is an oil-in-water emulsifying agent. It is a mixture of glyceryl stearate and poly ethylene glycol-75 stearate (PEG-75 stearate) [8,269]. It functions as a surfactant, solubiliser, thickening agent, emollient, spreading agent, wetting agent and dispersant in cosmetic and pharmaceutical formulations [270].

3.2.1.6. Glyceryl Monostearate (GMS)

GMS is a mixture of monoacylglycerols that consist mainly of monostearoylglycerol, with variable amounts of di- and tri-acylglycerols. It is a white to yellowish wax-like solid bead, flake or powder with a slight, agreeable fatty odour. It is a poor w/o emulsifier but it is a useful stabiliser for both o/w and w/o cream formulations. GMS has been reported to have emollient properties [33]. The addition of a surfactant to GMS systems has the effect of producing a self-emulsifying system that produces satisfactory o/w emulsions [33,268].

3.2.1.7. Cetostearyl Alcohol (Ceto-A)

Ceto-A is a mixture of solid aliphatic alcohols. It consists mainly of stearyl alcohol and cetyl alcohols and is a white or pale yellow wax-like mass, plate-like or flaky granule. Ceto-A is used as a stiffening agent and/or emulsion stabiliser in cream, ointment, and other topical preparations. Ceto-A is also used in conjunction with suitable hydrophilic substances such as emulsifying wax to produce an o/w emulsion that is stable over a wide pH range. Ceto-A is also used to improve the emollient properties of paraffin ointment formulations [33,268].

3.2.1.8. White Beeswax (WB)

WB is bleached yellow beeswax. It occurs as white or yellowish-white pieces or plates of polymer that are translucent when thin. WB has an odour similar to that of yellow beeswax. WB is usually used as a stiffening agent in ointments and creams and facilitates the incorporation of water into formulations to produce w/o emulsions [33,268].

3.2.1.9. Chlorocresol (CH)

CH occurs as a white or almost white, crystalline powder or as compacted crystalline pellets or colourless or white crystals that are soluble in hot water. CH is used as an anti-microbial preservative in many cosmetic creams containing water as a substantial part of the formulation. It is active against gram-positive and gram-negative bacteria in addition to a number of fungal species [33,268,271].

3.2.2. Melting Point Determination of Waxy Excipients

The melting point determination of each excipient that was likely to undergo melting during formulation and manufacturing processes was undertaken. The melting points of the excipients were determined using a Mettler-Toledo® Model FP62 GmbH melting point apparatus (Mettler-Toledo®, Schwerzenbach, Switzerland). Each sample was placed in a Mettler® ME-18552 melting point tube, ensuring that each tube was filled to a depth of at least 1 mm with the material to be tested. The melting point tube was then placed in the melting point apparatus to determine the melting point of the material.

Samples were analysed in triplicate (n=3) and two sets of data were generated since two different values for the starting temperature, heating rate and final temperature were selected. This was done to obtain an accurate melting point for each excipient. The first set of values commenced heating at a starting temperature of 30°C, with an applied heating rate of a 5°C/min to a final temperature of 70°C. The second set of data was generated using a starting temperature of 50°C, a heating rate of 1°C/min to a final temperature of 70°C. Samples of G-64, GMS, Ceto-A and WB were selected for evaluation since these materials are wax-like granules or flakes and they needed to undergo melting during formulation and manufacturing processes.

It was observed that the second set of melting point data produced small % RSD values, thereby displaying more accurate melting point results than the first set of melting point data, as shown in Table 3.3. The second set of data was therefore used in formulation development decisions. Since the melting points of the excipients fell between 50°C and 63°C, the melting point selected to manufacture the laboratory scale and scale-up cream formulations was set to 70°C.

Table 3.3. *First and second sets of melting point data*

	First melting point data (n=3)		Second melting point data (n=3)	
Excipients	**Melting point (°C)**	**%RSD**	**Melting point (°C)**	**%RSD**
G-64	57.23 ± 1.42	2.49	59.03 ± 0.47	0.80
GMS	57.26 ± 1.70	2.98	57.40 ± 0.10	0.17
Ceto-A	54.07 ± 1.28	2.46	52.77 ± 0.49	0.11
WB	63.63 ± 1.33	2.02	62.63 ± 0.05	0.78

3.2.3. Solubility of CP in PG

In general compounds with poor aqueous solubility precipitate during *in vitro* analyses, resulting in lower concentrations being measured than what could be predicted. Poor aqueous solubility of compounds is one of the major causes of low systemic exposure to a drug and consequently the lack of *in vivo* activity. Therefore it is imperative that the solubility of a molecule in a variety of solvents be investigated during early drug product development [272,273]. Since the formulation to be developed must deliver a topical corticosteroid, that is insoluble in water, propylene glycol (PG) was selected as a solvent of choice for formulation development purposes. PG was also considered for use as the receptor medium for *in vitro* analysis of drug release for laboratory scale and scale-up batches of CP cream formulations. Therefore the solubility of CP in different concentrations of PG was determined in aqueous mixtures *viz.,* 100% v/v, 80% v/v, 70% v/v, 50% v/v, 30% v/v and 0% v/v.

A saturation shake-flask solubility method was used to establish the saturation solubilities of CP in solutions of different concentration of PG [274,275]. An excess of CP (approximately 52 mg ± 1.5 mg) was weighed and placed into a 5 ml A-grade volumetric flask and a binary mixture of PG/water was added to produce a solution of 10.5 mg/ml. The binary mixtures were sonicated for five minutes to facilitate uniform mixing of the sample. Samples, in which a high proportion of PG was used *viz.,* greater than 70% v/v, were found to be viscous and the solubility equilibrium was further facilitated and achieved by shaking all binary mixtures on a Junior Orbit® Model 3520 shaker (Lab-Line Instruments Inc, Melrose Park, ILL) at ambient temperature (22°C) at 200 rpm for 24 hours. Aliquots of the solutions were filtered twice through a 0.45 μm hydrophilic PVDF syringe filter (Millipore® Co., Bedford, MA, USA) prior to analysis. The samples were diluted with MeOH, spiked with BV after filtration and were then analysed using HPLC with UV detection as described in § 2.5 and § 2.6 (Chapter 2). As expected, the greatest solubility of CP was observed in a vehicle that was comprised of 100% v/v PG and the lowest solubility was observed in HPLC grade water. The data are summarised in Table 3.4.

Table 3.4. *Solubility of CP in aqueous solutions of PG*

Propylene glycol content (%)	Mean saturation solubility ± SD (mg/ml)	%RSD
100	9.9027 ± 0.0272	0.27
80	1.0941 ± 0.0136	1.24
70	0.6355 ± 0.0015	0.23
50	0.1504 ± 0.0010	0.66
30	0.0814 ± 0.0008	0.98
0	$4.520 \times 10^{-3} \pm 5.593 \times 10^{-5}$	1.24

The solubility of CP in binary mixtures of PG and water increases as the PG content increases as shown in Figure 3.4.

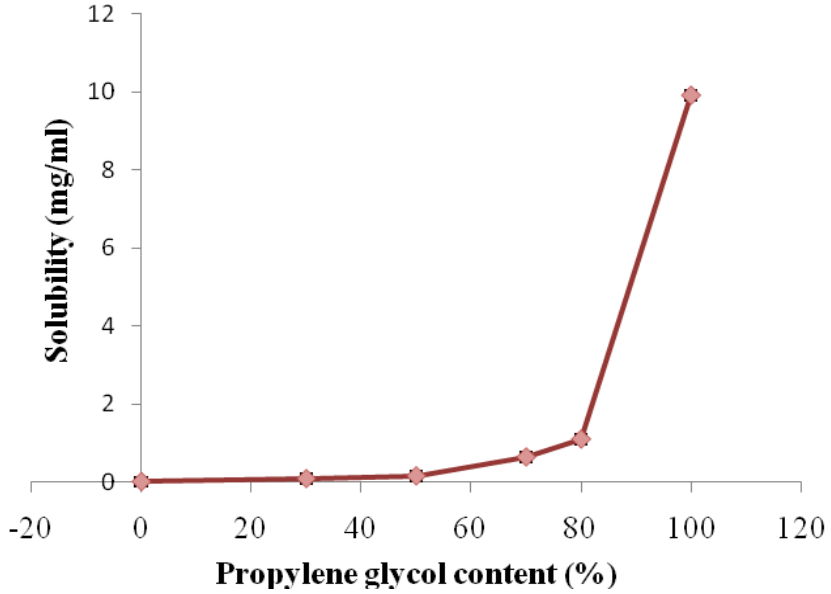


Figure 3.4. *Solubility of CP (mg/ml) in binary mixtures of varying propylene glycol content (%) on solubility of CP*

A Franz diffusion cell method is generally used for the *in vitro* analysis of drug release from topical formulations. This requires the use of a synthetic membrane, such as a 0.025 µm nitrocellulose membrane, placed between the donor and the receptor compartment [276-278]. Therefore some resistance between the membrane and the receptor chamber due to permeability barrier may occur [279]. One mechanism to overcome this resistance is to enhance drug permeation from the formulation into the vehicle by increasing the solubility of the API in the receptor medium. A 70:30% v/v PG:Water was chosen to facilitate the permeability of the API through the membrane, since CP has a high affinity for the solvent.

3.2.4. Stability of CP in PG:Water (70:30% v/v)

The stability of a compound in solution must be established to ensure the integrity of data generated in formulation development studies and that success is achieved at each stage of the drug product development process for the development of a successful formulation [280]. Therefore the stability of three different concentrations of CP *viz.*, high (12µg/ml), medium (6 µg/ml), low (3µg/ml) in binary mixtures of PG:Water (70:30% v/v) was investigated in order to evaluate the suitability of the vehicle

for *in vitro* diffusion studies of CP cream formulations. HPLC analysis of solutions was performed prior to storage of the solutions and following storage for a period of one week in order to establish the stability of the API in PG:Water (70:30% v/v). Since CP degrades in the presence of light, the solutions were protected from light using aluminium foil. The samples were kept in a refrigerator (4°C) until HPLC analysis was performed. No change in the levels of the three concentrations of CP (low, medium and high) was observed after a period of one week. This confirms that CP is stable in this binary composition of PG and water. No apparent decomposition of CP occurred after sampling and during storage, prior to analysis as shown in Figure 3.5.

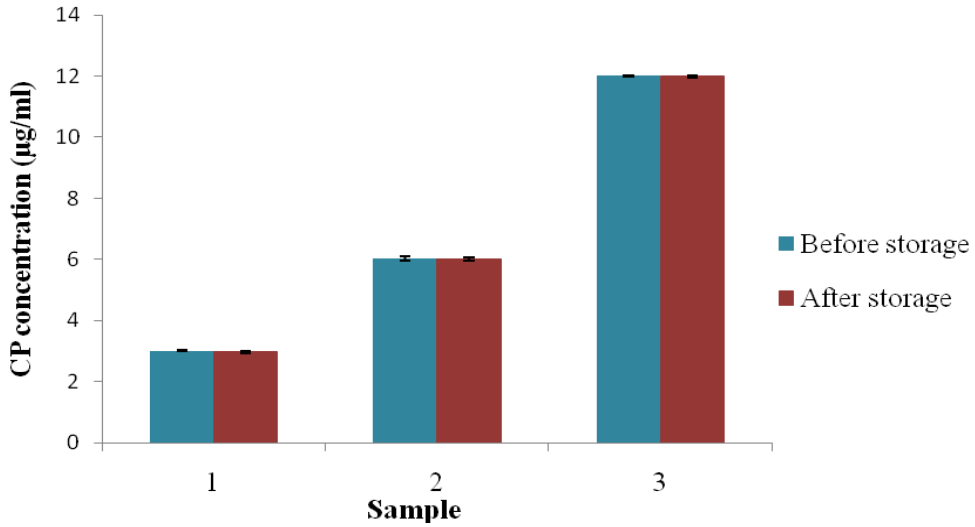


Figure 3.5. *CP stability in PG:Water (70:30% v/v) prior to and following storage at 4°C for one week*

3.2.5 Forced Degradation Studies of CP in PG under Different Conditions

The topical base used to deliver CP consists of propylene glycol and water as the primary components of the vehicle. Therefore forced degradation studies of CP in PG under different conditions such as the presence of a weak acid or base and with a preservative were undertaken. These degradation studies were performed to identify any potential drug-excipient interaction and to ensure that no degradation of CP had occurred.

3.2.5.1. Exposure to Weak Acid

Fifty ml of a 0.05% w/v CP in 100% v/v propylene glycol was added to 50 ml of a 0.05% w/v citric acid solution in a 100 ml A-grade volumetric flask. The mixture was sonicated using a Branson® B12 sonicator (Branson® Ltd, Shelton, Conn, USA) for two minutes to facilitate uniform mixing of the miscible solvents and was then refluxed at 70°C for eight hours. An aliquot of the solution was analysed using the HPLC method described in § 2.5 and § 2.6 (Chapter 2). Chromatographic analysis revealed that the peak for CP was observed at 6.4 minutes without the presence of additional peak(s). Thus there was no evidence of CP degradation having occurred. A typical chromatogram following analysis of this solution is shown in Figure 3.6.

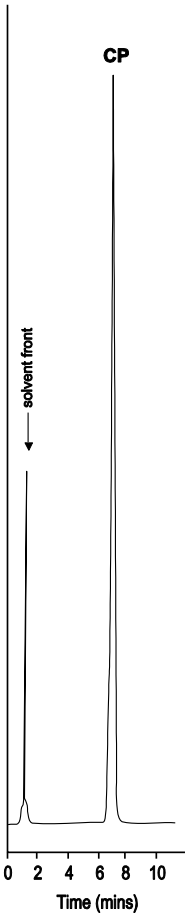


Figure 3.6. *Typical chromatogram showing no evidence of degradation of CP in PG after exposure to 0.05% w/v citric acid solution at a temperature of 70°C for eight hours*

3.2.5.2. Exposure to Weak Base

Fifty ml of a 0.05% w/v CP in 100% v/v propylene glycol was added to 50 ml of a 0.05% w/v sodium citrate solution in a 100 ml A-grade volumetric flask. The mixture was sonicated using a Branson® B12 sonicator (Branson® Ltd, Shelton, Conn, USA) for two minutes and was then refluxed at 70°C for eight hours. An aliquot of the solution was analysed using the HPLC method described in § 2.5 and § 2.6 (Chapter 2). Chromatographic analysis revealed that the peak for CP was observed at a retention time of 6.2 minutes, without the presence of additional peak(s). Thus there was no evidence of CP degradation having occurred. A typical chromatogram following analysis of this solution is shown in Figure 3.7.

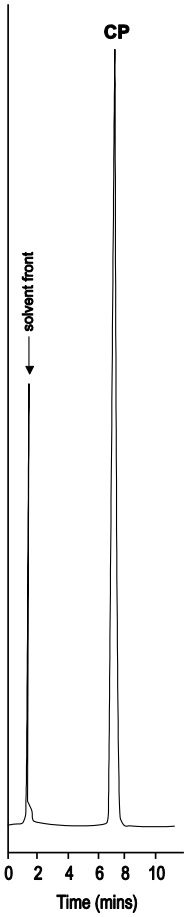


Figure 3.7. *Typical chromatogram showing no evidence of degradation of CP in PG after exposure to 0.05% w/v sodium citrate solution at a temperature of 70°C for eight hours*

3.2.5.3. Exposure to Chlorocresol

Fifty ml of a 0.05% w/v of CP in 100% v/v propylene glycol was added to 50 ml of 0.05% w/v of a hot chlorocresol (CH) solution in a 100 ml A-grade volumetric flask. The mixture was sonicated using a Branson® B12 sonicator (Branson® Ltd, Shelton, Conn, USA) for two minutes to facilitate mixing of the miscible solvents and then was refluxed at 70°C for eight hours. An aliquot of the solution was analysed using the HPLC method described in § 2.5 and § 2.6 (Chapter 2). Chromatographic analysis revealed that the peaks for CP and CH were observed at retention times of 2.8 and 6.4 minutes respectively, without the presence of extra peak(s). Thus there was no evidence of CP degradation having occurred. A typical chromatogram following analysis of this solution is shown in Figure 3.8.

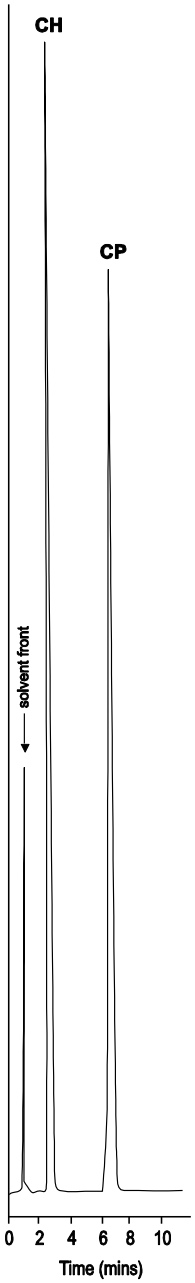


Figure 3.8. *Typical chromatogram showing no evidence of degradation of CP in PG after exposure to 0.05% w/v chlorocresol solution at a temperature of 70°C for eight hours*

3.2.6. Compatibility Studies

Studies of drug substance compatibility with excipients are an important component of preformulation testing and in early development studies for all dosage forms. In fact potential physical and chemical interactions between drugs and excipients can affect the chemical nature, the stability and bioavailability of an API. Consequently, the therapeutic efficacy and safety must be evaluated prior to commercialisation of a product. Infrared (IR) spectrophotometric analysis is a rapid analytical technique commonly used for evaluating drug-excipient interactions that are assessed by studying chemical bond shifts in an IR spectrum [251,281]. Other methods used for this purpose have included

Differential Scanning Calorimetry (DSC), Thermogravimetric Analysis (TGA), diffuse reflectance technique, x-ray powder diffractometry and hot stage microscopy [281,282].

IR spectrophotometric analysis was conducted using a Perkin-Elmer® FT-IR spectrum 2000 spectrophotometer (Perkin-Elmer® Ltd, Beaconsfield, England). Each powder excipient was mixed in a ratio of 1:1 with the API in a mortar and pestle, whereas each waxy excipient was melted with the API in a 1:1 ratio. Approximately 2 mg of each sample was mixed with 200 mg KBr and prepared for analysis as a disk and spectral data were collected over the IR spectrum region of 400-4000 cm^{-1}. The resultant chemical bond shifts were analysed for evidence of any possible drug-excipient interactions.

The IR spectrum of CP shown in Figure 1.4 (§ 1.2.8, Chapter 1) reveals the presence of characteristic peaks for CP occurring between 2000 cm^{-1} and 800 cm^{-1}. These peaks are assigned wavenumbers 1733 cm^{-1} for the C=O-O bond at position C-17, 1661 cm^{-1} for C=O at position C-20, 1606 cm^{-1} for C=C at positions C-1 to C-2 and C-4 and C-5, 1065 cm^{-1} for C-F at position C-9, 1009 cm^{-1} for C-O stretching at position C-11 and 888 cm^{-1} C-Cl at position C-21 respectively [39]. These bands were also observed when IR spectra of physical mixtures of excipient and CP were evaluated and revealed the same absorbance bands as shown in Figures 3.9–3.15. It is clear that no interaction between CP and any of the excipients listed in Table 3.2 was observed since no major chemical bond shifts were evident in the IR spectra.

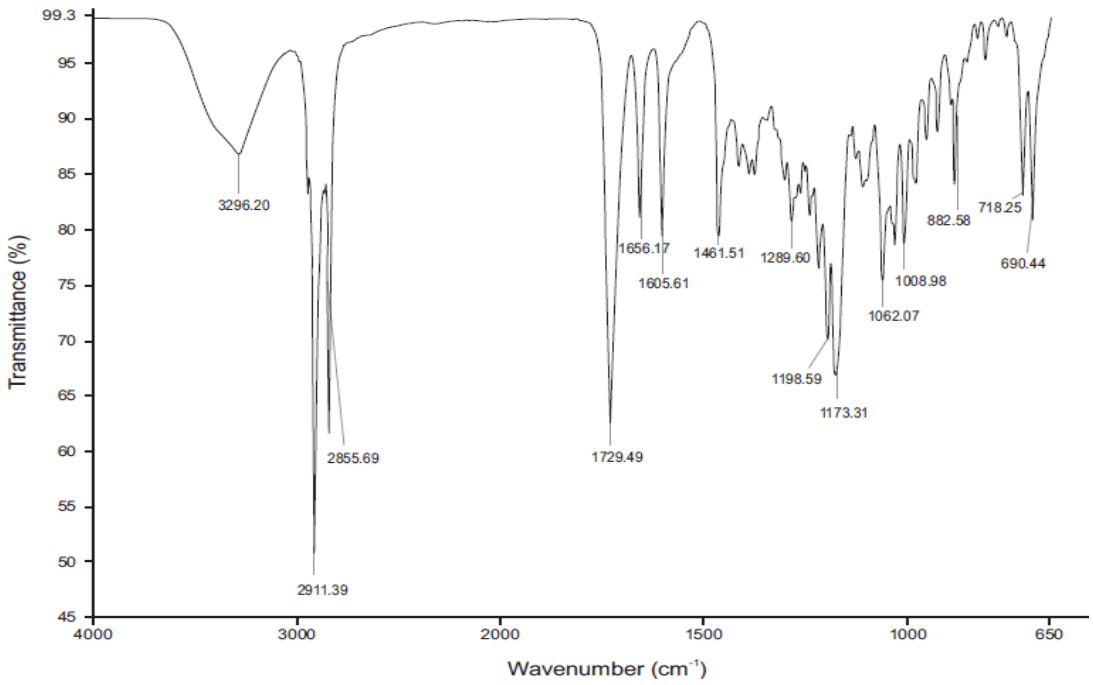


Figure 3.9. *Infrared spectrum of a physical mixture of CP and Glyceryl monostearate (1:1)*

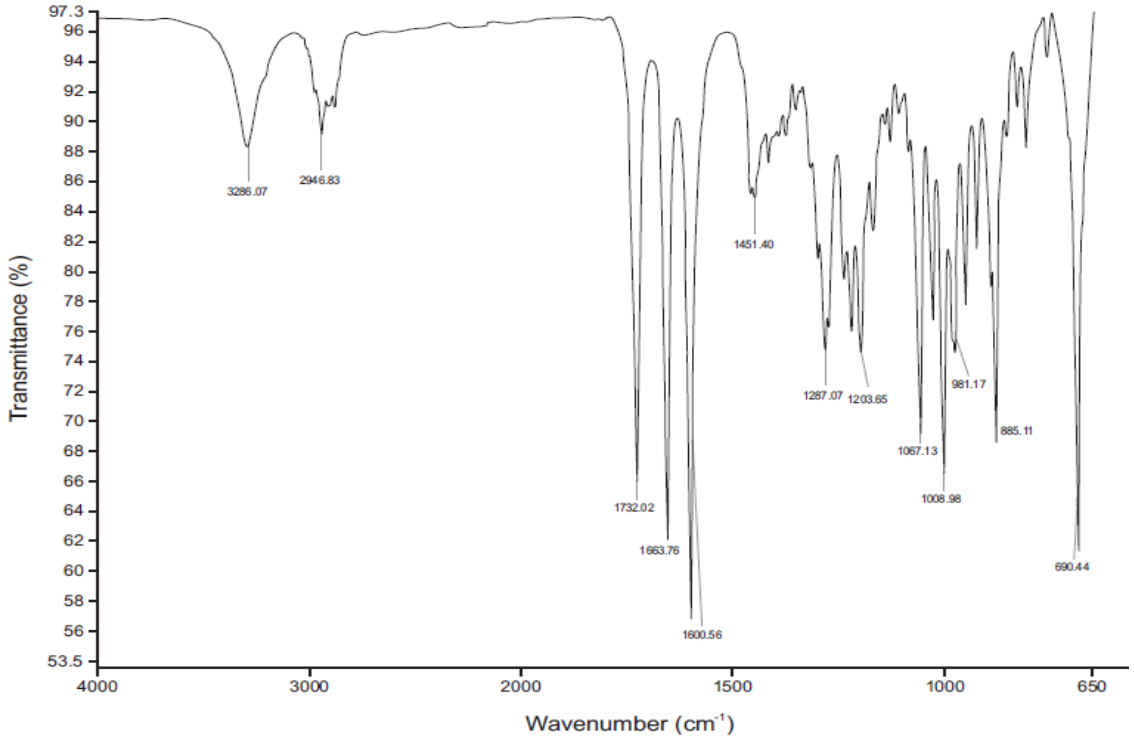


Figure 3.10. *Infrared spectrum of a physical mixture of CP and Chlorocresol (1:1)*

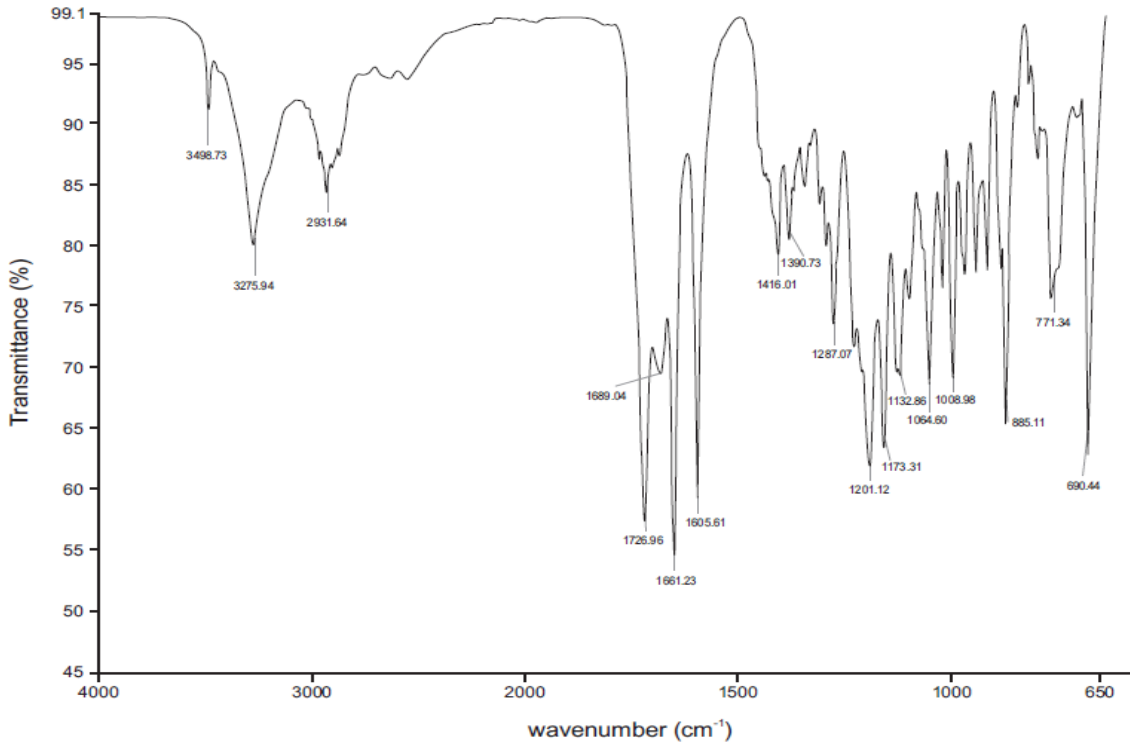


Figure 3.11. *Infrared spectrum of a physical mixture of CP and Citric acid (1:1)*

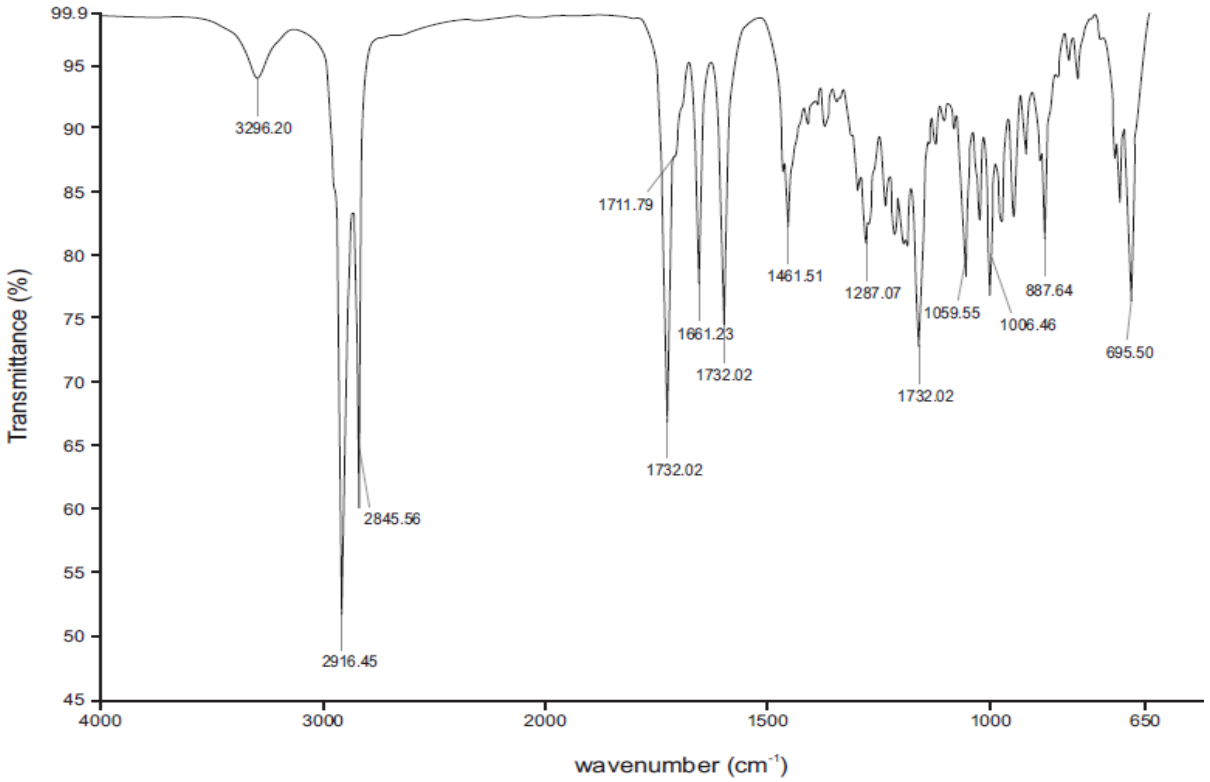


Figure 3.12. *Infrared spectrum of a physical mixture of CP and Cetostearyl alcohol (1:1)*

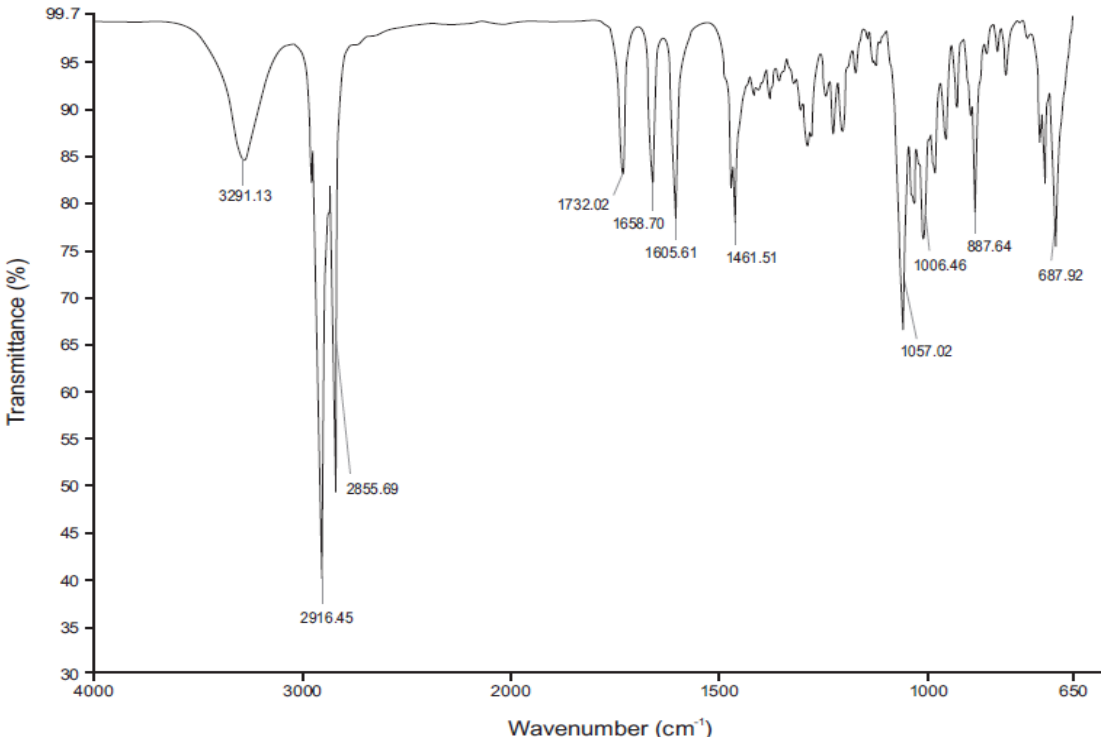


Figure 3.13. *Infrared spectrum of a physical mixture of CP and White beeswax (1:1)*

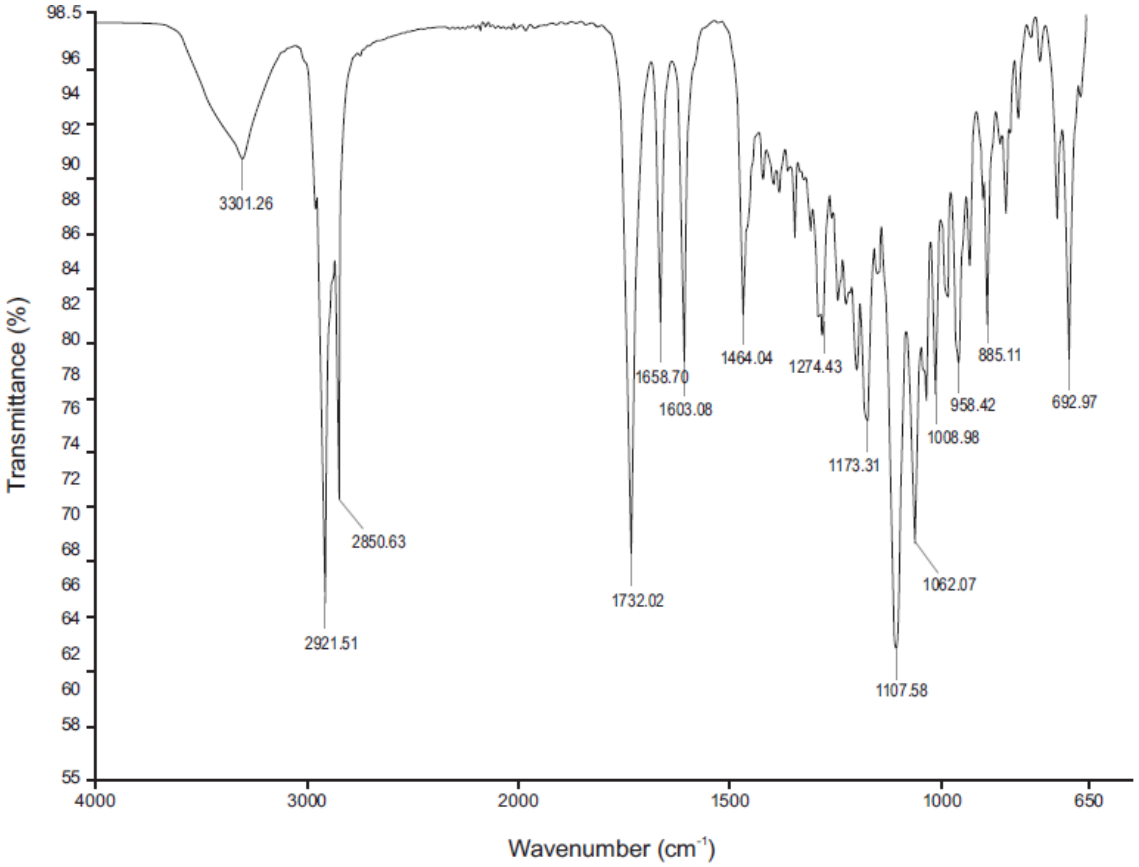


Figure 3.14. *Infrared spectrum of a physical mixture of CP and Gelot® 64 (1:1)*

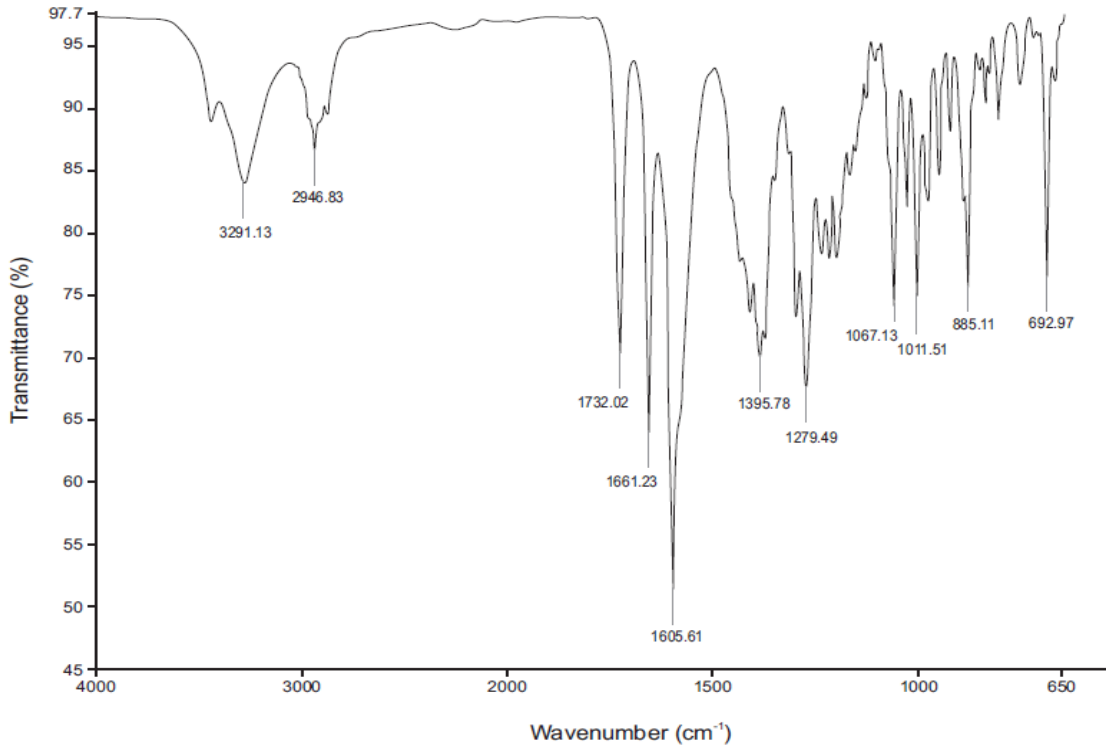


Figure 3.15. *Infrared spectrum of a physical mixture of CP and Sodium citrate (1:1)*

3.2.7. Thermogravimetric Analysis

Thermogravimetric Analysis (TGA) is a technique that has been applied in the pharmaceutical industry to reveal important information regarding the physicochemical properties of a drug and excipient molecules *viz.*, the presence of polymorphism, stability and formulation compatibility [283]. TGA is the first thermal analytical technique that is used to characterise a new molecule. It provides information about the composition of a material and its thermal or oxidative stability, through decomposition studies. TGA instruments use a sensitive analytical balance to measure weight changes as a sample is heated typically from room temperature to 100°C or greater [284,285].

TGA was used to determine the thermal response of CP from a temperature of 30°C to 650°C using a Perkin-Elmer® FT-IR thermogravimetric analytical (Perkin-Elmer® Ltd, Connecticut, USA). TGA measurement was performed using a platinum pan with a nitrogen flow rate of 20 ml/min. The weight of each sample was maintained between 1 mg and 2 mg so that the TGA instrument was able to support the weight of the platinum pan during thermal analysis. TGA measurements were performed at a heating rate of 10°C/min, with an initial temperature set to 40°C and the final temperature set at 650°C, with an atmosphere of 20 ml/min nitrogen. TGA studies were also undertaken to generate thermal profiles of 0.05% w/w CP laboratory scale cream formulations that are reported in § 4.4.7 (Chapter 4) *vide infra*.

The total percent weight of CP was observed to be 100% from a temperature of 30°C up to 200°C, revealing that there were no changes in the physical characteristics of CP under these conditions. At a temperature of above 200°C, it was observed that the total percent weight of CP decreased to a value of 26.1% at 650°C (Figure 3.16). At a temperature of 100°C, complete dehydration of the API might have occurred, followed by the melting of the CP in the range of 150-250°C, after which the decomposition of CP occurred at 225°C as shown in the Figure 3.17.

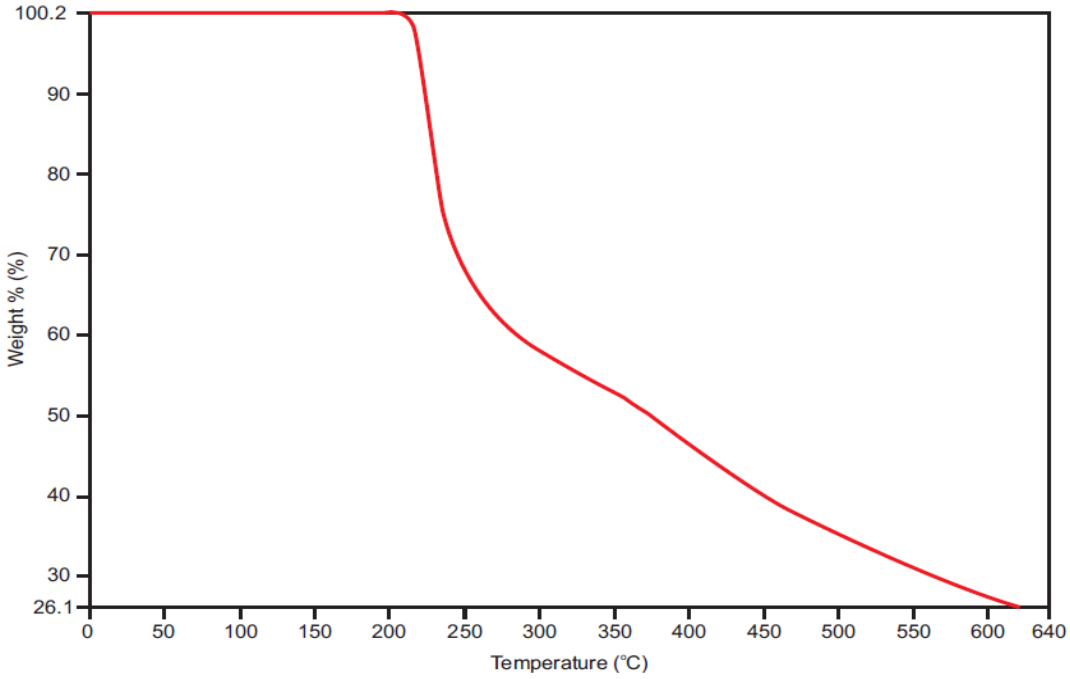


Figure 3.16. *Thermogravimetric curve for CP at a heating rate of 10°C/min*

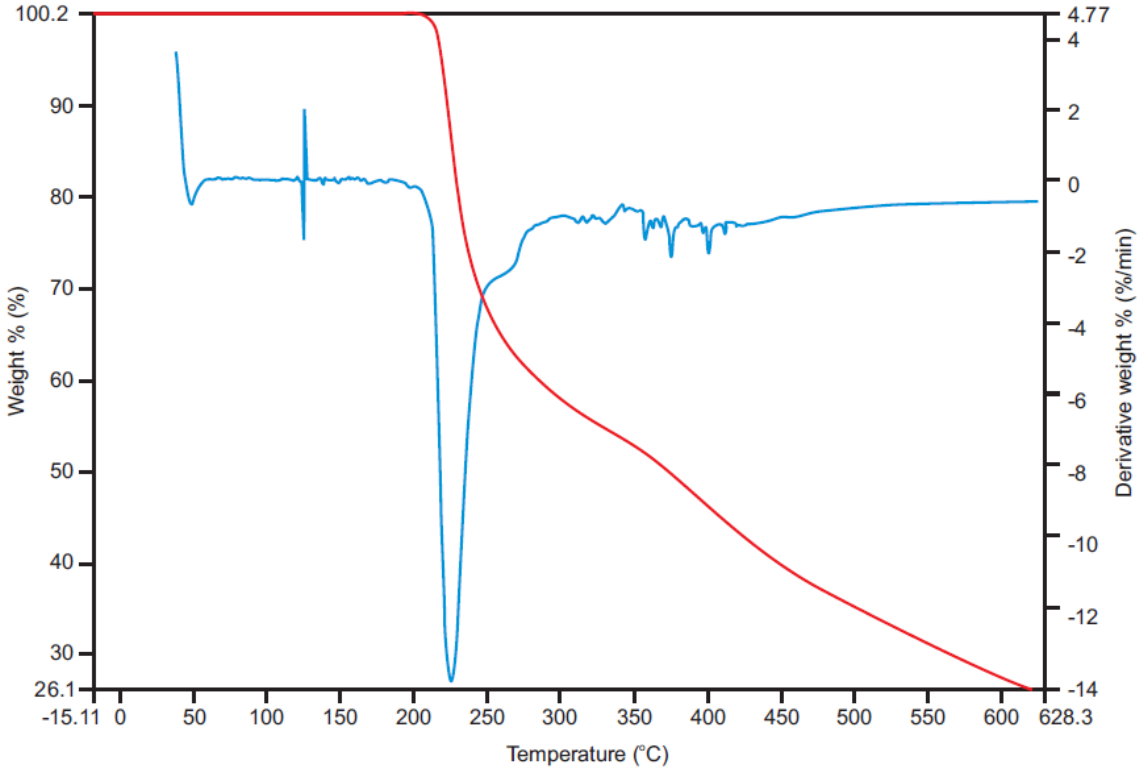


Figure 3.17. *Thermogravimetric (red curve) and its derivative curve (blue curve) for CP at a heating rate of 10°C/min*

3.3 CONCLUSION

Topical drug delivery systems are complex semi-solid systems that permit the delivery of an API, through intact or broken skin to exert local therapeutic effects [204]. Topical formulations are considered to be “rapid formulations” as they provide a relatively quick and easy method of applying medication for any condition. However, semi-solid systems are inherently thermodynamically unstable systems that require thorough investigation during formulation and process development [206]. The composition of a topical dosage form has a major influence on stability as well as the topical absorption of a compound contained in that formulation.

Consequently formulation design and preformulation studies are therefore important tools for the selection of specialised ingredients to produce an effective formulation of suitable quality. These approaches enable the formulation scientist to derive the necessary physicochemical parameters of an API in the solid and solution states, in addition to establishing compatibility with other excipients, prior to manufacture of large-scale batches of the product. Since the melting points of the waxy excipients fell between 50°C and 63°C, the melting point selected to manufacture the laboratory scale and scale-up cream formulations was set to 70°C. Furthermore, the solubility data of CP in different volumes of PG resulted in CP having the greatest solubility in a vehicle that was comprised of 100% v/v PG and a lowest solubility in HPLC grade water. A 70:30% v/v PG:Water was therefore deemed appropriate to be used as the receptor medium of the Franz cell diffusion system in order to facilitate the permeability of the API though the membrane. The stability of CP in 70:30% v/v PG:Water was then investigated in order to evaluate its suitability for *in vitro* diffusion studies of CP topical formulations. CP was found to be stable in this binary composition of PG and water during sampling and during storage, prior to analysis. The IR spectrophotometric analysis revealed that there were no possible drug-excipient interactions between CP and other excipients since no major chemical bond shifts were evident in the IR spectra. These results generated during the preformulation studies enabled the design, development and optimisation of a stable topical cream formulation for CP.

CHAPTER FOUR

DEVELOPMENT AND ASSESSMENT OF LABORATORY SCALE CLOBETASOL17-PROPIONATE FORMULATIONS

4.1 INTRODUCTION

4.1.1. Pharmaceutical Formulation Development

The pharmaceutical industry is an important part of the global health care system and the use of pharmaceutical products is extensive due to an increase in the incidence of acute and chronic diseases [286]. The cost of health care has escalated globally over the last two decades and this has prompted efforts in most countries to reduce the cost of medicines [287]. It is well-known that most interventions in health are associated with the use of medication. The current drug and formulation development arena requires several stages of research from candidate API selection to commercial dosage form production, when a pharmaceutical company seeks marketing authorisation for a new pharmaceutical product [251,288,289]. Pharmaceutical development is the first stage in the product development process, whereby molecules that have been discovered during the drug discovery phase are developed further to produce a pharmaceutical product [290,291]. Prior to initiating formulation development strategies to produce a commercially acceptable product, it is important to investigate the physicochemical properties of an API thoroughly in preformulation studies. This is done to acquire an excellent understanding of all physical, chemical and biopharmaceutical characteristics of that API [292,293]. The acquisition of such knowledge assists in making appropriate formulation choices to maximise the bioavailability of the API, which will result in an optimised therapeutic efficacy [294].

Innovator pharmaceutical companies produce hundreds of thousands of new molecular entities annually and it has been estimated that for every successful drug compound discovered and marketed, approximately 5000 to 10000 compounds have been introduced into the drug discovery pipeline [294,295]. Pharmaceutical companies invest significant revenue in Research and Development (R & D) and on average around 800 million USD and 10-15 years are necessary to develop and market a successful new drug product [294,296]. In 2004, statistical data revealed that the pharmaceutical industry spent approximately 1.25 billion USD for each molecular entity approved by the Food and Drug Administration (FDA). In 2005, worldwide expenditure in the pharmaceutical industry was reported to be 53 billion USD. The overall expenditure on R & D has been increasing steadily over years while new chemical entities are failing to reach the market [296-298]. Furthermore the expenditure on pharmaceuticals was expected to increase from 362 billion USD to 561 billion USD in price and this was an increase of 55% for the period 2000 to 2005. At the same time approximately 100 billion USD worth of products were facing patent expiry. This in conjunction with severe

budgetary constraints such as government-run health care systems, health insurance and government debt crisis, present an opportunity for generic companies to expand and build their capability to capture a sector of the global market [299].

Apart from the growing research expenditure, the pharmaceutical industry is facing tough challenges in keeping pace with the current business environment, in order to maintain and operate a successful and effective global pharmaceutical R & D organisation [296,300]. It is therefore crucial to maintain a pharmaceutical development process that manages cost effectiveness, in addition to developing pharmaceutical products of high quality, safety and efficacy [296,301].

4.1.2. Generic Pharmaceutical Formulation Development

Since the cost of pharmaceutical products has risen over the years, the contribution of drug costs to the overall cost of health care has received considerable attention from politicians [302,303]. A major strategy for lowering the costs of medication globally, thereby reducing the contribution to total health care costs, has been the introduction of multi-source interchangeable or generic medicines [290,299,304]. A generic pharmaceutical product is defined as a multi-source product that is interchangeable with the original/brand product and enters a pool of available substances when the original/branded product loses its exclusivity, through the expiry of patents and other intellectual property [299,305]. Consequently, generic products can be marketed when innovator or brand products are no longer protected by patents and which can be made available commercially as an 'unbranded' version of the original product. These types of generic products are called '*pure generics*' [299,306,307]. However over years, the categorisation of *'pure generics'* has been distorted, giving rise to the commercialisation of products that are not issued by an originator but may be linked to the name of the producer of the product. These products are referred to as *'branded generics'* [308]. A multi-source interchangeable/generic product must contain the same active ingredient in the same dosage form and quantity as the original product. It must thus own efficacy, safety, quality and bioequivalence profiles similar to the original product, to achieve the same therapeutic effect [306,309-312]. In other words, the generic is pharmaceutically equivalent to the original product [310].

Medications were historically used as a means to treat and alleviate symptoms of acute and chronic disease and minor ailments. However in the late 20th and early 21st centuries, medicines have also been used preventatively to modify and/or reduce health risks associated with chronic conditions [313]. Therefore prescription drug spending and utilisation have increased rapidly at a rate of over 10% per annum and currently represent 11% of all health care expenditure to satisfy global health care needs [314-316]. In the Unites States, the expenditure on prescription drugs has grown at an annual rate of 15% from 1996 to 2002, with the 178 billion USD spent in 2002 accounting for more than 11% of all health care expenditure. This increase was largely driven by a rise in the average price

of prescription medicines, which increased by more than 10% per annum from 1996 to 2001. These price increases are due mainly to an increase in the price of existing drug products and also by a shift to newly approved drugs, which tend to be more expensive than the drugs that were marketed first in the same therapeutic category [314]. In contrast, the South African health system consists of a large public sector and a small fast-growing private sector. The South African health care system ranges from basic primary health care, offered free by the government, to highly specialised high-technological health services available in the private sector [317]. Although the government contributes approximately 40% of all health care expenditure, the public health sector is under pressure to deliver health care services to 80% of the population. The South African public sector expenditure on pharmaceuticals rose by 58% per annum between 1995 and 1999 since the majority of the population relies on the public sector for access to health [318]. In 2000, approximately 8.25 billion Rand was spent on pharmaceuticals in South Africa, with the government spending only 24% of that total. Thus, R 59.36 per person was spent on medicines in the public sector as opposed to R 800.29 per person in the private sector [319].

For the period of 1997-2000, the national average savings through the use of generic drugs accounted for 9 billion USD or 11% of total prescription costs. At the same time, generic products have captured more than 65% of the global pharmaceutical market and approximately 70% of all prescriptions written in the Unites States are for generic products [287,304,320]. Increased generic substitution for original drug products has long been advocated as a means of decreasing consumer expenditure on prescription medicines. The majority of consumers realise major cost-saving when using generic products [304]. Moreover, the number of patents expiring between 2010 and 2014 has been estimated to put more than 209 billion USD of annual drug sales at risk, resulting in 113 billion USD of sales for innovator companies being lost to generic substitution [290].

Despite the importance and need for efficiency in generic product development, there is very little research directly relating to the generic pharmaceutical industry [290]. Manufacturers need to demonstrate the bioequivalence of a generic product to an original or reference product and the manufacturing process must comply with the FDA requirements, by producing a pharmaceutical product of acceptable quality, safety and efficacy [305,321,322]. The development process does not involve lengthy and costly clinical trials since generic manufacturers need to prove bioequivalence only [299,323]. On average, the development of generic drug products takes only three years as compared to the development time of branded products which takes up to seven years or more [289,324].

4.1.3. Phases in Pharmaceutical Formulation Development

The pharmaceutical product development process is multivariate in nature as the process differs substantially from one pharmaceutical company to another. There are no general or standard

processes that can be applied to all pharmaceutical companies in the industry [299]. However, the processes and procedures applied in formulation development for different types of dosage forms are similar. Prior to undertaking a pharmaceutical formulation development, the selection of an API and source must be identified. The physical characterisation of the API, preformulation studies and dosage form design must be established early in the process [264,293]. After generating all necessary physical and chemical information relating to the API of interest, the development of a prototype formulation takes place by adding appropriate quantities of excipients to form a small-scale batch of product with previously identified desirable product features. The manufacture of a small-scale production batch is essential to develop a facile manufacturing procedure for transfer to production-size batches. The batch size may start from as small as one hundred grams and it helps to observe, correct and improve the effects of important variables in the formulation and/or manufacturing process if necessary. Following the manufacture of a small-scale batch of the formulation, scale-up must commence if one is to be commercially successful. The scale-up process is the integration of previous phases of product development in addition to the transfer of technology to manufacture the desired formulation. Following successful scale-up and pharmaceutical formulation development, pre-clinical and clinical trials and the certification of a formulation, marketing must be approved. A summary of this process is depicted in Figure 4.1 [325-327].

The aim of pharmaceutical formulation development is therefore to design a quality product and a manufacturing process for that product in order to produce a dosage form that performs consistently *in vitro* and *in vivo*. The information and knowledge generated during pharmaceutical development studies and manufacturing provide a scientific understanding to support the establishment of a design space, specifications and manufacturing controls for a particular product [328].

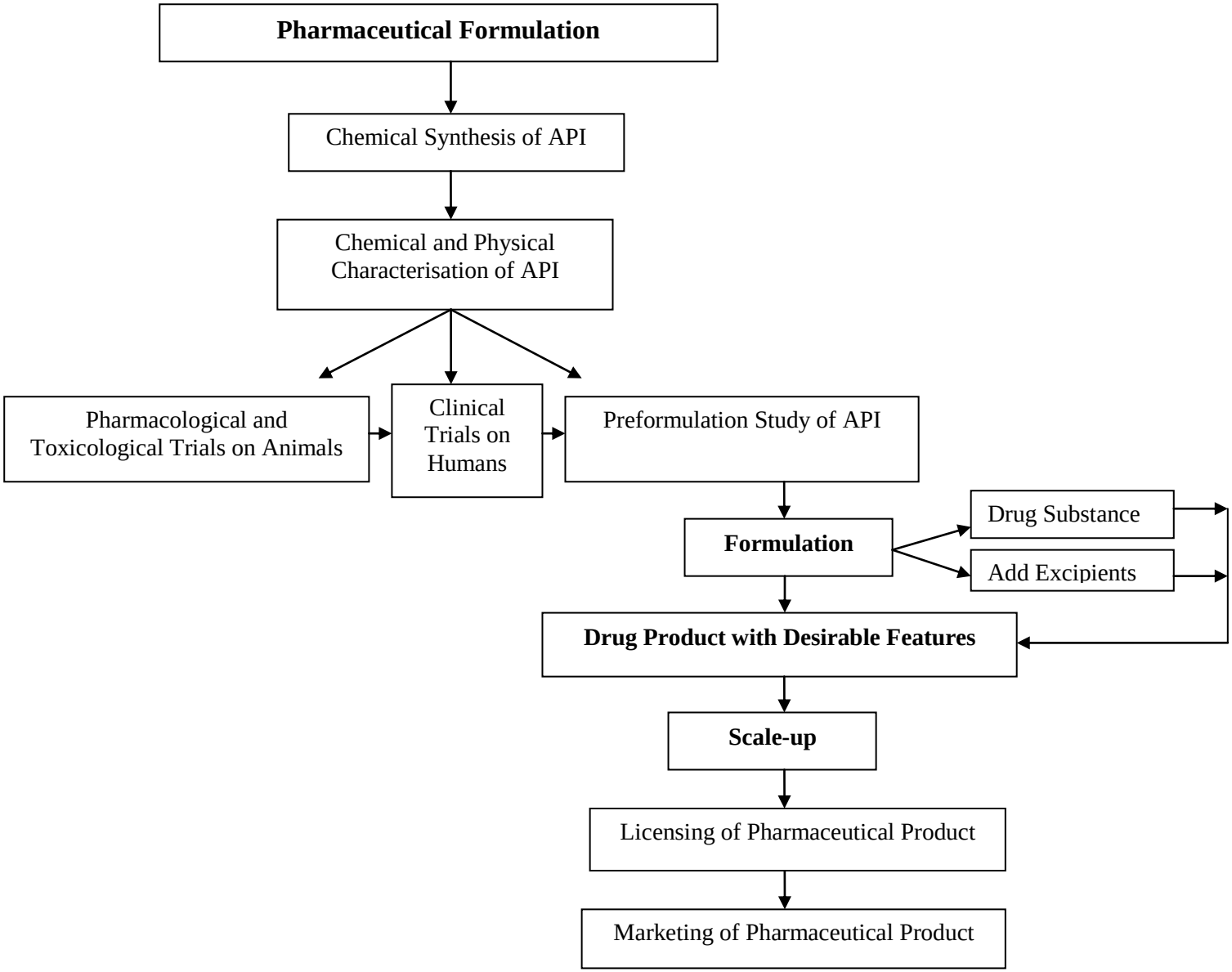


Figure 4.1. *Phases involved in the development of pharmaceutical formulations [325,326]*

4.2 FORMULATION

Formulation is that stage of product manufacture in which an API is combined with excipients to produce a dosage form suitable for the delivery of that API to a patient [329]. Formulation is often a major hurdle in drug product development [327,330]. Initially the development process requires the identification of a route of administration and target site for a molecule [331]. Any pharmaceutical product that is intended for human use must follow a quality process and relevant safety data must be generated for the product. In addition to the clinical application, the specific physicochemical properties of an API and excipients will influence the formulation options to be pursued [328,329]. Formulation scientists will encounter specific formulation challenges *viz.,* poor aqueous solubility, incompatibility of excipients and API, photodegradation and others when developing a pharmaceutical dosage form [48,332,333]. Therefore a variety of formulation parameters must be considered when developing a formulation, and the components of any formulation must be physically and chemically compatible with the API to be included in the product [327,334,335].

4.2.1. Topical Formulations

Topical drug delivery systems are usually thermodynamically unstable multi-component systems that often require the use of emulsifying agents to produce a stable product [209,336-338]. Topical corticosteroid formulations may require the incorporation of other components such as penetration enhancers that are found to improve the bioavailability of an API [67,71,72,217,235]. Furthermore the design of a cream, ointment and/or gel formulation for topical use varies according to the specific application and site of application for that product [66,339]. Sunscreens for instance are applications that must remain on the surface of the skin and block the penetration of UV radiation to the epidermis. They act as a filter and help to absorb and reflect high energy UV light. Sunscreens are used to prevent sunburn and they should be retained on the surface of the skin to minimise the need for multiple applications [340,341]. Topical corticosteroid cream formulations, on the other hand, act as drug delivery systems. A 0.05% w/w clobetasol 17-propionate (CP) cream is generally used to treat skin conditions such as chronic hyperkeratotic eczema of the hands and feet, and patches of chronic *lichen simplex* and chronic hyperkeratotic psoriasis of area(s) of the body (§ 1.6.2, Chapter 1) [18]. Topical steroid formulations are usually applied in a thin layer to the affected area(s) of the skin twice daily, preferably in the morning and evening, with gentle and complete rubbing into the skin [342]. Therefore the function of a topical corticosteroid drug delivery system is to ensure that the API penetrates the stratum corneum and that the API is delivered and released at the site it is required, to exert a therapeutic effect [203,217].

4.2.2. Physical Stability of Topical Formulations

Although different topical formulations are used for a diverse range of applications, there are common and important characteristics that all products should have, not the least of which being that they must

exhibit a high degree of stability [276,343,344]. When a topical formulation consists of several excipients blended together, there is a probability that the system will undergo destabilization [345,346]. The physical instability of topical formulations has been reported to occur through various time- and temperature-dependent physicochemical destabilizing mechanisms such as phase inversion [8,347].

Destabilization occurs in different phases. The first manifestation of instability is flocculation, during which there is close agglomeration of two or more individual droplets of a dispersed phase. The individual droplets then form loose assemblies or flocs, without the loss of the interfacial film that provides some degree of stabilization. In other words, the droplets form loose clusters in which each droplet is separated by a thin interfacial film [8,209,251,348]. Coalescence occurs when the interfacial film becomes so thin that it is unstable and the film is completely disrupted after which the flocculated droplets aggregate to form single large droplets [8,348,349]. The larger droplets continue to grow by a process called Oswald ripening, consuming smaller droplets due to differences in the solubility or the chemical potential of the small and large droplets [8,350,351]. The ultimate end-point of instability is creaming or sedimentation, in which the phases of the once-stable dosage form separate. When droplets rise to the top of the formulation, the process is called creaming, whereas if the dispersed droplets settle at the bottom of the container, the process is called cracking [8,251,352,353].

4.3 FORMULATION OF LABORATORY SCALE CLOBETASOL 17-PROPIONATE (CPLS) CREAM

4.3.1. General Methods of Preparation of Topical Formulations

Most topical formulations are produced by one of two general methods, whether the scale of manufacture is large or small [209]. Usually fusion and levigation techniques are used for the incorporation of some excipients and API into a base formulation [208,209,354-356]. The fusion method occurs at high temperatures, by blending all liquid or liquefied components together to produce a liquid emulsion. The solid components are then dispersed in the liquid and the formulation is allowed to cool and congeal [208,209,356]. This method is used only when all formulation components are stable at the temperatures at which fusion occurs. This system is used for the production of virtually all o/w creams [209,251,357]. Fusion is usually performed using steam-jacketed vessels (large-scale) or a porcelain dish (small-scale). The choice of an appropriate fusion temperature is important to avoid thermal degradation of the base or other components of the formulation and to prevent excessive evaporation of the aqueous phase during the fusion process [208,358,359].

The levigation method is also known as 'cold incorporation'. This method involves the simple mixing of a base and other components of the formulation over an ointment slab using a stainless steel spatula [208,209,360]. The levigation method is used when heat-labile materials are to be added to a previously manufactured ointment or cream system, or when the vehicle of a topical dosage form is heat-labile [209]. Small quantities of powders are incorporated into hydrocarbon bases, with the aid of a levigating agent such as liquid petroleum, which helps the wetting of powders. Uniform mixing is usually ensured by use of the geometric dilution method of mixing, which involves the stepwise addition of solids into the ointment base [208,361].The fusion method was preferred in this study for the development of a laboratory scale CP cream formulation to produce an o/w product that could be considered for scale-up in the next series of studies *vide infra* (§ 5.2.3, Chapter 5).

4.3.2. Manufacture of Laboratory Scale CP (CPLS) Cream Formulations

The fusion method for the manufacture of laboratory scale cream formulations is slightly more complicated than the manufacture of ointment and/or other semi-solid products. The technique usually involves the preparation of two phases *viz.*, an aqueous and an oily phase [209]. All CPLS formulations were manufactured using a modified procedure, adapted from a previously reported manufacturing method [362]. The composition of the CPLS formulations was selected on the data generated in preformulation studies (§ 3.2.1, Chapter 3) and the % composition for each batch of CPLS is shown in Table 4.1. All excipients were weighed using a Mettler® Model AE-163 top-loading analytical balance (Mettler® Instruments, Zurich, Switzerland).

Table 4.1. *Percentage composition (% w/w) of CPLS cream formulations tested*

No.	Excipients	Dermovate®	CPLS-01	CPLS-02	CPLS-03	CPLS-04	CPLS-05
1	CP	0.05	0.05	0.05	0.05	0.05	0.05
2	Propylene glycol	47.50	47.50	46.00	43.00	40.00	37.00
3	Sodium citrate	0.05	0.05	0.05	0.05	0.05	0.05
4	Citric acid	0.05	0.05	0.05	0.05	0.05	0.05
5	Arlacel® 165	1.50	-	-	-	-	-
6	Gelot® 64	-	1.50	3.00	6.00	9.00	12.00
7	Glyceryl monostearate	11.00	11.00	11.00	11.00	11.00	11.00
8	Cetostearyl alcohol	8.40	8.40	8.40	8.40	8.40	8.40
9	White beeswax	1.15	1.15	1.15	1.15	1.15	1.15
10	Chlorocresol	0.075	0.075	0.075	0.075	0.075	0.075
11	Distilled water	30.225	30.225	30.225	30.225	30.225	30.225

4.3.2.1. Aqueous phase

In general, the aqueous phase of a cream system should be heated to approximately 5°C above the temperature of the oil phase that is to be incorporated, in order to prevent premature solidification of the oily phase during the emulsification process [209,363,364].

Distilled water was heated to 75°C in a 500 ml beaker using a Colora® Model NB-34980 Ultra-Thermostat water bath (Colora®, Lorch, Germany). The temperature of the distilled water was maintained at 75°C using the water bath. Sodium citrate (SC) and Citric acid (CA) were added to the beaker containing the distilled water and were dissolved with the aid of a stirring rod. Propylene glycol (PG) was then added to the beaker containing this aqueous solution. CP was added to the solution while heating at 75°C, and the solution was sonicated using a Cole-Parmer® Model 8845-30 ultrasonic bath (Cole-Parmer® Instrument Co., Chicago, USA) for approximately 25 minutes until a clear solution had been produced.

4.3.2.2. Oil phase

As a general rule, the oil phase is heated to at least 5°C above the melting point of the highest-melting waxy ingredient to be incorporated into the formulation [209]. The highest melting point waxy compound to be included was white beeswax (WB) with a melting point of 63.63°C. Gelot® 64 (G-64) was melted with cetostearyl alcohol (Ceto-A), white beeswax (WB), glyceryl monostearate (GMS) and chlorocresol (CH) in a 1000 ml beaker heated to and maintained at 70°C using a water bath (Colora® Model NB-34980 Ultra-Thermostat water bath, Colora®, Lorch, Germany).

4.3.2.3. Cream Formulation

The warm aqueous phase was transferred to the hot oil phase with continuous manual stirring and the mixture was maintained at 70°C. The mixture was stirred at 70°C, with the aid of a glass rod, for five minutes, after which the beaker was removed from the water bath. The mixture was then homogenised using an AF Virtis® Model 6-105 homogeniser (Virtis® Co., Gardiner, USA) at approximately 3000 rpm for a further ten minutes while cooling. The resultant cream formulation was then packed into 500 g opaque containers and stored at room temperature (22°C) until required for further analysis. A schematic diagram of the manufacturing process of the CPLS formulations is given in Figure 4.2.

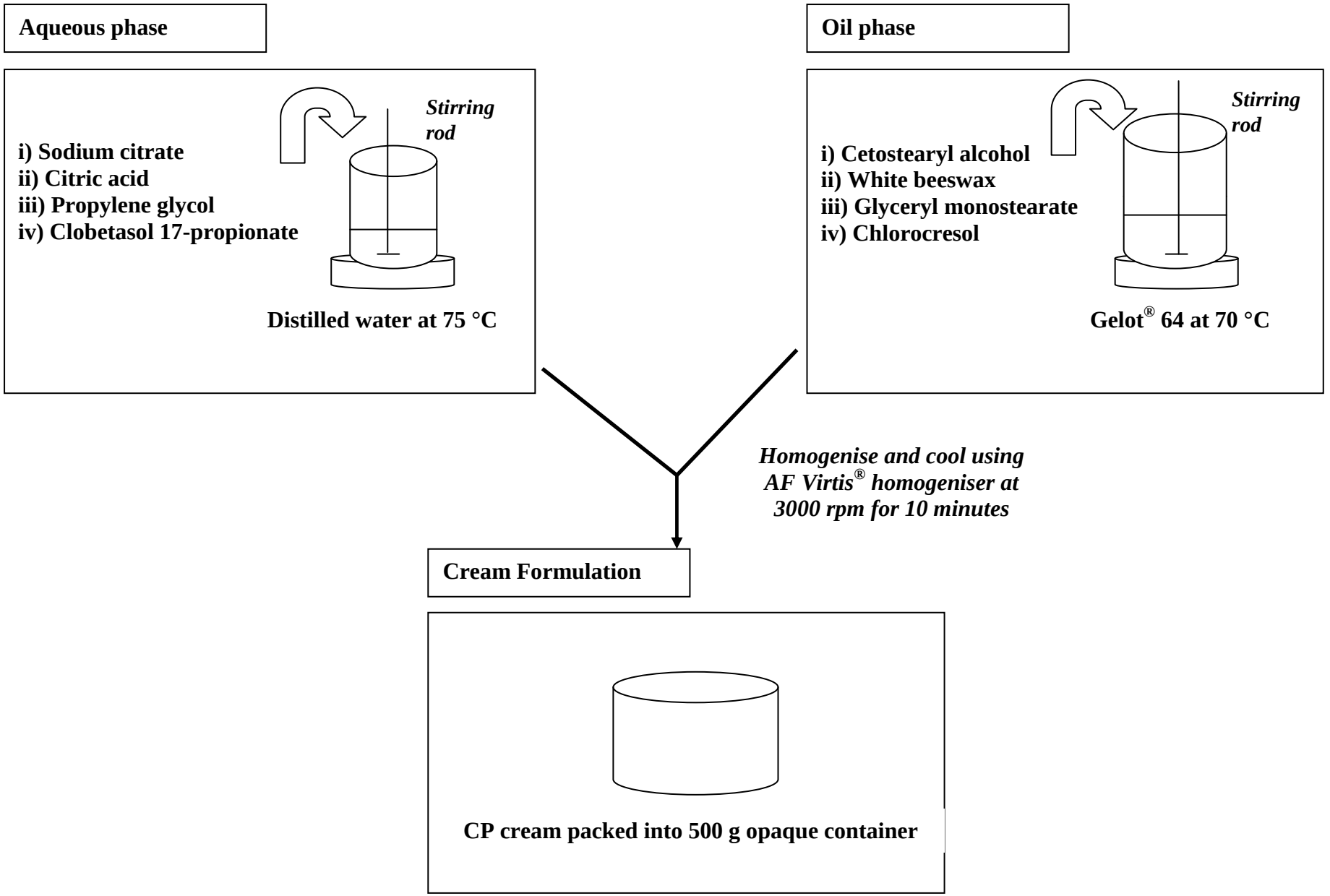


Figure 4.2. *Schematic diagram for the manufacture of CPLS formulations*

4.3.3. Effect of Different Concentrations of G-64 and PG on CPLS Formulations

Formulation scientists undertake topical formulation product development in order to manufacture a laboratory scale CP cream formulation. The laboratory scale CP cream formulation must show physical stability, must have the same physical and chemical characteristics and must ultimately produce the same *in vitro* release profiles as a commercially-available innovator reference product.

The CP-containing reference product on the South African market is Dermovate® (Glaxo® Wellcome Pharmaceuticals) [199,365,366]. The qualitative composition of the innovator cream reveals that the o/w emulsifier, Arlacel® 165 (A-165) is a major component of the product [199]. However Gelot® 64 (G-64) was used in the development of generic formulations. All the other excipients used in the development of generic formulations were the same as those in the Dermovate® product. Arlacel® 165 consists of a mixture of glyceryl monostearate and polyethylene glycol-100 stearate (PEG-100 stearate), whereas G-64 consists of glyceryl monostearate and polyethylene glycol-75 stearate (PEG-75 stearate) [8,269,367]. PEG-stearate is also known as macrogol stearate as there are two systems of nomenclature for these compounds. These substances have the general formula of $C_{17}H_{35}COO.[O.CH_2CH_2]_n.H$. The numbers reflected in the name of these compounds represent the average molecular weight of the recurring polymer chain. As the average molecular weight increases, the water solubility, hygroscopicity, and solubility of the polymer in organic solvents decrease and viscosity increases [33,368]. Therefore Dermovate® cream containing 1.5% w/w A-165 may exhibit different physical characteristics and *in vitro* profiles as compared to a formulation containing 1.5% w/w G-64. However, mixed emulsifiers such as G-64 have a 'self-bodying action'. A stable o/w emulsion of any consistency from mobile lotions to soft or stiff creams may be manufactured simply by altering the total concentration of mixed emulsifier in the formulation [368].

Most mixed emulsifying agents such as A-165 and G-64 usually exhibit marked crystalline polymorphic behaviour (α-crystalline polymorph) during the emulsion formation and manufacturing process. In the presence of water, the polymorph forms a hydrated crystal, in which the thickness of the water layers incorporated between the polar groups (~18A) is limited by the considerable strength of the Van der Waals attractive forces. Van der Waals forces usually balance the osmotic repulsive forces in the system, resulting in the formation of a swollen α-crystalline gel phase [369-371]. This gel phase is characterised by a lamellar structure of alternating bilayers of emulsifying wax separated by layers of water. However on heating, the gel phase is transformed to a lamellar liquid crystalline phase at a specific transition temperature, melting the hydrocarbon chains in a dynamic and disordered manner [369,372,373]. Most amphiphilic materials used in pharmaceutical systems transition from a gel to a liquid at temperatures of between 40°C and 50°C. The liquid crystalline phase converts back to a gel phase, once formulation has been completed and cooling takes place to room temperature, to form the multi-component visco-elastic gel network [368,374]. This visco-

elastic gel network that forms, after heating and cooling have been completed, may be composed of three phases *viz.*, i) crystalline hydrates of fatty amphiphiles, ii) a swollen lamellar α-crystalline gel phase of surfactant and fatty alcohol and iii) free water. The overall consistency of the topical formulation and the 'self-bodying action' of the emulsifying agent is ultimately related to the swelling properties of the material in addition to the amount of the α-crystalline gel phase [368,375-377]. PG has a dual action in the formulations of CP cream. It is a vehicle for the insoluble CP and further acts as a penetration enhancer. Therefore it was necessary to investigate the effect of varying concentrations of G-64 and PG on CPLS formulations (Table 4.1).

4.4 ANALYSIS OF TOPICAL DOSAGE FORM

As with any dosage form, quality testing procedures and associated acceptance criteria for topically administered products are used to assess the quality attributes and performance of topical formulations [209,378]. Topical dosage forms must undergo a variety of pharmacopeial and non-pharmacopeial tests to ascertain their physicochemical properties, *in vitro* and *in vivo* performance. This is done to ensure that quality standards are met and that the ultimate quality of a product is always assured. These tests must be performed frequently during topical formulation development, manufacturing and subsequent scale-up as they are vital in ensuring and retaining the quality attributes of a product and help in minimising batch-to-batch variability [208,215,378]. Furthermore results from such testing were used to select an optimised CPLS cream formulation for further development.

Following manufacture, all cream formulations were evaluated in terms of their physical appearance and skin feel properties. These subjective tests were performed as guide to evaluate topical dosage form properties, in relation to the desired properties that may be necessary for the treatment of specific skin conditions. Subjective assessment and expectations are also critical for a patient and a physician, in order to ensure that topical products that are sold may also improve the appearance and feel of the skin [247]. In addition to the subjective tests performed on cream formulations, physical tests such as viscosity, spreadability, pH, centrifugation and *in vitro* testing were performed to evaluate the performance of formulations. Viscosity and spreadability testing were performed on all CPLS cream batches as these primary parameters were used to evaluate complex flow behaviour of semi-solid systems. The viscosity and spreadability of a topical formulation can greatly influence the rate and extent of release of API and ultimately availability for absorption or therapeutic efficacy [109,379]. The pH of healthy human skin ranges between 4 and 6 [115,380]. The measurement of pH of topical formulations was necessary to monitor pH changes during long-term storage so that the component incompatibility or skin irritation is avoided [381]. The centrifugation test is of major interest since it provides rapid information, relating to the comparable physical stability properties of cream formulations [381,382]. Centrifugation is also an excellent tool for the evaluation of

accelerated deterioration of semi-solids over a short period of time [382]. Thermogravimetric analysis (TGA) is a useful technique that was successfully used to understand the behaviour of cream formulations, when external stress such as heating is applied to the system.

In vitro testing including monitoring of API and product homogeneity have become primary measures of equivalence in product uniformity performance *in vitro* [328,383]. Product homogeneity can be assessed in terms of content uniformity within units of a product formulation. Content uniformity is assessed using a specific stability-indicating test that can be used to measure product strength (content) throughout the formulation development process. In these studies this approach was used to confirm that the low concentration of CP of 0.05% w/w was uniformly distributed in each batch of product that was manufactured. This analytical approach also provided for overall specificity and evidence of any excipient interference during analysis [263,378,384]. The assessment of *in vitro* release rate of API from topical formulations is an important component of product development and has been documented in the Guidance for Industry for non-sterile semi-solid dosage forms (SUPAC-SS) [385]. Formulation scientists have devoted much attention to developing and validating *in vitro* release studies for topical formulations during formulation development [383,385]. *In vitro* release studies were performed on every batch of laboratory scale cream formulations manufactured, in order to assess the topical behaviour of these formulations *in vitro* and to evaluate, where possible, the effects of changes in formulation composition on CP release rates.

Product performance analysis was conducted on all CPLS creams in addition to the innovator product, Dermovate® to establish similarities and differences among these formulations and to select the most appropriate CPLS cream formulation to scale-up.

4.4.1. Visual Appearance and Skin Feel

All CPLS cream formulations, in individual clear jars, were placed on a dark background surface in our laboratory. Three observers were randomly selected to evaluate the physical characteristics of the CPLS products relative to Dermovate® cream. The three observers found that the cream formulations were white in colour, smooth in texture without any form of grittiness, similar to Dermovate® cream.

4.4.2. Centrifugation Analysis

Centrifugation analysis is one method of monitoring the stability of topical dosage forms, however this approach can also be used to assess the short-term physical stability of topical formulations [381,386]. The centrifugation technique is based on the theoretical principles reflected in Stokes Law and is one of the techniques used to predict the tendency of an emulsion to coalesce. Stokes Law [127] is shown in Equation 4.1.

$$V = \frac{(gr^2)(d_1 - d_2)}{9\mu}$$ ***Equation 4.1***

Where

V= Velocity of fall (cm sec^{-1})
g = Acceleration of gravity (cm sec^{-2})
r = "Equivalent" radius of dispersed particles (cm)
d_l = Density of dispersed particles (g cm^{-3})
d_2 = Density of dispersion medium (g cm^{-3})
μ = Viscosity of dispersion medium (dyne sec cm^{-2})

This test was performed by weighing two grams of each cream (n=3) in a centrifuge vial and centrifuging the sample at 3000 rpm using an Eppendorf® Model 5415 centrifuge (Geratebau Netheler+ Hinz GmbH 2000 Hamburg 63, West Germany) for 30 minutes [381]. An evaluation of the percent separation of the phases was performed immediately, one week and one month following weighing of the samples. The results for all batches revealed no evidence of phase separation or cracking, clearly demonstrating that the CPLS batches were stable over the period tested. The stability results for batches CPLS-01-CPLS-05 are included in Appendix II together with the batch summary records for these batches.

4.4.3. Viscosity

Rheological properties of a semi-solid dosage form such as viscosity can influence the delivery of an API from a vehicle significantly [387]. Viscosity may directly influence the diffusion rate of an API at the micro-structural level [388]. However semi-solids with comparatively high viscosities may exhibit high API diffusion rates, compared to topical products of comparatively low viscosity. These observations highlight the importance of assessing the impact of rheological properties, specifically viscosity, on drug product performance. The viscosity and rheological behaviour of a topical formulation may affect its application to treatment site(s), the consistency of treatment, and ultimately, the dose delivered. Therefore maintaining the reproducibility of the flow behaviour of the topical product at the time of drug release is an important product manufacturing control that formulation scientists should monitor as a demonstration of batch-to-batch consistency [378,389,390].

The viscosity of each batch of CPLS cream formulation was measured using a Brookfield® Model-RVDI+ Viscometer (Brookfield® ENG Labs Inc., Stoughton, USA). The viscometer was operated at 10 rpm using a T-F (code 96) spindle and a helipath stand. The T-F (code 96) spindle was selected so as to maintain a torque of between 10% and 90%. In order to obtain stable display readings, all viscosity measurements were recorded 60 seconds after the commencement of rotation of the spindle. Three readings (n=3) were recorded consecutively to obtain the average viscosity value for each batch. Viscosity readings were taken 24 hours following the manufacture of each CPLS formulation.
The results of viscosity measurement (Table 4.2) show that batch CPLS-02 had a viscosity similar to that measured for Dermovate®. Dermovate® and batch CPLS-02 were quite viscous compared to batch

CPLS-01 that had a viscosity half that of Dermovate®. This might be due to the fact that Dermovate® contained 1.5% w/w Arlacel® 165 (PEG-100 stearate) as an o/w emulsifier, while batch CPLS-01 which contained 1.5% w/w Gelot® 64 (PEG-75 stearate). Furthermore an increase in the % w/w content of G-64 resulted in an increase in the viscosity of batches CPLS-01 to CPLS-05, as shown in Figure 4.3. G-64 is a viscosity-modifying agent in topical formulations, therefore changing the % w/w content of G-64 in cream formulations can modify the rheological attributes of a product. The viscosity values for batches CPLS-01-CPLS-05 are included in Appendix II, together with the batch summary records for these batches.

Table 4.2. *Viscosity of CPLS cream batches*

No.	Batches	Viscosity ± SD (cP) (n=3)	%RSD
1	**Dermovate®**	44750 ± 50	0.11
2	**CPLS-01**	28500 ± 264	0.92
3	**CPLS-02**	44800 ± 862	1.92
4	**CPLS-03**	55500 ± 152	0.27
5	**CPLS-04**	69100 ± 416	0.60
6	**CPLS-05**	84500 ± 725	0.86

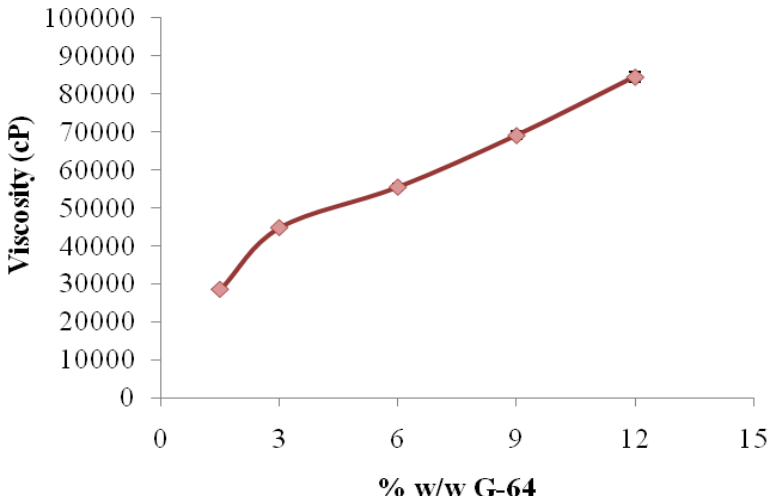


Figure 4.3. *Effect of increasing % w/w G-64 on the viscosity of CPLS cream formulations*

4.4.4. Spreadability

The parallel plate method is widely used for measuring and quantifying the spreadability of semi-solid formulations. This technique is simple, cheap, and the apparatus can be designed and fabricated according to individual requirements and the type of data that must be determined [263,391].

Two sheets of glass 15 x 15 cm were used to assess spreadability. One glass sheet was tarred on a Mettler® Model PM4600 balance (Mettler® Instruments, Zurich, Switzerland) and three grams of the cream formulation to be tested was placed in the centre of the glass square. The second sheet of glass

was placed on top of the formulation to spread the cream in a thin layer. The edges of both sheets were aligned and a 100 g weight was placed at each corner of the tiles as shown in Figure 4.4.

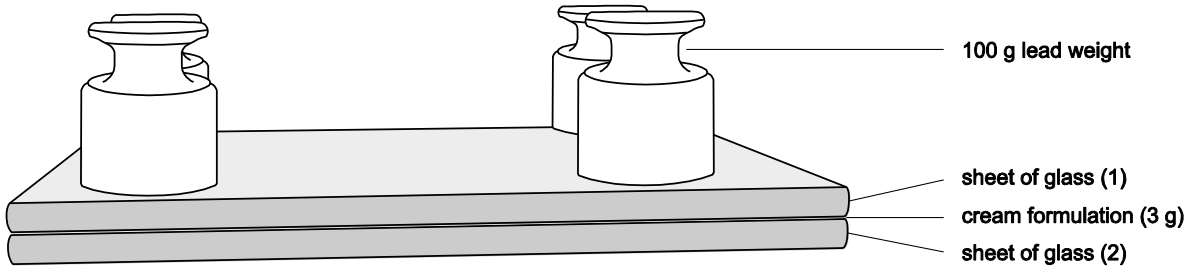


Figure 4.4. *Schematic of the apparatus used to measure spreadability*

The weights were left in place for three minutes and the radius of the spread formulation was measured for the each product. After calculation of the radius, the area covered by the product was calculated using Equation 4.2. Spreadability measurements were performed in triplicate (n=3) for each batch and the average recorded.

$$A=\Pi r^2 \qquad \textbf{\textit{Equation 4.2}}$$

Where

A = Area of the formulation spread on the surface of the tile (cm^2)
Π = Constant (3.141)
r = Radius of the formulation spread on the surface of the tile (cm)

The parallel plate method is not as precise and sensitive as other methods of measuring spreadability as the data generated must be manually interpreted [263]. However, the results offer an overall idea of the spreadability characteristics of a topical dosage form. Not surprisingly, the spreadability of batch CPLS-02 was similar to that of Dermovate®. Dermovate® and batch CPLS-02 were moderately spreadable compared to batch CPLS-01 with the highest spreadability of all cream formulations. An inverse trend was observed for spreadability as that for viscosity of the CPLS batches. Batch CPLS-01 with the lowest viscosity was the most spreadable of all cream formulations and the data are summarised in Table 4.3. In general, spreadability decreases with an increase in the % w/w G-64 and a decrease in % v/v PG, as shown in Figure 4.5. It can be said that the more the viscous the cream formulation, the less spreadable it becomes and by changing the % w/w content of G-64 in cream formulations, one can modify the spreadability of the topical product. The spreadability data for batches CPLS-01-CPLS-05 are included in Appendix II, together with the batch summary records for these batches.

Table 4.3. *Spreadability of CPLS creams*

No.	Batches	Spreadability ± SD (cm^2) (n=3)	%RSD
1	**Dermovate®**	25.22 ± 0.51	2.03
2	**CPLS-01**	58.77 ± 1.18	2.00
3	**CPLS-02**	26.12 ± 0.52	1.99
4	**CPLS-03**	20.03 ± 0.52	2.59
5	**CPLS-04**	15.56 ± 0.23	1.47
6	**CPLS-05**	10.38 ± 0.45	4.33

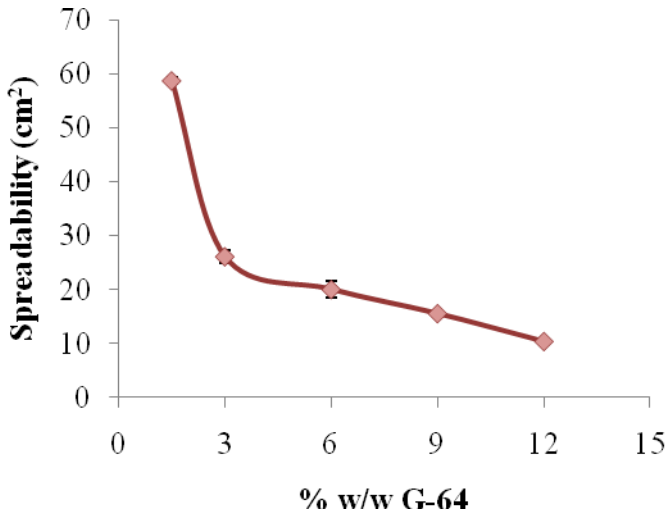


Figure 4.5. *Effect of increasing % w/w G-64 on the spreadability of CPLS cream formulations*

4.4.5. pH

Following topical formulation product development, the resultant dosage forms should be tested for pH at the time of batch release and at designated stability test time points thereafter. Most topical formulations usually contain a limited quantity of an aqueous phase, therefore pH measurements can be used as a quality control measure, where appropriate [378].

The pH of each cream sample was measured using a Crison® Model GLP 21 pH meter (Crison® Instruments, Barcelona, Spain) at room temperature (22°C). The pH measurements were determined in triplicate (n=3) on each 500 gram batch of the cream produced, within 24 hours of manufacture. The results of pH measurement revealed that the pH of all laboratory scale CP cream formulations were close to that of Dermovate® and these data are summarised in Table 4.4. An increase in the % w/w G-64 and a decrease in % v/v PG had no effect on the pH of the cream formulations, as shown in Figure 4.6. Since the cream batches fall within the range of the healthy skin pH (4-6), it is unlikely that any skin irritation will occur when these cream formulations are used. The pH ranges for batches CPLS-01-CPLS-05 are included in Appendix II, together with the batch summary records for these batches.

Table 4.4. *pH of CPLS creams*

No.	Batches	pH ± SD (n=3)	%RSD
1	**Dermovate®**	5.22 ± 0.03	0.59
2	**CPLS-01**	5.32 ± 0.02	0.37
3	**CPLS-02**	5.24 ± 0.05	0.95
4	**CPLS-03**	5.32 ± 0.02	0.37
5	**CPLS-04**	5.25 ± 0.06	1.35
6	**CPLS-05**	5.38 ± 0.01	0.19

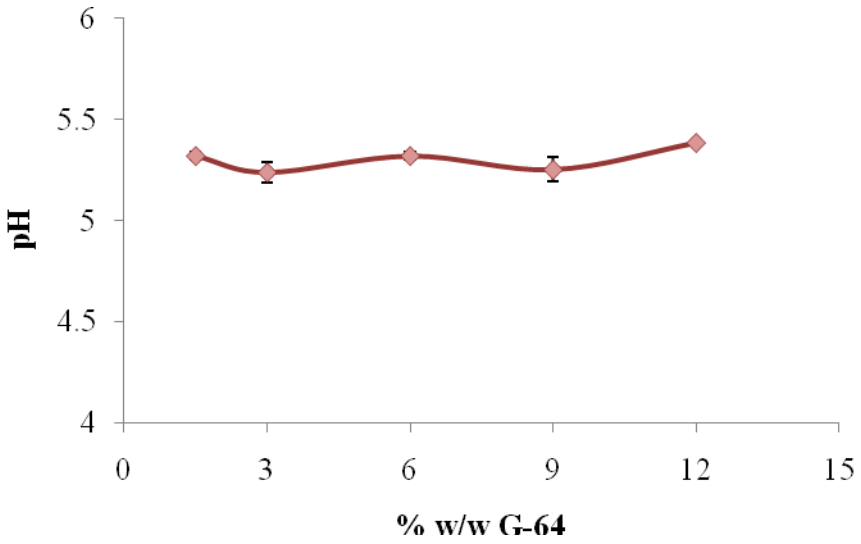


Figure 4.6. *Effect of increasing % w/w G-64 on the pH of CPLS cream formulations*

4.4.6. Content Uniformity

Content uniformity is the degree of uniformity in the amount of API within a batch of a formulation [392,393]. A low concentration of API *viz.*, 0.05% w/w CP was used to manufacture CPLS batches of cream. Therefore to establish the content uniformity, samples from the top, middle, and bottom of a container of cream were analysed for CP content. Topical formulations may show physical separation at different storage temperatures. Since emulsions, creams and topical lotions are prone to mild separation, due to the nature of the vehicle used to manufacture the product, the uniformity of distribution of an API in that formulation may be affected [208,378,394]. The following procedure was used to test API blend uniformity in the cream formulations manufactured on a laboratory scale.

The content of each CPLS cream formulation was determined by dissolving an accurately weighed 1200 mg of cream taken from the top/middle/bottom of the opaque container in 50 ml of a methanolic solution containing betamethasone 17-valerate (BV) as internal standard. The mixture was placed in a Colora® Model NB-34980 Ultra-Thermostat water bath (Colora®, Lorch, Germany) and agitated at 2000 rpm for 10 minutes, at 70°C until all components of the cream had melted. The solution was placed in 10 ml centrifuge tubes, sealed and placed in a freezer (-20°C) for 20 minutes, after which the sample was centrifuged at 2000 rpm for a further 15 minutes. A 4.0 ml aliquot of the supernatant was filtered through a 0.45 µm HVLP Millipore® filter membrane (Millipore® Co., Bedford, MA,

USA) at room temperature (22°C), prior to HPLC analysis at 239 nm. This analytical procedure was performed in triplicate (n=3) for each of the samples removed from the top/middle/bottom sections of the packaged batches. A detailed extraction procedure and a schematic diagram of the extraction procedure are presented in § 2.6.8.2 (Chapter 2) and Figure 2.20 respectively.

The United States Pharmacopoeia (USP) [45] specifies that CP cream formulations should contain not less than 90.0% w/w and not more than 115.0% w/w of the labelled amount of CP. The % drug content for each cream batch is summarised in Table 4.5 and all results indicate that the cream formulations tested comply with USP standards [45]. The % content uniformity data for batches CPLS-01-CPLS-05 are included in Appendix II together, with the batch summary records.

Table 4.5. *Content uniformity of CPLS creams*

No.	Batches	Drug content ± SD (%) TOP (n=3)	Drug content ± SD (%) MIDDLE (n=3)	Drug content ± SD (%) BOTTOM (n=3)
1	**Dermovate®**	99.67 ± 0.01	99.89 ± 0.02	100.01 ± 0.01
2	**CPLS-01**	101.54 ± 0.01	101.40 ± 0.02	100.05 ± 0.01
3	**CPLS-02**	99.99 ± 0.02	100.18 ± 0.02	99.91 ± 0.01
4	**CPLS-03**	99.85 ± 0.03	99.23 ± 0.01	100.23 ± 0.03
5	**CPLS-04**	99.17 ± 0.01	100.35 ± 0.05	99.87 ± 0.02
6	**CPLS-05**	99.80 ± 0.02	99.86 ± 0.01	99.24 ± 0.05

4.4.7. Thermogravimetric Analysis

Thermogravimetric Analysis (TGA) of 0.05% w/w CPLS creams was performed using a Perkin-Elmer® FT-IR thermogravimetric analytical instrument (Perkin-Elmer® Ltd, Connecticut, USA), as described in § 3.2.6 (Chapter 3). Approximately 1 mg to 2 mg of each sample was weighed into a platinum pan. The TGA measurement was performed at a heating rate of 5°C/min with the initial and final temperatures set at 40°C and 150°C respectively, with an atmosphere of 20 ml/min nitrogen. TGA measurements were performed on each CPLS cream formulation. Each formulation, despite differences in composition, produced similar results and these are shown in Figure 4.7.

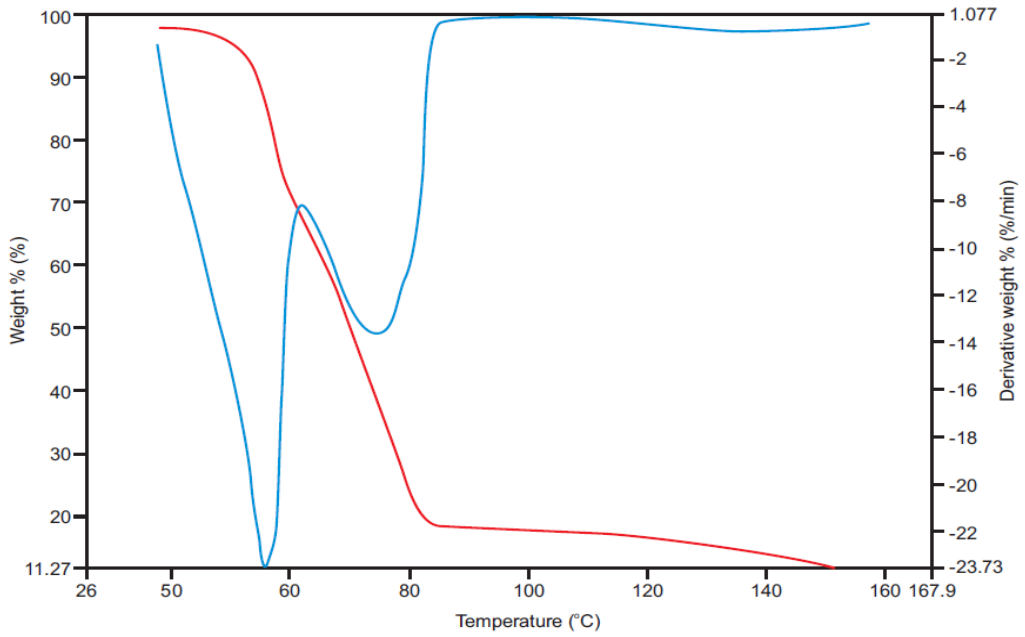


Figure 4.7. *Thermogravimetric (red curve) and its derivative curve (blue curve) for one CLPS cream formulation at a heating rate of 5°C/min*

The TG curve represents the weight loss of a sample as a function of temperature, while the Derivative of TG (DTG) curve represents the rate of evaporation of water incorporated in the formulation [395,396]. As the temperature increased from 28 °C to 80 °C, a rapid loss of the total weight of the cream was observed. This weight loss was probably due to an increase in the evaporation rate of the bulk water phase and volatile substances such as PG. Below 50°C, the bulk water started to evaporate, and at 58°C, the TG-curve shows a point of inflection corresponding to a minimum in the DTG-curve. At 58°C, CA and GMS, which make up the bulk of the waxy materials, had undergone melting. In the temperature range of 58-65°C, the evaporation of bulk water was surpassed by the evaporation of semi-hydrated water from the CA and GMS and by evaporation of water that was mechanically entrapped in the visco-elastic CA-GMS gel network. At 75°C, following melting of the visco-elastic phase, the TG-curve shows a second inflection point corresponding to a minimum in the DTG-curve, which reflects the weight loss due to the evaporation of interlamellary fixed water. Over the temperature interval of 84°C-150°C, the interlamellary fixed hydration water was released.

4.5 *IN VITRO* ANALYSIS

Numerous experimental methods have been developed to investigate the bioavailability of API from topical vehicles and the percutaneous absorption of topically applied products [66,224,397-399]. Following application of a topical corticosteroid formulation, release of the API from the topical product must take place prior to a pharmaco-therapeutic effect being observed. The API initially is in

contact with the stratum corneum and must partition through this layer prior to diffusion through other layers of the skin, to the site of action [400-402]. The use of *in vitro* methods to assess the release of API from semi-solid dosage forms is gaining increasing attention from the pharmaceutical industry and regulatory authorities as it is a valuable tool for monitoring product reproducibility when compositional or process manufacturing changes and batch scale-up are undertaken [262,328]. *In vitro* release testing is often selected for use as it is relatively simple to set up and maintains appropriate experimental conditions. The resulting *in vitro* data are also derived rapidly at low cost. It is also suitable to evaluate the release of an API from different batches of a formulation at the same time and therefore can provide a useful indication of what may occur in bioequivalence testing of different formulations [403-405].

4.5.1. *In Vitro* Release Testing of CP from CPLS Topical Formulations

One of the most widely used designs for studying *in vitro* diffusion and drug release from topical corticosteroid formulations is the Franz diffusion system cell [107,229,385,405-409]. A glass Franz diffusion cell system (Crown Glass Company Inc., Branchburg, NJ, USA), consisting of six cells with a diffusional surface area of 2.063 cm^2 was used in these studies. Franz diffusion cells consist of a donor compartment and a receptor compartment and a schematic representation of this system is given in Figure 4.8.

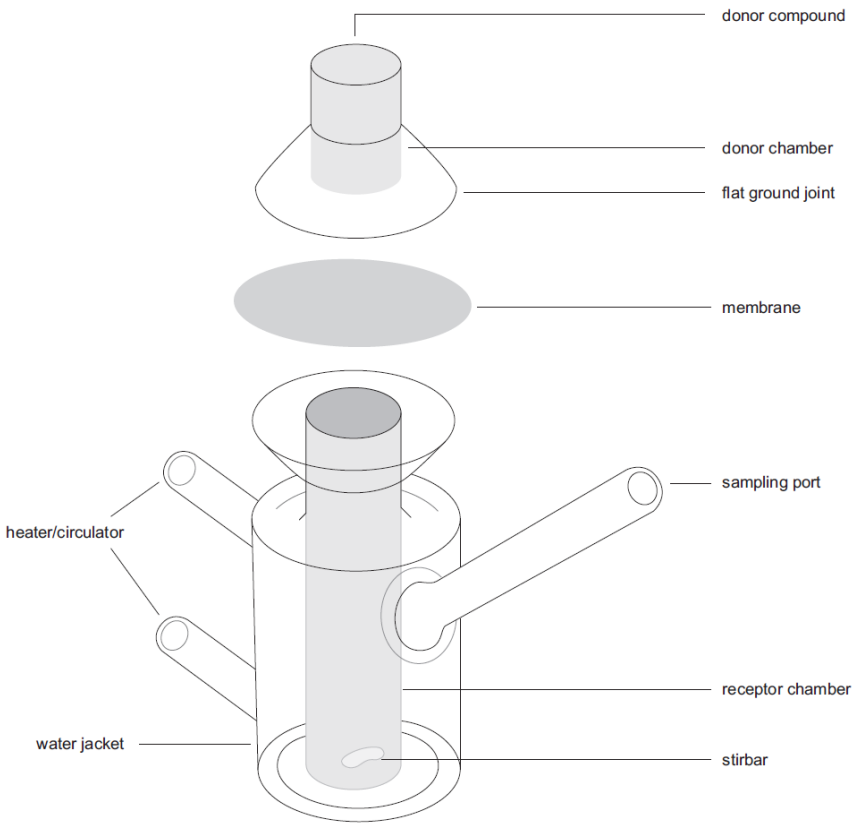


Figure 4.8. *Schematic representation of a glass Franz diffusion cell and its essential components [410]*

A 0.025 μm nitrocellulose MF-Millipore® membrane (Millipore® Co., Bedford, MA, USA) was mounted between the donor and receptor chambers and the two compartments were clamped together. Pre-treatment of the nitrocellulose membrane was undertaken by soaking the membrane in the receptor fluid for two hours, to allow the membrane to equilibrate with the receptor medium. A 300 mg aliquot of the topical formulation to be tested was placed on the surface of the membrane using a glass rod and spread evenly across the surface of the membrane. The receptor chamber was filled with 12.6 ml of receptor fluid and maintained at 32 ± 0.5°C using a circulating water heater to pump water through the jacketed cells. Since temperature may affect the release of API from topical formulations, the temperature was maintained at 32 ± 0.5°C, which is the temperature of the skin surface [411-413]. The receptor medium was a mixture of PG:Water in a ratio of 70:30% v/v (§ 3.2.3, Chapter 3). The donor compartment was then covered with Parafilm®, to prevent evaporation of the vehicle. The receptor solution was continuously stirred using a 10 mm x 2.5 mm rectangular magnetic stirrer bar (Merck® Chemicals Ltd, Darmstadt, Germany). Receptor solution samples were withdrawn through the sampling port at 2, 4, 8, 12, 24, 48 and 72 hours. After each sample removal, the cells were refilled with the same volume of fresh receptor medium that had been maintained at 32°C. Each formulation was tested in triplicate (n=3) and the diffusion cell conditions are summarised in Table 4.6.

Table 4.6. *Franz cell diffusion conditions for in vitro release of CP cream formulations*

Apparatus	**Glass Franz diffusion cell system**
Sample number	6 cells
Average diffusional surface area (n=6)	2.063 ± 0.104 cm^2
Average receptor volume (n=6)	12.61 ± 0.25 ml
Temperature	32.0 ± 0.5°C
Synthetic membrane	0.025 μm nitrocellulose
Dosing conditions	300 mg
Sample occlusion/ non-occlusion	Occlusion
Magnetic stirrer	10 mm x 2.5 mm rectangular magnetic stirrer bar
Sampling time	2, 4, 8, 12, 24, 48, 72 hours
Sample analysis	HPLC with UV detection 239 nm

The CP content of the samples in the receptor compartment was analysed by HPLC (§ 2.4, Chapter 2). The *in vitro* release profiles for CP were plotted as cumulative amount released per unit area of CP versus time, in addition to cumulative amount released per unit area of CP versus square root of time for each formulation. Similarities or differences in the *in vitro* release rates of CP from the test formulations (CPLS) were compared to that obtained for Dermovate® cream using statistical/mathematical modelling. The *in vitro* release profile data for batches CPLS-01-CPLS-05 are included in Appendix II, together with the batch summary records for these batches.

4.5.2. Assessment of CP Cream Formulations on the South African Market

Dovate® (Aspen® Pharmacare, Port-Elizabeth, South Africa) is currently the most widely available generic CP cream formulation on the South African market and Dermovate® (Glaxo® Wellcome Pharmaceuticals) is the innovator product [33,339,414]. Dovate® was the first generic to receive market authorisation for South Africa, when the patent for Dermovate® expired in the 1990s [38,415]. *In vitro* analysis was performed on the Dovate® and Dermovate® creams using the glass Franz diffusion cell system (§ 4.5.1, Chapter 4). All *in vitro* determinations were performed in triplicate (n=3) and the *in vitro* release profiles of the cream formulations currently on the South African market are shown in Figure 4.9.

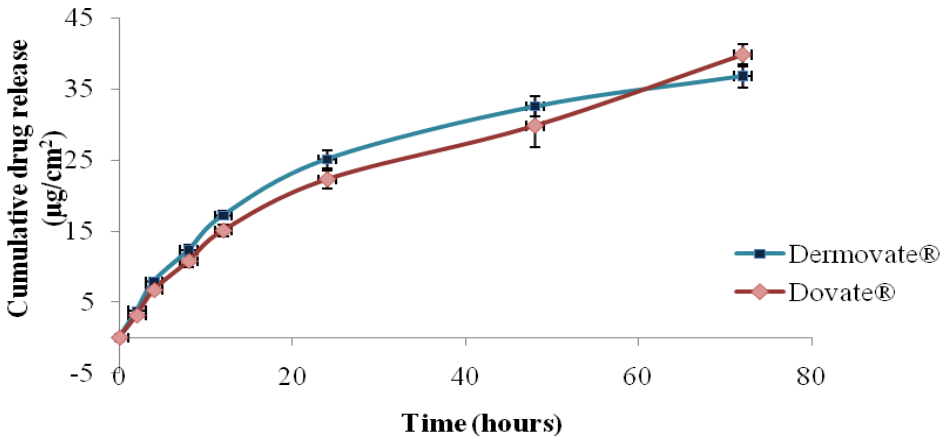


Figure 4.9. *In vitro release profiles for Dermovate® and Dovate®*

The *in vitro* release profile of CP from Dermovate® was higher than that for Dovate® for the period 6 hours to 48 hours. Dovate® showed an initial decrease in the release of CP from 6 to 48 hours but the release of CP started to increase after 48 hours (Figure 4.9). Therefore these topical products were further investigated, in respect of their physical characteristics, by analysis of viscosity, spreadability and drug content. These results are summarised in Table 4.7.

Table 4.7. *Analysis of Dovate® and Dermovate®*

No.	Formulations	Viscosity (cP) (n=3)	Spreadability (cm^2) (n=3)	Drug content ± SD (%) (n=3)
1	**Dermovate®**	44675 ± 210	25.67 ± 0.64	100.41 ± 0.01
2	**Dovate®**	51 450 ± 419	20.40 ± 0.40	99.05 ± 0.02

Although Dovate® appeared to be less viscous than Dermovate®, Dovate® was found to have higher viscosity and lower spreadability than Dermovate® (Table 4.7). This may be due to the fact that different excipients in its formulation composition resulted in the formation of a strong visco-elastic gel-like network, forming at the oil-water interface, and producing a rigid sub-microscopic network structure [416,417]. The drug content is within the range described in the USP [45] for CP cream formulations, thereby showing that the API was homogeneously distributed throughout the topical

formulation. The significant difference in release rates of CP, viscosity and spreadability from these two cream formulations manufactured by two different suppliers, may be due to the differences in the formulation composition, industrial equipment, changes in the manufacturing process or storage conditions for each product. The significant decrease in the *in vitro* release profile of CP from Dovate® compared to Dermovate® may be associated with the high viscosity of Dovate® and these two formulations were taken further into investigation *vide infra* (§ 6.2, Chapter 6).

4.5.3. Assessment of CPLS Cream Formulations

The shape of an *in vitro* release curve, especially that describing drug release from topical formulations, is affected by phases of the liberation process from a formulation, since the properties of the API and vehicle may affect the release process [48]. The cumulative amount of drug released from batch CPLS-01 exhibited the highest rate and extent of CP release, whereas that from batch CPLS-05 exhibited the lowest rate and extent of CP over a 72 hour period (Table 4.8). The cumulative % drug released, in addition to the flux over a 72 hour period, was the highest for batch CPLS-01 compared to other CPLS formulations. The cumulative amount of drug release per unit area, cumulative % release of CP over 72 hour period and the flux demonstrated that Dermovate® and batch CPLS-02 exhibited similar rate and release of CP as shown in Table 4.8. Furthermore the two curves of Dermovate® and batch CPLS-02 were virtually superimposable, as shown in Figure 4.10.

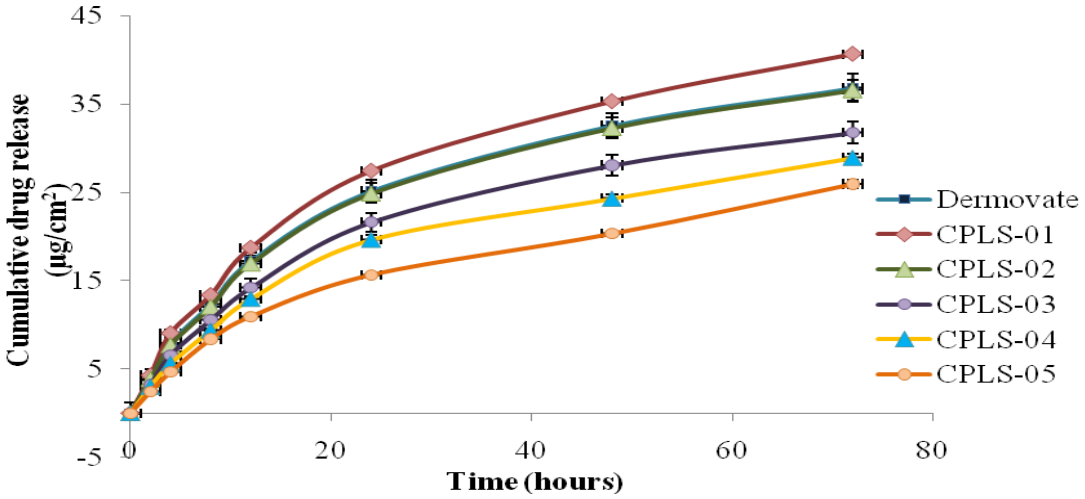


Figure 4.10. *In vitro release profiles of CPLS formulations, in comparison with Dermovate®*

A highly viscous formulation is likely to retard the release of CP from its topical base therefore requiring a longer time for the API to come into contact with the synthetic membrane, prior to drug diffusion into the receptor. One parameter affecting the liberation process of an API from a topical cream base is the melting rate of the bases in which the API is formulated. This causes a lag phase prior to the release of API. Therefore the lag time corresponds to a delay in the movement of molecules of API and subsequent release from a topical dosage form, and therefore diffusion across the synthetic membrane, if the membrane itself is not rate-limiting in nature [387]. Batches CPLS-04

and CPLS-05 exhibited the longest lag phase, which can be attributed to their composition as listed in Table 4.8. Least squares linear regression analysis was used to establish the linearity of the *in vitro* release profiles of CP over a 72 hour period. The resultant correlation coefficients (R^2) of 0.98-0.99 (Table 4.8) reveal that the plots of cumulative amount of drug released per unit area versus square root of time (flux) were linear for all CPLS formulations.

Table 4.8. *Comparison of drug release kinetics from CPLS creams*

No.	Formulation	Q (μg/cm^2) over 72 hr	Cumulative % release over 72 hr	Flux (μg/cm^2/hr$^{1/2}$)	Lag time (hr$^{1/2}$)	Correlation coefficient (R^2)
1	**Dermovate®**	36.84	48.23	4.34	0.80	0.98
2	**CPLS-01**	40.67	55.44	4.79	0.70	0.99
3	**CPLS-02**	36.59	48.88	4.31	0.80	0.98
4	**CPLS-03**	31.77	43.31	3.74	0.81	0.98
5	**CPLS-04**	28.96	39.48	3.41	0.85	0.98
6	**CPLS-05**	25.67	35.39	3.60	0.85	0.99

The differences in the release rates of the cream formulations (Figure 4.10) may well be due to the different % w/w G-64 and % v/v PG used to manufacture the different batches, ultimately resulting in the production of creams of different viscosities. The 'self-bodying action' of a mixed emulsifying system is normally based on the swelling properties and concentration of the α-crystalline gel phase in such systems. At low emulsifying agent concentrations, an increase in the volume of PG in the formulation that is incorporated between the bilayers of the gel and crystalline hydrate phases forms a lotion-like product. However at higher mixed emulsifier concentrations, the crystalline and gel phases link together to form a visco-elastic gel network that becomes a viscous semi-solid cream on cooling [368]. An increase in the % w/w of G-64 and a decrease in the % v/v PG in a formulation resulted in a decrease in the rate and extent of CP release over a 72 hour period, due to the increase in viscosity of the formulations under investigation.

4.6 STATISTICAL COMPARISON AND MATHEMATICAL MODELLING OF *IN VITRO* RELEASE PROFILES

During topical formulation development and analysis, *in vitro* release profiles and statistical data are used to assess the quality and performance of topical corticosteroid-containing dosage forms [418,419]. These data are used to compare the impact of different formulation compositions on resultant drug release rates in addition to choosing an optimised formulation for further development and evaluation. The comparison of *in vitro* release profiles has extensive application throughout the product development process and can be used to develop *In Vitro-In Vivo* Correlations (IVIVC), which may help reduce the cost and speed up of the product development process [420,421]. A comparison of these parameters may also be useful to establish specifications for *in vitro* testing of dosage forms and to establish the similarity of formulations, for which the composition,

manufacturing site, scale of manufacturing process and/or equipment may have been modified [418,422].

In a number of recent Guidance documents, the FDA has placed emphasis on the meaningful comparison of *in vitro* release profiles and statistical data from the testing of different dosage forms [423-425]. For instance the FDA SUPAC Guidance document indicates that similar *in vitro* profiles for approved and modified formulations may be acceptable for the justification of certain levels of change, without prior FDA approval or the need to perform additional bioequivalence studies [385,418]. As a result, there is increasing interest amongst pharmaceutical scientists focused on developing methodologies for the comparison of *in vitro* release profile and associated data [422]. Although qualitative interpretations and statistical data for the comparison of release rate profiles are essential tools in providing crucial information about the release of an API from a formulation, mathematical modelling of release rate data from different dosage forms is also useful [422,423,426,427]. This approach allows for the prediction of release kinetics prior to the large-scale production of a formulation [428]. Furthermore, mathematical modelling enables the measurement of important physical parameters, such as the diffusion coefficient of an API from model-fitting of experimental release data [428,429]. Thus, mathematical modelling requires comprehension of all phenomena affecting the release kinetics of an API and has important significance for in-process optimisation of the manufacture of such formulations [429,430].

4.6.1. Modelling of Drug Release Data

Many kinetic models can be used to describe the overall rate and extent of drug release from dosage forms. Qualitative and quantitative changes of a formulation may alter the rate and extent of drug release and ultimately the *in vivo* performance of the dosage form [422,423,427,429,431]. The development and use of tools such as mathematical and statistical models facilitate product development by reducing the necessity of certain bioequivalence studies [429,432]. In this regard, the use of *in vitro* dissolution data to predict *in vivo* performance of a drug product can be considered a rational approach for the development of controlled release dosage forms. The methods used to investigate the kinetics of drug release from controlled release formulation can be classified into three categories *viz.*, i) statistical methods that include exploratory data analysis, repeated measures design, analysis of variance and multivariate analysis of variance, ii) model-independent methods including difference (f_1) and similarity (f_2) factors, and iii) model-dependent methods including zero order, first order and Higuchi models [428,429].

4.6.1.1. Exploratory Data Analysis

The exploratory data analysis method is a statistical analytical approach used for the statistical and mathematical comparison of *in vitro* release profiles. Although such methods are currently not endorsed by the FDA, this statistical/mathematical approach can be used to obtain a clear

understanding of the *in vitro* release profiles of formulations from a graphical and numerical perspective. Therefore the use of this approach during drug product development is recommended [422,433]. The release profile data are depicted graphically by plotting the mean amount of drug released from each formulation under investigation at each time point, and should include error bars extending to at least two standard errors. The data generated following drug release may also be summarised numerically to represent the mean and standard deviation of the release data at each time point for each formulation. A 95% confidence interval for the differences in the mean release profile at each time point may then be evaluated [422,433]. The exploratory data analysis approach was used for the comparison of *in vitro* rate profiles in these studies and the results of the graphical methods are shown in Figure 4.11. The numerical release data are summarised in Tables 4.9 and 4.10.

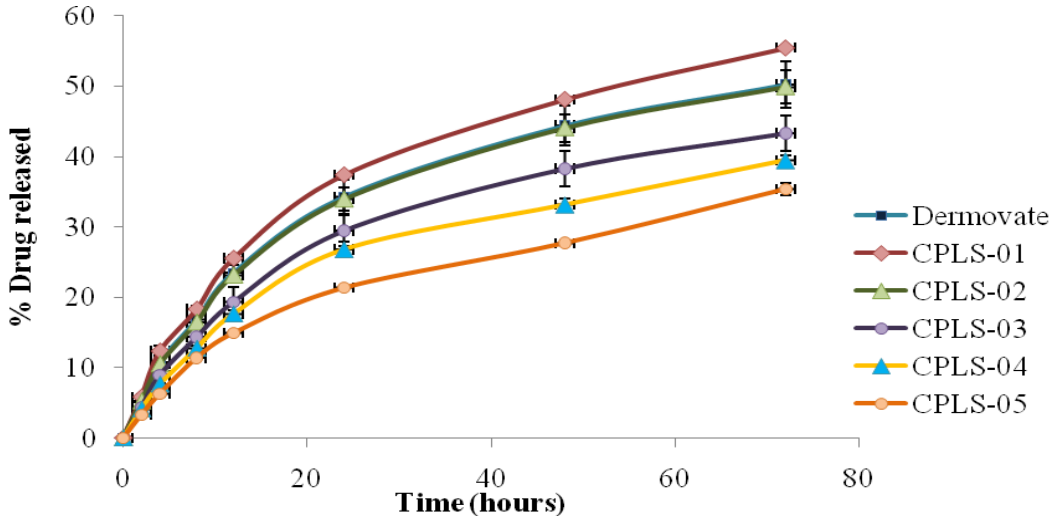


Figure 4.11. *Statistical comparison of the percent drug released per unit area profiles for CPLS (Tests) formulations in comparison with Dermovate® (Reference). Note: Error bars are extended to twice the standard errors on either side of the mean at each time point for comparative purposes.*

Table 4.9. *Summary statistics for the percent drug released per unit area (% DR) over 72 hours for the Test formulations and Dermovate®*

Time (hrs)	Dermovate® %DR Mean±(SD) (n=3)	CPLS-01 % DR Mean±(SD) (n=3)	CPLS-02 % DR Mean±(SD) (n=3)	CPLS-03 % DR Mean±(SD) (n=3)	CPLS-04 % DR Mean±(SD) (n=3)	CPLS-05 % DR Mean±(SD) (n=3)	Diff* (R-01)	Diff * (R-02)	Diff * (R-03)	Diff* (R-04)	Diff * (R-05)
0	0	0	0	0	0	0	0	0	0	0	0
2	5.14(0.01)	5.83(0.18)	5.25(0.03)	4.39(0.02)	4.10(0.07)	3.36(0.18)	-0.69	-0.11	0.75	1.04	1.78
4	10.79(0.48)	12.34(0.38)	10.51(0.27)	8.98(0.13)	7.65(0.04)	6.34(0.19)	-1.55	0.28	1.81	3.14	4.45
8	16.81(0.69)	18.31(0.27)	15.45 (0.25)	14.37(0.09)	12.78(0.16)	11.42(0.12)	-1.50	1.36	2.44	4.03	5.39
12	23.48(0.59)	25.53(0.21)	23.14 (0.16)	19.35(1.06)	17.66(0.28)	14.94(0.10)	-2.05	0.34	4.13	5.82	8.54
24	34.28(1.24)	37.41(0.08)	33.92 (0.84)	29.48(1.07)	26.81(0.52)	21.36(0.14)	-3.13	0.36	4.80	7.47	12.65
48	44.40(1.39)	48.13(0.17)	44.04 (0.96)	38.28(1.24)	33.17(0.44)	27.72(0.18)	-3.73	0.36	6.12	11.23	16.68
72	49.79 (1.61)	55.44(0.13)	48.78 (1.16)	43.31(1.25)	39.48(0.36)	35.39(0.46)	-5.65	-0.29	6.48	10.31	14.40

***Difference = % Drug released per unit area of Dermovate®- % Drug released per unit area of CPLS**

The graphical data in Figure 4.11 reveal that there were no significant differences in the mean amount of CP released per unit area or percentage CP released per unit area between Dermovate® and the CPLS formulations from time 0 to 8 hours. From 8 to 72 hours, different amounts of CP were released compared to Dermovate®. Furthermore, batch CPLS-02 was shown to have a drug release profile similar to Dermovate®, since the two graphs are almost superimposable at the specific time points. However the summary data from numerical release shown in Table 4.9 reveal that Dermovate® displayed a slightly higher mean amount of CP released per unit area compared to batch CPLS-02 over the 72 hour test period. The mean amount of API released per unit area from batch CPLS-01 at times 0, 2, 4, 8 and 12 hours was similar to that of batch CPLS-02 at those times. However at later times, up to 72 hours, the rate of CP release from CPLS-01 formulation increased significantly (Figures 4.10 and 4.11). The mean amount of CP released per unit area from CPLS-03 to CPLS-05 was much lower than Dermovate® as can be seen in the *in vitro* release plots and that the numerical data (Table 4.9) are in close agreement with the graphically represented results.

The degree of overlapping of error bars in the graphic plots of the release profile gives an indication of whether the release profiles may be considered to differ from each other, and whether the difference is significant. The reason is that the error bars at each dissolution time may be considered an approximate 95% confidence interval (Table 4.10).

Table 4.10. *Summary statistics for the 95% Confidence Interval for difference (LL, UL) for the Test formulations and Dermovate®*

Time (hrs)	95% Confidence interval for difference LL, UL CPLS-01	95% Confidence interval for difference LL, UL CPLS-02	95% Confidence interval for difference LL, UL CPLS-03	95% Confidence interval for difference LL, UL CPLS-04	95% Confidence interval for difference LL, UL CPLS-05
0	-1.258, 1.258	-0.345, 0.345	-1.684, 1.684	-2.841, 2.841	-4.225, 4.225
2	-1.948, 0.568	-0.455, 0.235	-0.934, 2.434	-1.801, 3.891	-2.445, 6.605
4	-2.808, 2.808	-0.065, 0.625	0.125, 3.494	0.298, 5.981	0.224, 8.675
8	-2.785, -0.242	1.015, 1.705	0.755, 4.124	1.188, 6.871	1.164, 9.615
12	-3.308, -0.792	-0.005, 0.685	2.445, 5.814	2.978 , 8.061	4.314, 12.765
24	-4.388, -1.872	0.015, 0.705	3.115, 6.484	4.628, 10.311	8.435, 16.875
48	-4.988, -2.472	0.015, 0.705	4.435, 7.804	8.388, 14.071	12.454, 20.905
72	-6.908, -4.392	-0.635, 0.055	4.795, 8.164	7.468, 13.151	16.174, 18.625

Therefore, if the confidence intervals for the two formulations at a given time point do not overlap, the mean release profiles at that time may be significantly different from each other [422]. The error bars for Dermovate® and batch CPLS-02 (Figure 4.11) clearly overlapped at all time points, however the error bars of the other CPLS formulations *viz.*, batches CPLS-01, CPLS-03-CPLS-05 at each release time point did not. The release profiles for batch CPLS-02 and Dermovate® were therefore considered to be similar.

A summary of the numerical data complements the graphical data for drug release and represents the mean and standard deviation of drug release data at each time point for drug release testing of CPLS

and Dermovate® formulations. If the 95% confidence interval for the mean difference at a given time point does not contain zero, then the difference in release at that time point is considered to be significantly different at a 5% level of significance [422,429]. The 95% confidence interval, constructed to establish whether the release profiles were different at specific times, reveals that zero was included in most cases for batch CPLS-02 than other CPLS formulations, as shown in Table 4.10. This is an indication that there is no difference between these two curves *viz.*, Dermovate® and batch CPLS-02, and thus the release rate data for both cream formulation batches may be considered similar at these times with a 95% level of confidence.

Exploratory data analysis is not intended to be comprehensive, and can be deficient for a number of reasons. For instance it is difficult to definitively conclude that API releases for the two formulations under comparison are significantly different, as the error bars for these may overlap at some time points. The 95% confidence interval for the difference between the mean of the test and reference formulations may contain zero at some time points and not at others. Furthermore if there are too many formulations under comparison, graphical representation of the mean dissolution profiles may be too cluttered in─ particular if error bars are included (Figure 4.11). Consequently, it may be easier to interpret graphical plots of fewer sets of dissolution data at one time to obtain a better understanding of the *in vitro* release profiles, as shown in Figure 4.12.

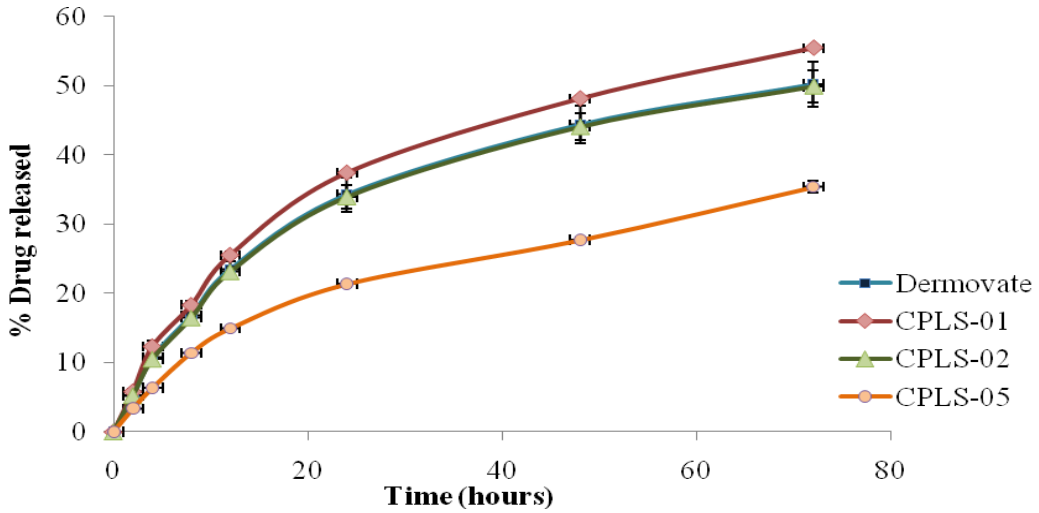


Figure 4.12. *Statistical comparison of the cumulative amount of drug released per unit area for fewer CPLS (Test) formulations in comparison to Dermovate® (Reference). Note: Error bars are extended to twice the standard errors on either side of the mean at each time point for comparative purposes.*

Similarly, the numerical summary table would be quite large if more than two formulations are to be compared. This is due to the large number of columns for the difference, and the corresponding 95% confidence interval that is constructed for that difference for each formulation to be compared. Nevertheless exploratory data analysis is a first and useful tool in obtaining an improved understanding of the release rate profile data generated in such studies [422].

4.6.1.2 Analysis of Variance (ANOVA)

Statistical methods, unlike mathematical methods go some way towards taking into account the statistical properties of variability and correlation of *in vitro* release profile data in the comparison of those data [422,434]. Analysis of variance methods may be divided into one-way analysis/univariate (ANOVA) and two-way analysis of variance/multivariate (MANOVA) [422,429,433,434]. ANOVA or MANOVA methods are not mentioned in the FDA Guidance documents [422]. However ANOVA analysis may be used to assess the difference between mean amount of API released at a single time point in *in vitro* testing, whereas MANOVA methods may be used to evaluate data derived in multiple time points in *in vitro* testing [435,436]. Data generated following ANOVA analysis are more informative and easier to interpret and analyse, than data arising from MANOVA analysis, in which the transformation of data for repeated measurement makes the statistical results difficult to interpret and analyse [435,437].

Since ANOVA explains separate statistical comparisons of mean *in vitro* release data at a specific time, it is equivalent to a t-test in the case in which the *in vitro* release data for two formulations are being compared. While this method of comparing *in vitro* release rate data takes the variability of the data into account at each time point, it ignores the correlation between the time points. The data at each time point are evaluated independently of any other data point [422]. Different *post hoc* tests can be used to determine the exact points of difference between *in vitro* profiles. These include the Least Significant Difference Test [438,439], Bonferroni Test [440,441], Tukey's Multiple Range Test [439,442], Scheffé Method [435] and Newman-Keuls Test [443,444].

ANOVA analysis was used to compare the *in vitro* release profiles of CP between CPLS formulations and Dermovate® to detect and analyse the difference, if any, between the *in vitro* release data generated from the formulations under investigation. The main advantage of using ANOVA analysis for this purpose is that this statistical approach permits the detection of differences between *in vitro* release profiles at individual time points. A univariate ANOVA analysis was performed using GraphPad® Prism software Version 5.00 for Windows (GraphPad® Software, San Diego, California, USA, www.graphpad.com) to assess whether differences between time points of *in vitro* release profiles existed for each of the formulations tested. A Bonferroni test, with $P < 0.05$ as significance level, was used as a *post hoc* test to assess which of the formulations were different at the individual time points evaluated during *in vitro* dissolution tests. The results of ANOVA analysis, performed to compare the *in vitro* release profiles for the different laboratory scale formulations, using Dermovate®, as reference, are summarised in Table 4.11.

Table 4.11. *Summary of ANOVA analysis of in vitro release profiles for CPLS and Dermovate®*

Time (hrs)	Comparison*	Mean Difference** (R-CPLS)	95% Confidence interval for mean difference		P-value	Summary
			Lower Limit	Upper Limit		
2	**R vs 01**	-0.6733	-0.9315	-0.4151	P < 0.0001	Significant
	R vs 02	-0.1800	-0.4382	0.7821	P > 0.05	Not significant
	R vs 03	0.7833	0.5251	1.042	P < 0.0001	Significant
	R vs 04	0.0600	-0.1982	0.3182	P > 0.05	Not significant
	R vs 05	1.7400	1.482	1.998	P < 0.0001	Significant
4	**R vs 01**	-1.660	-2.168	-1.152	P < 0.0001	Significant
	R vs 02	0.1800	-0.3275	0.6875	P > 0.05	Not significant
	R vs 03	2.013	1.506	2.521	P < 0.0001	Significant
	R vs 04	3.080	2.572	3.588	P < 0.0001	Significant
	R vs 05	4.333	3.862	4.841	P < 0.0001	Significant
8	**R vs 01**	-1.483	-1.957	-1.010	P < 0.0001	Significant
	R vs 02	0.1833	-0.2900	0.6567	P > 0.05	Not significant
	R vs 03	2.537	2.063	3.010	P < 0.0001	Significant
	R vs 04	4.137	3.663	4.610	P < 0.0001	Significant
	R vs 05	5.257	4.783	5.730	P < 0.0001	Significant
12	**R vs 01**	-1.733	-2.917	-0.5497	P < 0.01	Significant
	R vs 02	0.5500	-0.6336	1.734	P > 0.05	Not significant
	R vs 03	4.253	3.070	5.437	P < 0.0001	Significant
	R vs 04	6.683	5.500	7.867	P < 0.0001	Significant
	R vs 05	8.980	7.796	10.16	P < 0.0001	Significant
24	**R vs 01**	-3.143	-3.956	-2.330	P < 0.0001	Significant
	R vs 02	0.6733	-0.1396	1.486	P > 0.05	Not significant
	R vs 03	5.010	4.197	5.823	P < 0.0001	Significant
	R vs 04	7.677	6.864	8.490	P < 0.0001	Significant
	R vs 05	13.39	12.57	14.20	P < 0.0001	Significant
48	**R vs 01**	-3.677	-4.132	-3.222	P < 0.0001	Significant
	R vs 02	0.3267	-0.1283	0.7816	P > 0.05	Not significant
	R vs 03	5.863	5.408	6.318	P < 0.0001	Significant

Continued...

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	R vs 04	11.31	10.86	11.76	P < 0.0001	Significant
	R vs 05	16.54	16.09	16.99	P < 0.0001	Significant
72	**R vs 01**	-5.115	-5.667	-4.562	P < 0.0001	Significant
	R vs 02	0.3567	-0.1955	0.9088	P > 0.05	Not significant
	R vs 03	6.857	6.305	7.409	P < 0.0001	Significant
	R vs 04	10.65	10.10	11.21	P < 0.0001	Significant
	R vs 05	14.52	13.96	15.07	P < 0.0001	Significant

***Comparison = Dermovate® (R) vs CPLS (-01, -02, -03, -04 and -05)**

****Mean Difference = % Drug released Reference - % Drug released CPLS**

ANOVA analysis of data generated using different formulation compositions, summarised in Table 4.11, reveal that for the initial 2 hours of *in vitro* release of CP, no discrimination between batch CPLS-02 ($P > 0.05$) and Dermovate®, in addition to batch CPLS-04 ($P > 0.05$) and Dermovate®, was observed. However discrimination was observed for all the other CPLS batch formulations *viz.*, CPLS-01 (****$P < 0.0001$), CPLS-03 (****$P < 0.0001$) and CPLS-05 (****$P < 0.0001$) that were used. However after 2 hours, ANOVA results indicate that the *in vitro* release profile for batch CPLS-02 ($P > 0.05$) was similar to that for Dermovate®, although other CPLS batch formulations revealed significant *in vitro* differences at t= 4, 8, 12, 24, 48 and 72 hours respectively.

4.6.1.3. Model-Independent Methods

In order to establish *in vitro* data equivalence, the FDA recommends that a model-independent approach, based on the calculation of difference (f_1) and similarity (f_2) factors, be used [423,445,446]. Moore and Flanner [447] proposed the use of difference and similarity factors, f_1 and f_2 respectively, for the comparison of *in vitro* release profiles and these factors are known as fit factors [447]. The main advantage of using the f_1 and f_2 approach is that it provides a relatively simple means of comparing *in vitro* release data. The calculation of f_1 using Equation 4.3 establishes the percent difference between two curves at each time point and is a measurement of the relative error between *in vitro* release profiles of the test and the reference product at all time points.

$$f_1 = \left\{ \frac{\left[\sum_{i=1}^{P} |R - T|\right]}{\left[\sum_{i=1}^{P} R\right]} \right\} X\ 100 \qquad \textit{Equation 4.3}$$

Where

f_1= Difference factor
R=*In vitro* measurement for the reference formulation at P time points
T= *In vitro* measurement for the test formulation at P time points
P= Number of time points

The calculation of f_2, using Equation 4.4, determines the logarithmic reciprocal square root transformation of the sum of the squared error, and is a measure of the similarity in percent releases between the *in vitro* release profiles of the test and reference product at all time points [423,429,434].

$$f_2 = 50 \log \left\{ \left[1 + \left(\frac{1}{P}\right) \sum_{i=1}^{P} (R - T)^2 \right]^{-1/2} X\ 100 \right\} \qquad \textit{Equation 4.4}$$

Where

f_2= Similarity factor
R=*In vitro* measurement for the reference formulation at P time points
T= *In vitro* measurement for the test formulation at P time points
P= Number of time points

A shortcoming is that both factors do not account for the variability or correlation structure of the data under investigation and they are sensitive to the number of data points used in this analysis. This

model-independent method is most suitable for *in vitro* release profile comparison, when the data from three to four or more sample points are available for analysis. From a statistical point of view, this method appears to be less discriminating than methods such as ANOVA and model-dependent methods [434,445,448].

A value between 0 and 15 for f_1 and between 50 and 100 for f_2 is indicative of the sameness or equivalence between the two *in vitro* release profiles being evaluated [421,445,449]. The percent error (f_1) is zero (0) when the *in vitro* release profiles of the test and reference product are identical and this value increases proportionally as *in vitro* profiles become dissimilar. An f_2 value close to 100 indicates that the *in vitro* profiles of a test and reference product are identical [447]. The main advantages of using f_1 and f_2 factors are that they are easy to compute and provide a single number for the determination of closeness between two resultant *in vitro* release profiles. However there are limitations to the use of this method that could affect the outcome of the analysis. Both f_1 and f_2 values are sensitive to the number of sampling time points used [448,450,451]. Furthermore the *in vitro* release data that have been correlated at specific sample time points are estimates which are complicated, in that the variation of the estimates is difficult to calculate, and the estimates may be biased [422,452]. The f_2 factor is also insensitive to the shape of the *in vitro* release curve and does not take into account the unequal spacing between sampling time points. Therefore it is impossible to determine the frequency and prevalence of false positive and false negative rates of decision for approval of pharmaceutical products on these factors [453].

A newer model-independent mathematical approach can be used for the comparison of *in vitro* profiles of a test and reference product. This model uses a similarity factor, S_d for comparison purposes [454]. The Gohel similarity factor [455] can be calculated using Equation 4.5.

$$S_d = \frac{\sum_{t=1}^{n-1} \left| \log \left(\frac{AUC_{Rt}}{AUC_{Tt}} \right) \right|}{n-1} \qquad \textit{Equation 4.5}$$

Where

n= Number of data points collected during an *in vitro* release test
AUC_{Rt}= Area under the curve of the reference product at time t
AUC_{Tt}= Area under the curve of the test product at time t

The primary advantage of the similarity factor S_d over the similarity factor f_2 is the simplicity and flexibility of determination of S_d. The data used for the determination of S_d can be expressed as either the amount of API dissolved or as the percent API dissolved. Two *in vitro* release profiles are considered similar when S_d is zero (0) or close to 0 [454,455]. The S_d factor was calculated for the comparison of CPLS formulation to Dermovate® and was compared to the f_2 factor to determine whether or not a relationship between these two parameters could be established to determine the

similarity in the *in vitro* releases. The S_d values were calculated using AUC_{Rt} and AUC_{Tt} values that had been determined by the trapezoidal rule [456].

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The f_1 and f_2 difference and similarity factors, and the Gohel similarity factor, were used to compare the *in vitro* release profiles generated for CP from CPLS cream formulations of different formulation composition. For assessing the discriminatory behaviour of the *in vitro* release test, Dermovate® cream was used as the reference product and the results of these analyses are summarised in Table 4.12.

Table 4.12. *f_1, f_2 and S_d values for CPLS formulations and Dermovate®*

Comparison*	f_1	f_2	S_d
R vs 01	10.0	78.0	0.0063
R vs 02	1.1	99.3	0.0006
R vs 03	14.2	70.9	0.0174
R vs 04	23.7	60.1	0.0187
R vs 05	33.9	52.5	0.0207

***Comparison = Dermovate® (R) vs CPLS (-01, -02, -03, -04 and -05)**

On consideration of the difference factor alone, it is not possible to determine whether CP release from batches CPLS-01, CPLS-02 and CPLS-02 were similar to that of Dermovate® as the values for f_1 that were calculated are < 15 for each comparison. However batch CPLS-02, with a 3.00% w/w G-64 and 46.00% v/v PG showed the least difference for the *in vitro* release profile generated compared to Dermovate®, as the f_1 factor is close to zero [447]. Batches CPLS-03, CPLS-04 and CPLS-05 with f_1 values $\geq$ 15.0 showed significant differences in their *in vitro* release profiles compared to Dermovate®. CPLS formulations with increased % w/w G-64 and decreased % v/v of PG content compared to the reference product, resulted in those batches showing significant differences in their *in vitro* release profiles.

The similarity factor f_2, for the comparison of the CPLS formulations, indicates that all batches showed some similarity in their *in vitro* release profile, as the f_2 values are > 50. However, an f_2 value of close to 100 indicates that the *in vitro* profiles of a test and reference product are identical. The release profile for batch CPLS-02 was most similar to that of Dermovate®, as the f_2 is 99.3. It can be clearly seen from the data listed in Table 4.11 that there seems to be a relationship between the f_2 values and the similarity factor, S_d. Batches that showed values of f_2 greater than 50 resulted in S_d values of $\leq$ 0.02. However it can be observed that as the f_2 value increases, the value for S_d decreases. Batch CPLS-05, with a 12.00% w/w G-64 and 37.00% v/v PG content, showed the least similar *in vitro* release profile to Dermovate®, with the lowest f_2 value (52.2) and highest S_d value (0.0207). Therefore it is vital to evaluate the performance of different formulation compositions of generic laboratory scale formulations, as they can influence the *in vitro* release profiles of CP from its topical base. It is therefore important to calculate the difference and similarity factors for test formulations in relation to those generated for a commercially available innovator product for comparison.

4.6.1.4. Model-Dependent Methods

Model-dependent methods are based on the use of mathematical functions that are used to describe an *in vitro* release profile. A mathematical function for a dosage form under investigation is selected for evaluation and the *in vitro* release profiles are subsequently fitted to the function, after which the model is evaluated against previously established model parameters [429]. Commonly used model-dependent approaches included fitting data to the zero order, first order, Higuchi and regression models [428,429,434,445,448]. These models may be used to evaluate the mechanism of release of an API from a delivery system. They are useful for understanding which formulation parameters alter an *in vitro* release of that API from the dosage form, thereby providing a means of optimising the formulation development process [421]. In general the release of an API from topical dosage forms may be characterised by a mechanism of release, which is primarily diffusion-controlled [360,457].

4.6.1.4.1. Zero Order Model

Zero order models can be used to describe the *in vitro* release profiles from several types of modified or sustained release dosage forms including transdermal drug delivery systems. This model is used for *in vitro* release from dosage forms that do not disaggregate and release an API slowly [429]. A zero order reaction is independent of the concentration of the reactants, and is mathematically shown in Equation 4.6. To evaluate CP release kinetics, the release profiles generated during *in vitro* drug release studies were plotted as a cumulative amount of API released versus time [451,458].

$$Q_t = Q_0 + K_0 t$$

Equation 4.6

Where

Q_t= Amount of API dissolved in time t
Q_0= Initial amount of API in the solution (most times, $Q_0 = 0$)
K_0= Zero order release rate constant
t= Time

4.6.1.4.2. First Order Model

First order kinetic models may be used to describe the release of an API from a delivery system in which the rate of release is dependent on the concentration of the reactants under ideal or “sink” conditions. This type of model has also been used to describe absorption and/or elimination of drugs from the biological system, although it is difficult to conceptualise this mechanism on a theoretical basis [429,459,460]. The release of an API which follows first order kinetics can be mathematically expressed using Equation 4.7.

$$\ln Q_t = \ln Q_0 - K_1 t$$

Equation 4.7

Where

Q_t= Amount of API dissolved in time t
Q_0= Initial amount of API in the solution (most times, $Q_0 = 0$)
K_1= First order release rate constant
t= Time

4.6.1.4.3. Higuchi Model

The first example of a mathematical model aimed at describing API release from a matrix system was proposed by Higuchi in 1961 [429]. Initially conceived for planar systems, it was then extended to evaluating drug release from systems of different geometry and porosity. The Higuchi model is based on the hypotheses that i) the initial concentration of an API in the matrix is much higher than the solubility of the API in that matrix, ii) diffusion of an API takes place only in one dimension that is, the edge effect must be negligible, iii) particles of an API are much smaller than the system thickness, iv) swelling and dissolution of the matrix are negligible, v) drug diffusivity is constant and vi) perfect sink conditions are always maintained in the environment in which the drug release occurs [428,429].

In 1963, Higuchi described the release of an API from an insoluble matrix and confirmed that the drug release is directly proportional to the square root of time, when based on the principles of Fickian diffusion. The model also describes the mechanism of release of water-soluble and water-insoluble compounds that are uniformly dispersed and incorporated into semi-solid matrices. A simplified version of the Higuchi model is described in Equation 4.8 [429,445,460].

$$Q_t = K_H t^{1/2}$$

Equation 4.8

Where

Q_t= Amount of drug released at time t
K_H= Higuchi dissolution constant
t= Time

The Higuchi model is based on Fick's first law of diffusion, and drug release is considered to be diffusion-controlled if the release profile for that drug in a matrix is square root time dependent [427,461]. In the cases where semi-solid matrices are used, the penetration of the API into the skin tends to follow Fick's first law of diffusion, which describes the rate of transfer of solutes as a function of the concentration of the various ingredients, the size or surface area of treatment and the permeability of the skin [399]. Fick's first law of diffusion [462] is shown in Equation 4.9.

$$J_A = -D \frac{dC_A}{dx}$$ ***Equation 4.9***

Where

J_A= Flux of AP
D= Drug diffusion coefficient
C_A= Concentration of API

4.6.1.5. Model-Dependent Results

CP release data from the CPLS batches and Dermovate® were fitted to the zero, first order and the Higuchi models to establish the mechanism of drug release from these formulations and the results are summarised in Table 4.13. The data were also plotted for each model and the release profiles are shown in Figures 4.13 and 4.14 for the zero and Higuchi models respectively.

Table 4.13. *Model-dependent parameters generated of CPLS (Tests) and Dermovate® (Reference)*

Formulation	**Zero order**		**First order**		**Higuchi**	
	K_0	**R^2**	**K_1**	**R^2**	**K_H**	**R^2**
Dermovate®	0.66	0.97	-0.0129	0.94	6.31	0.99
CPLS-01	0.72	0.98	-0.0131	0.95	6.95	0.98
CPLS-02	0.67	0.97	-0.0128	0.93	6.32	0.99
CPLS-03	0.58	0.98	-0.0133	0.93	5.50	0.98
CPLS-04	0.51	0.99	-0.0132	0.94	4.93	0.98
CPLS-05	0.45	0.98	-0.0135	0.93	4.29	0.99

The zero order model may be considered suitable to describe the mechanism of release of CP from cream formulations as the R^2 values for the model fit are close to 0.99 for all cream formulations (Table 4.13). Generally zero order models are useful for describing the mechanisms of drug release if the dosage forms being tested do not disaggregate during *in vitro* testing. Since most cream formulations do not disaggregate during *in vitro* testing when using Franz cell apparatus, the zero order model is suitable to describe the mechanism of CP release from these cream formulations. The release of CP from its topical base was considered to be a linear function of time for each CPLS formulation (Figure 4.13), therefore following a zero order release kinetic model. However this model was not sufficient to provide a detailed mechanism of CP release from this matrix and other models were therefore considered.

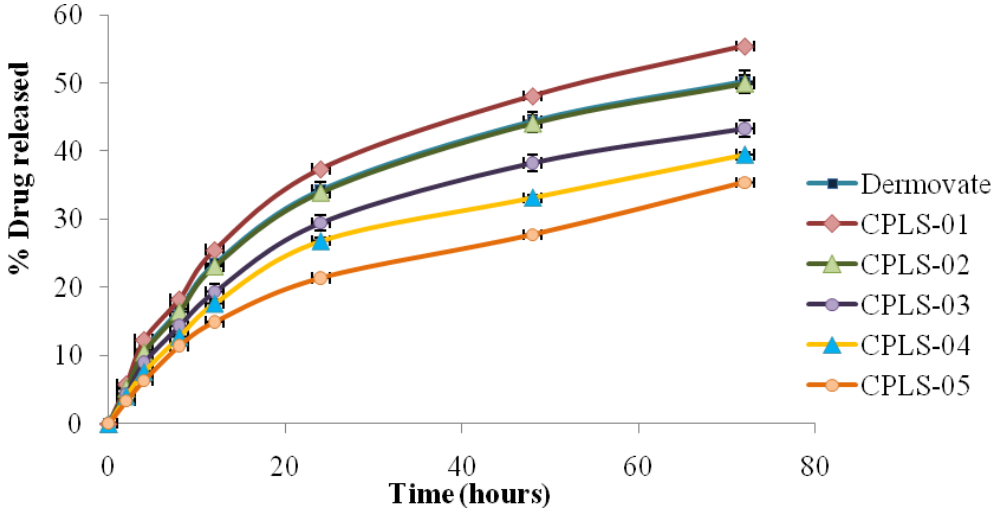

Figure 4.13. *Zero order plot of in vitro release kinetics of CPLS batches and Dermovate®*

The R^2 values obtained following fitting of CP release data to a first order model suggest that the mechanism of release may follow a first order kinetic process, since most R^2 values for these cream formulations are between 0.93 and 0.95. However, first order kinetics are most applicable for describing the release of water-soluble drugs from porous matrices. These cream formulations contain CP, which is a poorly water-soluble API and the cream base is a visco-elastic gel network structure that does not form a porous matrix to facilitate drug release by a first order type mechanism. Therefore other models such as that proposed by Higuchi were used to carry out further investigations of the mechanism of drug release from the cream.

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When the amount of API released was plotted against the square root of time for each cream formulation, a linear relationship was observed, with resultant $R^2 > 0.99$ for all the curves, as shown in Figure 4.14. CP release from the creams was best described by the Higuchi model, where the rate-controlling step for drug release is diffusion through the topical base.

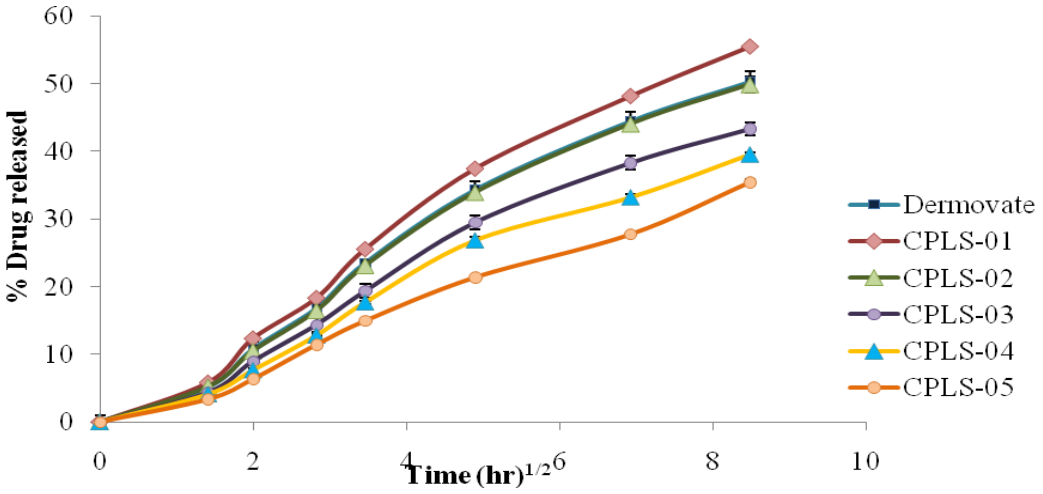

Figure 4.14. *Higuchi plot of in vitro release kinetics of CPLS batches and Dermovate®*

This relationship exists for formulations in which the API is fully dissolved, or where the drug is formulated as a suspension [387]. The synthetic membrane has no effect on drug release and in this case, it is the properties of the formulation that control the release of CP. Batch CPLS-01, which contained 1.50% w/w G-64 and 47.50% v/v PG, showed the highest rate of release, suggesting that the PG content may completely solubilise the CP in the delivery vehicle, thereby facilitating release of API. Therefore solubility of CP in PG is an important factor that determines the extent and rate of release of API, so that therapeutic efficacy can be achieved [387,463]. PG acts as a co-solvent for the highly lipophilic CP, and an increase in the % v/v PG increases the transport of the API from the topical base via the synthetic membrane to the receptor medium, located in the receptor compartment of the Franz diffusion cell. The results also reveal the potential penetration enhancement capability of the PG. The flux increases as the affinity of CP for PG rises and the data are summarised in Table 4.8. An increase in PG content in the formulation composition also resulted in a decreased lag time. The *in vitro* release profile of CP from all formulations is best described by fitting the data to the Higuchi model. Since all R^2 are 0.98 or 0.99, CP release from cream formulations is diffusion-controlled.

4.7 CONCLUSION

In an era where there is an increasing demand for pharmaceuticals, innovator pharmaceutical companies are experiencing tough challenges, in respect of increasing research expenditure [296,300]. There is an opportunity for generic companies to expand their offerings onto a global market and generic medicines are currently extensively prescribed due to their cost-effectiveness [287]. A formulation scientist faces many challenges when developing and manufacturing semi-solid dosage forms due to their micro-structural complexity [336,337,464]. Therefore research on topical formulations is performed to sustain the high demand for the development and production of generic topical products. Prior to a generic product being commercialised, it must be subjected to some

development to prove it is bioequivalent to an original/brand product [306,309]. The development phases may not be lengthy, but the final generic formulation must conform to the same standards of efficacy, safety and quality as the original product [289,324].

Topical dosage form analysis is an important tool for product quality assurance. This analytical tool permits the formulation scientist to select the most appropriate generic formulation relative to the innovator product for further development [209,378]. All laboratory scale CP cream formulations were white, smooth and stable without any form of grittiness. The pH of the creams ranged between 5 and 6, confirming that there is little or no potential for skin irritation to occur when these formulations are applied. The cream batches in these studies complied with the USP requirements for content uniformity and batch CPLS-02 was the only laboratory scale formulation that showed similar viscosity and spreadability characteristics to Dermovate®. Therefore this formulation was selected as a potential generic formulation for further development. The results obtained from dosage form analysis were further investigated to explain the *in vitro* release characteristics of CP from these laboratory scale cream formulations.

A Franz cell diffusion apparatus was successfully used to assess the release of CP from the topical formulations of the five different CPLS formulations. The results of these studies serve as a guide for the selection of an optimal CP cream generic formulation, in particular with respect to the concentration of mixed emulsifier and penetration enhancer required for optimal *in vitro* performance. A comparison of the results of *in vitro* release studies revealed that batch CPLS-01, in which 1.50% w/w G-64 and a 47.50% v/v PG were used, released CP faster and to a greater extent over a 72 hour period than batch CPLS-05, in which 12.00% w/w G-64 and a 37.00% v/v PG were used, and from which the smallest amount of CP was released. Batches CPLS-04 and CPLS-05 had the highest viscosities of all CPLS formulations, and therefore exhibited the longest lag phase due to the formulation composition. Batch CPLS-02, in which 3.00% w/w G-64 and a 46.00% v/v PG were used, demonstrated an *in vitro* release profile similar to that of Dermovate®. These studies confirmed that the vehicle and the emulsifying agent in a topical formulation have a significant influence on the release of CP. The use of G-64 as a viscosity modifying agent in addition to an o/w emulsifying agent, and PG acting as the penetration enhancer and vehicle for insoluble CP, is clearly important.

The pharmaceutical topical dosage forms manufactured in these studies had been critically assessed by use of model-dependent and model-independent methods of analysis. The release characteristics of CP from topical formulations were found to be affected by factors such as the base, vehicle, emulsifying agent, physicochemical properties of CP and test parameters such as sample size and/or lag times. Exploratory data analysis was used as an initial approach for the statistical comparison of CP profiles from CPLS cream formulations, and the results were presented graphically and numerically. Although the method appeared to be very useful in evaluating the *in vitro* release of CP

from each CPLS formulation, a definitive conclusion to determine the similarity and differences in the release profiles at specific time points could not be made. The use of the difference (f_1) and similarity (f_2) factors to characterise the *in vitro* release profiles of CP from formulations with different % w/w G-64 and % v/v PG revealed that batch CPLS-02 showed the least difference in *in vitro* release compared to Dermovate®, as the value for f_1 was close to zero (0) and f_2 was close to 100. The Gohel and Panchal similarity factor S_d, confirmed that batch CPLS-02 demonstrated the most similarity to the reference product in respect of the *in vitro* release profile, with a value for S_d of 0.0006. The CP *in vitro* release profiles from each of the formulations under investigation were also fitted to the zero, first order and Higuchi models. The model with the highest R^2 was deemed the best fit. It was apparent that the Higuchi plots showed the best fit, with resultant R^2 values of 0.98 and 0.99. Therefore a diffusion-controlled mechanism appeared to predominate in CP release from these laboratory scale formulations.

The role, concentration and ratio of excipients in a formulation are crucial when producing a successful formulation as a small change in concentration of an emulsifying agent─ for example in semi-solid products─ can result in the manufacture of an unstable topical formulation. Therefore the formulation and manufacture of laboratory scale batches and analysis of these formulations to investigate their performance characteristics, prior to proceeding to industrial scale manufacture, is vital in the early stages of the drug product development process. When manufacturing generic formulations, these preliminary studies serve as a means of selecting the most suitable topical product from a laboratory perspective, for further formulation development and manufacture of scale-up batches.

CHAPTER FIVE

DEVELOPMENT AND ASSESSMENT OF SCALE-UP CLOBETASOL17-PROPIONATE CREAM FORMULATIONS

5.1 INTRODUCTION

During the course of product development, progressive refinements in formulation procedures, formulation *per se* and manufacturing processes are made [288,465]. Research and Development (R & D) personnel put a considerable amount of time and effort into developing successful pharmaceutical dosage forms on a laboratory scale, with exact specifications that guarantee adequate physical and chemical stability. These pharmaceutical products are designed to deliver and release an API, according to specific criteria and up to this point, such products are manufactured on a laboratory scale, using laboratory equipment [207,466,467]. The use of small-scale production equipment is essential to develop an appropriate manufacturing procedure for large-scale production. A formula is transformed into a feasible, robust product by the development of a facile, reliable and practical method of manufacture that effects the orderly transition from the laboratory scale to routine processing in a full-scale production facility [207]. The scale-up of a process or batch is studied to determine the operating conditions applicable to large-scale production batches, with the goal of obtaining products of the same quality, based on the previously optimised laboratory scale experiments [468,469]. Therefore scale-up can be defined as the process of increasing the size of production batches from gram to kilogram quantities. The scale-up process usually involves the integration of previous phases of formulation development, in addition to technology transfer for realising the large-scale manufacture of a product [468,470]. This stage of formulation development is crucial, since many of the process limitations which are not apparent at the laboratory scale very often become significant on a larger scale [207,288,465].

In moving from R & D to full-scale production, it is essential to manufacture intermediate-size batches. In practice, the transition from a laboratory scale to a large production plant is not direct or linear. The pharmaceutical product is commonly manufactured on an intermediate scale, usually larger in size than the initial development batches but smaller than that envisaged for the industrial-scale [207,470,471]. This is done to enhance the production of products as much as possibly to optimise the operating parameters, prior to commencing large-scale manufacture. The manufacture of intermediate-scale batches also makes possible the production of enough pharmaceutical product for clinical testing and samples for marketing. However, inserting an intermediate step between R & D and full-scale production does not necessarily guarantee a smooth transition from laboratory to factory [470,472]. Scale-up procedures are based on well-prepared technical transfer documents that will assure ease of transition, effective product quality, overall economy and timely achievement of market

readiness [288,465]. During intermediate industrial-scale manufacture, the production equipment selected must be compatible with the formulation in addition to producing a cost-effective, simple and reliable product. Raw materials that meet the required specifications for quality must be used during scale-up efforts and production and process controls must be evaluated, validated and finalised. The principles of Good Manufacturing Practice (GMP) must be applied and controlled throughout formulation processing to maintain quality assurance [207,473,474].

Scale-Up and Post-Approval Changes (SUPAC) are of special interest to the FDA, as a growing number of regulatory documents has been released over the past few years by the Center for Drug Evaluation and Research (CDER). These documents include the Immediate Release Solid Oral Dosage Forms (SUPAC-IR), Modified Release Solid Oral Dosage Forms (SUPAC-MR), and Semi-Solid Dosage Forms (SUPAC-SS) guidelines [385,450,470,475]. Any significant change in a process of making a pharmaceutical dosage form is a regulatory concern. The FDA, in collaboration with the pharmaceutical industry and academics in this research area, have recently launched a number of initiatives, under the framework of the Product Quality Research Institute (PQRI) [470,476]. Scale-up issues may require post-approval changes to a formulation that alter the composition, site of manufacture, the manufacturing process and/or equipment. From the regulatory standpoint, scale-up and scale-down approaches must be subjected to the same degree of scrutiny [477]. In a typical drug product development cycle, once a set of clinical studies has been completed, it becomes very difficult to change the product or the process of manufacture of that product to accommodate specific production needs. Such needs may include changes in batch size and manufacturing equipment or processes. Post-approval changes in the size of a batch from small-scale to larger-scale production batch should be submitted as additional information in an application, with a specific requirement that the new batches are to be produced using equipment of the new operating principles. Batches should be manufactured in full compliance with GMP and existing Standard Operating Procedures (SOP) at that production plant. The manufacturing changes may require new stability, dissolution and *in vivo* bioequivalence testing [305,470].

5.2 MANUFACTURE AND FORMULATION OF SCALE-UP CLOBETASOL 17-PROPIONATE (CPSU) CREAMS

5.2.1. Topical Scale-Up Formulation

Creams, ointments, gels and pastes are often semi-solids of usually high viscosity [207,247]. When manufacturing topical formulations, the rate of mixing, stirring and temperature of operation are critical for both laboratory and industrial-scale manufacture [288]. The effects of nine different manufacturing variables on the quality of laboratory scale formulation of semi-solid paraffin in water, prepared with two non-ionic surfactants, steareth 21 and sorbitan oleate were investigated [478]. The

manufacturing process variables that were investigated were i) order and ii) method of addition of materials, iii) emulsification time, iv) addition rate, v) homogenisation speed, vi) homogenisation time, vii) cooling time, viii) agitation speed and ix) agitation temperature. Homogenisation speed was the most important manufacturing factor for the production of a stable emulsion and longer homogenisation times resulted in improved stability of the topical formulations [478].

On a laboratory scale, an electrically operated homogeniser is usually used for thorough mixing and stirring. The homogeniser can be manually adjusted and positioned in the laboratory vessel to achieve maximum turbulence. The angle of entry of the propeller shaft and the depth of the propeller can be adjusted to prevent aeration [207,479]. However, the control of the mixing process and stirring rate is limited with large-scale production equipment used for the manufacture of semi-solid products, since industrial equipment consists of a fixed bowl vessel, with an immovable propeller shaft and homogeniser. High-speed agitation of the homogeniser may introduce air into the pharmaceutical product, while slow mixing may result in the production of an unsatisfactory formulation [480]. Such problems often occur in large-scale production but are not apparent when small quantities of product are manufactured using a beaker and a laboratory homogeniser. Furthermore, the mixing equipment must be capable of effectively and continuously moving the viscous semi-solid mass from the outside walls of a mixing bowl vessel to the centre of the vessel and from the bottom to the top of the bowl. This action is required to ensure the distribution of the ingredients and to bring about rapid and efficient heat transfer to and from the product during the heating and cooling steps of the manufacturing process. Therefore pharmaceutical equipment used for the mixing and homogenisation of semi-solid materials include agitator and shear mixers which may be high-shear mixers, homogenisers and colloid mills [207,473,481-483]. Scale-up issues with respect to topical formulations can easily occur when transferring from small-size batches to large commercial-size batches. Formulation scientists must be aware of the potential scalability problems that may be encountered and take cognisance of them when developing a formulation for scale-up and the manufacturing processes involved [470].

The main focus of this study was to scale-up an o/w CP cream formulation developed from 500 g laboratory batches to a 5 kg batch size using a Wintech® cream/ointment mixer (Model WLP/13/2006-2007, Wintech® Pharmachem Equipment PVT. Ltd, Mumbai, India). Since the Wintech® cream/ointment mixer was a new manufacturing plant, it was important to evaluate the different process parameters of that equipment that would affect the ultimate formulation characteristics.

5.2.2. Wintech® Cream/Ointment Mixer

The Wintech® cream/ointment mixer consists of several parts that are essential for the manufacturing of semi-solid dosage forms and a schematic diagram of the plant is shown in Figure 5.1.

The essential components of the Wintech® cream/ointment mixer are the mixing bowl vessel, homogeniser, anchor with attached anchor blades, water jacket, heater, sight glass, operating panel and pressure vent. The mixing bowl vessel is a round stainless steel bowl which can handle a minimum of 5 kg and a maximum of 20 kg of semi-solid materials. The charging port is used for the introduction of all excipients and API into the mixing bowl vessel during manufacture. The co-centric homogeniser is used for homogenising, dispersing and emulsification purposes [207,484] while the anchor with the attached anchor blades is used for thorough and continuous batch mixing [207,485]. The water jacket located on the outside of the mixing bowl vessel is a metal sheath that permits water intake and has outlet vents to allow water to be pumped through the void space**.** On heating, the water jacket can provide an even application of heat and can help to provide a regulated and constant temperature for manufacture. If necessary, the water jacket can also be used for the cooling of semi-solid products [403]. A heater is used to heat the water in the jacket to the desired temperature. The sight glass is an area where the formulation scientist can observe the formulation while manufacturing takes place. All instructions *viz.*, the appropriate homogenisation time, homogenisation speed, anchor speed, anchor time and temperature are PLC controlled through an operating panel fitted to a touch screen. The pressure vent is used to release the pressure that may build up during and after the manufacture of a semi-solid product.

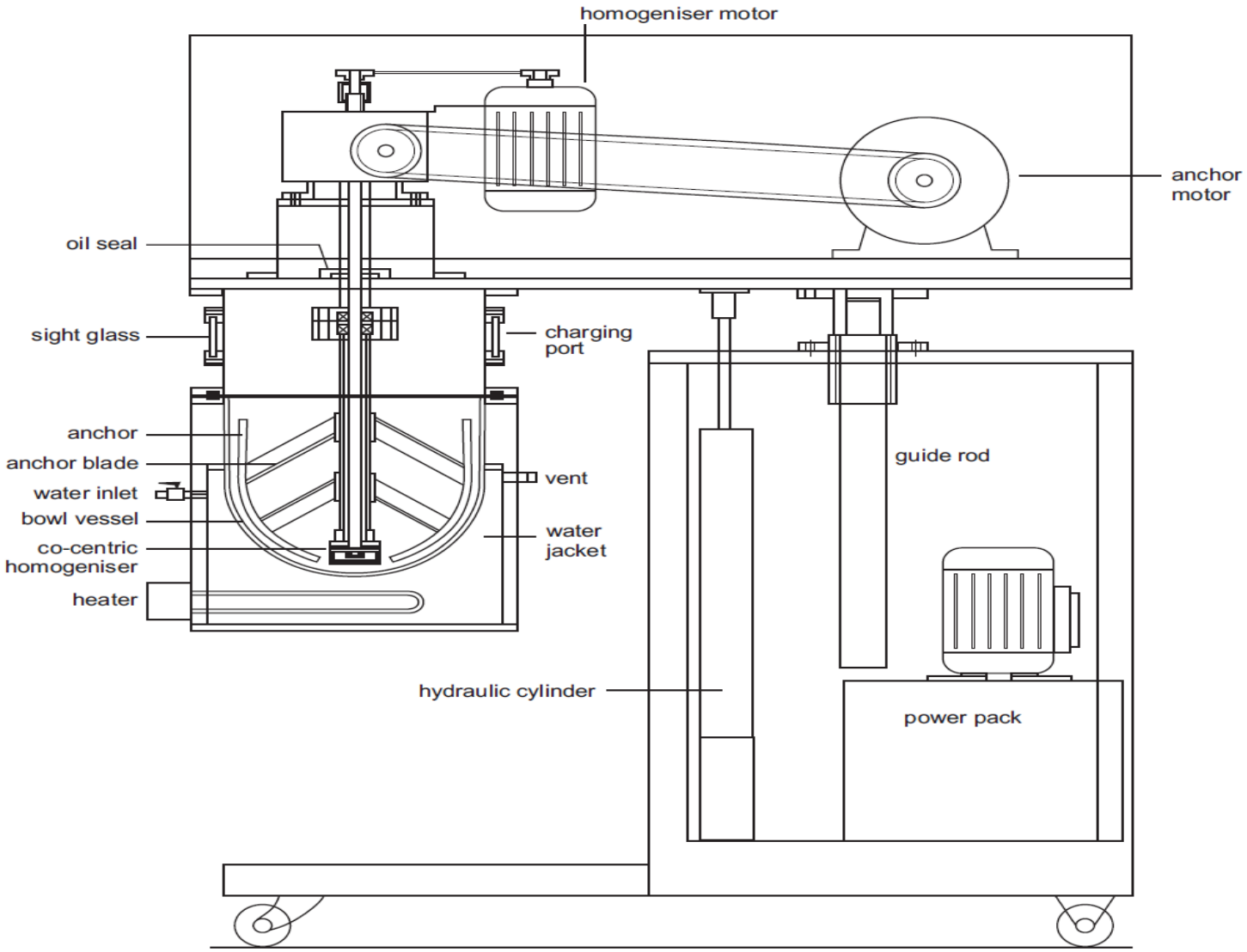

Figure 5.1. *Schematic diagram of the Wintech® cream/ointment mixer and its essential components*

5.2.3. Manufacture of Scale-Up CP (CPSU) Cream Formulations

The fusion method was selected as the preferred approach for the manufacture of a scale-up CP (CPSU) cream formulation. Batch CPLS-02 was found to have the desirable formulation characteristics similar to the reference product Dermovate®, in respect of viscosity, spreadability and *in vitro* release on a laboratory scale. Therefore it was necessary to investigate whether a formulation with the same % w/w composition would exhibit similar performance, following manufacture on a larger scale. However, large-scale manufacture is a more controlled process than laboratory scale manufacture as the large-scale limitations in terms of homogenisation speed, homogenisation time and anchor speed are set [208]. The composition of the initial formulation used to manufacture CPSU cream is summarised in Table 5.1.

Table 5.1. *Batch quantity of scale-up CP cream formulations developed and assessed in these formulation studies*

No.	Excipients	% w/w	Batch Quantity (g)
1	CP	0.05	2.50
2	Propylene glycol	46.00	2300.00
3	Sodium citrate	0.05	2.50
4	Citric acid	0.05	2.50
5	Gelot® 64	3.00	150.00
6	Glyceryl monostearate	11.00	550.00
7	Cetostearyl alcohol	8.40	420.00
8	White beeswax	1.15	57.50
9	Chlorocresol	0.075	3.75
10	Distilled water	30.225	1511.25
	TOTAL	100.00	5000.0

The following manufacturing process was used for the manufacture of all CPSU batches. A total of 30 CPSU batches was manufactured using the Wintech® cream/ointment mixer, using different homogenisation speed, anchor speed, homogenisation time and cooling time, as explained *vide infra* (§ 5.3) The mixing bowl vessel was cleaned with detergent and distilled water prior to manufacture, to avoid any cross-contamination from the previous batch that was manufactured. Following cleaning, the mixing bowl was drained, dried and allowed to equilibrate to room temperature (22°C). The room temperature of the cream manufacturing facility and the temperature of the Wintech® cream/ointment mixer were recorded respectively in a batch production record (Appendix III). The water jacket surrounding the bowl vessel was then filled with distilled water until the water jacket was full, for heating and cooling purposes. All excipients were weighed on a Mettler® Model PM460 balance (Mettler® Instruments, Zurich, Switzerland) and were introduced into the mixing bowl vessel through the charging port in an ordered manner. Propylene glycol and distilled water were first added to the bowl vessel, followed by sodium citrate, citric acid, CP, glyceryl monostearate, cetostearyl alcohol, white beeswax, Gelot® 64 and chlorocresol respectively.

Following charging of the mixing bowl vessel, the heater was activated and allowed to rise to 70°C. The anchor was programmed to operate at a set anchor speed to mix all excipients for two hours. Once thorough mixing had taken place and all waxy excipients had melted, the co-centric homogeniser was activated at a set homogenisation speed and time to commence the emulsification process. After a predetermined time the co-centric homogeniser was switched off. The formulation in the mixing bowl vessel was mixed at a set anchor speed and distilled water (± 20°C) was allowed to pass through the water inlet into the water jacket for a specific cooling time. The temperature of the distilled water was also recorded in the batch manufacturing record and the water inlet was then closed after the required set cooling time. Slow continuous agitation of the anchor was permitted until the temperature on the operating panel touch screen displayed between 30°C and 35°C. The anchor was finally stopped and the pressure build up in the mixing bowl vessel was released by activating the pressure vent.

The final CP cream was then stored in an opaque container until quality control tests *viz.*, viscosity, spreadability, content uniformity and *in vitro* release analysis were completed. The creams were then packed into 50 g aluminium cream/ointment tubes. The dosage form analysis was usually performed immediately, following storage in the opaque bulk containers and prior to packaging. This is considered to be an "in-process" control until the cream product has been packaged into appropriate cream/ointment tubes. This is a practical way of analysing semi-solids since they have a tendency to exhibit an increase in viscosity on storage and such products cannot be stored for any length of time [207].

5.2.4. Packaging of CPSU Cream Formulations

Packaging is used to contain, protect and preserve products during their distribution, storage and handling. In addition packaging permits ease of identification, adequate instructions and promotion for use. Pharmaceutical semi-solids have the ability to cling to a surface of application for a reasonable period of time. Therefore the packaging of semi-solids into appropriate containers is critical to ensure that their adhesion due to their rheological behaviour permits semi-solid materials to retain their shape, until acted upon by an external force in which they deform and subsequently flow [486]. The USP [45] recommends packaging and storage requirements for official ointments and creams. The USP specifies that CP creams must be packaged into collapsible tubes or tight containers and stored in either a cool place or at controlled room temperature. Special storage conditions are also recommended to ensure that the quality, safety and efficacy of topical dosage forms are not compromised. Therefore instructions such as protect from light since the API is photodegradable, avoid exposure to direct sunlight and do not refrigerate may be included [207,208]. An ideal container should protect the pharmaceutical product from the external environment such as temperature, humidity and particulate contamination, and it should be non-reactive with the product components. It

should also be easy to use, light in weight and economical. As tubes made of aluminium and plastic meet most of these qualities, they are used extensively for the packaging of semi-solid products. Aluminium tubes with special internal epoxy coatings are commercially available. These tubes often reduce incompatibility, thus enhancing the stability of semi-solid products [208].

The final cream was stored in 5 kg opaque containers and was finally packed into 50 g collapsible aluminium cream/ointment tubes using a manual Shreeji® collapsible tube filling machine (Shreeji® Pharmaceutical Scientific and Laboratory Instruments, Mumbai, India) that is depicted in Figure 5.2. The collapsible tube filler is a hand-operated machine. The 5 kg CP cream bulk was placed into the stainless steel hopper and a 50 g collapsible aluminium tube was placed at the bottom of the nozzle at each tube filling time. The hand lever was used to fill the collapsible aluminium tube with the desired quantity of viscous semi-solid material. The tube filling process was performed in a continuous manner to avoid entrapment of air in the topical formulation. Once completely filled, a crimping system was used to form an air-tight seal and the tubes were then stored at room temperature (22°C).

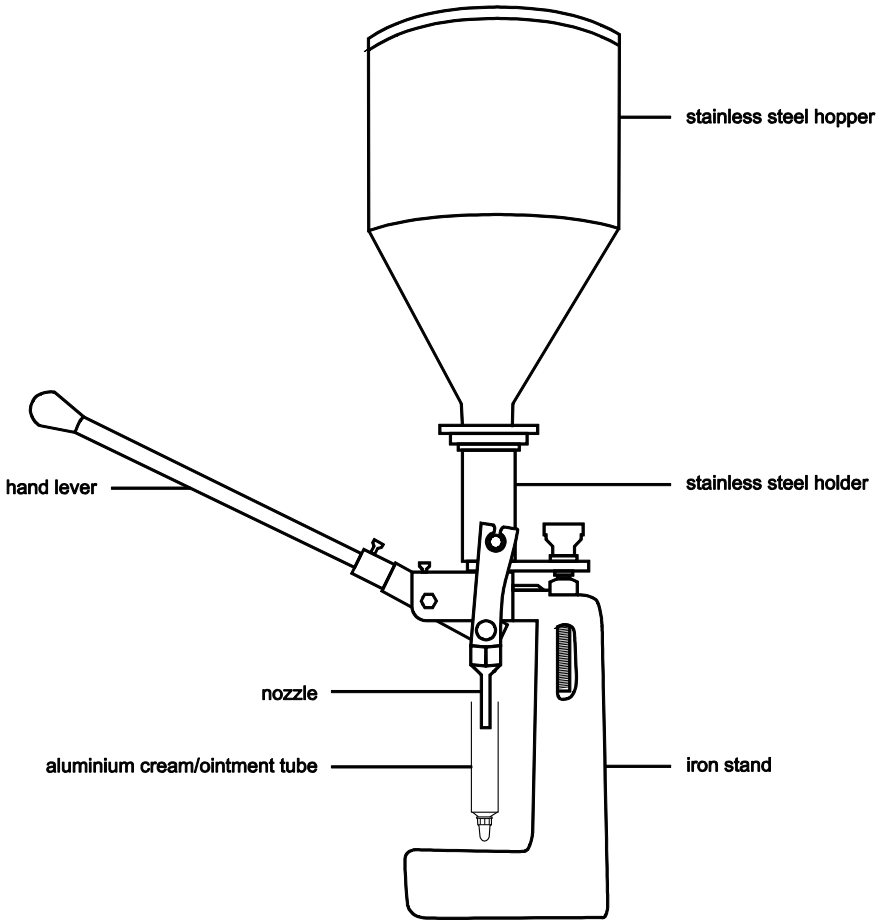

Figure 5.2. *Schematic diagram of collapsible tube filling machine and its essential components*

5.2.5. Minimum Fill of CPSU Cream Formulations

One of the USP specifications for CP cream formulation after packaging is the minimum fill test [45,208]. This test is performed to compare the weight or volume of the product filled into each container with their labelled weight or volume. A minimum fill test is applied only to containers that contain not more than 150 g or ml of a formulation [208].

Since the 5 kg CP cream formulations were packed into 50 g collapsible aluminium cream/ointment tubes, the minimum fill test was performed after filling ten tubes with each batch of CPSU formulation. The labels from the ten 50 g aluminium cream/ointment tube were first removed and their weights (W_1) were recorded. The entire formulation was then removed from tube and after cleaning and drying the tubes, the weight (W_2) of the empty tubes was recorded again. The difference in the total weight (W_1) and the empty-tube weight (W_2) is the weight of the fill for each CPSU formulation. The average content (n=10) of the collapsible cream/ointment tubes is summarised in Table 5.2.

Table 5.2. *Average content of CPSU formulations*

Formulation	Average content ± SD (n=10)	Formulation	Average content ± SD (n=10)
CPSU-01	95.08 ± 1.05	**CPSU-16**	93.06 ± 1.89
CPSU-02	96.20 ± 1.21	**CPSU-17**	95.08 ± 1.03
CPSU-03	95.09 ± 1.98	**CPSU-18**	97.30 ± 1.39
CPSU-04	92.08 ± 2.90	**CPSU-19**	92.10 ± 2.10
CPSU-05	95.06 ± 1.11	**CPSU-20**	98.05 ± 1.00
CPSU-06	92.09 ± 1.99	**CPSU-21**	95.59 ± 1.76
CPSU-07	96.90 ± 1.00	**CPSU-22**	95.06 ± 1.01
CPSU-08	92.30 ± 1.90	**CPSU-23**	97.99 ± 1.55
CPSU-09	90.09 ± 1.20	**CPSU-24**	96.89 ± 1.89
CPSU-10	93.10 ± 1.89	**CPSU-25**	95.09 ± 1.20
CPSU-11	96.09 ± 1.09	**CPSU-26**	96.20 ± 1.32
CPSU-12	98.15 ± 2.89	**CPSU-27**	94.10 ± 2.89
CPSU-13	97.01 ± 1.99	**CPSU-28**	95.24 ± 2.90
CSPU-14	95.09 ± 2.01	**CPSU-29**	98.56 ± 1.76
CPSU-15	97.29 ± 3.01	**CPSU-30**	93.19 ± 1.09

The USP specifications [45] recommend that the average net content of ten containers should not be less than the labelled amount and if the product weight is less than 60 g or ml, the net content of any single container should not be less than 90% of the labelled amount [208]. Since the average content for each batch was ≥ 90% of the labelled amount, all batches met the minimum fill test specifications.

5.2.6. Congealed Material

During the manufacturing development of the batches of CPSU cream formulations, one major production issue occurred at the beginning of the cooling process. The presence of congealed material was found to occur at specific sites of the Wintech® mixing bowl vessel as shown in Figure 5.3.

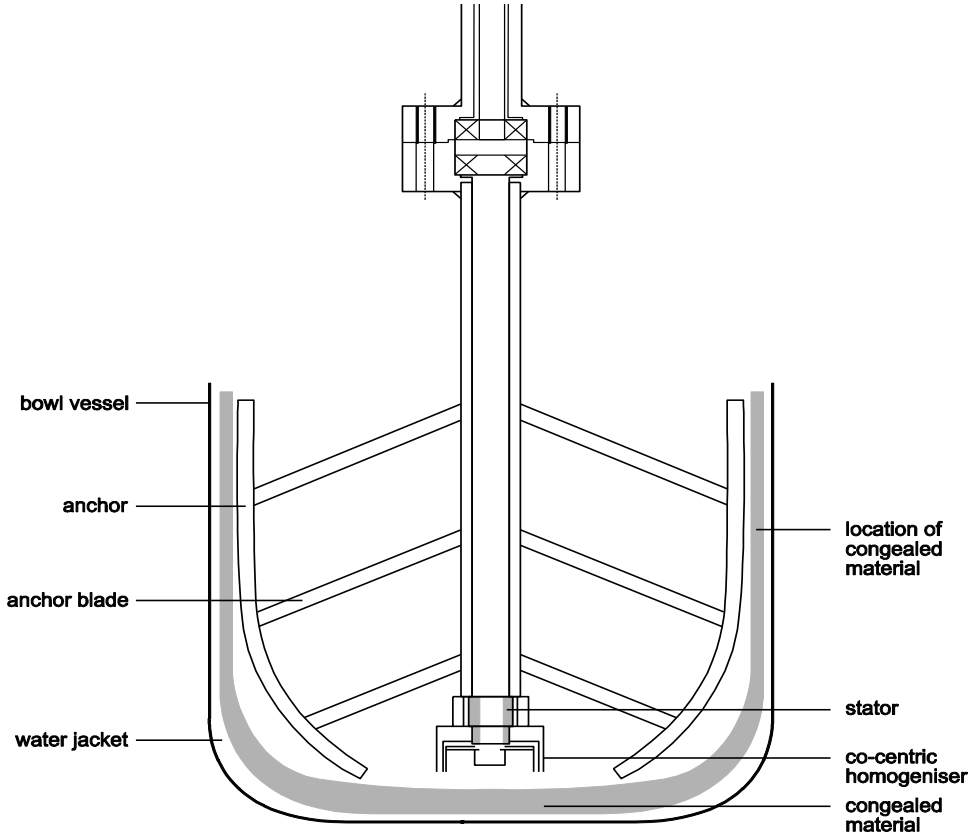

Figure 5.3. *Location of congealed material in the Wintech® cream/ointment mixing bowl vessel*

This congealed material was a solid wax-like stiff substance that was observed initially on the inner surface of the bowl vessel, which is in direct contact with the water jacket. However, this waxy solid material was found mostly between the walls of the mixing vessel and the anchor blades and apparently formed during the cooling procedure. The specific sites where the congealed material was formed are called "cold spots" since these sites are in close contact with the cold water passing through the water jacket [470,487]. This phenomenon was however not visible during the manufacture of the 500 g laboratory scale batches. The reason for the presence of the congealed material formed during the scale-up process might be primarily due to the presence of a water jacket on the outside of the Wintech® mixing bowl vessel. When distilled water is allowed to pass through the water jacket to cool the cream formulation, the cream formulation which is on the inner surface of the bowl vessel gets cooled more rapidly and solidifies more quickly than the formulation found in the middle of the mixing vessel. This gives rise to the presence of congealed material on the inner wall of the bowl vessel. In contrast, laboratory scale batches were made in glass beakers. The cooling of the CPLS cream formulation occurred in a less controlled manner and without the introduction of a cooling cycle to permit the formulation to set.

A second reason to the formation of congealed material in the mixing bowl vessel could be the significant difference in cooling times for different batch sizes. Cooling times for the 500 g laboratory

batches were approximately ten minutes, whereas cooling times for the 5 kg batches ranged between 40 minutes and 120 minutes, depending on the batch manufacturing requirements for that product. Longer cooling times allow for the waxy excipients to solidify in the formulation, precipitating the formation of congealed material. A third possible reason could be that the cold spots were located in areas inaccessible to the agitation anchor blades and homogeniser. Therefore the cold spots in the mixing vessel were areas for precipitation of the solid waxy materials that cannot easily be reincorporated with the rest of the bulk cream. Laboratory scale batches have little or no cold spots or areas where the homogeniser or spatula could not reach. It is important to investigate whether the congealed material formed on the inner surface of the mixing bowl vessel had an effect on the degree of uniformity of distribution of CP in the formulation. Therefore dosage form analysis such as blend uniformity testing was performed on each batch at the top/middle/bottom sections of the product whilst it was still in the mixing bowl vessel.

5.3 DEVELOPMENT AND FORMULATION OF A GENERIC CPSU CREAM FORMULATION USING AN EXPERIMENTAL DESIGN

5.3.1. Background

Similar to any other manufacturing procedure, machine process parameters may contribute to the total variability in the ultimate output or formulation performance. To our knowledge, little information has been published relating to the effect of machine process parameters on the topical formulations. Traditionally a one-factor-at-a-time technique (univariate method) is used to optimise a variable system. This system is not only time-consuming but also often easily confuses the alternative effect between the components. The univariate method optimises conditions one-by-one by varying levels of one condition while keeping others constant at unspecified levels. Therefore it requires a larger number of experiments to be conducted in order to determine the optimum levels for a process or formulation [488,489]. The drawbacks can be eliminated by optimising all parameters that may affect a product collectively, by using an experimental design approach which includes factorial design and Response Surface Methodology (RSM). Experimental design is a multivariate approach recommended for the development of analytical methods. This approach is applied to produce a large amount of data that can easily be interpreted to examine the main and interaction effects of experimental conditions on the efficacy of methods. It can also be used to optimise simultaneously experimental conditions regarding their interaction with each other by a minimum number of experiments [488,489].

RSM is a collection of statistical and mathematical techniques that are useful for developing, improving and optimising processes. It provides a means for relating changes in the measured "responses" of a physical system to changes in the conditions that make up that system. It is a set of

procedures involving experimental strategy and mathematical methods that collectively enable an investigator explore a physical system efficiently. In particular these procedures relate the change in a measured response of a system to the changes in the variables defining that system [490,491]. In this study, the most important applications of this methodology are in scenarios where a number of input factors related to the use of the Wintech® cream/ointment mixer might have a significant effect on the *in vitro* and *in vivo* performance of the cream formulations that were manufactured. The experimental strategy of RSM consists of factorial-type experimental designs. These experimental designs vary widely in specific details, but all of them have a common basic structure in which a series of experiments is performed with the variables at specific values. The experimental design to be selected depends on the specific application and the aim is therefore to obtain the maximum amount of information from a reduced number of experimental runs needed to provide sufficient information for statistically acceptable result [490,492]. The Central Composite Design (CCD), Box-Behnken Design (BBD) and three-level factorial design are among the most popular experimental designs used for this purpose. This popularity is attributable to their statistical efficiency, especially when augmented with centre runs [488,493].

The main objective of this study was to investigate the effect of the Wintech® cream/ointment mixer process variables *viz.*, homogenisation speed, anchor speed, homogenisation time and cooling time on the formulation responses *viz.*, viscosity, spreadability, pH , % drug content and cumulative % drug released per unit area over 72 hour period. This was performed with the intention of obtaining a minimum number of experimental runs to identify and define a CPSU formulation with responses similar to those of the innovator product, Dermovate®.

5.3.2. Process Variables

The process variables selected for evaluation were based on literature data [478,494] and the manufacturing procedure of the laboratory scale CP cream. The four process variables selected for consideration were i) homogenisation speed (X_1) , ii) anchor speed (X_2) , iii) homogenisation time (X_3) and iv) cooling time (X_4). Each process variable was investigated at two levels, -1 and +1 and the design of the study is shown in Table 5.3.

Table 5.3. *Actual and coded values of the process variables used for CCD*

Variable	**Symbol**	**Real values of coded levels**				
		$-\alpha^a$	**-1**	**0**	**+1**	**$+\alpha^a$**
Homogenisation speed (rpm)	X_1	100	700	1300	1900	2500
Anchor speed (rpm)	X_2	5	15	25	35	45
Homogenisation time (minutes)	X_3	40	60	80	100	120
Cooling time (minutes)	X_4	40	60	80	100	120

[a] α=2.0 (star or axial point in the case of four independent variables)

These levels were selected to cover the range of settings within which the cream/ointment mixer could be operated. In other words the study covers the entire operational region and where optimal levels are

likely to exist. The independent variables are coded to the (-1, +1) interval where the low and high levels are coded as -1 and +1 respectively. The axial points are located at (±α, 0, 0), (0, 0, ±α) and (0, 0, ±α), where α is the distance of the axial point from the center point and this makes the design rotatable. In these studies, the α value was fixed at 2.0 (rotatable). The experimental sequence was randomised in order to minimise the effects of uncontrolled factors. Other process variables that were not evaluated in these studies were considered as held-constant factors.

5.3.3. Formulation Responses

Each batch was monitored for performance by evaluating the physical characteristics of the CPSU cream formulation. The four formulation responses monitored included i) viscosity (Y_1), ii) spreadability (Y_2), iii) pH (Y_3), iv) % drug content (Y_4) and v) cumulative % drug released per unit area over 72 hours (Y_5).

The viscosity of the batches was determined using a Brookfield® Model-RVDI+ Viscometer (Brookfield® ENG Labs Inc., Stoughton, USA) as described in § 4.4.3 (Chapter 4). The spreadability was measured using a parallel plate method, as described in § 4.4.4 (Chapter 4). The pH of each CPSU cream formulation was measured at room temperature (22°C) as described in § 4.4.5 (Chapter 4). Blend uniformity was determined by removing cream samples in triplicate (n=3) from the top, middle and bottom of the mixing bowl vessel to evaluate the degree of uniformity of distribution of CP within each batch of product, as shown in Figure 4.4.6 (Chapter 4). Analysis of samples was performed in triplicate (n=3) and an average of the % drug content calculated. The viscosity, spreadability, pH and % drug content data for batches CPSU-01-CPSU-30 are included in Appendix IV, together with the batch summary records for these batches. A Franz diffusion cell system (Crown Glass Company Inc., Branchburg, NJ, USA) was used for the *in vitro* release testing of CPSU batches. The *in vitro* release profiles for CP were plotted as cumulative % drug released per unit area versus time. Three parallel determinations (n=3) for each batch were performed and the Franz diffusion cell conditions that were used are listed in § 4.5.1 (Chapter 4).

5.3.4. Experimental Design

Central Composite Design (CCD) is an experimental approach that can be applied to develop a second-order response model with few factors (n) *viz.*, $2 \leq n \leq 6$ efficiently. CCD is a good choice for robustness testing since it is highly efficient with respect to the number of experimental runs required [495,496]. An experimental design consists of three distinct sections. These include i) full (2^n), ii) fractional ($2^{n-z} \geq n+1$) design, where the factor levels are coded to the usual low (-1) and high (+1) values and iii) axial points or "star" points on the axis of each variable at a distance α from the designated center of the design. The center points are included as replicates to provide an estimate of the experimental error variance [495]. Generally the CCD is explained by 2^n factorial runs, with $2n$ axial runs and n center runs *viz.*, six replicates [493,497-499]. As the number of factors, n, increases,

the number of runs for a complete replicate of the design increases dramatically [497]. A CCD was generated to evaluate the response surface models for the batches produced using different process variables to study the effect of the experimental factors on the responses of interest and the CCD is summarised in Table 5.4.

CCD is suitable for fitting of models to a quadratic surface and can be used to optimise the effective parameters, with a minimum number of experiments as well as to analyse the interaction between these parameters [499]. Therefore a 2^4 full factorial CCD was used for four independent variables consisting of sixteen factorial points, eight axial points and six replicates at the center points, resulting in 30 experiments required to be conducted, as shown in Equation 5.1.

$$N = 2^n + 2n + n_c \qquad \textbf{\textit{Equation 5.1}}$$
$$= 2^4 + 2 \times 4 + 6 = 30$$

Where

N =Total number of experiments required

n =Number of factors

Based on the CCD method, each response was used to develop an empirical model that correlated the responses of each batch to the four process variables using a second-degree polynomial equation as given by Equation 5.2.

$$Y = b_0 + \sum_{i=1}^{n} b_i x_i + \left(\sum_{i=1}^{n} b_{ii} x_i\right)^2 + \sum_{i=1}^{n=1} \sum_{j=i+1} b_{ij} x_i x_j \qquad \textbf{\textit{Equation 5.2}}$$

Where

Y = Predicted response of the process

b_0 = Constant coefficient

b_i = Linear coefficients

b_{ii} = Interaction coefficients

x_i, x_j = Coded levels of the factors *viz.,* independent or control variables

Table 5.4. *CCD for evaluating CPSU cream formulation responses using Wintech® cream/ointment mixer variables*

Run	Variables								Responses				
	Homogeniser speed (X_1)		**Anchor speed (X_2)**		**Homogenisation time (X_3)**		**Cooling time (X_4)**		**VSC* (Y_1)**	**SPD* (Y_2)**	**pH (Y_3)**	**% DC* (Y_4)**	**% DR 72 hr* (Y_5)**
	Level	**rpm**	**Level**	**rpm**	**Level**	**min**	**Level**	**min**	**(cP)**	**(cm^2)**	**-**	**(%)**	**(%)**
1	0	1300	0	25	0	80	0	80	33033	28.7	5.24	100.14	61.23
2	0	1300	0	25	-2	40	0	80	30766	30.5	5.68	99.75	63.09
3	0	1300	0	25	0	80	-2	40	30600	30.2	5.60	99.93	63.40
4	+1	1900	+1	35	+1	100	+1	100	45000	24.6	5.39	98.79	50.49
5	0	1300	0	25	0	80	0	80	33466	29.3	5.48	101.42	60.64
6	0	1300	0	25	0	80	0	80	33633	31.4	5.29	98.00	60.61
7	+2	2500	0	25	0	80	0	80	81566	18.9	5.48	99.69	42.22
8	+1	1900	+1	35	-1	60	+1	100	51966	21.0	5.60	99.86	54.23
9	-1	700	+1	35	+1	100	+1	100	29933	30.2	5.75	97.96	62.21
10	+1	1900	-1	15	-1	60	-1	60	42333	30.8	5.72	99.82	57.23
11	0	1300	0	25	+2	120	0	80	36316	21.1	5.51	98.39	58.03
12	-1	700	-1	15	+1	100	+1	100	22733	28.6	5.35	98.59	64.97
13	+1	1900	+1	35	-1	60	-1	60	50133	24.9	5.65	98.86	57.42
14	0	1300	0	25	0	80	0	80	32800	28.6	5.66	99.93	61.04
15	+1	1900	-1	15	+1	100	-1	60	43933	24.9	5.89	98.10	55.50
16	0	1300	+2	45	0	80	0	80	35833	26.1	5.68	98.67	55.93
17	+1	1900	-1	15	-1	60	+1	100	43600	25.5	5.73	99.77	55.09
18	-1	700	-1	15	-1	60	-1	60	18333	35.0	5.34	98.82	67.05
19	0	1300	0	25	0	80	0	80	33566	28.9	5.57	97.25	60.61
20	-1	700	-1	15	+1	100	-1	60	20833	32.4	5.55	99.49	66.19
21	-2	100	0	25	0	80	0	80	10800	41.1	5.94	98.89	75.21
22	-1	700	-1	15	-1	60	+1	100	20900	33.5	5.49	99.62	65.76
23	0	1300	-2	5	0	80	0	80	29633	31.5	5.62	99.49	68.89
24	+1	1900	-1	15	+1	100	+1	100	48506	23.9	5.17	99.39	54.62
25	+1	1900	+1	35	+1	100	-1	60	52966	22.1	5.13	98.78	52.55
26	0	1300	0	25	0	80	0	80	33766	28.3	5.47	98.89	61.23
27	0	1300	0	25	0	80	+2	120	36166	30.1	5.31	98.79	58.15
28	-1	700	+1	35	-1	60	-1	60	25866	33.5	5.72	99.07	63.36
29	-1	700	+1	35	+1	100	-1	60	27600	32.5	5.56	98.89	65.30
30	-1	700	+1	35	-1	60	+1	100	27066	32.5	5.59	100.32	64.89

***Responses: VSC= Viscosity (cP); SPD= Spreadability (cm^2); DC= % Drug content; % DR 72hr= Cumulative % drug released per unit area over a 72 hour period**

5.3.5. Model Fitting and Statistical Analysis

The statistical analysis of the data was performed by fitting the data to models using Design-Expert® software (version 7.0, Stat-Ease Inc., Minneapolis, USA). Fitting of the data to the models *viz.*, linear, two factorial, quadratic and cubic, and their subsequent Analysis of Variance (ANOVA), showed that the correlation between process variables and the batch responses was most suitably described by a quadratic polynomial model. Therefore a quadratic polynomial equation was generated for each response from the CCD. ANOVA analysis and the Regression Coefficient (R^2) were used to analyse data statistically and normal plots of residuals, contour plots and 3-dimensional response surface plots were used to visualise the data graphically. The regression methods were used to develop the appropriate response surfaces to characterise build performance. The resulting surfaces were used to identify settings for the experimental factors that were required to achieve target performance levels [491] and the contour plots indicate the general shape of the response surface [490].

5.3.6. Quadratic Polynomial Equations and their Regression Coefficients

The complete design matrix together with the values of the multiple responses obtained from the experimental work is summarised in Table 5.3. The viscosity of the cream formulations ranged between 10 800 cP and 81566 cP, spreadability between 18.1 cm^2 and 41.1 cm^2, pH between 5.18 and 5.89, % drug content between 97.25% and 101.42% and thecumulative % drug released per unit area over a 72 hours ranged between 42.44% and 68.89%. The six experiments performed at the center points were used to determine the experimental error of the quadratic models. According to the sequential model sum of squares, the models were selected, based on their highest order of polynomials where the terms were significant. A quadratic model was selected for each formulation response using the Design-Expert software. The final empirical models in terms of coded factors for viscosity (Y_1), spreadability (Y_2), pH (Y_3), % drug content (Y_4) and cumulative % drug released over 72 hours (Y_5) are shown in Equations 5.3-5.7.

Viscosity (Y_1) = $33377.33 + 14146.04X_1 + 2806.62X_2 + 1166.96X_3 + 1018.29X_4 + 3513.30X_1^2 - 299.20X_2^2 - 97.20X_3^2 - 136.70X_4^2 - 23.31X_1X_2 - 60.06X_1X_3 - 168.31X_1X_4 - 298.19X_2X_3 - 456.69X_2X_4 - 26.69X_3X_4$ ***Equation 5.3***

Spreadability (Y_2) = $29.20 - 4.88X_1 - 1.00X_2 - 0.25X_3 - 0.18X_4 - 0.10X_1^2 - 0.40X_2^2 - 0.016X_3^2 - 0.066X_4^2 - 0.44X_1X_2 + 0.74X_1X_3 + 0.54X_1X_4 + 0.49X_2X_3 + 0.14X_2X_4 - 0.044X_3X_4$ ***Equation 5.4***

pH (Y_3) = $6.45 - 0.041X_1 + 0.011X_2 - 0.058X_3 - 0.045X_4 + 0.054X_1^2 + 0.039X_2^2 + 0.025X_3^2 - 9.688E\text{-}003X_4^2 - 0.10X_1X_2 - 0.074X_1X_3 - 0.032X_1X_4 - 0.026X_2X_3 + 0.064X_2X_4 - 0.028X_3X_4$ ***Equation 5.5***

% Drug content (Y_4) = $99.27 + 0.092X_1 - 0.11X_2 - 0.37X_3 + 7.917\ E\text{-}3X_4 - 6.563E\text{-}003X_1^2 - 0.059X_2^2 - 0.062X_3^2 + 0.011X_4^2 - 0.032X_1X_2 - 0.022X_1X_3 + 0.13X_1X_4 - 0.077X_2X_3 + 0.012X_2X_4 - 0.22X_3X_4$ ***Equation 5.6***

Cumulative % drug released over 72 hours (Y_5) = $60.89 - 6.19X_1 - 1.75X_2 - 0.97X_3 - 0.95X_4 - 0.68X_1^2 + 0.24X_2^2 - 0.22X_3^2 - 0.16X_4^2 - 0.026X_1X_2 - 0.52X_1X_3 - 0.26X_1X_4 - 0.34X_2X_3 - 0.081X_2X_4 - 0.13X_3X_4$ ***Equation 5.7***

These polynomial equations comprise the coefficients for intercept, first order main effects, interaction terms and higher order effects. The sign and magnitude of the main effects signify the

relative influence of each factor on the response that is the average value of changing one factor at a time from its low to high value. The interaction terms *viz.*, X_1X_2, X_1X_3, X_1X_4, X_2X_3 X_2X_4 and X_3X_4 show how the formulation response changes when two factors were changed simultaneously and the quadratic terms *viz.*, X_1^2, X_2^2, X_3^2 and X_4^2 are included to investigate non-linearity within the set of results. A positive sign (+) in front of a term indicates a synergistic effect whereas a negative sign (-) indicates an antagonistic effect [357,499,500].

The quality of the quadratic response surface model developed was based on the regression coefficient and standard deviation values. The closer the R^2 value is to unity and the smaller the standard deviation, the more accurate the response that could be predicted by the model. If an R^2 value is > 0.9, it indicates that there is a good correlation between the experimental and predicted responses [500]. A summary of the R^2 values is listed in Table 5.5.

Table 5.5. *Model summary statistics of the appropriate quadratic response surface models*

Response factor	**Quadratic Response Surface Model**							
	SD*	**F-value**	**Prob > F**	**R^{2*}**	**Adj R^{2*}**	**Pred R^{2*}**	**Adeq Prec***	**C.V (%)***
Y_1	4273.13	21.22	< 0.05	0.9519	0.9071	0.8976	19.07	11.95
Y_2	0.55	18.57	< 0.05	0.9455	0.8946	0.7275	17.83	1.39
Y_3	1.18	1.49	> 0.05	0.5818	0.1915	-0.9163	4.76	2.72
Y_4	1.61	0.36	> 0.05	0.2500	-0.4500	0.8720	2.53	3.02
Y_5	2.50	12.11	< 0.05	0.9187	0.8467	0.7167	15.98	4.15

***SD= Standard deviation, R^2 = Regression coefficient, Adj R^2 = Adjusted R^2, Pred R^2 = Predicted R^2, Adeq Prec = Adequate precision, C.V (%) = coefficient of variation**

The regression coefficient indicates that 95.19%, 94.55%, 58.16%, 25% and 91.87% of the total variation in Y_1, Y_2, Y_3, Y_4 and Y_5 can be attributed to the experimental variables, respectively. The R^2 values of 0.5818 and 0.2500 for Equations 5.5 and 5.6 were deemed inappropriate to validate the fit which might lead to large variation in the pH and % drug content predicted when using this model. However, for Equations 5.3, 5.4 and 5.7, the R^2 of 0.9519, 0.9455 and 0.9187 were considered relatively high as their values are close to unity, indicating good agreement between the experimental and predicted viscosity (Y_1), spreadability (Y_2) and cumulative % drug released over a period of 72 hours (Y_5), for this model.

In addition to these models, the predicted R^2 value must be in reasonably close agreement with the adjusted R^2 value, indicating the reliability of models. The viscosity (Y_1), spreadability (Y_2) and cumulative % drug released over 72 hours (Y_5) data showed some degree of reliability in the quadratic response surface models as their adjusted and predicted R^2 values were in close agreement (Table 5.4). Furthermore the higher values > 4 of adequate precision indicate adequate signal. All responses showed adequate signal since their values are > 4, except for the % drug content [500]. The relatively low values for the coefficient of variation indicate precision and reliability of the experiments performed and spreadability had the lowest coefficient of variation, showing a precise and reliable quadratic response surface model had been developed.

The standard deviation (SD) for Equations 5.3-5.7 (Table 5.4) reveals that the predicted value for spreadability (Y_2) was more accurate and closer to its actual value as compared to the other responses. A possible reason for the lower superiority and higher error of estimation given by the other equations (5.3, 5.5-5.7) could be that there are other parameters that may affect viscosity, pH, % drug content or cumulative % drug released over 72 hours, other than the four variables *viz.*, homogenisation speed, anchor speed, homogenisation time and cooling time that were evaluated in this work. The adequacy of the models was further justified through an analysis of variance.

5.3.7. Analysis of Variance (ANOVA)

The ANOVA analysis of the quadratic regression models was used to check the significance of the quadratic response surface models and the models were tested at a 0.05 level of significance. A summary of the ANOVA analysis for the quadratic models for the five responses is listed in Tables 5.6-5.10 respectively.

5.3.7.1. ANOVA analysis of theResponse Surface Quadratic Model for Viscosity (Y_1)

ANOVA analysis for the response surface quadratic model for viscosity of the cream formulations resulted in an F-value of 21.22 for this model, implying that the model is significant. The P-value is a tool that can be used to check the significance of each coefficient, which also indicates the strength of interaction between each independent variable. The smaller the P-value, the more evidence there is to support rejecting the null hypothesis [488]. Therefore a value of Prob > F < 0.05 indicates that the model terms were significant. In this case, X_1, X_2 and X_1^2 were significant model terms whereas X_3, X_4, X_2^2, X_3^2, X_4^2, X_1X_2 , X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 were all insignificant to the response, as can be seen in the data summarised in Table 5.6.

Table 5.6. *ANOVA analysis for the response surface quadratic model of viscosity*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	5.424 E+009	14	3.875 E+008	21.22	< 0.0001[a]
X_1	4.803 E+009	1	4.803 E+009	263.01	< 0.0001[a]
X_2	1.891 E+008	1	1.891 E+008	10.32	0.0058[a]
X_3	3.268 E+007	1	3.268 E+007	1.79	0.2009[b]
X_4	2.489 E+007	1	2.489 E+007	1.36	0.2613[b]
X_1^2	3.386 E+008	1	3.386 E+008	18.54	0.0006[a]
X_2^2	2.455 E+006	1	2.455 E+006	0.13	0.7190[b]
X_3^2	2.591 E+005	1	2.591 E+005	0.014	0.9068[b]
X_4^2	5.125 E+005	1	5.125 E+005	0.028	0.8692[b]
X_1X_2	8695.56	1	8695.56	4.762 E-004	0.9829[b]
X_1X_3	57720.06	1	57720.06	3.161 E-003	0.9559[b]
X_1X_4	4.533 E+005	1	4.533 E+005	0.025	0.8769[b]
X_2X_3	1.423 E+006	1	1.423 E+006	0.078	0.7840[b]
X_2X_4	3.337 E+006	1	3.337 E+006	0.18	0.6751[b]
X_3X_4	11395.56	1	11395.56	6.241 E-004	0.9804[b]
Residual	2.739 E+008	15	1.826 E+007	-	-

[a] Significant at "Prob > F" less than 0.05 ; [b] Insignificant at "Prob > F" more than 0.05

Significant factors affecting the response Y_1 were the synergistic effects of the linear contribution of X_1, X_2, X_3, X_4 and the quadratic contributions of X_1^2 while Y_1 was affected in an antagonistic manner by the quadratic contributions of X_2^2, X_3^2 and X_4^2 and the interaction effects of X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 (Equation 5.3). Residuals are the difference between actual and predicted values for each point and show how well the model satisfies the assumptions of the analysis of variance [501,502]. A normal plot of residuals, also known as a normal probability plot, is used to visualise the performance of the response surface quadratic model. This graphical technique indicates whether the residuals follow a normal distribution and for a normal distribution set of data, the points should form an approximately straight line [503,504]. Most of the points for the viscosity normal probability plot of residual formed a linear pattern, indicating that a normal distribution is a good model for this set of data. However after a certain range of parameters, the minimum and maximum viscosity values were completely out of the linearity region, as shown in Figure 5.4.

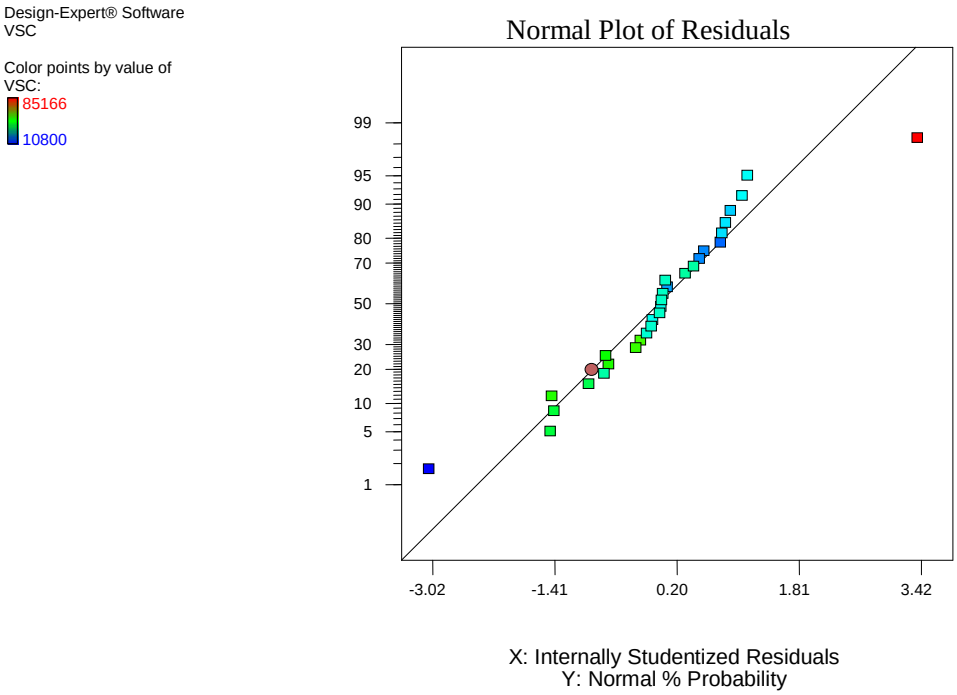

Figure 5.4. *Normal probability plot of residuals for viscosity*

5.3.7.2. ANOVA analysis of the Response Surface Quadratic Model for Spreadability (Y_2)

ANOVA analysis of the response surface quadratic model for spreadability of the cream formulations resulted in an F-value of 18.57 for the model implying that the model is significant. The significant model terms were X_1 and X_2 since the values of Prob > F were < 0.05. The insignificant model terms to the response were X_3, X_4, X_1^2, X_2^2, X_3^2, X_4^2, X_1X_2 , X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 as summarised in Table 5.7.

Table 5.7. *ANOVA analysis for the response surface quadratic model of spreadability*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	622.95	14	44.50	18.57	<0.0001[a]
X_1	571.35	1	571.35	238.49	<0.0001[a]
X_2	23.80	1	23.80	9.93	0.0066[a]
X_3	1.45	1	1.45	0.61	0.4486[b]
X_4	0.77	1	0.77	0.32	0.5790[b]
X_1^2	0.29	1	0.29	0.12	0.7320[b]
X_2^2	4.46	1	4.46	1.86	0.1927[b]
X_3^2	6.696 E-003	1	6.696 E-003	2.795 E-003	0.9585[b]
X_4^2	0.12	1	0.12	0.049	0.8273[b]
X_1X_2	3.15	1	3.15	1.32	0.2694[b]
X_1X_3	8.85	1	8.85	3.69	0.0738[b]
X_1X_4	4.73	1	4.73	1.97	0.1803[b]
X_2X_3	3.90	1	3.90	1.63	0.2214[b]
X_2X_4	0.33	1	0.33	0.14	0.7155[b]
X_3X_4	0.031	1	0.031	0.013	0.9115[b]
Residual	35.94	15	2.40	-	-

[a] Significant at "Prob > F" less than 0.05 ; [b] Insignificant at "Prob > F" more than 0.05

Significant factors affecting the response Y_2 were the synergistic effects of interaction contribution of X_1X_3, X_1X_4, X_2X_3 and X_2X_4 while Y_2 was affected in an antagonistic manner due to a linear contribution of X_1, X_2, X_3, X_4, quadratic contributions of X_1^2, X_2^2, X_3^2, X_4^2 and interaction effects of X_1X_2 and X_3X_4. The normal probability plot of residuals for spreadability shows that the data are linear and follow a normal distribution as most points for spreadability fell on a straight line. The minimum spreadability value falls on the straight line whereas the maximum value is totally out of the linearity region, as shown in Figure 5.5.

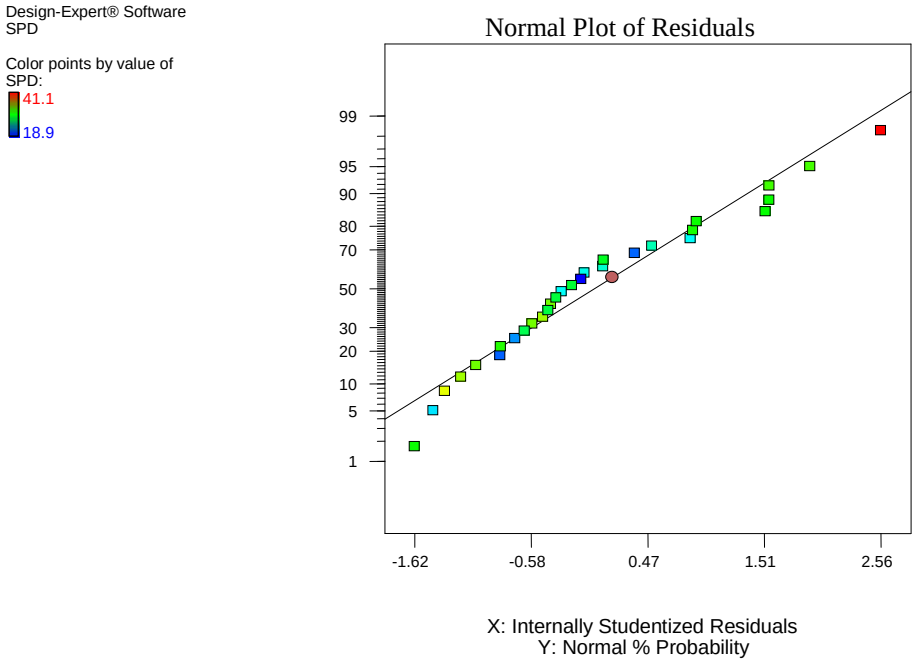

Figure 5.5. *Normal probability plot of residuals for spreadability*

5.3.7.3. ANOVA analysis of the Response Surface Quadratic Model for pH (Y_3)

ANOVA analysis of the response surface quadratic model for pH of the cream formulations resulted in an F-value of 1.49 for the model, implying that the model is insignificant. The only significant model term was X_1X_2 since the value of Prob > F was < 0.05. The insignificant model terms to this response were X_3, X_4, X_1^2, X_2^2, X_3^2, X_4^2, X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4 as summarised in Table 5.8.

Table 5.8. *ANOVA analysis for the response surface quadratic model of pH*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	0.66	14	0.047	1.49	0.2261[b]
X_1	0.041	1	0.041	1.29	0.2738[b]
X_2	3.037 E-003	1	3.037 E-003	0.096	0.7610[b]
X_3	0.081	1	0.081	2.54	0.1316[b]
X_4	0.048	1	0.048	1.51	0.2385[b]
X_1^2	0.080	1	0.080	2.53	0.1323[b]
X_2^2	0.042	1	0.042	1.32	0.2682[b]
X_3^2	0.018	1	0.018	0.56	0.4677[b]
X_4^2	2.574 E-003	1	2.574 E-003	0.081	0.7794[b]
X_1X_2	0.17	1	0.17	5.25	0.0369[a]
X_1X_3	0.089	1	0.089	2.80	0.1152[b]
X_1X_4	0.016	1	0.016	0.51	0.4846[b]
X_2X_3	0.011	1	0.011	0.33	0.5731[b]
X_2X_4	0.066	1	0.066	2.09	0.1684[b]
X_3X_4	0.013	1	0.013	0.40	0.5367[b]
Residual	0.47	15	0.032	-	-

[a] Significant at "Prob > F" less than 0.05 ; [b] Insignificant at "Prob > F" more than 0.05

Although this response surface quadratic model was insignificant, the significant factors affecting the response Y_3 were still investigated using Equation 5.5. The synergistic effects that affected the pH response were a linear contribution of X_2, quadratic contribution of X_1^2, X_2^2 and X_3^2 and interaction effects of X_2X_4, whereas the antagonistic effects were a linear contribution of X_1, X_3 and X_4, quadratic contribution of X_4^2 and interaction effects of X_1X_2, X_1X_3, X_1X_4, X_2X_3 and X_3X_4. The normal probability plot of residuals indicates that the residuals follow a normal distribution, in which case most pH points including the minimum and maximum pH values fell into a straight line, as shown in Figure 5.6.

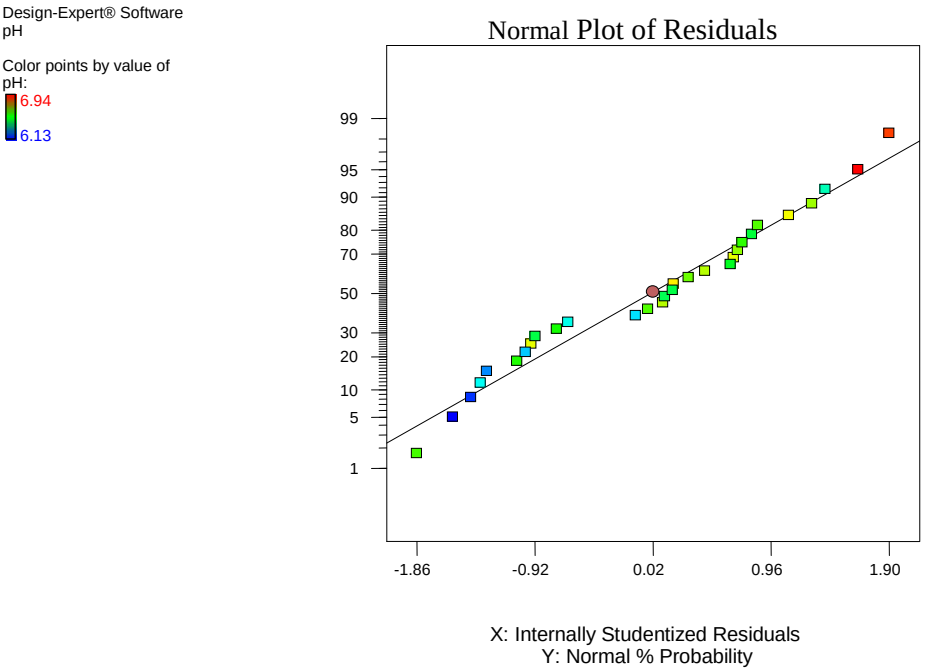

Figure 5.6. *Normal probability plot of residuals for pH*

5.3.7.4. ANOVA analysis for the Response Surface Quadratic Model of % Drug Content (Y_4)

ANOVA analysis of the response surface quadratic model for % drug release of CPSU cream formulations resulted in an F-value of 0.36 for the model, implying that the model is insignificant. All model terms to this response were insignificant as the Prob > F values were > 0.05, as can be seen in Table 5.9.

Table 5.9. *ANOVA analysis for the response surface quadratic model of % drug content*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	5.14	14	0.37	0.36	0.9692[b]
X_1	0.20	1	0.20	0.20	0.6628[b]
X_2	0.31	1	0.31	0.30	0.5934[b]
X_3	3.28	1	3.28	3.19	0.0944[b]
X_4	1.504 E-003	1	1.504 E-003	1.463 E-003	0.9700[b]
X_1^2	1.181 E-003	1	1.181 E-003	1.149 E-003	0.9734[b]
X_2^2	0.096	1	0.096	0.093	0.7645[b]
X_3^2	0.10	1	0.10	0.10	0.7549[b]
X_4^2	3.281 E-003	1	3.281 E-003	3.191 E-003	0.9557[b]
X_1X_2	0.016	1	0.016	0.016	0.9016[b]
X_1X_3	7.656 E-003	1	7.656 E-003	7.446 E-003	0.9324[b]
X_1X_4	0.26	1	0.26	0.25	0.6240[b]
X_2X_3	0.095	1	0.095	0.092	0.7659[b]
X_2X_4	2.256 E-003	1	2.256 E-003	2.194 E-003	0.9633[b]
X_3X_4	0.78	1	0.78	0.76	0.3978[b]
Residual	15.42	15	1.03	-	-

[a] Significant at "Prob > F" less than 0.05 ; [b] Insignificant at "Prob > F" more than 0.05

Although this response surface quadratic model was insignificant, the factors affecting the response Y_4 were investigated using Equation 5.6. The synergistic effects that affected the % drug content

response were a linear contribution of X_1 and an interaction contribution of X_1X_4 and X_2X_4, whereas the antagonistic effects were affected by other linear, quadratic and interaction contribution of this model. The normal probability plot of residuals of the % drug content indicates that the residuals follow a normal distribution, in which case most of the % drug content points followed a straight line. However the minimum and the maximum % drug content values fell outside the linearity region, as denoted in Figure 5.7.

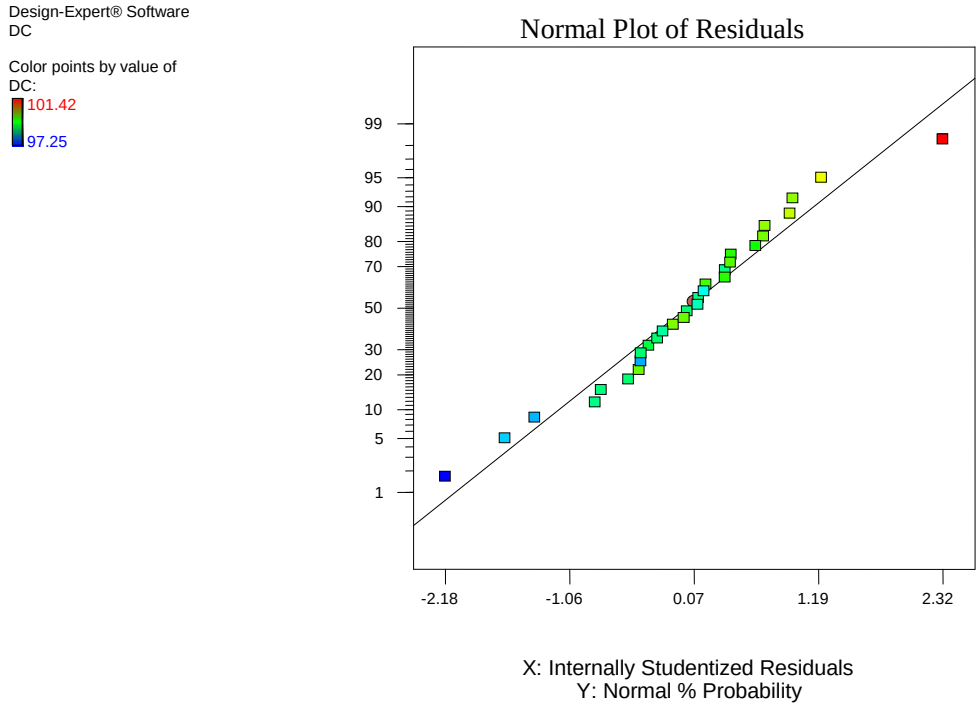

Figure 5.7. *Normal probability plot of residuals for % drug content*

5.3.7.5. ANOVA analysis for the Response Surface Quadratic Model of Cumulative % Drug Released over 72 hours (Y_5)

ANOVA analysis of the response surface quadratic model for cumulative % drug released from the cream formulations over 72 hours resulted in an F-value of 12.11 of the model, implying that the model is significant. The significant model terms were X_1 and X_2 since the Prob > F values were < 0.05. The insignificant model terms to the response were X_3, X_4, X_1^2, X_2^2, X_3^2, X_4^2, X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4, as summarised in Table 5.10.

Table 5.10. *ANOVA analysis for the response surface quadratic model of cumulative % drug released over 72 hours*

Source	Sum of squares	Degree of Freedom	Mean Square	F-value	Prob >F
Model	1061.81	14	75.84	12.11	<0.0001[a]
X_1	919.59	1	919.59	146.83	<0.0001[a]
X_2	73.22	1	73.22	11.69	0.0038[a]
X_3	22.62	1	22.62	3.61	0.0768[b]
X_4	21.66	1	21.66	3.46	0.0827[b]
X_1^2	12.66	1	12.66	2.02	0.1756[b]
X_2^2	1.64	1	1.64	0.26	0.6165[b]
X_3^2	1.32	1	1.32	0.21	0.6527[b]
X_4^2	0.74	1	0.74	0.12	0.7356[b]
X_1X_2	0.011	1	0.011	1.760 E-003	0.9671[b]
X_1X_3	4.39	1	4.39	0.70	0.4157[b]
X_1X_4	1.08	1	1.08	0.17	0.6836[b]
X_2X_3	1.90	1	1.90	0.30	0.5895[b]
X_2X_4	0.11	1	0.11	0.017	0.8984[b]
X_3X_4	0.29	1	0.29	0.046	0.8336[b]
Residual	93.94	15	6.26	-	-

[a] Significant at "Prob > F" less than 0.05 ; [b] Insignificant at "Prob > F" more than 0.05

Significant factors that affected the response Y_5 were the synergistic effect of the quadratic contribution of X_2^2 only, while Y_5 was shown to exhibit an antagonistic effect of the linear contribution X_1, X_2, X_3 and X_4, quadratic contributions of X_1^2, X_3^2 and X_4^2 and interaction effects of X_1X_2, X_1X_3, X_1X_4, X_2X_3, X_2X_4 and X_3X_4. The normal probability plot of residuals for the cumulative % drug released over 72 hours shows that all data points fit closely to a straight line and the model follows a normal distribution. The minimum value of the cumulative % drug released from one of the CPSU batches almost fell onto the straight line, whereas the maximum value fell completely out of the linearity region, as shown in Figure 5.8.

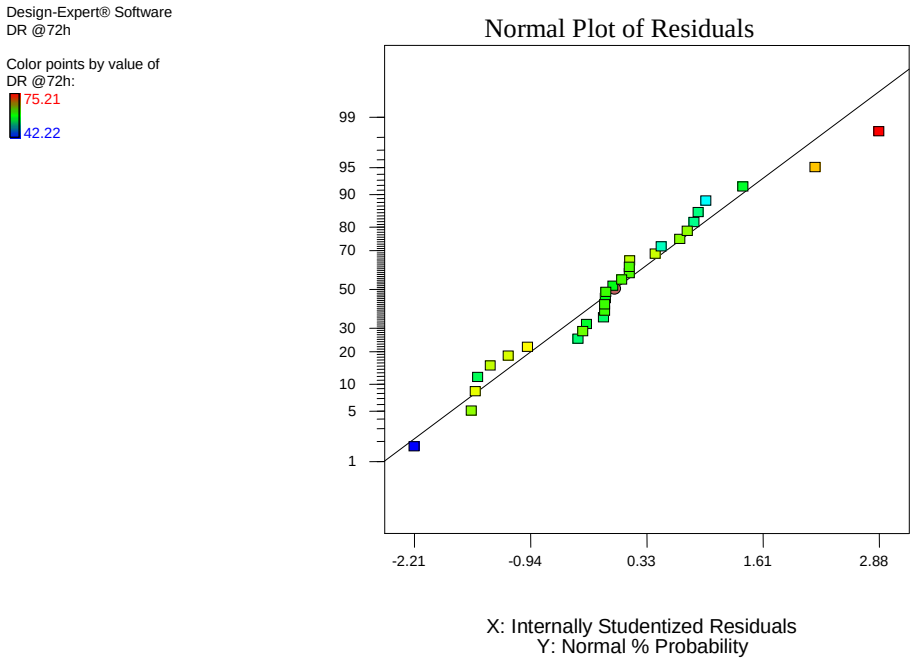

Figure 5.8. *Normal probability plot of residuals for the cumulative % drug released over 72 hours*

5.3.8. Effect of Process Variables

The relationship between coded variables and responses can be better understood by examining the experimental design data with a series of surface plots. These graphical representations facilitate the examination of the effects of the experimental factors on the responses. Contour and response surface plots between factors can be generated using Design-Expert® software [488,493]. Contour plots are two-dimensional (2D) plots whereas response surface plots are three-dimensional (3D) in nature. The response surface plot usually displays three variables and one variable must be set at an arbitrary value and the response surface plotted with the other two variables on the axes. In other words, the response surfaces display the variation of two factors while a third is kept constant [488,490,505].

5.3.8.1. Viscosity

The largest F-value of 263.01 was observed for homogenisation speed (X_1), indicating that this factor has the most significant effect on the viscosity of the cream formulations when compared to the other process variables. The anchor speed (X_2) was another variable that was seen to have some effect on the viscosity of the cream formulations since the F-value for this parameter was 10.32 (Table 5.5). The effects of homogenisation time (X_3) and the cooling time (X_4) on the viscosity were quite similar with F-values of 1.79 and 1.36, respectively, clearly indicating that these parameters had the least effect on the viscosity of the resultant cream formulations. The quadratic effect of homogenisation speed (X_1^2) on the viscosity of the formulations was also significant. Furthermore, it was observed that all four variables studied had a synergistic effect on the viscosity of the creams.

The contour and 3D response surface plots that were constructed to show the interaction effects of the process variables on the viscosity (Y_1) of the cream formulations are depicted in Figures 5.9 and 5.10. The homogenisation speed and anchor speed were found to have a significant effect on the formulation response. The contour plot shows that anchor speed has no effect on the viscosity of the creams that were produced while the homogenisation speed has the most significant effect on this formulation parameter (Figure 5.9).

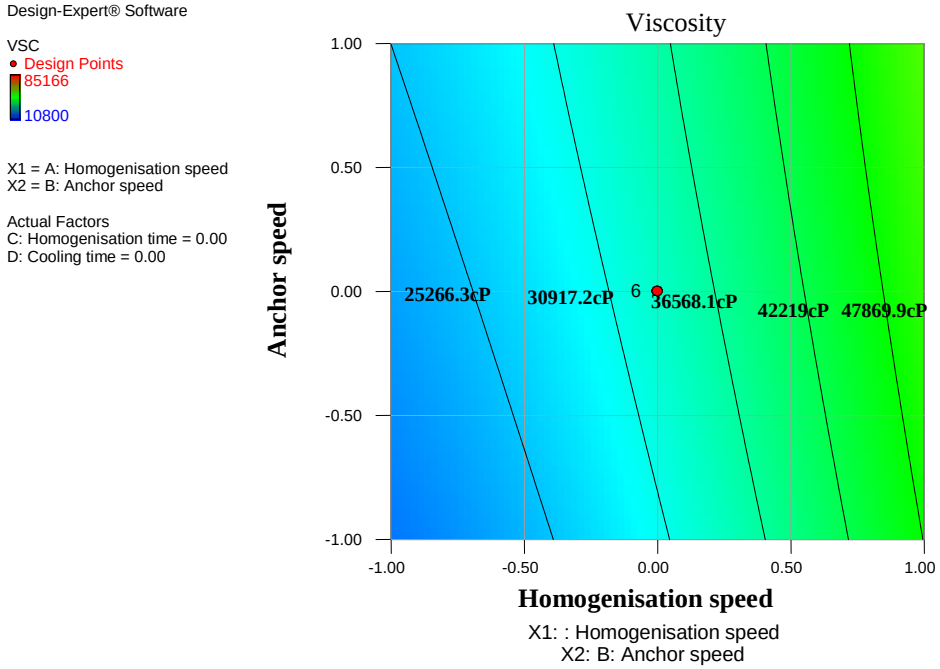

Figure 5.9. *Contour plot showing the effects of homogenisation speed and anchor speed on cream viscosity*

An increase in the homogenisation speed resulted in an increase in the viscosity of the cream formulations from 25 266 cP to 47 869 cP, indicating that the homogenisation speed has by far the greatest impact on the final quality of semi-solid products [478,494]. This suggests that the mixing efficiency of manufacturing equipment will have a profound effect on the quality of the final product. With increased homogenisation speeds, the higher shearing forces disrupt the hydrocarbon chains of the oil and wax droplets exposing surfactant chains to the water with the formation of an additional gel phase. There is also an increase in the number of emulsified particles. The 3D response surface plot shows that an increase in anchor speed resulted in a slight increase in viscosity from 10 000 cP to 26 000 cP although an increase in homogenisation speed was found to have the major and significant effect on the viscosity with values for this parameter ranging between 10 000 cP and 54 000 cP. The maximum viscosity value was determined when both the homogenisation speed and the anchor speed were at their highest settings as shown in Figure 5.10.

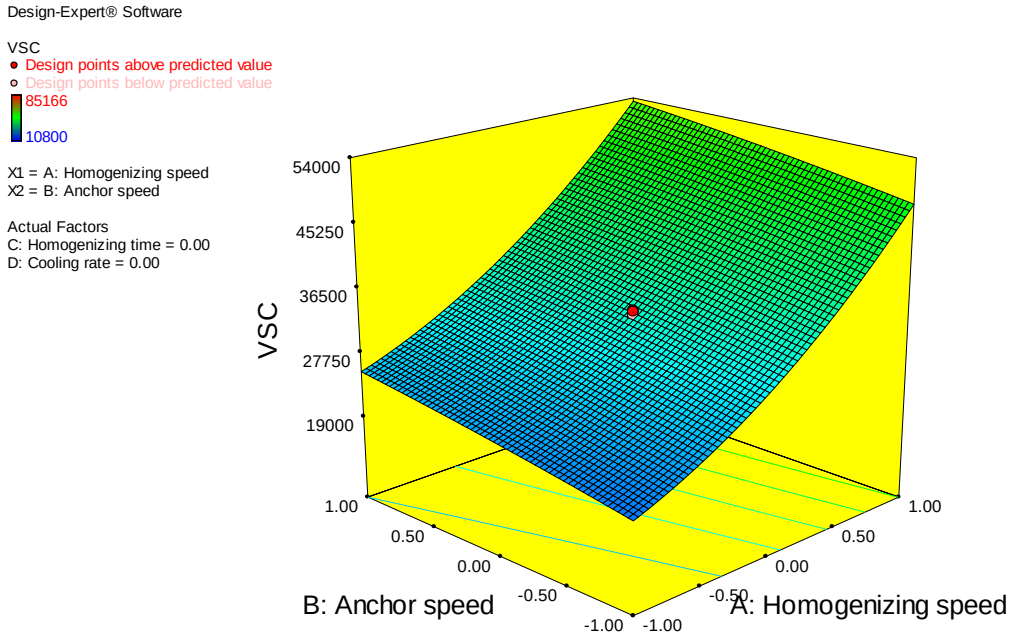

Figure 5.10. *3D response surface plot showing the effects of homogenisation speed and anchor speed on cream viscosity*

5.3.8.2. Spreadability

The largest F-value of 238.49 was observed for the homogenisation speed (X_1), indicating that this parameter had the most significant effect on spreadability of the cream formulations when compared to the other process variables evaluated. The anchor speed (X_2) also had a significant effect on the cream formulations, since the F- and P-values were 9.93 and < 0.05 respectively (Table 5.6). The homogenisation time (X_3) and cooling time (X_4) had the least effect on spreadability, since the F-values for these parameters were 0.61 and 0.32, respectively. Furthermore, it was observed that all four independent variables had an antagonistic effect on the spreadability of the cream batches. The interaction contribution of X_1X_3, X_1X_4, X_2X_3 and X_2X_4 showed some positive synergistic effects on the spreadability.

The contour and 3D response surface plots that were constructed to show the interaction effects of process variables on the spreadability (Y_2) of the cream formulations are shown in Figure 5.11 and 5.12. The effects of homogenisation speed and anchor speed were studied as they were found to have significant effects on this response. The contour plot shows that the anchor speed has no effect on the spreadability of the CPSU creams whereas the homogenisation speed has the most significant effect on these parameters as shown in Figure 5.11.

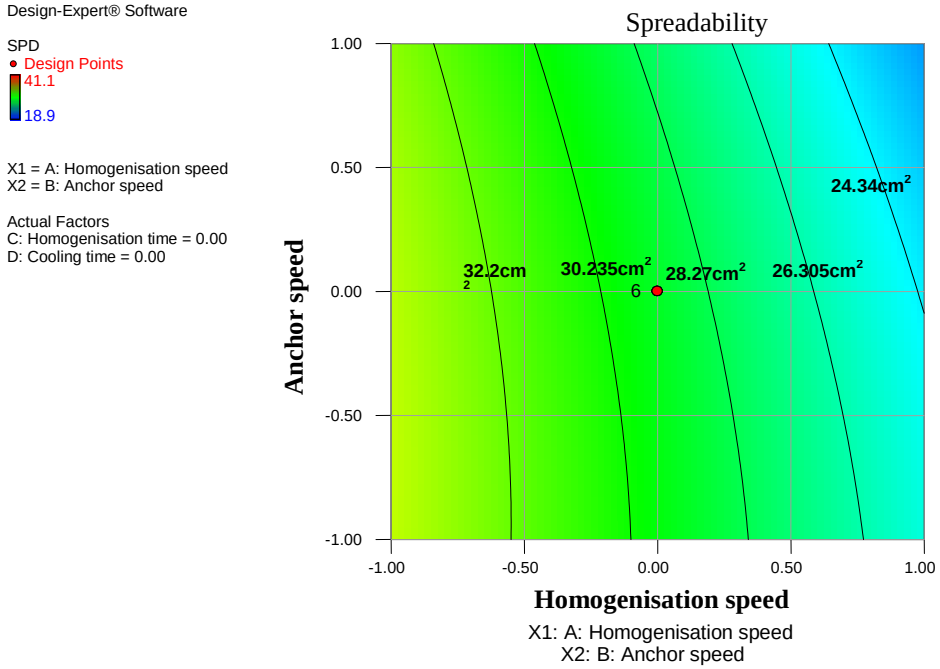

Figure 5.11. *Contour plot showing the effects of homogenisation speed and anchor speed on cream spreadability*

An increase in the homogenisation speed caused a decrease in the spreadability of the cream formulations with the value for this parameter reduced from 32.2 cm^2 to 24.3 cm^2. Viscous semi-solids have an increased number of emulsified particles which in turn, increases the particle-particle interactions thereby, restricting the movement of the molecules, resulting in a reduction in the spreadability of the creams [391]. The 3D response surface plot reveals that an increase in homogenisation speed produced a decrease in spreadability from 32 cm^2 to 25cm^2. However an increase in anchor speed had the opposite effect and an increase in the spreadability from 27 cm^2 to 32.5 cm^2 was observed. The overall combined effect of homogenising and anchor speeds had a significant decrease in the spreadability of the formulations and thus the minimum value of spreadability was observed when the homogenisation speed and the anchor speed were at their highest value, as shown in Figure 5.12.

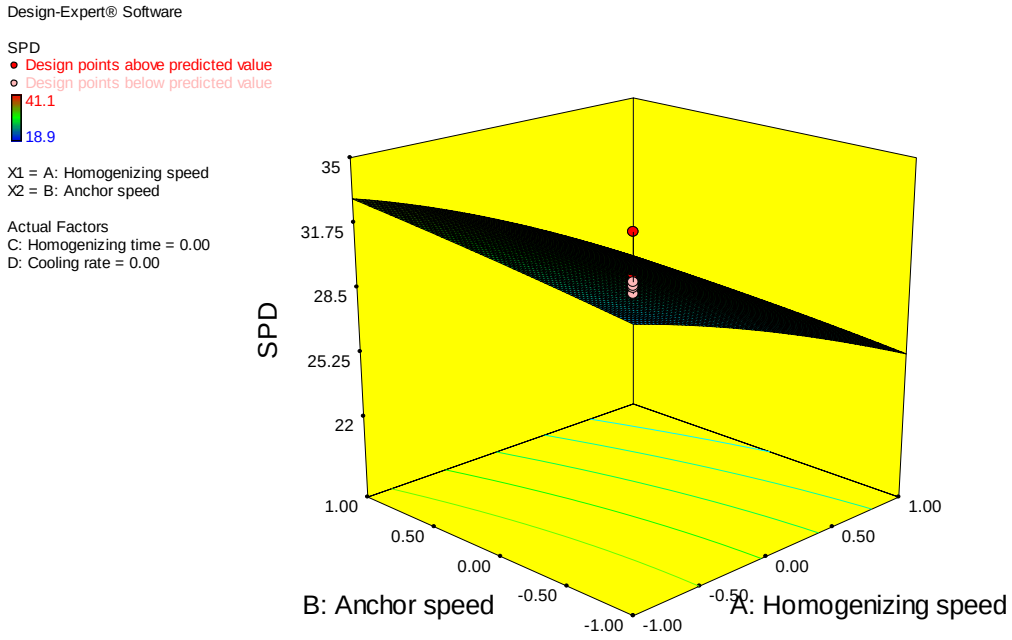

Figure 5.12. *3D response surface plot showing the effects of homogenisation speed and anchor speed on cream spreadability*

5.3.8.3. pH

All process variables for this parameter (X_1, X_2, X_3 and X_4) revealed an F value < 5.0, indicating that there was no significant effect on the pH of the formulations. Furthermore, it was observed that three of the independent variables studied had an antagonistic effect on the pH of the cream batches and variables X_2, X_1^2, X_2^2, X_3^2 and X_2X_4 contributed to a synergistic effect on the pH of the batches of cream. The variables X_1, X_3, X_4, X_4^2, X_1X_2, X_1X_3, X_1X_4, X_2X_3 and X_3X_4 demonstrated an antagonistic effect and the contour and 3D response surface plots constructed to show the interaction effects of process variables on the pH (Y_3) of the cream formulations are shown in Figure 5.13 and 5.14.

The effects of homogenising and anchor speeds were studied as they had the most significant effects on the majority of the responses. The anchor and homogenisation speeds have no effect on the pH of the CPSU cream batches (Figures 5.13 and 5.14). An increase in homogenisation and anchor speeds resulted in a similar pH for all cream batches that ranged between 5.50 and 5.65, with no extreme minimum and maximum values. Therefore it is unlikely that any skin irritation will occur following topical application of these cream formulations.

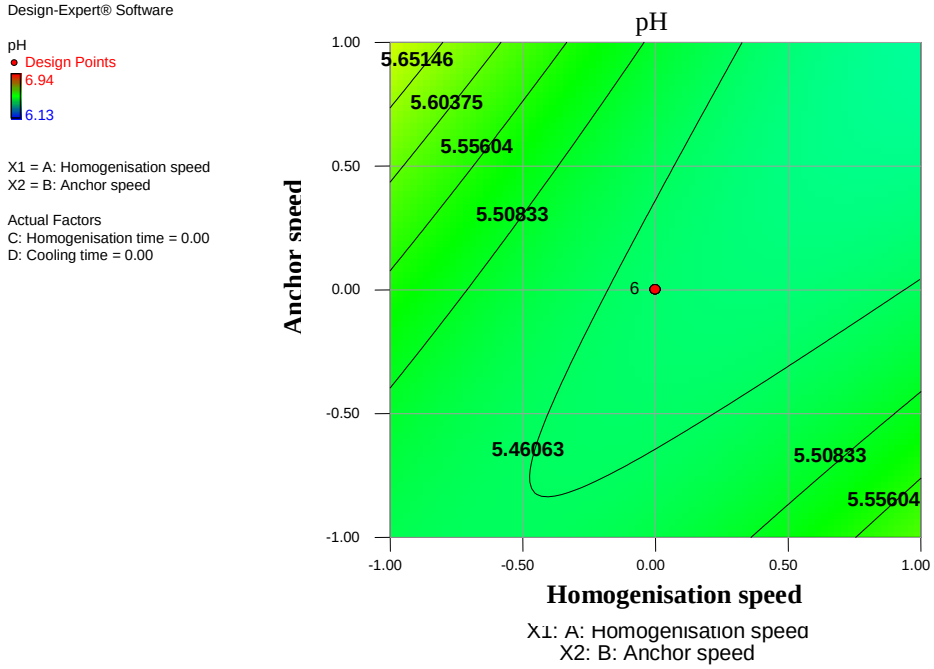

Figure 5.13. *Contour plot showing the effects of homogenisation speed and anchor speed on cream pH*

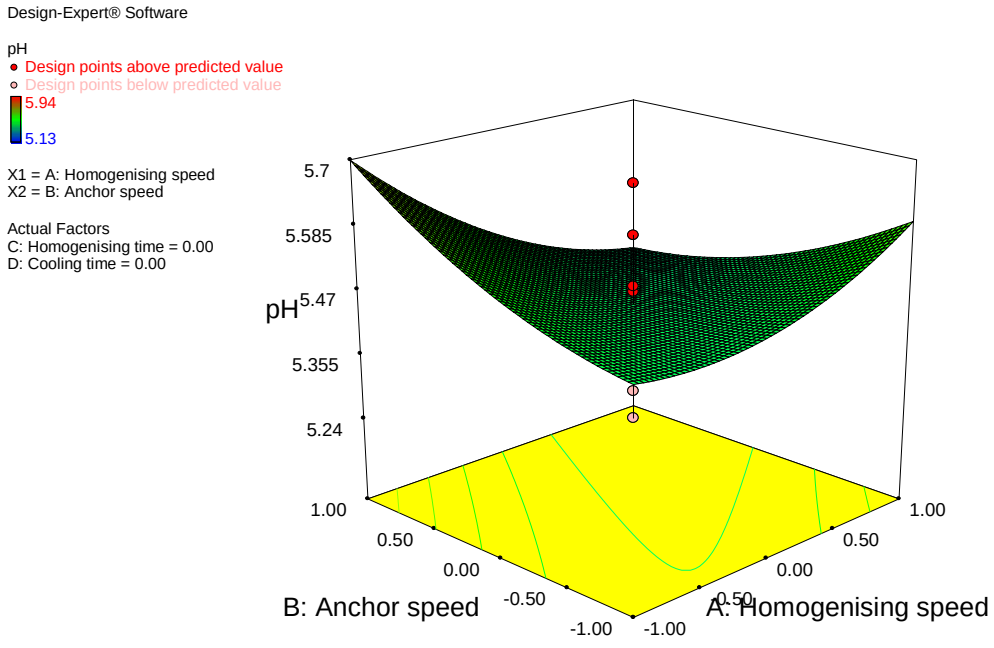

Figure 5.14. *3D response surface plot showing the effects of homogenisation speed and anchor speed on cream pH*

5.3.8.4. Percent (%) drug content

All process variables for this parameter *viz.*, X_1, X_2, X_3 and X_4 produced an F-value of < 5.0, indicating that there was no significant effect on the % drug content of the creams. The synergistic effects of % drug content were contributed by the linear effects of X_1 and X_4, quadratic effect of X_4^2 and the

interaction effects of X_1X_4 and X_2X_4 while the antagonistic effects were mainly due to X_2, X_3, X_1^2, X_2^2, X_3^2, X_1X_2, X_1X_3, X_2X_3 and X_3X_4 respectively.

The contour and the 3D response surface plots which were constructed to show the interaction effects of process variables on the % drug content (Y_4) of the cream formulations are shown in Figures 5.15 and 5.16. The effects of homogenisation and anchor speeds were studied as they had the most significant effects on the majority of the responses. The anchor and homogenisation speeds had no effect on the % drug content of the CPSU batches (Figures 5.15 and 5.16). An increase in homogenisation and anchor speeds resulted in uniform % drug content for all cream batches that ranged between 99.10% and 99.37% with no extreme minimum and maximum values. Therefore formulations contained uniformly distributed CP despite the different homogenisation and anchor speeds for all 30 batches investigated.

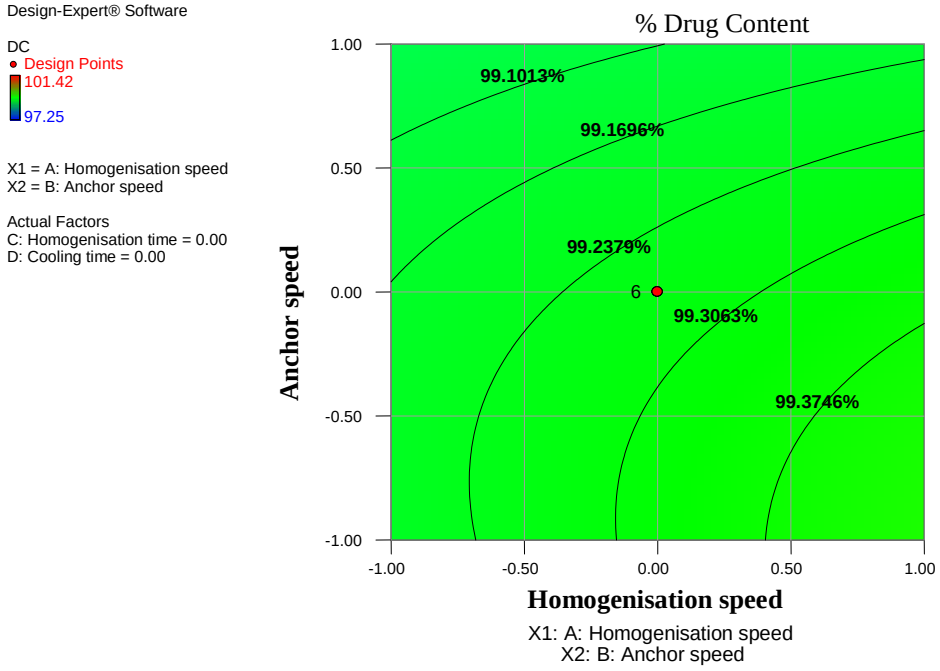

Figure 5.15. *Contour plot showing the effects of homogenisation speed and anchor speed on % drug content of creams*

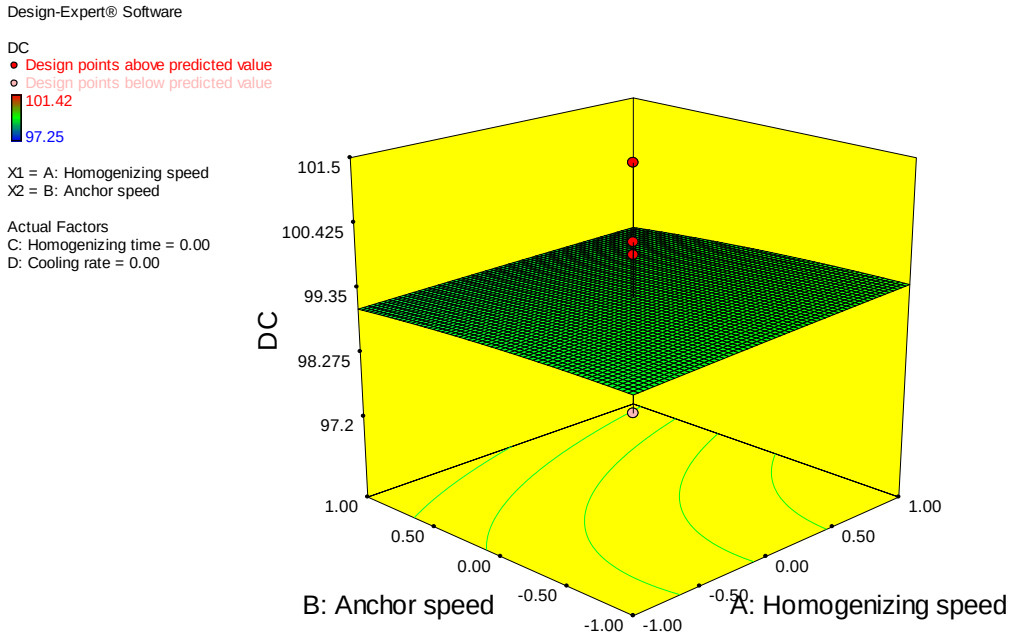

Figure 5.16. *3D response surface plot showing the effects of homogenisation speed and anchor speed on % drug content of creams*

5.3.8.5 Cumulative percent drug released over 72 hours

The largest F-value of 146.83 was observed for homogenisation speed (X_1), indicating that this parameter had a significant effect on the cumulative % drug released over 72 hours from the CPSU cream formulations. The anchor speed (X_2) was another variable that was seen to have some effect on the drug release from the cream formulations, since the F-value for this parameter was 11.69 (Table 5.9). The effects of homogenisation time (X_3) and cooling time (X_4) were quite similar, with F-values of 3.61 and 3.46 respectively and therefore these parameters had the smallest impact on the cumulative % drug released from the test formulations in 72 hours. All four process variables showed an antagonistic effect on the % release of drug from the cream batches, while the synergistic effects were contributed from X_1^2 and X_2^2.

The contour and the 3D response surface plots constructed to show the interaction effects of process variables on the cumulative % drug released from the cream formulations over a period of 72 hours (Y_5) are depicted in Figures 5.17 and 5.18 respectively. The effects of homogenisation and anchor speeds were studied as they were found to have a significant effect on this response. The contour plot shows that anchor speed had a slight effect on the cumulative % drug released, while homogenisation speed had the most significant effect on this formulation parameter (Figure 5.17).

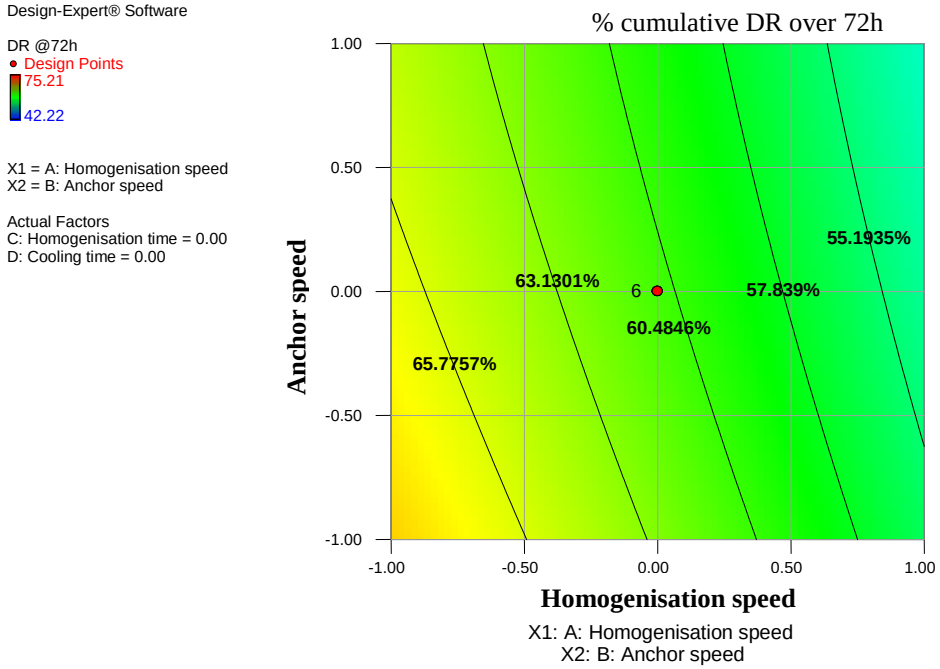

Figure 5.17. *Contour plot showing the effects of homogenisation speed and anchor speed on the cumulative % drug released over 72 hours*

An increase in the homogenisation speed resulted in a decrease in the % drug released over 72 hours, from 65% to 55%. The decrease in % drug released due to the increase in homogenisation speed might be due to the increase in viscosity of the CPSU cream formulations. The strong and rigid visco-elastic gel microstructure situated in viscous formulations hinders the ability of an API to diffuse from the base [506,507]. The 3D response surface plot shows that an increase in anchor speed produced an increase in the % drug released, from 58% to 64%, although an increase in the homogenisation speed caused a decrease in the % drug released from 58% to 55%. The lowest cumulative % drug released over the 72 hour test period occurred when both the homogenising and anchor speeds were set at the maximum settings (Figure 5.18).

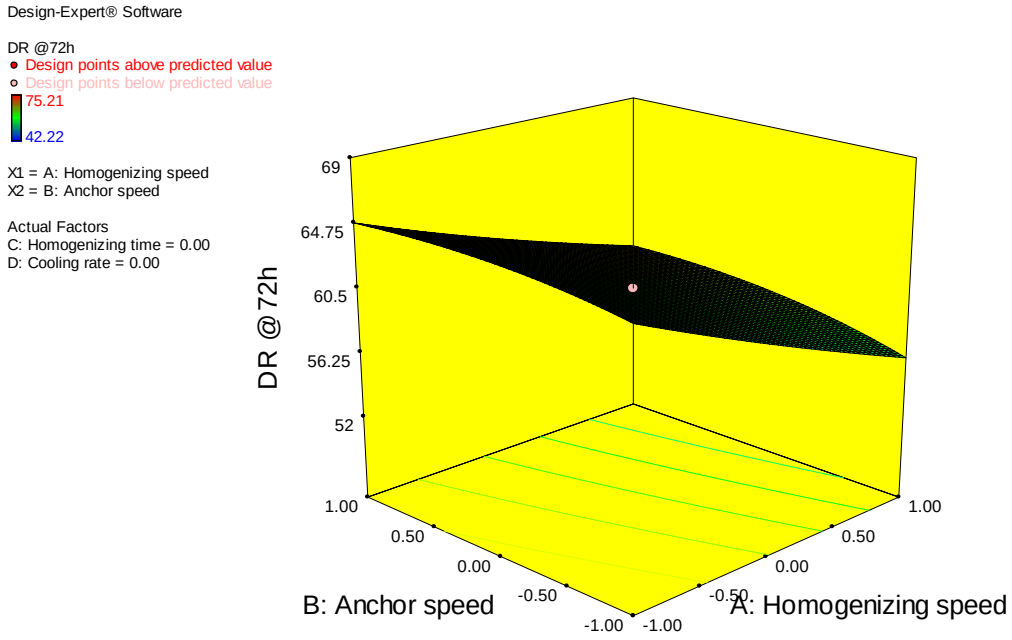

Figure 5.18. *3D response surface plot showing the effects of homogenisation speed and anchor speed on the cumulative % drug released over 72 hours*

5.4 FORMULATION OPTIMISATION

It is essential that a scale-up of a generic formulation that exhibits similar physical and *in vitro* release properties to an innovator product such as Dermovate® is produced. It is clear that the different formulation responses monitored can be manipulated by modification of process variables such as homogenisation speed, anchor speed, homogenisation time and cooling time. The optimal conditions for the manufacture of a generic CPSU formulation were predicted using the optimisation function of the Design-Expert® software. Numerous methods are available to optimise a multi-response process and in this study, numerical optimisation was selected as the preferred approach. Numerical optimisation presents a comprehensive and up-to-date description of the most effective methods in a continuous optimisation process and responds to the growing interest in optimisation in engineering, business and science, by focusing on the methods that are best suited to practical problems [498].

The target was to achieve viscosity (Y_1), spreadability (Y_2), and a cumulative % drug released over 72 hours (Y_5), of a test formulation that would be similar to that of the reference formulation, Dermovate®. The predicted values for the process variables *viz.*, homogenisation speed (X_1), anchor speed (X_2), homogenisation time (X_3), cooling time (X_4) in addition to the respective formulation responses obtained from numerical optimisation, are summarised in Table 5.11.

Table 5.11. *Predicted values for process variables and respective formulation responses*

Process Variables				Formulation Responses			
X_1 (rpm)	X_2 (rpm)	X_3 (min)	X_4 (min)	Y_1 (cP)	Y_2 (cm^2)	Y_5 (%)	Desirability
1899.70	33.34	100.00	98.98	44600	25.7	50.4	0.92

The optimal conditions were found in the desirability zone as the desirability value of the model is 0.92. Therefore the optimal generic formulation was manufactured by using a homogenisation speed of 1900 rpm, anchor speed of 35 rpm, homogenisation time of 100 minutes and cooling time of 100 minutes. The optimal formulation had a viscosity of 44 600 cP, a spreadability of 25.7cm^2 and a cumulative % drug released of 50.4% after 72 hours, as summarised in Table 5.12.

Table 5.12. *The predicted and experimental responses at the preliminary and optimised conditions*

Experimental Responses			Predicted Responses			% Prediction Error		
Y_1	Y_2	Y_5	Y_1	Y_2	Y_5	Y_1	Y_2	Y_5
44600	25.7	50.4	43800	25.5	48.9	0.17	0.77	2.97

The optimal variables were found to be near the high level of homogenisation speed, anchor speed, homogenisation time and cooling time, thus permitting the production of an appropriate formulation. The low magnitude of difference reported as a percentage prediction error indicates the robustness of the mathematical modelling and high prognostic ability of RSM, suggesting that RSM is an efficient tool for optimisation [500].

5.5 STATISTICAL COMPARISON AND MATHEMATICAL MODELLING OF *IN VITRO* RELEASE PROFILES

During formulation and product development and analysis, *in vitro* release profiles are essential tools for the assessment of batch-to-batch quality and performance of topical corticosteroid-containing dosage forms. *In vitro* release is also used to ensure product sameness for semi-solid dosage forms when SUPAC-related changes are made to the formulation and/or manufacturing process [385]. *In vitro* release testing is usually performed at the early stages of formulation development, especially following the manufacture of laboratory scale formulations [421,470]. The rate and extent of API release from the same formulation composition may vary after a scale-up process, due to the different process variables and equipment used for large scale manufacture. Therefore *in vitro* testing becomes important when attempting to identify similarities and/or differences between study formulations, in order to assess scale-up challenges qualitatively and quantitatively. In the case of generic formulation development, statistical comparison of the *in vitro* profiles of a test and the reference product is vital to identify a formulation that will best reflect the ultimate *in vivo* behaviour [508].

Statistical data generated from *in vitro* analyses can be used to compare the effect of different process variables on the resultant drug release rates, in addition to choosing an optimised formulation for

further evaluation [245,418]. An *in vitro* release profile for CP release was generated for each formulation manufactured and these were compared to that generated for Dermovate® and Dovate®. Each *in vitro* release profile was plotted as the cumulative amount of CP released per unit area versus time, and the *in vitro* release data for batches CPSU-01-CPSU-30 are also included in Appendix IV, together with a batch summary record for these batches. Qualitative interpretation and statistical data for the comparison of release rate profiles of CP were essential and provided crucial information about the release of CP from the scale-up formulations [422,423,426,427]. Furthermore the use of mathematical modelling permitted the prediction of release kinetics from those formulations and enabled the measurement of important physical parameters, such as the diffusion coefficient of the API, following model fitting of experimental release data to the models selected for evaluation [428,429].

5.5.1. Exploratory Data Analysis

The exploratory data analysis approach was used initially to compare the *in vitro* release profiles of five formulations as shown in § 4.6.1.1 (Chapter 4). This method was successfully used to determine the similarity or difference between the release of CP from test formulations and Dermovate®, by extending the error bars to two standard errors at the specific time points used to generate each *in vitro* release profile. Furthermore, the mean and standard deviation for drug release at each time point was calculated for each formulation in order to construct a 95% confidence interval to establish whether differences in the mean release profile existed. This method is feasible to compare a small number of *in vitro* release profiles, however in cases where a large number of formulations *viz.*, CPSU-01-CPSU-30 are under comparison, the graphical representation of the mean *in vitro* release profiles will not be appropriate as the release profiles will be too cluttered and the error bars will not be easily interpreted. This approach was deemed inappropriate to get an understanding of the release rate profiles of the formulations in comparison to Dermovate®.

5.5.2. Analysis of Variance (ANOVA)

Although the FDA Guidance does not mention use of ANOVA analysis for dissolution curve comparison purposes, ANOVA analysis was used to establish a statistical comparison of the difference or similarity of the mean amount of CP released at a single time point of the *in vitro* test for the purposes of comparing the test formulations and Dermovate® as reported in § 4.6.1.2 (Chapter 4). This statistical approach was also used to compare the test and reference products at individual time points *viz.*, 2, 4, 8, 12, 24, 48 and 72 hours respectively for the 30 test formulations. A summary of the statistical analysis at individual times of 2, 8, 24 and 48 hours is listed in Table 5.13. The complete ANOVA table for each CPSU formulation with all time points is reported in Appendix V.

ANOVA analysis of the data generated reveals that for the initial 2 hours of *in vitro* release testing, there were no significant differences between batches CPSU-11, CPSU-14, CPSU-15, CPSU 20,

CPSU-21, CPSU-23, CPSU-24, CPSU-25, CPSU-28, CPSU-29 and CPSU-30, and the innovator product, Dermovate®. However the different batches exhibited different experimental lag times, that are possibly dependent on the physical characteristics of the formulation and at a time of 2 hours, the batches may have exhibited different melting rates of the topical base prior to the liberation of the API to the receptor medium. These initial results may not produce the appropriate *in vitro* release data at that specific point. Therefore it was vital to evaluate the similarity and difference of the formulations at the specific time points, in order to gain a better understanding of the release of CP from the scale-up formulations. At a time of 4 hours, it was observed that batches CPSU-04, CPSU-10, CPSU-13, CPSU-15, CPSU-17, CPSU-18 and CPSU-19 exhibited an *in vitro* release profile similar to Dermovate® and at the later times, 8, 12, 24 and 48 hours, fewer batches (~3 batches) were similar to Dermovate®. A clear discrimination was observed between the test formulations and Dermovate® that was only observed at the later times of *in vitro* release testing. Batch CPSU-04 was the only formulation for which the *in vitro* release profile was similar to that of Dermovate® at most of the time points under investigation.

Table 5.13. *Summary of ANOVA analysis of in vitro release profiles for CPSU and Dermovate®*

Time (hrs)	Comparison*	Mean Difference** (R-CPLS)	95% Confidence interval for mean difference		P-value	Summary
			Lower Limit	Upper Limit		
2	**R vs 01**	0.3867	0.07684	0.6965	P < 0.01	Significant
	R vs 02	-1.473	-1.783	-1.164	P < 0.0001	Significant
	R vs 03	-1.377	-1.686	-1.067	P < 0.0001	Significant
	R vs 04	0.3233	0.01351	0.6332	P < 0.05	Significant
	R vs 05	1.557	1.247	1.866	P < 0.0001	Significant
	R vs 06	0.5867	0.2768	0.8965	P < 0.0001	Significant
	R vs 07	0.3867	0.07684	0.6965	P < 0.001	Significant
	R vs 08	0.8633	0.5535	1.173	P < 0.0001	Significant
	R vs 09	0.4800	0.1702	0.7897	P < 0.0001	Significant
	R vs 10	0.3133	0.003507	0.6232	P < 0.05	Significant
	R vs 11	0.1800	-0.1298	0.4894	P > 0.05	Not significant
	R vs 12	0.4300	0.1202	0.7398	P < 0.001	Significant
	R vs 13	0.6633	0.3535	0.9732	P < 0.0001	Significant
	R vs 14	0.2667	-0.04316	0.5765	P > 0.05	Not significant
	R vs 15	0.1767	-0.1332	0.4865	P > 0.05	Not significant
	R vs 16	0.3233	0.01351	0.6332	P < 0.05	Significant
	R vs 17	0.3367	0.02684	0.6465	P < 0.05	Significant
	R vs 18	-1.163	-1.473	-0.8535	P < 0.0001	Significant
	R vs 19	0.3433	0.03351	0.6532	P < 0.05	Significant
	R vs 20	0.003333	-0.3065	0.3132	P > 0.05	Not significant
	R vs 21	0.1167	-0.1932	0.4265	P > 0.05	Not significant
	R vs 22	-0.5800	-0.8898	-0.2702	P < 0.0001	Significant
	R vs 23	-0.2000	-0.5098	0.1098	P > 0.05	Not significant
	R vs 24	0.3033	-0.006493	0.6132	P > 0.05	Not significant
	R vs 25	-0.02000	-0.3298	0.2898	P > 0.05	Not significant
	R vs 26	0.7533	0.4435	1.063	P < 0.0001	Significant
	R vs 27	2.250	1.940	2.560	P < 0.0001	Significant
	R vs 28	0.1900	-0.1198	0.4998	P > 0.05	Not significant
	R vs 29	0.1000	-0.2098	0.4098	P > 0.05	Not significant
	R vs 30	0.2500	-0.05983	0.5598	P > 0.05	Not significant
8	**R vs 01**	3.078	2.725	3.428	P < 0.0001	Significant

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	R vs 02	-3.720	-4.071	-3.369	P < 0.0001	Significant
	R vs 03	-3.830	-4.181	-3.479	P < 0.0001	Significant
	R vs 04	-0.100	-0.4513	0.2513	P > 0.05	Not Significant
	R vs 05	1.113	0.7621	1.465	P < 0.0001	Significant
	R vs 06	1.483	1.132	1.835	P < 0.0001	Significant
	R vs 07	2.537	2.185	2.888	P < 0.0001	Significant
	R vs 08	1.097	0.7454	1.448	P < 0.0001	Significant
	R vs 09	0.4200	0.06874	0.7713	P < 0.01	Significant
	R vs 10	-1.516	-1.868	-1.165	P < 0.0001	Significant
	R vs 11	1.970	1.619	2.321	P < 0.0001	Significant
	R vs 12	-0.08000	-0.4313	0.2713\	P > 0.05	Not significant
	R vs 13	-0.8833	-1.235	-0.5321	P < 0.0001	Significant
	R vs 14	1.667	1.315	2.018	P < 0.0001	Significant
	R vs 15	0.2930	-0.05826	0.6443	P > 0.05	Not significant
	R vs 16	-0.4600	-0.8113	-0.1087	P < 0.001	Significant
	R vs 17	-0.3940	-0.7453	-0.04274	P < 0.01	Significant
	R vs 18	-3.270	-3.721	-3.019	P < 0.0001	Significant
	R vs 19	3.423	3.072	3.775	P < 0.0001	Significant
	R vs 20	-1.977	-2.328	-1.625	P < 0.0001	Significant
	R vs 21	0.6667	0.3154	1.018	P < 0.0001	Significant
	R vs 22	1.117	0.7654	1.468	P < 0.0001	Significant
	R vs 23	-2.717	-3.068	-2.365	P < 0.0001	Significant
	R vs 24	1.160	0.8087	1.511	P < 0.0001	Significant
	R vs 25	0.01333	-0.3379	0.3646	P > 0.05	Not significant
	R vs 26	2.390	2.039	2.741	P < 0.0001	Significant
	R vs 27	3.097	2.745	3.448	P < 0.0001	Significant
	R vs 28	-1.593	-1.945	-1.242	P < 0.0001	Significant
	R vs 29	-0.6533	-1.005	-0.3021	P < 0.0001	Significant
	R vs 30	-0.3800	-0.7313	-0.02874	P < 0.05	Significant
24	**R vs 01**	3.623	2.891	4.356	P < 0.0001	Significant
	R vs 02	-7.187	-7.919	-6.454	P < 0.0001	Significant
	R vs 03	-7.237	-7.969	-6.504	P < 0.0001	Significant
	R vs 04	0.4267	-0.3058	1.159	P > 0.05	Not significant
	R vs 05	3.787	3.054	4.519	P < 0.0001	Significant
	R vs 06	1.790	1.058	2.522	P < 0.0001	Significant
	R vs 07	6.087	5.355	6.819	P < 0.0001	Significant
	R vs 08	0.8067	0.07421	1.539	P < 0.05	Significant

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	R vs 09	-1.777	-2.509	-1.044	P < 0.0001	Significant
	R vs 10	-3.843	-4.575	-3.111	P < 0.0001	Significant
	R vs 11	4.190	3.458	4.922	P < 0.0001	Significant
	R vs 12	-0.4833	-1.216	0.2491	P > 0.05	Not significant
	R vs 13	-4.353	-5.086	-3.621	P < 0.0001	Significant
	R vs 14	1.557	0.8242	2.289	P < 0.0001	Significant
	R vs 15	-1.320	-2.052	-0.5875	P < 0.0001	Significant
	R vs 16	2.146	1.414	2.879	P < 0.0001	Significant
	R vs 17	-2.008	-2.739	-1.264	P < 0.0001	Significant
	R vs 18	-6.583	-7.136	-5.851	P < 0.0001	Significant
	R vs 19	1.030	0.2975	1.762	P < 0.001	Significant
	R vs 20	-5.237	-5.969	-4.504	P < 0.0001	Significant
	R vs 21	-4.970	-5.702	-4.238	P < 0.0001	Significant
	R vs 22	3.777	3.044	4.509	P < 0.0001	Significant
	R vs 23	-8.120	-8.852	-7.388	P < 0.0001	Significant
	R vs 24	-1.868	-2.601	-1.136	P < 0.0001	Significant
	R vs 25	0.0500	-0.6825	0.7825	P > 0.05	Not significant
	R vs 26	2.430	1.698	3.1263	P < 0.0001	Significant
	R vs 27	-3.550	3.608	5.072	P < 0.0001	Significant
	R vs 28	-4.250	-4.282	-2.818	P < 0.0001	Significant
	R vs 29	-4.003	-4.982	-3.518	P < 0.0001	Significant
	R vs 30	-10.810	-4.736	-3.271	P < 0.0001	Significant
48	**R vs 01**	-1.370	-1.742	-0.9983	P < 0.0001	Significant
	R vs 02	-5.737	-5.745	-5.003	P < 0.0001	Significant
	R vs 03	-5.027	-5.398	-4.655	P < 0.0001	Significant
	R vs 04	-0.1100	-0.4817	0.2617	P > 0.05	Not significant
	R vs 05	-1.573	-1.945	-1.202	P < 0.0001	Significant
	R vs 06	-2.160	-2.522	-1.778	P < 0.0001	Significant
	R vs 07	8.493	8.122	8.865	P < 0.0001	Significant
	R vs 08	-1.277	-1.648	-0.9049	P < 0.0001	Significant
	R vs 09	-6.140	-6.512	-5.868	P < 0.0001	Significant
	R vs 10	-3.530	-3.902	-3.158	P < 0.0001	Significant
	R vs 11	-0.4900	-0.8617	-0.1183	P < 0.0001	Significant
	R vs 12	-10.45	-10.82	10.08	P < 0.0001	Significant
	R vs 13	-7.173	-7.545	-6.802	P < 0.0001	Significant
	R vs 14	-2.153	-2.525	-1.785	P < 0.0001	Significant
	R vs 15	-2.893	-3.265	-2.522	P < 0.0001	Significant

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R vs 16	0.2200	-0.1517	0.5917	P > 0.05	Not Significant
R vs 17	-3.863	-4.235	-3.492	P < 0.0001	Significant
R vs 18	-13.63	-14.00	-13.25	P < 0.0001	Significant
R vs 19	-2.377	-2.748	-2.005	P < 0.0001	Significant
R vs 20	-11.12	-11.50	-10.75	P < 0.0001	Significant
R vs 21	-17.49	-17.86	-17.12	P < 0.0001	Significant
R vs 22	-1.410	-1.782	-1.038	P < 0.0001	Significant
R vs 23	-12.75	-13.12	-12.38	P < 0.0001	Significant
R vs 24	-3.277	-3.648	-2.905	P < 0.0001	Significant
R vs 25	-2.210	-2.582	-1.838	P < 0.0001	Significant
R vs 26	-4.113	-4.485	-3.742	P < 0.0001	Significant
R vs 27	0.7167	0.3449	1.088	P < 0.0001	Significant
R vs 28	-9.760	-10.13	-9.388	P < 0.0001	Significant
R vs 29	-13.33	-13.70	-12.96	P < 0.0001	Significant
R vs 30	-10.54	-10.91	-10.17	P < 0.0001	Significant

***Comparison = Dermovate® (R) vs CPSU (-01 to -30)**
****Mean Difference = % Drug released Reference - % Drug released CPSU**

5.5.3. Model-Independent Methods

FDA [509] has endorsed the use of model-independent methods to calculate the difference (f_1) and similarity (f_2) factors for comparing *in vitro* release profiles of formulations [423,446,510]. The use of f_1 and f_2 provides a single value from a number of data points to establish the closeness between two resultant *in vitro* release profiles [422]. This approach was used to compare each test formulation to Dermovate®, as described in § 4.6.1.3 (Chapter 4). Since the factor f_2 is insensitive to the shape of the *in vitro* release curve, and does not take into account the unequal spacing between sampling time points, a similarity S_d factor proposed by Gohel and Panchal [454,455] was also used to compare the *in vitro* release profiles of the test formulations and Dermovate® by using a new model-independent approach. The f_1, f_2 and S_d factors were also used to assess the similarities and differences in the CPSU cream formulation relative to Dermovate® and the results are listed in Table 5.14.

Table 5.14. *f_1, f_2 and S_d values for CPSU formulations and Dermovate®*

Comparison*	f_1	f_2	S_d	Comparison*	f_1	f_2	S_d
R vs 01	15.0	66.7	0.0130	**R vs 16**	6.2	80.7	0.0316
R vs 02	18.9	62.9	0.0108	**R vs 17**	7.7	79.6	0.0729
R vs 03	19.2	62.4	0.0040	**R vs 18**	27.5	53.9	0.0807
R vs 04	2.9	95.9	0.0001	**R vs 19**	14.2	67.3	0.0650
R vs 05	14.0	68.5	0.0004	**R vs 20**	23.5	56.3	0.0307
R vs 06	12.4	68.8	0.0012	**R vs 21**	34.7	46.8	0.1220
R vs 07	16.7	66.5	0.0169	**R vs 22**	15.3	61.1	0.0520
R vs 08	6.8	84.7	0.0244	**R vs 23**	27.2	53.1	0.0889
R vs 09	16.0	64.1	0.0234	**R vs 24**	8.3	80.8	0.0884
R vs 10	9.9	74.9	0.0566	**R vs 25**	4.0	90.9	0.0119
R vs 11	10.4	73.6	0.0798	**R vs 26**	16.7	66.0	0.0112
R vs 12	21.1	58.0	0.0123	**R vs 27**	13.4	71.8	0.0352
R vs 13	12.5	70.0	0.0345	**R vs 28**	19.9	60.1	0.0865
R vs 14	12.3	68.8	0.0045	**R vs 29**	22.9	55.8	0.0150
R vs 15	7.0	80.2	0.0454	**R vs 30**	20.7	58.1	0.0105

On consideration of the difference factor, batches CPSU-04, CPSU-08, CPSU-10, CPSU-11, CPSU-13, CPSU-14, CPSU-15, CPSU-16, CPSU-17, CPSU-19, CPSU-24, CPSU-25 and CPSU-27 were considered similar to the *in vitro* release profile of Dermovate® as the f_1 values that were calculated were < 15 for these comparisons. The other CPSU batches had f_1 values > 15.0 showing that they are different with respect to the *in vitro* release profiles generated in comparison to the reference product. Batch CPSU-21 produced data, in which discrimination between the test and reference products was the greatest as the f_1 value was 34.5. Therefore this scale-up formulation has the greatest difference in *in vitro* release compared to Dermovate®. These CPSU formulations were manufactured using different homogenisation speed, anchor speed, homogenisation time and cooling time and resulted in the production of formulations with different physical properties and *in vitro* release profiles. The similarity factor, f_2 for comparison of the CPSU formulations indicates that all batches except batch CPSU-21 with an f_2 value of 46.8 showed similarity to the *in vitro* release profile of the reference product as all f_2 values were > 50. An f_2 value of close to 100 indicates that the *in vitro* profiles of a

test and reference product are identical. Batch CPSU-04 had an f_2 value of 95.9 and an S_d value of 0.0001 and had an *in vitro* release profile that was most similar to that observed for Dermovate®. Furthermore a relationship between the f_2 values and the Gohel and Panchal similarity factor S_d could not be established for the scale-up batches as some CPSU batches that had values of $f_2 > 50$ resulted in calculated S_d values of ≥ 0.02. This may be due to the fact that four different process variables contributed to the differences in formulation responses and *in vitro* release profiles, compared to the laboratory scale formulations, where the only formulation composition was changed. Batch CPSU-21 was the least similar to Dermovate® in terms of the *in vitro* release profile with a resultant f_2 value of 46.8 and S_d value of 0.1220. It was therefore important to evaluate the performance of different formulations of the generic scale-up dosage forms in order to establish which of the CPSU formulations were most similar to the Dermovate® product.

5.5.4. Model-Dependent Methods

Zero, first and Higuchi models are model-dependent methods based on mathematical functions that can be used to describe the *in vitro* release profiles derived from experimental formulations. These mathematical models were used to evaluate the mechanism of CP release from the laboratory scale dosage forms and to gain an understanding of which that formulation parameters may alter the *in vitro* release of CP as described in § 4.6.1.5 (Chapter 4). An evaluation of the *in vitro* release profiles for CP from the 30 scale-up batches was undertaken to determine whether an equal amount of API would be released per unit time, and that the drug release would therefore follow a zero-order kinetic process. The Higuchi model was also used to determine whether the release of API from each CPSU formulation was directly proportional to the square root of time, based on the principles of Fickian diffusion as shown in Table 5.15.

Table 5.15. *Model-dependent parameters generated for CPSU formulations and Dermovate®*

Formulation	Zero order		First order		Higuchi	
	K_0	R^2	K_1	R^2	K_H	R^2
Dermovate®	0.66	0.97	-0.0129	0.94	6.31	0.99
CPSU-01	0.71	0.90	-0.0221	0.97	6.91	0.98
CPSU-02	0.82	0.96	-0.0219	0.96	7.45	0.99
CPSU-03	0.88	0.92	-0.0228	0.93	8.32	0.99
CPSU-04	0.66	0.97	-0.0116	0.95	6.41	0.99
CPSU-05	0.84	0.97	-0.0229	0.93	7.65	0.97
CPSU-06	0.78	0.96	-0.0230	0.95	7.15	0.98
CPSU-07	0.90	0.91	-0.0230	0.96	8.50	0.98
CPSU-08	0.88	0.95	-0.0221	0.95	8.21	0.99
CPSU-09	0.74	0.90	-0.0223	0.96	7.02	0.98
CPSU-10	0.80	0.95	-0.0221	0.95	7.39	0.98
CPSU-11	0.88	0.90	-0.0216	0.97	6.50	0.99
CPSU-12	0.77	0.96	-0.0222	0.93	7.10	0.98
CPSU-13	0.80	0.95	-0.0222	0.95	7.39	0.98
CPSU-14	0.81	0.95	-0.0226	0.95	6.50	0.98
CPSU-15	0.80	0.88	-0.0223	0.96	7.10	0.99
CPSU-16	0.74	0.90	-0.0222	0.97	7.39	0.98
CPSU-17	0.73	0.93	-0.0227	0.96	6.50	0.98
CPSU-18	0.75	0.89	-0.0231	0.97	7.19	0.99
CPSU-19	0.85	0.92	-0.0236	0.96	8.04	0.98
CPSU-20	1.03	0.96	-0.0228	0.94	9.52	0.98
CPSU-21	0.84	0.94	-0.0231	0.96	7.77	0.99
CPSU-22	0.88	0.93	-0.0223	0.96	8.24	0.98
CPSU-23	0.72	0.91	-0.0235	0.97	6.85	0.97
CPSU-24	0.92	0.91	-0.0227	0.97	8.68	0.98
CPSU-25	0.80	0.88	-0.0231	0.97	7.68	0.99
CPSU-26	0.90	0.92	-0.0223	0.96	8.41	0.99
CPSU-27	0.83	0.96	-0.0277	0.96	7.63	0.98
CPSU-28	0.54	0.86	-0.0255	0.94	7.44	0.98
CPSU-29	0.73	0.88	-0.0225	0.97	8.01	0.98
CPSU-30	0.78	0.89	-0.0235	0.97	6.88	0.98

For comparing the results of the model fitting, using the value of R^2 as a good fit criterion, the Higuchi model was found to be the model that the data were best fitted to, in order to describe the *in vitro* release of CP from all CPSU batches. The R^2 values for this mathematical model ranged between 0.98 and 0.99 for all 30 batches of creams, indicating that diffusion was the predominant factor controlling the release of CP from these formulations.

The zero order model may also be considered an appropriate model to describe the mechanism of release of CP from all CPSU cream formulations as the R^2 values for the model fit range between 0.88 and 0.97. The release of CP from the base is considered to be linear as a function of time for cream formulations that produce a R^2 value > 0.95. Of the 30 batches of cream produced, 11 batches released CP, according to a zero-order kinetic model. The R^2 values that were obtained following fitting of CP release data to a first order model, suggested that all CPSU formulations followed a first order kinetic process since the R^2 values for these cream formulations range from 0.93 to 0.97.

5.6 GENERIC CPSU FORMULATION

The optimised scale-up formulation was selected on the basis of the most significant formulation responses *viz.,* viscosity, spreadability and cumulative % drug released per unit area generated using the CCD in addition to evaluation of the statistical and mathematical data generated during data analysis. Each significant response for the test formulation was compared to that of Dermovate®. The scale-up formulation that showed the most similar viscosity, spreadability and cumulative % drug released per unit area data to that of Dermovate®, was selected as the most appropriate generic formulation for use in *in vivo* studies. A comparison of the results revealed that batch CPSU-04, for which the process variables were a homogenisation speed of 1900 rpm, anchor speed of 35 rpm, homogenisation time of 100 minutes and cooling time of 100 minutes, was selected as the most desirable CPSU formulation following evaluation of the data and the data are summarised in Table 5.16.

Table 5.16. *Analysis of Dermovate® and CPSU formulations*

Formulation	VSC* ± SD (cP) (n=3)	SPD* ± SD (cm^2) (n=3)	% DR*± SD (n=3)	Formulation	VSC* ± SD (cP) (n=3)	SPD* ± SD (cm^2) (n=3)	% DR*± SD (n=3)
Dermovate®	44675 ± 210	25.6 ± 0.6	50.23 ± 0.21	**CPSU-16**	35833 ± 602	26.1 ± 0.5	55.93 ± 1.00
CPSU-01	33033 ± 896	28.7 ± 0.8	61.23 ± 0.30	**CPSU-17**	43600 ± 556	25.5 ± 0.8	55.09 ± 0.78
CPSU-02	30766 ± 550	30.5 ± 0.6	63.09 ± 0.22	**CPSU-18**	18333 ± 832	35.0 ± 0.5	67.05 ± 0.04
CPSU-03	30600 ± 953	30.1 ± 0.9	63.40 ± 0.55	**CPSU-19**	33566± 1250	29.3 ± 1.7	60.61 ± 0.60
CPSU-04	44600 ± 602	25.7 ± 0.8	50.49 ± 0.60	**CPSU-20**	20833 ± 802	32.4± 0.7	66.19 ± 0.90
CPSU-05	33466 ± 1250	29.3 ± 1.7	60.64 ± 0.06	**CPSU-21**	10800 ± 65	41.1± 2.8	75.21 ± 1.48
CPSU-06	33633 ± 1000	31.4 ± 1.0	60.61 ± 0.22	**CPSU-22**	20900 ± 624	33.5 ± 1.5	65.76 ± 0.98
CPSU-07	81566 ± 1001	18.9 ± 0.7	42.22 ± 0.87	**CPSU-23**	29633 ± 404	31.5 ± 1.5	68.89 ± 0.70
CPSU-08	51966 ± 378	20.9 ± 0.9	54.23 ± 0.89	**CPSU-24**	48506± 1732	23.9 ± 0.7	54.62 ± 0.09
CPSU-09	29933 ± 404	30.1 ± 0.9	62.21 ± 0.78	**CPSU-25**	52966± 2513	22.1 ± 2.5	52.55 ± 0.10
CPSU-10	42333 ± 896	24.6 ± 0.1	57.23 ± 0.90	**CPSU-26**	33766 ± 862	28.3 ± 0.1	61.23 ± 0.09
CPSU-11	36316 ± 652	30.2 ± 0.5	58.03 ± 0.76	**CPSU-27**	36166 ± 569	30.1 ± 0.9	58.15 ± 1.90
CPSU-12	22733 ± 379	28.6 ± 0.2	64.97 ± 0.82	**CPSU-28**	25866 ± 550	33.5 ± 0.6	63.36 ± 0.70
CPSU-13	50133 ± 351	21.1 ± 0.2	57.42 ± 0.09	**CPSU-29**	27600 ± 556	32.5 ± 0.5	65.30 ± 0.70
CPSU-14	32800 ± 700	28.6 ± 0.6	61.04 ± 0.10	**CPSU-30**	27066 ± 802	32.5 ± 0.6	64.89 ± 0.98
CPSU-15	43933 ± 737	24.9 ± 0.5	55.50 ± 1.20				

***Responses: VSC= Viscosity (cP); SPD= Spreadability (cm^2); % DR= cumulative % drug released over 72 hours**

The use of the difference and similarity factors to characterise the *in vitro* release profiles of CP from CPSU formulations revealed that batch CPSU-04 exhibited similar physical characteristics and *in vitro* release profiles for CP to that of Dermovate®. This scale-up formulation also followed the Higuchi model as following modelling and an R^2 value of > 0.99 was obtained. Therefore a diffusion-controlled mechanism appeared to be predominant when describing CP release from batch CPSU-04 and Dermovate®, respectively.

5.7 CONCLUSION

The development and production of a successful dosage form occurs in a number of stages of the formulation development chain *viz.,* from the manufacture of batches of laboratory scale to intermediate batches [465,470]. Laboratory scale manufacturing of semi-solid products mostly involves the use of laboratory equipment which permits better control over the formulation process when compared to large-scale manufacturing where agitator and high-shear mixers are used. These items of large production equipment are usually fitted with a fixed propeller shaft and an immovable bowl and thus scale-up issues can easily occur when transferring a small batch size to a production plant for the manufacture of a commercial batch [207,466,470]. A Wintech® cream/ointment mixer (Wintech® Pharmachem Equipments PVT. Ltd, Mumbai, India) was used to scale-up an o/w cream formulation from a 500 g to 5 kg batch size. The process of scaling-up the CP cream formulations was critical in order to establish whether a laboratory scale cream formulation with an exact composition would produce a product at large-scale using different process parameters that performed in the same way.

The formation of congealed material was discovered at specific sites of the mixing bowl vessel and these sites are called "cold spots". The presence of a water jacket on the outside of the Wintech® mixing bowl vessel may tend to solidify the inner wall surface formulation more quickly than the formulation found in the middle of the bowl vessel. Also the cooling times of the 5 kg batches varied from 40 minutes to 120 minutes, and longer cooling times have a tendency to facilitate the formation of congealed material, or the "cold spots" may not be accessible to the anchor blades and the homogeniser. Therefore the solidified waxy material on the inner surface of the bowl vessel could not be incorporated into the bulk cream readily.

A Central Composite Design was used to investigate the effect of process variables on the CPSU cream formulation. The CCD approach was used with the intention of using a minimum number of experimental runs to produce a CPSU formulation with responses that were similar to the innovator product, Dermovate®. The process variables selected for evaluation based on data in literature and the manufacturing procedure of the laboratory scale CP formulations were homogenisation speed, anchor speed, homogenisation time and cooling time. The 30 CPSU cream formulations were analysed in terms of viscosity, spreadability, pH, % drug content and cumulative % drug released per unit area. A model fitting technique using Design-Expert® software (version 7.0, Stat-Ease, Inc., Minneapolis, USA) showed that a correlation between the process variables and the CPSU cream responses was most suitably described with quadratic polynomial models. A quadratic polynomial equation was generated for each response from the CCD. Statistical analysis was undertaken using ANOVA, evaluation of the regression coefficient, graphically using a normal plot of residuals, contour and 3-dimensional response surface plots. The viscosity, spreadability and cumulative % drug released per

unit area generated R^2 values > 0.90, indicating that there was good agreement between the experimental and the predicted values for these responses. However the pH and % drug content generated R^2 values < 0.90 suggesting that the fit to these models was poor as there was a large variation in the experimental data compared to the predicted values from these models. The viscosity, spreadability and cumulative % drug released over a period of 72 hours also showed some reliability in the quadratic response surface models as their adjusted and predicted R^2 values were in close agreement. Furthermore all responses except the % drug content demonstrated higher values of adequate precision (> 4), thereby revealing that all responses showed adequacy in the signal results. The spreadability was found to have the lowest coefficient of variation and was the most precise parameter with a reliable quadratic response surface model. The standard deviation for each quadratic model showed that the predicted value for spreadability was more accurate and closer to its actual value as compared to the other responses.

The ANOVA of the quadratic regression models at a 95% level of significance showed that homogenisation speed and anchor speed had the most significant effect on viscosity, spreadability and cumulative % drug released per unit area. However the process parameters showed no effect on the pH and % drug content (P-value > 0.05). Since the pH of the scale-up cream batches fell within the range of that for healthy skin between 4 and 6, it was unlikely that any skin irritation would occur during and following application of these cream formulations. The blend uniformity of the 30 batches of CPSU formulations ranged from 99.10% – 99.37% and therefore CP could be considered to be uniformly distributed throughout the CP cream formulations, despite of the presence of congealed material on the inner surface of the mixing bowl vessel, for all the batches investigated.

The normal plot of residuals indicated that all responses followed a linear pattern indicating that the data was normally distributed and the model was appropriate for this set of data. The contour and 3D response surface plots confirmed that homogenisation speed was the process variable that had the most significant effect on the quality of the CPSU formulations. An increase in homogenisation speed resulted in an increase in viscosity, a decrease in spreadability and a decrease in the cumulative % drug released per unit area. The anchor speed also contributed a secondary effect to the viscosity, spreadability and cumulative % drug released per unit area. Statistical analysis of the *in vitro* release data for CP release from the test batches was undertaken to compare the effects of the different process variables on the resultant drug release rates in addition to facilitating the selection of an optimised formulation for further evaluation. Qualitative interpretation and statistical data analysis using ANOVA, the difference (f_1) and similarity (f_2 and S_d) factors were used to compare the release rate profiles of CP from the scale-up formulations. The use of ANOVA, f_1, f_2 and S_d used to characterize the *in vitro* release profiles of CP from CPSU formulations revealed that batch CPSU-04 released CP at a similar rate and extent to Dermovate®. Batch CPSU-04 had a P-value of < 0.05 at

most times, an f_1 value of 2.9, an f_2 value of 95.9 and an S_d value of 0.0001, revealing this batch was similar to Dermovate®. This scale-up formulation also followed Higuchi type release kinetics as a R^2 value of > 0.99 was generated for the fit and therefore a diffusion-controlled mechanism appeared to be the dominant factor controlling drug release for this formulation.

The optimised generic CPSU formulation was therefore chosen in terms of the most significant responses affecting the cream formulations in comparison to Dermovate®. A comparison of the results revealed that batch CPSU-04 with a homogenisation speed of 1900 rpm, anchor speed of 35 rpm, homogenisation time of 100 minutes and cooling time of 100 minutes was selected as the most desirable generic formulation. This scale-up formulation had a viscosity of 44600 cP, a spreadability of 25.7 cm^2 and a cumulative % drug released per unit area over 72 hours of 50.49%. Batch CPSU-04 was further investigated in an *in vivo* human skin blanching assay to establish whether the formulation was bioequivalent to Dermovate®.

CHAPTER SIX

BIOEQUIVALENCE (BE) ASSESSMENT OF CLOBETASOL 17-PROPIONATE CREAM FORMULATIONS USING THE HUMAN SKIN BLANCHING ASSAY

6.1 INTRODUCTION

Following the expiry of patents on many corticosteroid products just over 20 years ago, and the increasing appearance of generic topical corticosteroid formulations, proof of bioequivalence (BE) of such products to an innovator product has become a pre-requisite for market authorisation by regulatory authorities [23,511,512]. Currently the patents on all initial topical corticosteroid products that were registered and sold, have expired, resulting in the availability of a significant number of generic corticosteroid formulations on the South African market [512]. Corticosteroid molecules display a unique property of inducing skin whitening or blanching at the site of application and this has been used as a criterion to determine the bioavailability (BA) of corticosteroids formulated into topically applied dosage forms [513-515]. The skin whitening/blanching effect is one of the pharmacological side effects of topical corticosteroids and it is thought that this blanching effect is due to the local vasoconstrictive properties of these molecules [512,516,517]. In South Africa, all medicines must be registered by the Medicines Control Council (MCC), the local equivalent of FDA, prior to approval for commercial use. The measurement of the blanching effect of a generic topical corticosteroid formulation in comparison to an innovator product is acceptable for the purposes of establishing BE [512]. The human skin blanching assay (HSBA) is an accepted method for the assessment of BE of corticosteroid formulations and is a reliable, qualitative and comparative measure of topical corticosteroid BA and potency [19-22,110,111,408,511,517,518].

The HSBA is also known as the vasoconstrictor assay (VCA) and has been used for over three decades as a tool for the evaluation of corticosteroid release from topical dosage forms [19,23,110,519]. The skin blanching or skin whitening response used for this assessment, following application of topical corticosteroid products to the skin, was first observed in 1950 [339]. This assay was then applied for BA/BE assessment by Mckenzie and Stoughton in 1962 [516] and was used to evaluate the degree of skin blanching, following application of the corticosteroid [511]. The application of corticosteroids makes use of the response of whitening/blanching of the human skin and the intensity of blanching is directly related to the clinical efficacy of the formulation [19,110,512,519]. However it is well established in the natural, medical and pharmaceutical sciences that due to inherent biological variability among individuals, repeated measurements of the same response do not necessarily produce the same result [19]. The application of corticosteroid molecules to human skin may not induce pallor in all individuals and the thickness of the stratum corneum from the same region of the arm may differ between individuals, depending on the number of cell layers

present which may range from 12 to 30. Since the HSBA measures the rate and extent to which a topically applied corticosteroid diffuses from a formulation into and through the stratum corneum to the dermis, the difference in the physiological properties of human skin will produce different blanching results in different individuals, following application of a corticosteroid [19,22,520,521].

The blanching phenomenon has been accepted by regulatory authorities as being a valid surrogate measure for the comparison of different formulations containing the same corticosteroid molecules. The FDA Office of Generic Drugs [522] issued a Guidance document in 1995, in which aspects relating to the conduct of *in vivo* BE assessment of topical dermatological corticosteroids was documented [523]. The FDA [522] also mentions the use of a pharmacodynamic approach, based on an update of the Stoughton-McKenzie vasoconstrictor bioassay, to assess the bioequivalence of topically applied corticosteroids. The intensity of blanching observed is also correlated with the inherent potency of a corticosteroid and is dependent on the class into which the corticosteroid is placed *viz.*, weak, medium, strong or very strong [512]. Variations of the vasoconstriction assay retain one essential parameter *viz.,* the intensity of a drug-induced blanching side-effect, which is quantified by one method or another [517].Whilst the methodology of the HSBA is generally well accepted, there are different methods of assessing the intensity of blanching. The subjective nature of the visual assessment of blanching was the first scientific approach to the bioassay and was developed by McKenzie and Stoughton to assess the skin blanching effect of topical corticosteroid formulations [19,110,511,516,517]. The objective assessment techniques that are used as alternative methods of assessing skin blanching include the measurement of reflectance, thermographic and laser Doppler velocimetry techniques that attempt to record the degree of skin blanching at each application site in a more quantitative manner [19,22,524]. The use of a chromameter for the assessment of blanching measures the skin colour by reflectance. This approach has been in use for the last decade and objective measurements of skin colour, following exposure to a topical corticosteroid-containing formulation can be readily undertaken [110,513,517,519,525-527].

6.1.1. Visual Assessment

McKenzie and Stoughton [516] developed the first documented, single reading time visual assessment procedure for comparing corticosteroid performance [517,522,528]. The assessment of the intensity of corticosteroid-induced skin blanching was performed visually by one observer using an ordinal data scale and this approach was criticised due to the lack of reproducibility, sensitivity and precision of the visual assessment data [110]. This methodology was refined and improved over a number of decades to the point where a multiple-reading, visual-assessment protocol, conducted by more than one trained and experienced observer was considered. This approach is considered to be sensitive and accurate for the evaluation of topical corticosteroid delivery, *in vivo*. Subjective assessment should always be performed with care and some internal monitoring of the ability of an observer is vital to

provide for consistent evaluation of the degree of blanching [19]. The use of visual methodology to compare two formulations-containing the same corticosteroid in the same concentration or for comparing different corticosteroids for clinical potency ranking has been valuable. The human eye is an organ that can discriminate subtle differences in similar colours and is able to compare the skin colour of a site that has been exposed to the steroid (application site) and the surrounding un-medicated skin, simultaneously [339,517]. This visual assessment approach takes into account different factors *viz.*, inherent skin pigmentation, hirsutism and mottling, subconsciously. These are fundamental facets of the assay that instrumental and/or computational systems would have to duplicate. Refinement of the visual assay methodology has markedly improved the credibility of the technique. In addition the intensity of blanching produced by the same formulation depends on the forearm position to which it is applied and varies between left and right arms [517].

6.1.2. Chromameter Assessment

The FDA has recommended that an alternative measurement system be used to circumvent the perceived subjectivity of the visual assessment methodology for *in vivo* corticosteroid activity assessment. This approach makes use of a chromameter to monitor corticosteroid-induced skin blanching for BE assessment purposes and it is an objective instrumental method [110,513,517,519,525,529]. The FDA [522] has released a Guidance document recommending the use of the chromameter for this purpose. Currently the FDA [522] recommends that the degree of blanching be assessed preferably using a chromameter and/or by visual assessment [511]. The Guidance document details the chromametric procedures that must be followed for the determination of topical corticosteroid BE. The chromameter is a compact portable instrument used for the assessment of surface colour based on tristimulus analysis of a reflected pulse of xenon light. The skin colours are measured using CIE 1976 *viz.*, *L*a*b* system which simulates the sensitivity and selectivity of the human eye. The reflected light is usually quantified in terms of three colour indices *viz.*, *a*- (red-green) and *b*- (yellow-blue) values are the chromaticity co-ordinates, while the *L*-value (light-dark) is the lightness variable [110,522]. These three values define a unique point in a three-dimensional (3D) colour map, defining the colour of the measured surface absolutely. The instrument performs accurately, precisely and reproducibly for solid, planar surfaces that are completely uniform in colour and topography. The FDA Guidance document [522] suggests the analysis of the *a*-scale index data only, for pilot and pivotal trials with baseline correction and unmedicated-site readings. The *a*-scale value appears to show the greatest sensitivity to colour change over the progression of the blanching response, followed by the *L*-scale and *b*-scale data [517,522,523]. However, similarly to the use of visual assessment, training and experience in the use of a chromameter is essential if reproducible and reliable results are to be generated [511].

6.2 OBJECTIVE

The use of both visual and chromameter assessments for HSBA is an FDA regulated method for the assessment of BE of topical corticosteroid products [522]. This method was used to evaluate the BE of Dermovate®, Dovate® and two scale-up CP formulations. Since the *in vitro* release studies of Dermovate® and Dovate® showed that there was a significant difference in their *in vitro* release profiles, as described in § 4.5.2 (Chapter 4), an investigation of the *in vivo* BE of these two formulations that are available on the South African market was undertaken. Therefore two CPSU formulations were selected for comparative purposes on the basis of the physical properties and *in vitro* release profiles of the innovator and generic CP formulations. The objective of the study was to evaluate the *in vivo* BE of 0.05% w/w CP cream formulations with respect to Dermovate® and Dovate® in healthy human volunteers, using the skin blanching effect.

6.3 METHODS AND PROCEDURES

6.3.1. Study Population and Criteria for Participation

6.3.1.1. Number of Subjects

The subjects considered for this study were aged between 18 and 60 and were in general good health. The subjects were selected on the basis of a pre-screening test that was performed prior to the study. Fourteen subjects were enrolled for this study and dropouts were not replaced during the study. The data for all subjects who completed the study were included in the statistical analysis of the information.

6.3.1.2. Conditions for Participation in This Study

Volunteers had to complete a written informed consent form indicating that they are willing to participate fully in the study. They had to undergo a pre-screening test prior to the study in order to assess any possibility or sensitivity to CP and to evaluate whether they exhibited any blanching. Volunteers had to fulfil the inclusion and exclusion criteria that are summarised in § 8.3 and § 8.4 (Appendix V). The volunteers had to be fully committed to the study and abide by all restrictions required for the subjects, as listed in § 8.5 (Appendix V).

6.3.2 Study Products

The study products were four 0.05% w/w CP cream formulations, of which two were commercially available on the South African market *viz.*, Dermovate® and Dovate®. The other two CP formulations were selected from those that were developed and manufactured, specifically for the purposes of this research, as described in Chapter 5. The reference product, Dermovate® cream and the generic product, Dovate® cream were purchased in sufficient quantities from the respective pharmaceutical manufacturers and stored at a temperature of 22°C. Two scale-up test products *viz.*, batches CPSU-04

and CPSU-16 that produced similar formulation responses and *in vitro* release profiles to Dermovate® and Dovate® creams, were manufactured in the Faculty of Pharmacy at Rhodes University using the principles of Good Manufacturing Practice (GMP). The CPSU cream formulations were packed into 50 g collapsible aluminium cream/ointment tubes and stored at 22°C prior to use.

6.3.3. Study Design

Prior to the study, a pre-screening test was conducted and the subjects were selected, based on the definition of a "responder" as described by the FDA Guidance document [522]. A "responder" is defined as a subject that shows a response to a corticosteroid after exposure to a single dose duration of a Reference Listed Drug (RLD), under the same conditions to be used in a pilot and/or pivotal study [522]. One application site located on the upper forearm was demarcated using a pre-punched adhesive template that exposed an area of 1.1 cm^2 skin for the application of the reference product, Dermovate®. Approximate 10 µl of the formulation that is equivalent to 11 mg was applied to the application site using a 10 µl Eppendorf® micropipette (Merck® Chemicals Pty Ltd, Germiston, South Africa) and was spread uniformly over the site using a glass rod. The dose duration for this exposure was 40 minutes after which the product was gently removed from the skin by three consecutive swabs using wet and dry cotton wool. Visual assessment was performed six hours after product removal to establish if any skin blanching had occurred. Any subject exhibiting blanching on a multiple unit scale of 1-4 where, 1 is an intermediate degree of blanching and 4 is intense blanching, was selected for inclusion in this study.

Fourteen healthy male and female subjects (male and female) that exhibited a positive skin blanching response in the pre-screening test were included in the study. Fourteen application sites were used per forearm and were demarcated using pre-punched adhesive labels that exposed a square of 1.1 x 1.1 cm^2 for the application of the reference, generic and the two CPSU products. Four 0.05% w/w cream formulations *viz.*, Dermovate® (R_1), Dovate® (R_2), batches CPSU-04 (T_1) and CPSU-16 (T_2) were used in this study. Approximately 10 µl of cream that was equivalent to 11 mg of cream was applied to the designated application sites on each forearm using a 10 µl Eppendorf® micropipette and were then uniformly spread in the demarcated area using a glass rod.

The FDA Guidance document [522] recommends three different dose durations *viz.*, ED_{50}, D_1 and D_2 be used in these studies. The ED_{50} is the dose duration at which half of the skin blanching effect is achieved, D_1 is the dose duration equal to half that of the ED_{50} and D_2 is the dose duration that is double that of ED_{50}. The dose duration value for CP cream reported by Au *et al.* was 40 minutes and this was used to assess the bioequivalence of the CP topical creams. The reference product, Dermovate® was applied to application sites demarcated as D_1 (20 minutes) and D_2 (80 minutes) respectively for the determination of "detectors" amongst the subjects included in the study. A representative application chart for the creams that were evaluated in the study is shown in Figure 6.1.

In addition, four control sites were randomly selected from the 14 application sites and were demarcated as UNT.

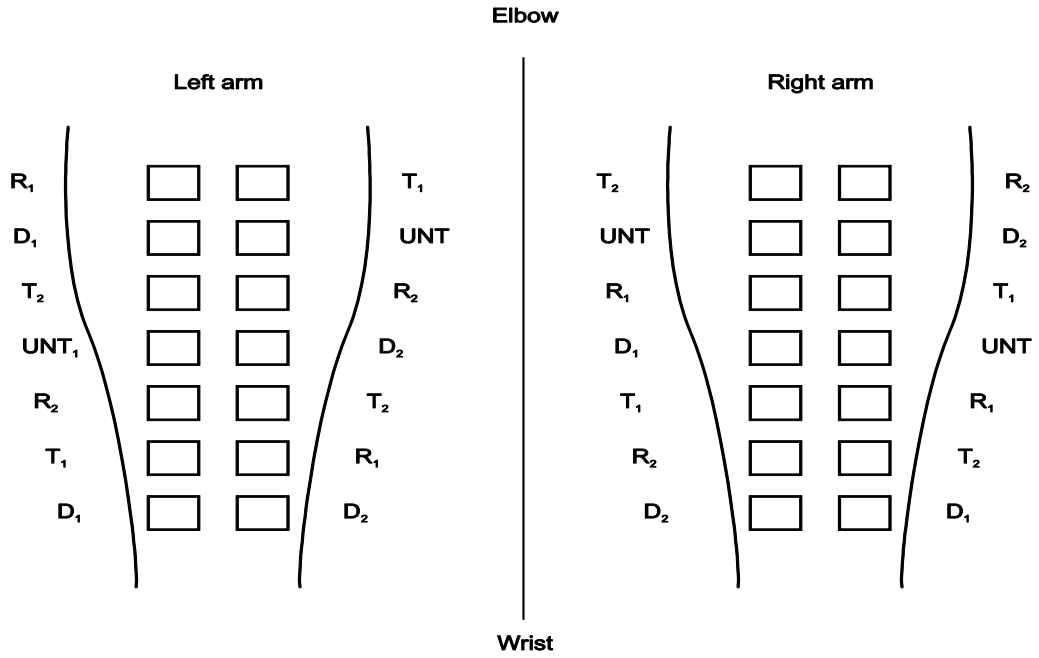

Figure 6.1. *Schematic diagram indicating the application sites on both forearms for the human skin blanching study*

Key	Formulation	Duration	Key	Formulation	Duration
R_1	Reference	40 minutes	D_1	Reference	20 minutes
R_2	Generic	40 minutes	D_2	Reference	80 minutes
T_1	Test 1	40 minutes	UNT	Unmedicated	-
T_2	Test 2	40 minutes			

The application sites, for different dose durations, on the ventral forearm were randomly assigned and the sites were protected from accidental abrasion following application of the formulations using a plastic guard. Prior to evaluation of the pharmacodynamic response at the end of the application period, the remaining cream was gently removed by swabbing three consecutive times at each application site, with wet and dry cotton swabs. The skin blanching response was then measured using visual and chromameter assessment methods, at times = 2, 4, 6, 8, 10, 12, 14, 22, 24 and 30 hours after the removal of the products. The subjects were housed in the Biopharmaceutics Research Institute Clinic at Rhodes University, maintained at 22°C and a relative humidity of 55 ± 3%. Standard overhead lightning was used during the pre-screening test and for the duration of the study.

6.3.4. Skin Blanching Assessments

6.3.4.1. Visual Assessment

The degree of blanching was measured visually by two trained observers using a multiple unit scale of 0-4 where 0 indicates no blanching and a score of 4 indicates intense blanching and scores of 1, 2, and 3 represent intermediate grades of blanching. Each application site was assigned a blanching score by

comparing the extent of blanching of the treated skin to the surrounding skin and un-medicated sites. The blanching response results were calculated as a percentage total possible score (% TPS), according to the method described by Haigh and Kanfer [21] as shown in Equation 6.1.

$$\text{Percent total possible score (\% TPS)} = \frac{\text{Actual Score}}{\text{TPS}} \times 100 \qquad \textit{Equation 6.1}$$

The maximum score per site = 4

The number of independent observers = n

The number of sites per preparation per arm = S

The number of subjects = V

Total possible score (TPS) = 4 x n x S x V

The % TPS was calculated for each formulation at each application site for each observer and a plot of the average % TPS was plotted against time to generate a blanching profile for each formulation. The trapezoidal rule was then used to calculate the area under the effect curve (AUEC) for each formulation.

6.3.4.2. Chromameter Assessment

The chromameter assessment was performed using a Minolta® Model CR 400 chromameter (Minolta®, Osaka, Japan) for the quantification of the intensity of blanching at each individual application site. The chromameter was calibrated using a white calibration plate, prior to the evaluation of the blanching response at each time. Baseline readings at zero time and all reading times included the measurement of the untreated or control sites. The chromameter provided readings based on three scales *viz.*, *L*-scale, *a*-scale and *b*-scale. However the FDA [522] recommends that only the *a*-scale be used to calculate the area under the effect curve (AUEC). Furthermore only the data obtained from subjects defined as "detectors" *viz.*, individual subjects whose AUEC values at D_1 and D_2 are both negative and that meet the dose duration-response criterion, should be included in the data analysis. The FDA Guidance document [522] also states that a "detector" is a "responder" if the blanching data for the subjects meet the criterion established by use of Equation 6.2.

$$\frac{\text{AUEC at } D_2}{\text{AUEC at } D_1} \geq 1.25 \qquad \textit{Equation 6.2}$$

Where,

AUEC at D_2 = 0.5 [AUEC at D_2 (left arm) + AUEC at D_2 (right arm)]

AUEC at D_1 = 0.5 [AUEC at D_1 (left arm) + AUEC at D_1 (right arm)]

At each time of observation, the *a*-value recorded for each application site was baseline corrected by subtracting the baseline or zero time values that were recorded to yield baseline-corrected values *viz.*, Δ*a*-values. The mean Δ*a*-values of all formulations were calculated and plotted against time. The area under the Δ*a*-curve (AUEC) was calculated for each formulation using the trapezoidal rule.

6.3.4.3. Data Analysis

The AUEC values calculated for the left and right forearms were averaged for both the visual and chromameter data. Statistical analysis was performed using Locke's method to calculate a confidence interval (CI), based on Fieller's Theorem for the visual and chromameter AUEC data as required in the Guidance document [522,530]. AUEC data sets for all formulations *viz.*, reference (R_1), generic (R_2) and the two test formulations (T_1 and T_2) were compared to calculate an exact CI and to evaluate the BE of the formulations under investigation.

6.4 RESULTS AND DISCUSSION

Of the 14 subjects included in the study, eight were Caucasian and six were Indian. The study group included eight females and six males. All 14 subjects who exhibited some blanching on the multiple unit scale of 1-4 used during pre-screening, were included in the pivotal study. In addition, all 14 subjects showed some degree of blanching during the study. The blanching response of one of the 14 subjects following application of four CP cream formulations *viz.*, Dermovate® (R_1), Dovate® (R_2), CPSU-04 (T_1) and CPSU-16 (T_2) is shown in Figure 6.2.

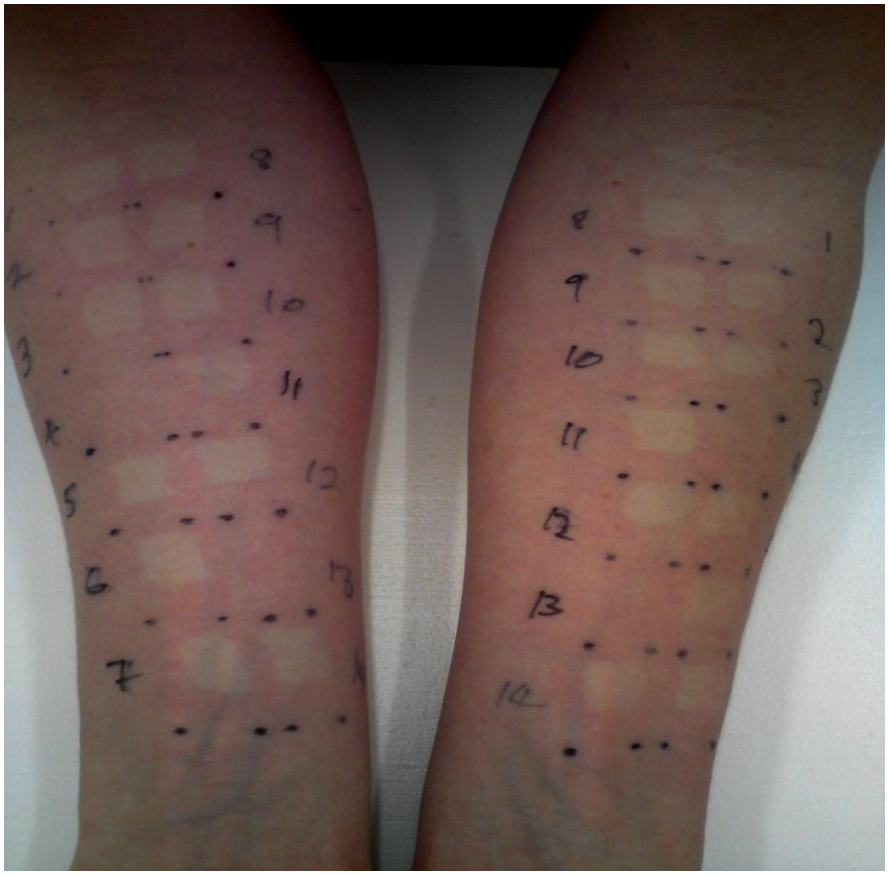

Figure 6.2. *Blanching response after application of the four CP cream formulations*

The mean visual and chromameter blanching profiles (n=14) of the reference product, Dermovate® at different dose durations *viz.*, D_2 (80 minutes), ED_{50} (40 minutes) and D_1 (20 minutes) are shown in Figures 6.3 and 6.4.

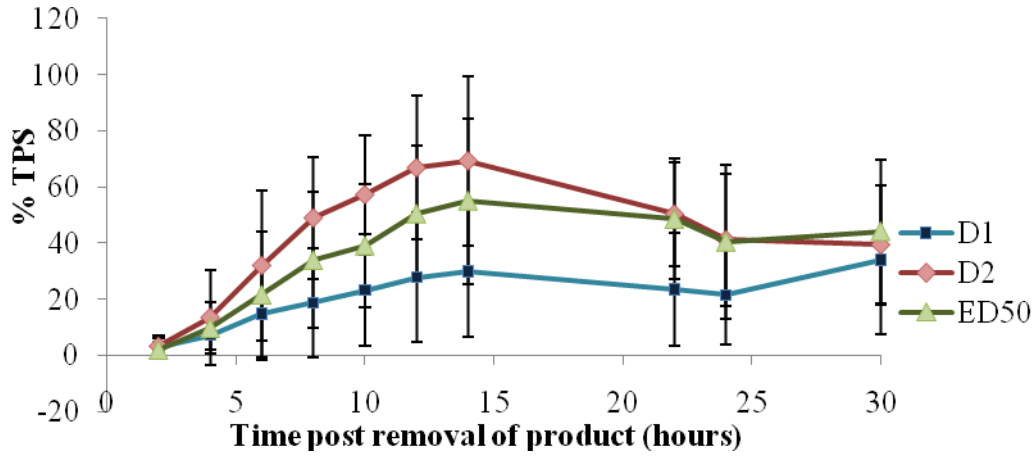

Figure 6.3. *Mean visual blanching profiles ± SD (n=14) of the reference product, Dermovate® at dose duration, D_2 (80 minutes), ED_{50} (40 minutes) and D_1 (20 minutes)*

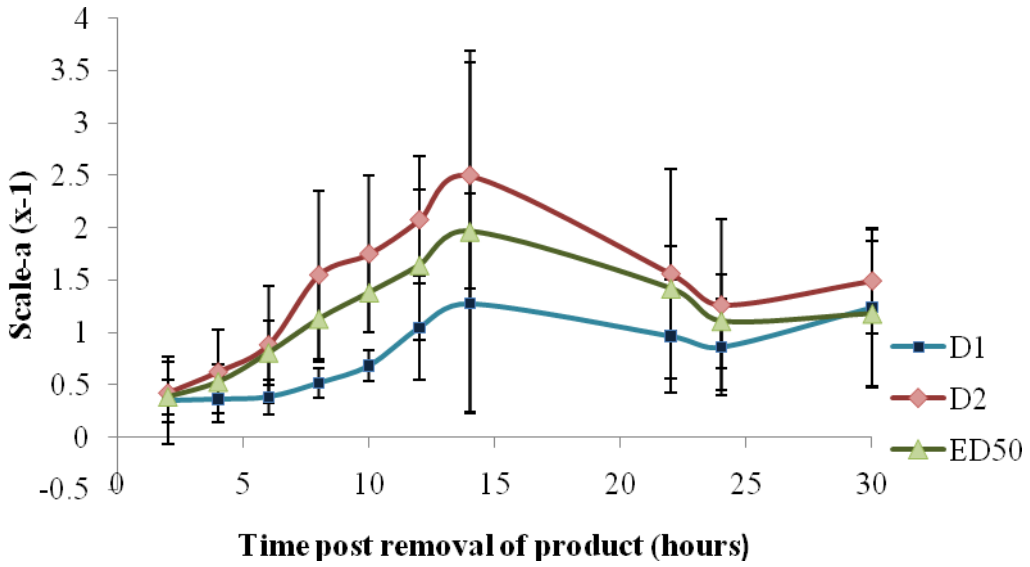

Figure 6.4. *Mean chromameter blanching profiles ± SD (n=14) of the reference product, Dermovate® at dose durations, D_2 (80 minutes), ED_{50} (40 minutes) and D_1 (20 minutes) on the a-scale*

The mean visual and chromameter blanching profiles of the reference product at different dose durations reveal that the ED_{50}, or dose duration of 40 minutes, showed an intermediate blanching profile that fell between the profiles for dose durations of 20 minutes (D_1) and 80 minutes (D_2). As expected, a higher intensity of blanching was observed for D_2 that was the longest dose duration of 80 minutes, and the lowest blanching intensity was observed for D_1 with dose duration of 20 minutes (Figures 6.3 and 6.4). The dose duration has an effect on the intensity of blanching and as dose duration increases, the intensity of blanching increases. The AUEC of the dose duration profiles *viz.*, D_1 and D_2 were calculated using the Trapezoidal rule for each subject and the ratio of AUEC

D_2/AUEC D_1 was calculated to establish which subjects could be defined as "detectors". These data are summarised in Table 6.1.

Table 6.1. *Determination of "Detectors" for the 14 subjects included in the study*

Subjects	Visual Ratio $\frac{AUEC\ D2}{AUEC\ D1} \geq 1.25$	Summary	Chromameter Ratio $\frac{AUEC\ D2}{AUEC\ D1} \geq 1.25$	Summary
1	2.171	Detector	2.056	Detector
2	2.213	Detector	1.403	Detector
3	1.896	Detector	1.682	Detector
4	1.845	Detector	1.557	Detector
5	5.791	Detector	1.571	Detector
6	3.812	Detector	2.023	Detector
7	2.910	Detector	1.959	Detector
8	2.167	Detector	4.009	Detector
9	2.367	Detector	2.503	Detector
10	1.589	Detector	1.776	Detector
11	2.256	Detector	1.888	Detector
12	2.484	Detector	2.843	Detector
13	1.593	Detector	2.780	Detector
14	1.121	Non detector	1.233	Non detector

Thirteen of the fourteen subjects were found to be "detectors" as the ratio of the AUC D_1/AUC $D_2 \geq$ 1.25 for the visual and chromameter assessments. The mean visual blanching profile for the CP cream formulations for the group of detectors (n=13) is shown in Figure 6.5 respectively.

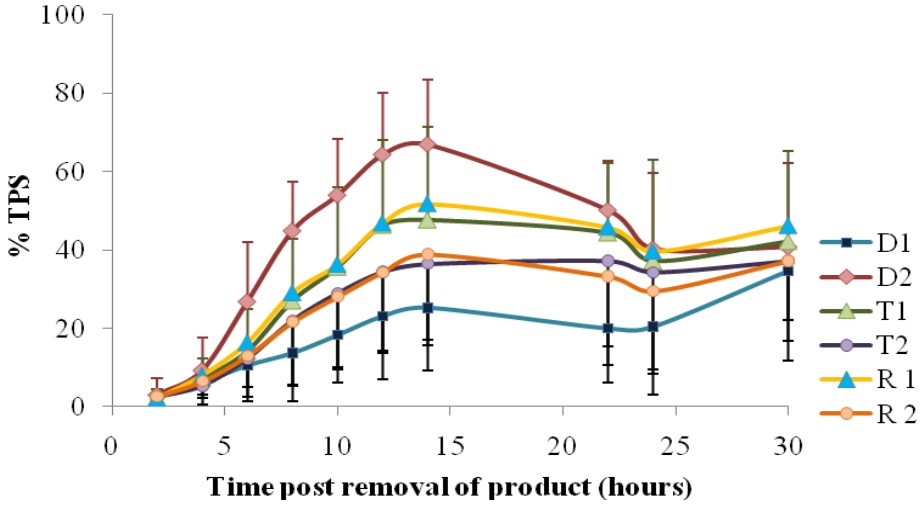

Figure 6.5. *Visual data for "detectors" subjects (n=13)*

The mean visual blanching profile for the CP creams indicates that peak blanching occurred at 14 hours following product removal. Formulation T_1 exhibited similar blanching effects to Formulation R_1 and Formulation T_2 exhibited similar blanching intensity to Formulation R_2. However formulations R_1 and T_1 had higher blanching profiles than that observed for formulations R_2 and T_2. Since the use of the visual method and human eye is a subjective method of evaluating skin blanching, it was vital to measure the blanching using a chromameter which is considered an objective tool for the assessment

of BE. The mean chromameter blanching profile for the CP cream formulations for the group of "detectors" (n=13) is shown in Figure 6.6.

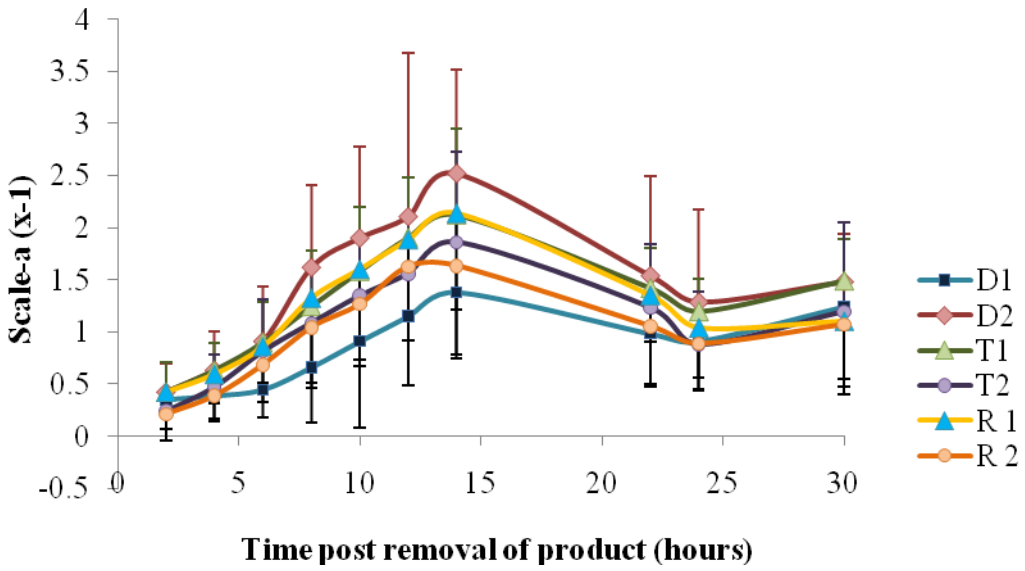

Figure 6.6. *Chromameter data for all "detectors" subjects (n=13)*

The mean chromameter blanching profile for the "detectors" reveals that the degree of blanching peaked at 14 hours after product removal. Formulation R_1 demonstrated a similar blanching effect to formulation T_1 and the degree of blanching exhibited for formulation R_2 was similar to that observed for formulation T_2. Formulations R_1 and T_1 exhibited higher blanching profiles than that observed for formulations R_2 and T_2, showing that a higher blanching intensity occurred for these two formulations. In comparison to the visual blanching profile, the chromameter blanching profile depicted in Figure 6.6 clearly demonstrated larger standard deviations about the mean values at each time point, clearly showing the poor precision of these instrumental data. Furthermore the AUEC for each profile was calculated to investigate the blanching effect of these formulations further. The visual and chromameter blanching profiles of formulation R_1 are similar to that of formulation T_1, therefore the blanching effect of these two formulations may be the same at different times post removal of the products. Similarly, the AUEC of the visual and chromameter blanching profiles of formulation R_2 is similar to that of formulation T_2 therefore the blanching effects of these two formulations may be similar (Table 6.2). Both the visual and chromameter AUEC data show that formulations R_1 and T_1 exhibited higher degrees of blanching effect than formulations R_2 and T_2, demonstrating that these formulations are different from each other, as summarised in Table 6.2.

Table 6.2. *AUEC values for the visual and chromameter curves for "detectors" (n=13)*

	Visual				Chromameter			
	R_1	R_2	T_1	T_2	R_1	R_2	T_1	T_2
Mean AUEC	1062.269	803.945	1002.779	822.606	18.7305	15.3932	19.865	16.5613
SD	300.641	263.225	321.003	250.641	8.582	7.096	7.590	8.111
CV%	28.2	36.0	32.0	30.4	45.8	46.1	38.2	48.9

Locke's method was used to establish a CI based on Fieller's Theorem for the visual and chromameter AUEC data, as required in the FDA Guidance [522,530]. A 90% CI was calculated for the ratio of the average AUEC response of the test product and the reference product using Locke's method. The exact CI obtained for the CP formulations was then used to evaluate the BE of these products and these results are shown in Table 6.3.

Table 6.3. *90% CI calculated using Locke's method for visual and chromameter data*

Locke's method for "detectors" (n=13)								
Formulation		**Visual**			**Chromameter**			
T/R	**Mean Ratio %**	**% CV**	**90% CI**	**Summary**	**Mean Ratio %**	**% CV**	**90% CI**	**Summary**
R_1/R_2	133.5	13.3	119.6-150.9	Not BE	121.7	21.7	114.4-130.9	Not BE
T_2/R_2	103.6	11.5	97.1-111.43	BE	107.6	34.0	98.6-116.6	BE
T_2/R_1	78.0	16.1	72.9-82.1	Not BE	88.4	23.2	77.6-98.8	Not BE
T_1/R_1	94.4	13.8	90.5-98.4	BE	106.1	12.9	98.9-113.2	BE
T_1/R_2	125.5	11.6	113.8-141.4	Not BE	129.1	29.2	122.1-137.5	Not BE
T_1/T_2	118.8	10.2	111.4-126.3	Not BE	119.9	29.8	113.3-128.2	Not BE

The FDA Guidance document [522] mentions that the Office of Generic Drugs has not determined a CI that can be used to state categorically, whether two formulations are BE. However, the Office recognizes that a CI between 80 to 125% can be used as a public standard for the assessment of BE of topical corticosteroid products [522,531]. A 90% CI was calculated in order to compare the four CP cream formulations and to establish whether they were BE to each other. The visual and chromameter data for subjects classed as "detectors" revealed that only formulations T_2 and R_2 and T_1 and R_1 had a point estimate that fell in the 80-125% range. The comparison of other formulations reveals that the products did not meet the BE requirements for those comparisons. The percentage coefficient of variation shown in Table 6.4 demonstrates that the chromameter data obtained in this study are highly variable and reveals the relatively poor precision of this data [25,110,517,532]. The visual assessment however demonstrated a low percentage coefficient of variation with smaller standard deviations and error bars.

6.5 CONCLUSION

The HSBA with both the visual and chromameter approaches, was used to assess the BE of commercially available topical products containing 0.05% w/w CP to the scale-up CP test formulations. Since the *in vitro* release studies of Dermovate® and Dovate® showed that there was a significant difference in their *in vitro* release profiles, an investigation of the *in vivo* BE of these two

formulations that are available on the South African market was undertaken. Therefore two scale-up formulations were selected for comparative purposes on the basis of the physical properties and *in vitro* release profiles of the innovator and generic CP formulations. This approach, in which 14 subjects with 12 application sites per arm for formulations and 2 control sites, was used to establish formulations that were BE. The dose duration applied for the reference product (Dermovate®) were 20 minutes (D_1), 40 minutes (ED_{50}) and 80 minutes (D_2). The mean visual and chromameter blanching profiles for the reference product for the different dose durations showed, as expected, that the intensity of blanching increased as dose duration increased. Therefore a maximum blanching effect was noted, following a dose duration of 80 minutes at those specific application sites compared to that observed for 20 minutes, following product application, where the intensity of blanching was minimum. The ratio of the AUEC D_2/D_1 for all 14 subjects showed that 13 subjects could be classified as "detectors" as the ratio for this parameter was ≥ 1.25. The mean visual and chromameter blanching profiles for all CP cream formulations peaked at 14 hours following product removal. Graphical representation of the blanching curves showed that formulation T_1 exhibited a similar blanching profile to formulation R_1 while formulation T_2 showed a similar blanching profile to formulation R_2.

Locke's method was used to calculate a 90% CI to confirm BE of the four CP formulations tested in this study. The visual and chromameter data generated for the "detectors" showed that batch CPSU-11 was found to be bioequivalent to Dovate® and batch CPSU-04 to Dermovate® respectively. However the BE criteria for Dermovate® and Dovate® were not met with both the visual and chromameter data. One of the possible reasons for this difference is that the use of shorter dose durations is more discriminatory than when longer dose durations of 6 hours are applied to assess the BA and BE of topical corticosteroid cream formulations. Longer dose duration will result in the API reaching its maximum blanching effect after 6 hours of product application [19,20,110,517,532,533]. Therefore no discrimination between profiles could be observed as a maximum blanching effect would be achieved due to saturation of the system. Nowadays, shorter and discrete dose durations, for example 40 minutes, are used to assess the BE of topical corticosteroid products. These shorter dose durations may help to provide appropriate discrimination between corticosteroid formulations as the intensity of blanching for different formulations will be directly related to the clinical efficacy of the formulation. The large standard deviation error bars, wide BE intervals and the high percentage coefficient of variation of the chromameter assessment data demonstrate that the chromameter method is highly variable. This instrumental technique was therefore found to exhibit poor precision in its data compared to the visual assessment data [25,110,517,525]. Therefore the visual assessment of skin blanching, although subjective, continues to appear more reliable, robust, effective and precise than current chromameter techniques when evaluating different formulations containing the same corticosteroid.

CHAPTER SEVEN
CONCLUSIONS

CP is a super-high potency corticosteroid that possesses anti-inflammatory, anti-pruritic, vasoconstrictive, immunosuppressive and anti-proliferative properties and is used to treat a wide range of dermatological conditions. Potent innovator corticosteroids are expensive and therefore there is a need to develop and manufacture a generic CP product for topical application. There are several challenges regarding the industrial-scale manufacture of cream formulations and the objectives of this research were to identify and overcome some of these challenges.

An HPLC method was developed, optimised and validated according to the ICH guidelines for the *in vitro* quantitation of CP in cream dosage forms. The method was found to be linear over a concentration range of 0.15 μg/ml to 15 μg/ml and precise with % RSD values of ≤ 5%. The method was also found to be accurate with a % RSD that was determined to be ≤ 2%. In addition, the method was considered selective for the detection and quantitation of CP in the presence of excipients and degradation products and therefore the method can be considered stability-indicating.

The melting points of all the waxy materials to be considered for use, in the formulation of laboratory and scale-up CP creams, were determined. The solubility of CP in PG revealed that this compound was most soluble in a binary mixture PG:Water, in a ratio of 70:30% v/v and this solution was selected as the receptor medium for *in vitro* release studies, using the Franz diffusion cell method. CP was found stable in a 70:30% v/v PG:Water solution, following storage at 4°C for one week. IR spectrophotometric analysis revealed that no major chemical bond shifts were observed, thereby suggesting that no interaction between CP and the excipients to be used in the formulation was likely to occur.

Dermovate® is the innovator CP product whereas Dovate® is currently the most widely available generic CP formulation on the South African market. Dosage form analysis of these two formulations revealed that Dovate® cream formulation had higher viscosity and lower spreadability than Dermovate® and the *in vitro* release of CP from the generic formulation was lower than that of the reference product. Laboratory scale CP creams were manufactured using different concentrations of Gelot® 64 and propylene glycol in order to establish a composition that would produce a formulation with the same physical and chemical characteristics and *in vitro* release profile, as the innovator product, Dermovate®. The laboratory scale CP creams were then assessed in terms of their viscosity, spreadability, pH, centrifugation and content uniformity. All batches were physically stable, had a pH that ranged between 5 and 6 and complied with the USP requirements for content uniformity. Batch CPLS-02 was the only laboratory scale formulation that showed similar viscosity and spreadability characteristics to Dermovate®. The results obtained from dosage form analysis were further

investigated to explain the *in vitro* release characteristics of CP from these laboratory scale cream formulations. A Franz cell diffusion apparatus was successfully used for this purpose and a comparison of the *in vitro* release profiles revealed that CP release from batch CPLS-01 in which 1.50% w/w G-64 and 47.50% v/v PG were used, was faster and greater in extent over a 72 hour period as compared to batch CPLS-05 in which 12.00% w/w G-64 and 37.00% v/v PG were used and from which the lowest amount of CP was released. Batch CPLS-02 in which 3.00% w/w G-64 and 46.00% v/v PG were used, released CP in a similar manner to Dermovate®. The release of drug from the topical dosage forms was then critically assessed by use of model-dependent and model-independent methods of analysis. Model independent parameters such as the difference, f_1 or similarity, f_2 factors to characterise the *in vitro* release profiles of CP suggested that drug release from batch CPLS-02 was similar to that observed for Dermovate® as the values for f_1 was close to zero and f_2 was close to 100. The Gohel and Panchal similarity factor, S_d confirmed that batch CPLS-02 demonstrated the most similarity with respect to the *in vitro* release profile to the reference product with a value for S_d of 0.0006. The Higuchi plots of drug release versus the square root of time were linear with resultant R^2 values of 0.98 and 0.99. Therefore a diffusion-controlled mechanism appears to control CP release from these laboratory scale formulations. The formulation and manufacture of laboratory scale batches and subsequent dosage form analysis were vital to investigate the performance characteristics of formulations prior to proceeding to scale-up manufacture.

Batch CPLS-02 was further investigated to ascertain whether the formulation with the same % w/w composition would exhibit similar performance, following manufacture on a larger scale. A Wintech® cream/ointment mixer (Wintech® Pharmachem Equipments PVT. Ltd, Mumbai, India) was used to scale-up the o/w cream formulation from 500 g to a 5 kg batch size. It was important to evaluate the process parameters, using this piece of equipment that may have an impact on the ultimate formulation characteristics. A Central Composite Design was used to investigate the effect of process variables on the performance of the scale-up cream formulations. This approach uses a minimum number of experimental runs to produce an optimum process and formulation that would be similar to that of Dermovate®. The homogenisation speed, anchor speed, homogenisation time and cooling time were process variables that were likely to have the greatest impact on the quality of the product produced. Thirty scale-up batches were manufactured and analysed in terms of their viscosity, spreadability, pH, % drug content and cumulative % drug released per unit area over 72 hours. Model fitting using Design-Expert® software (version 7.0, Stat-Ease, Inc., Minneapolis, USA) was undertaken and revealed that a correlation between the process variables and the cream responses was most suitably described by quadratic polynomial relationships. A quadratic polynomial equation was generated for each response following CCD studies. The results were statistically evaluated using ANOVA analysis and regression coefficient determination and graphically by plotting normal plots of residuals, contour and 3-dimensional response surface plots. The viscosity, spreadability and

cumulative % drug released per unit area produced R^2 values > 0.90, indicating that there was a good agreement between the experimental and predicted values for these responses. However pH and % drug content had R^2 values < 0.90 which seemed to invalidate the fit for these models as there was a large variation in the experimental values when compared to the data predicted from these models.

The ANOVA test on the quadratic regression models generated revealed that homogenisation speed and anchor speed had the most significant effect on the viscosity, spreadability and cumulative % drug released per unit area for all formulations. However these process parameters had no impact on the pH and % drug content. The normal plot of residuals indicated that all responses followed a linear pattern showing that a normal distribution was an appropriate model for this set of data. Contour and 3D response surface plots confirmed that homogenisation speed was the process variable that had the most significant effect on the quality of the scale-up formulations. An increase in homogenisation speed resulted in an increase in viscosity, a decrease in spreadability and a decrease in the cumulative % drug released per unit area. The anchor speed had a secondary effect on the measured responses for the formulations investigated. The qualitative interpretation and statistical analysis of the *in vitro* release data from the scale-up formulations using ANOVA and the f_1, f_2 and S_d factors revealed that batch CPSU-04 released CP at a similar rate and extent to Dermovate®. Batch CPSU-04 had a P-value of < 0.05 at most time points, an f_1 value of 2.9, an f_2 value of 95.9 and an S_d value of 0.0001, revealing this batch released CP in a manner that was similar to Dermovate®. A diffusion-controlled mechanism appeared to be predominant in these test formulations as they followed a Higuchi type model with resultant R^2 values > 0.99 for the relationship. The optimised generic scale-up formulation was therefore selected for further evaluation on the basis of viscosity, spreadability and cumulative % drug released per unit area in addition to statistical and mathematical data. Batch CPSU-04 was the only scale-up formulation that showed similar viscosity, spreadability and cumulative % drug released per unit area to that of Dermovate® and was therefore selected for evaluation in *in vivo* studies.

The human skin blanching study using both visual and chromameter assessment was used to establish the bioequivalence of the scale-up formulations to Dermovate® and Dovate®. Two scale-up formulations were selected for comparative purposes on the basis of the physical properties and *in vitro* release profiles of the innovator and generic CP formulations. The *in vitro* release studies of Dermovate® and Dovate® showed that there was a significant difference in their *in vitro* release profiles and therefore an investigation of the *in vivo* bioequivalence of these two formulations found on the South African market was undertaken. Batch CPSU-11 was the scale-up formulation that showed similar viscosity, spreadability and cumulative % drug released per unit area to that of Dovate® and was therefore selected for *in vivo* assessment. Fourteen subjects participated in the study and fourteen application sites were used with two control sites per arm. Thirteen subjects were classified as "detectors" as the ratio for AUEC D_2/D_1 was ≥ 1.25. The mean visual and chromameter

blanching profiles for all CP cream formulations peaked at 14 hours following product removal and at this time maximum blanching intensity was observed. Graphical representation of the blanching curves showed that formulation CPSU-04 exhibited a similar blanching profile to Dermovate® while formulation CPSU-11 showed a similar blanching profile to formulation Dovate®. Locke's method was used to calculate a 90% CI and confirmed that batch CPSU-11 was bioequivalent to Dovate® and batch CPSU-04 was bioequivalent to Dermovate®. Furthermore Dermovate® and Dovate® were not found to be bioequivalent when using both the visual and chromameter data. The possible reason for this difference is that longer dose durations of up to 6 hours may have been used to assess the bioavailability and bioequivalence of corticosteroid cream formulations [19,20,517,532]. Longer dose durations would result in maximum blanching effect of the topical corticosteroid, showing no discrimination between profiles compared to shorter dose durations which are used to provide appropriate discrimination between corticosteroid formulations. The chromameter although an objective method of evaluation of colour changes due to the blanching effect, did not produce data as precise as the visual assessment data [25,110,517,532]. Therefore further bioequivalence studies may be conducted using other blanching assessment techniques such as the digital image analysis.

The studies conducted and reported in this thesis define a pathway for the design, development, manufacture and assessment of generic topical formulations. Although Dovate® is a generic CP cream formulation on the South African market, this project looked at some important laboratory scale and scale-up formulation development and assessment aspects that are vital before licensing and marketing of additional pharmaceutical products. Following the manufacture of the same composition of a topical formulation on a laboratory scale and scale-up level, Batch CPSU-04 performed in a similar manner to Dermovate® *in vitro* and *in vivo*. Since a bioequivalence relationship exists for CP following topical administration of this generic cream formulation, Batch CPSU-04 has the potential to be commercialised for the South African market. This topical cream formulation also exhibits similar physical characteristics *viz.*, viscosity and spreadability to Dermovate®, showing that this generic cream formulation can clearly aid in the treatment of dermatological conditions. Future studies that would be necessary for progression of the project would be manufacturing and assessing batch sizes > 5 kg of CP cream formulations using the same equipment, to investigate any possibility of additional scale-up challenges and to identify process limitations of this manufacturing process.

APPENDIX I

BATCH PRODUCTION RECORD

LABORATORY SCALE MANUFACTURE

A sample batch production record for Batch CPLS-01, a laboratory scale CP cream formulation is included. The batch production records for the other batches of the laboratory scale CP cream formulation batches are available on request.

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate laboratory scale cream Page 1 of 3
Batch number:CPLS-01 **Batch size:**500 g

MANUFACTURING APPROVALS

Batch record issued by:_____ Date:_____

Master record issued by:_____ Date:_____

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Grahamstown 6140, SOUTH AFRICA
BATCH PRODUCTION RECORD

Product Name: Clobetasol 17-propionate laboratory scale cream
Batch Number:CPLS-01 **Batch Size:**500 g
Temperature:70°C

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	0.25 g			
2	Propylene glycol	RM000181	47.50	237.50 ml			
3	Sodium citrate	RM000183	0.05	0.25 g			
4	Citric acid	RM000185	0.05	0.25 g			
5	Gelot® 64	RM000177	1.50	7.50 g			
6	Glyceryl monostearate	RM000182	11.00	55.00 g			
7	Cetostearyl alcohol	RM000184	8.40	42.00 g			
8	White beeswax	RM000142	1.15	5.75 g			
9	Chlorocresol	RM000186	0.075	0.375 g			
10	Distilled water	N/A	30.225	151.125 ml			

EQUIPMENT VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Weighing balance	Model 500C PJ Precisa®		
Water bath	Model NB-34980 Colora® Ultra-Thermostat		
Homogeniser	Model 6-105 AF Virtis® “23”		
Digital thermometer	Model TM-902C Lutron®		

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA
BATCH PRODUCTION RECORD

Product name: Clobetasol 17-propionate laboratory scale cream
Batch number:CPLS-01 **Batch size:**500 g

MANUFACTURING PROCEDURE				
Steps	**Procedure**	**Time**	**Done by**	**Checked by**
1	Weigh all materials accurately using a balance			
2	Heat distilled water to 75°C in a 500 ml beaker using a water bath. Maintain the temperature of the distilled water at 75°C			
3	Add and dissolve sodium citrate and citric acid in the same 500 ml beaker containing the distilled water			
4	Add propylene glycol to the same beaker containing the aqueous solution			
5	Slowly add CP in the 500 ml beaker while continuously stirring and heating at 75°C and sonicate the solution for further 25 minutes until a clear solution is obtained [Aqueous phase]			
6	In another 1000 ml beaker, melt Gelot® 64 followed by cetostearyl alcohol, white beeswax, glyceryl monostearate and chlorocresol at a temperature of 70°C using a water bath [Oil phase]			
7	Add the heated aqueous phase to the heated oil phase and maintain a temperature of 70°C while stirring with a glass rod for 5 minutes			
8	Remove the 1000 ml beaker containing the mixture from the water bath and homogenise using a homogeniser at 3000 rpm for another 10 minutes			
9	Pack the final cream into 500 g opaque containers and store at room temperature (22°C) until further use for analysis			

SIGNATURE AND INITIAL REFERENCE			
Full name (Print)	**Signature**	**Initials**	**Date**

APPENDIX II

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

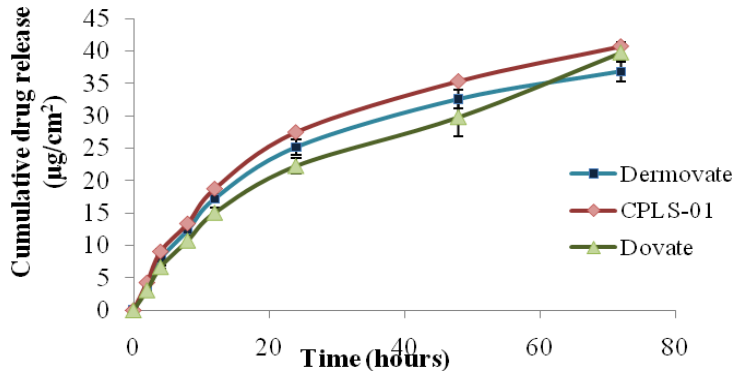
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate laboratory scale cream
Batch Number: CPLS-01
Date of Manufacture: 01/04/2010
Melting Temperature: 70°C
Batch Size: 500 g

Excipient Name	Original Formula (% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	0.25 g	RM000150
Propylene glycol	47.50	237.50 ml	RM000181
Sodium citrate	0.05	0.25 g	RM000183
Citric acid	0.05	0.25 g	RM000185
Gelot® 64	1.50	7.5 g	RM000177
Glyceryl monostearate	11.00	55.0 g	RM000182
Ceteostearyl alcohol	8.40	42.0 g	RM000184
White beeswax	1.15	5.75 g	RM000142
Chlorocresol	0.075	0.375 g	RM000186
Distilled water	30.225	151.125 ml	N/A

Production Equipments Used	
Water bath	Model NB-34980 Colora® Ultra-Thermostat water bath
Digital thermometer	Model TM-902C Lutron®
Homogeniser	Model 6-105 AF Virtis® "23" homogeniser

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	28500 ± 265	0.93
Spreadability (cm^2)	58.77 ± 1.18	2.01
Centrifugation	Stable	-
pH	5.32 ± 0.02	0.37
CP content (%)	101.50 ± 0.01	0.09

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, slightly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

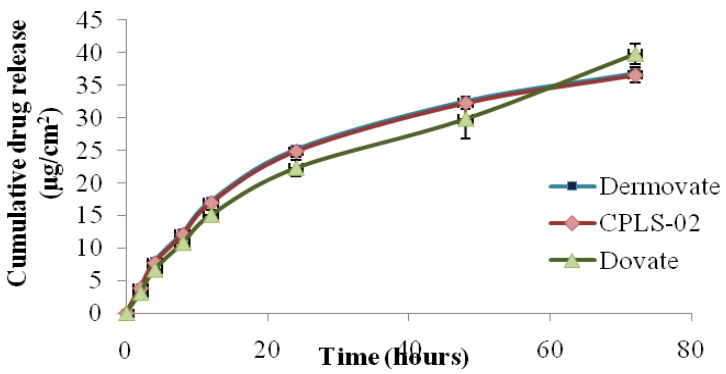
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate laboratory scale cream
Batch Number: CPLS-02
Date of Manufacture: 01/04/2010
Melting Temperature: 70°C
Batch Size: 500 g

Excipient Name	Original Formula (% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	0.25 g	RM000150
Propylene glycol	46.00	230.0 ml	RM000181
Sodium citrate	0.05	0.25g	RM000183
Citric acid	0.05	0.25 g	RM000185
Gelot® 64	3.00	15.0 g	RM000177
Glyceryl monostearate	11.00	55.0 g	RM000182
Ceteostearyl alcohol	8.40	42.0 g	RM000184
White beeswax	1.15	5.75 g	RM000142
Chlorocresol	0.075	0.375 g	RM000186
Distilled water	30.225	151.125 ml	N/A

Production Equipments Used	
Water bath	Model NB-34980 Colora® Ultra-Thermostat water bath
Digital thermometer	Model TM-902C Lutron®
Homogeniser	Model 6-105 AF Virtis® "23" homogeniser

Tests Performed	Mean ± SD	%RSD
Viscosity (cP)	44800 ± 862	1.92
Spreadability (cm^2)	26.12 ± 0.52	1.95
Centrifugation	Stable	-
pH	5.24 ± 0.05	0.95
CP content (%)	99.98 ± 0.02	0.13

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Batch Summary Record

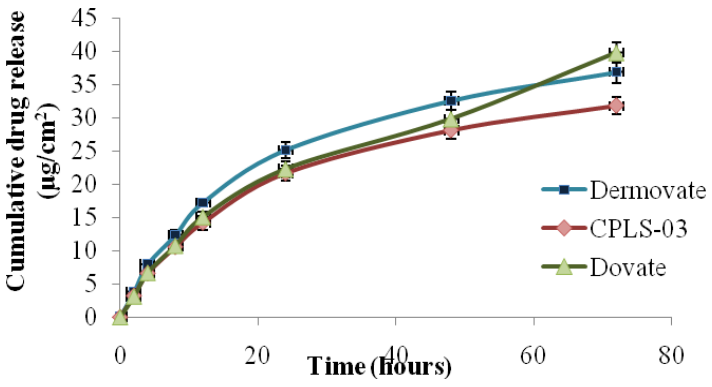
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate laboratory scale cream
Batch Number: CPLS-03
Date of Manufacture: 02/04/2010
Melting Temperature: 70°C
Batch Size: 500 g

Excipient Name	Original Formula (% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	0.25 g	RM000150
Propylene glycol	43.00	215.0 ml	RM000181
Sodium citrate	0.05	0.25g	RM000183
Citric acid	0.05	0.25 g	RM000185
Gelot® 64	6.00	30.0 g	RM000177
Glyceryl monostearate	11.00	55.0 g	RM000182
Ceteostearyl alcohol	8.40	42.0 g	RM000184
White beeswax	1.15	5.75 g	RM000142
Chlorocresol	0.075	0.375 g	RM000186
Distilled water	30.225	151.125 ml	N/A

Production Equipments Used	
Water bath	Model NB-34980 Colora® Ultra-Thermostat water bath
Digital thermometer	Model TM-902C Lutron®
Homogeniser	Model 6-105 AF Virtis® "23" homogeniser

Tests Performed	Mean ± SD	%RSD
Viscosity (cP)	55500 ± 153	0.34
Spreadability (cm^2)	20.03 ± 0.52	2.59
Centrifugation	Stable	-
pH	5.32 ± 0.02	0.37
CP content (%)	99.71 ± 0.03	0.25

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogeneous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

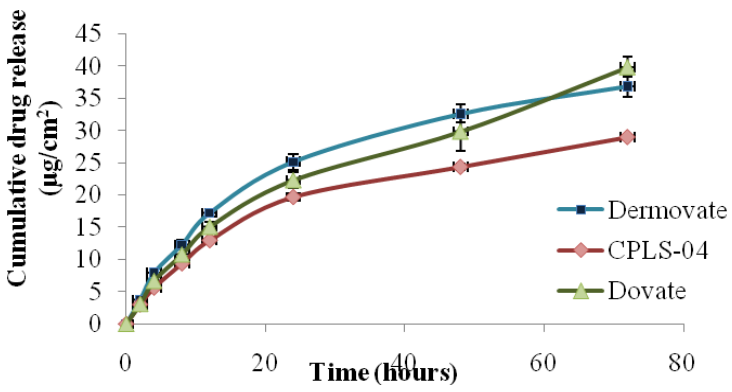
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate laboratory scale cream
Batch Number: CPLS-04
Date of Manufacture: 02/04/2010
Melting Temperature: 70°C
Batch Size: 500 g

Excipient Name	Original Formula (% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	0.25 g	RM000150
Propylene glycol	40.00	200.0 ml	RM000181
Sodium citrate	0.05	0.25g	RM000183
Citric acid	0.05	0.25 g	RM000185
Gelot® 64	9.00	45.0 g	RM000177
Glyceryl monostearate	11.00	55.0 g	RM000182
Ceteostearyl alcohol	8.40	42.0 g	RM000184
White beeswax	1.15	5.75 g	RM000142
Chlorocresol	0.075	0.375 g	RM000186
Distilled water	30.225	151.125 ml	N/A

Production Equipment Used	
Water bath	Model NB-34980 Colora® Ultra-Thermostat water bath
Digital thermometer	Model TM-902C Lutron®
Homogeniser	Model 6-105 AF Virtis® "23" homogeniser

Tests Performed	Mean ± SD	%RSD
Viscosity (cP)	69100 ± 416	0.60
Spreadability (cm^2)	15.56 ± 0.23	1.47
Centrifugation	Stable	-
pH	5.25 ± 0.06	1.35
CP content (%)	98.77 ± 0.07	0.60

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogeneous cream was produced
- The cream was smooth, highly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Batch Summary Record

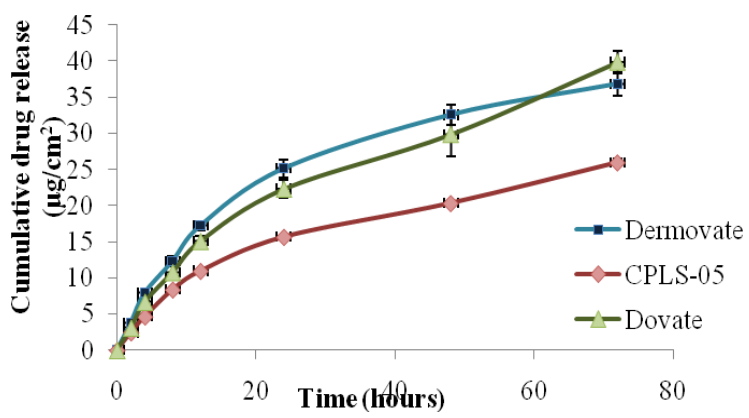
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate laboratory scale cream
Batch Number: CPLS-05
Date of Manufacture: 03/04/2010
Melting Temperature: 70°C
Batch Size: 500 g

Excipient Name	Original Formula (% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	0.25 g	RM000150
Propylene glycol	37.00	185.0 ml	RM000181
Sodium citrate	0.05	0.25g	RM000183
Citric acid	0.05	0.25 g	RM000185
Gelot® 64	12.00	60.0 g	RM000177
Glyceryl monostearate	11.00	55.0 g	RM000182
Ceteostearyl alcohol	8.40	42.0 g	RM000184
White beeswax	1.15	5.75 g	RM000142
Chlorocresol	0.075	0.375 g	RM000186
Distilled water	30.225	151.125 ml	N/A

Production Equipment Used	
Water bath	Model NB-34980 Colora® Ultra-Thermostat water bath
Digital thermometer	Model TM-902C Lutron®
Homogeniser	Model 6-105 AF Virtis® "23" homogeniser

Tests Performed	Mean ± SD	%RSD
Viscosity (cP)	84500 ± 725	0.86
Spreadability (cm^2)	10.35 ± 0.45	4.33
Centrifugation	Stable	-
pH	5.38 ± 0.01	0.19
CP content (%)	99.73 ± 0.03	0.26

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogeneous cream was produced
- The cream was smooth, highly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

APPENDIX III

BATCH PRODUCTION RECORDS

SCALE-UP MANUFACTURE

A sample of one batch production record for Batch CPSU-01 manufactured using a Wintech® cream/ointment mixer for the CP scale-up cream formulation is included here. The batch production records for the other 29 CP scale-up cream formulation batches, *viz.*, batches CPSU-02 to CPSU-30 are available on request.

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BATCH PRODUCTION RECORD

Product Name: Clobetasol 17-propionate scale-up cream Page 1 of 3

Batch Number:CPSU-01 **Batch Size:**5000 g

MANUFACTURING APPROVALS

Batch record issued by:_____ Date:_____

Master record issued by:_____ Date:_____

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

BATCH PRODUCTION RECORD

Product Name : Clobetasol 17-propionate scale-up cream
Batch Number :CPSU-01 **Batch Size :**5000 g

MASTER FORMULA AND BATCH FORMULA							
Item no.	**Material**	**Rhodes No.**	**Quantity (% w/w)**	**Amount/ Batch**	**Amount Dispensed**	**Dispensed by**	**Checked by**
1	CP	RM000150	0.05	2.50 g			
2	Propylene glycol	RM000181	46.00	2300.00 ml			
3	Sodium citrate	RM000183	0.05	2.50 g			
4	Citric acid monohydrate	RM000185	0.05	2.50 g			
5	Gelot® 64	RM000177	3.00	150.00 g			
6	Glyceryl monostearate	RM000182	11.00	550.00 g			
7	Cetostearyl alcohol	RM000184	8.40	420.00 g			
8	White beeswax	RM000142	1.15	57.50 g			
9	Chlorocresol	RM000186	0.075	3.75 g			
10	Distilled water	N/A	30.225	1511.25 ml			

WINTECH® CREAM/OINTMENT MIXER VERIFICATION			
Description	**Type**	**Verified by**	**Confirmed by**
Bowl vessel	Stainless steel		
Co-centric homogeniser	Teflon and stainless steel		
Anchor and impellers	Stainless steel		
Water inlet	Stainless steel		
Water jacket	Stainless steel		
Charging port	Stainless steel		
Water immersion heater	NA		
PT-100 sensor	NA		
Operating panel touch screen	LCD		

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BATCH PRODUCTION RECORD

Product Name : Clobetasol 17-propionate scale-up cream
Batch Number :CPSU-01 **Batch Size :**5000 g

MANUFACTURING PROCEDURE				
Steps	**Procedure**	**Time**	**Done by**	**Checked by**
1	Clean the bowl vessel with detergent and distilled water, tilt the bowl and drain water and allow to equilibrate at room temperature Room temperature: _____°C Machine temperature: _____°C			
2	Fill the jacket with distilled water			
3	Weigh all materials accurately using a balance			
4	Introduce all excipients starting with propylene glycol and distilled water, followed by sodium citrate, citric acid, clobetasol17-propionate, glyceryl monostearate, cetostearyl alcohol, white beeswax, Gelot® 64 and chlorocresol through the charging port in the bowl vessel			
5	Activate the heater to a temperature of 70°C using the panel touch screen. Allow the mixture to mix using the anchor at the set anchor speed for two hours			
6	Once melted, activate the co-centric homogeniser at a set homogeniser speed and time for emulsification purposes and thorough mixing			
7	After the homogeniser is switched off, allow the anchor to continue mixing at a set anchor speed and allow distilled water to pass though the water jacket for a set cooling time Temp of cold water=____°C			
8	After cooling, close the water inlet and allow slow continuous agitation of the anchor until the temperature on the operating panel displays 30°C			
9	Stop the anchor and release the pressure built up in the bowl vessel by the use of the pressure vent			
10	Store the final CP cream in 5 kg opaque containers until quality control tests are completed and then package into 50 g collapsible aluminium cream/ointment tubes			

SIGNATURE AND INITIAL REFERENCE			
Full name (Print)	**Signature**	**Initials**	**Date**

APPENDIX IV

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

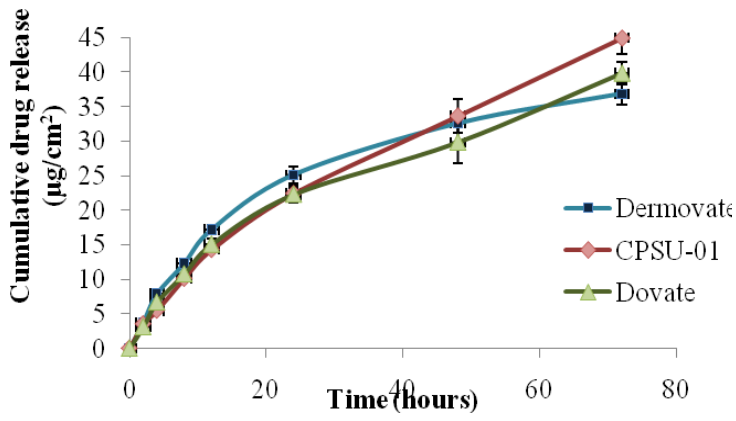
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-01
Date of Manufacture: 21/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	33033 ± 896	3.89
Spreadability (cm^2)	28.7 ± 0.8	2.78
Centrifugation	Stable	-
pH	6.24 ± 0.07	1.00
CP content (%)	100.14 ± 1.69	1.69

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

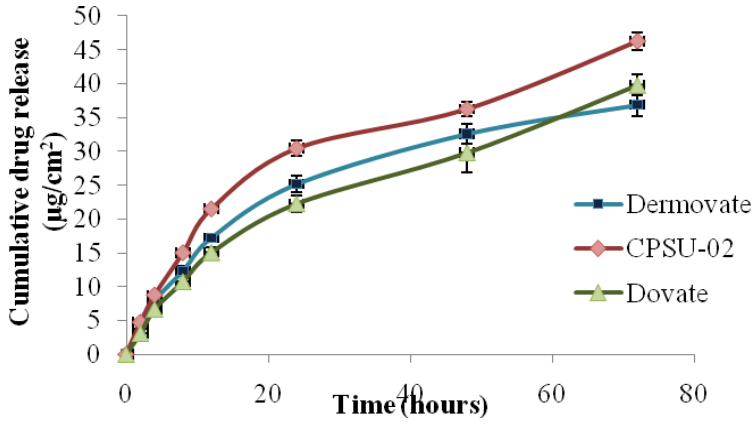
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-02
Date of Manufacture: 08/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	40
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	30766 ± 550	1.79
Spreadability (cm^2)	30.5 ± 0.6	1.85
Centrifugation	Stable	-
pH	6.68 ± 0.08	1.27
CP content (%)	99.75 ± 0.33	0.33

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

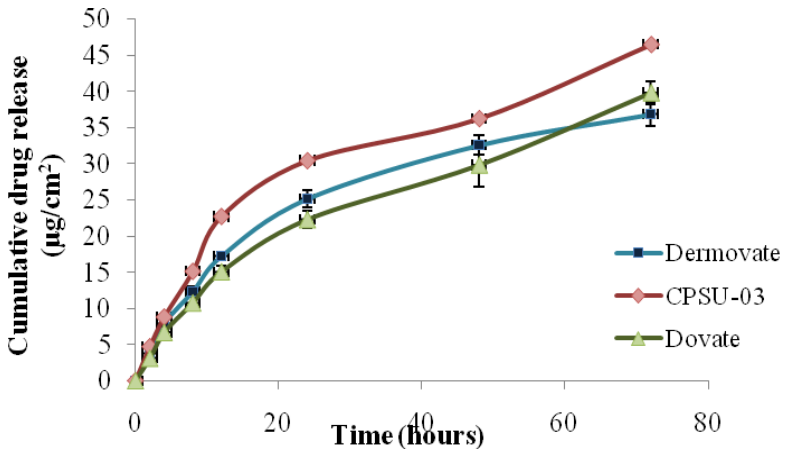
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-03
Date of Manufacture: 20/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	40

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	30600 ± 953	3.11
Spreadability (cm^2)	30.1 ± 0.9	3.22
Centrifugation	Stable	-
pH	6.60 ± 0.05	0.68
CP content (%)	99.93 ± 1.50	1.50

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

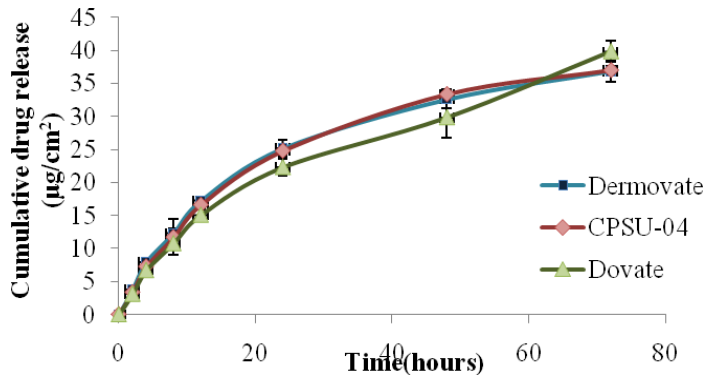
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-04
Date of Manufacture: 27/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	35
Homogenisation time (min)	100
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	50600 ± 602	1.04
Spreadability (cm^2)	24.6 ± 0.8	3.14
Centrifugation	Stable	-
pH	6.39 ± 0.04	0.63
CP content (%)	98.79 ± 1.56	1.58

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

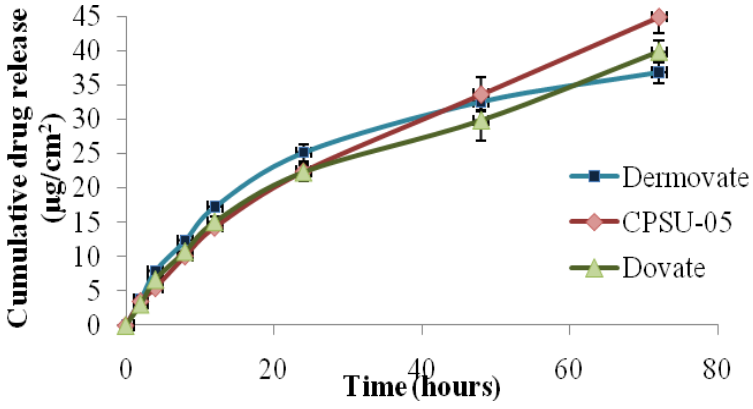
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-05
Date of Manufacture: 24/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	33466 ± 1250	5.32
Spreadability (cm^2)	29.3 ± 1.7	5.78
Centrifugation	Stable	-
pH	6.48 ± 0.05	0.79
CP content (%)	101.42 ± 0.31	0.31

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

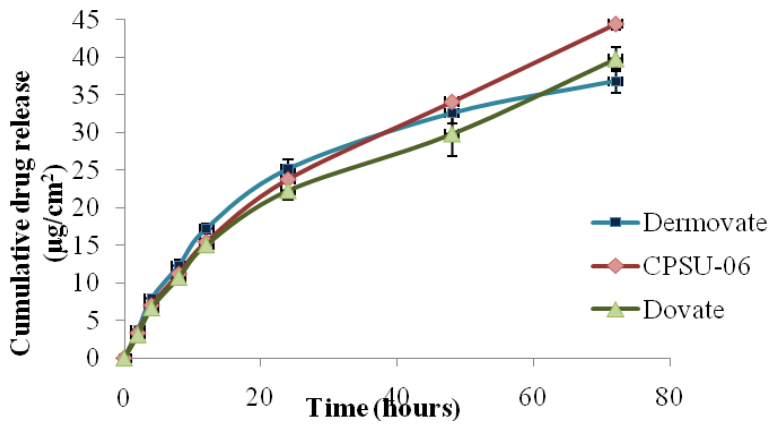
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-06
Date of Manufacture: 27/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	33633 ± 1000	4.29
Spreadability (cm^2)	31.4 ± 1.0	3.22
Centrifugation	Stable	-
pH	6.29 ± 0.03	0.46
CP content (%)	98.00 ± 0.84	0.85

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

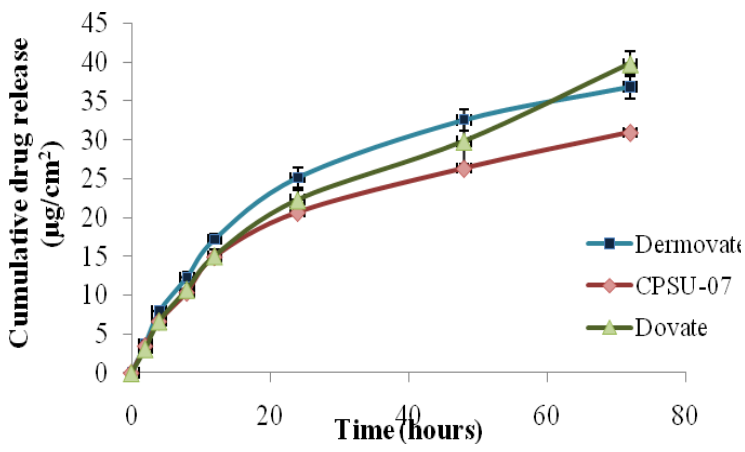
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-07
Date of Manufacture: 23/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	2500
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	85166 ± 1001	1.17
Spreadability (cm^2)	18.8 ± 0.7	4.07
Centrifugation	Stable	-
pH	6.48 ± 0.02	0.35
CP content (%)	99.69 ± 0.33	0.33

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, highly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

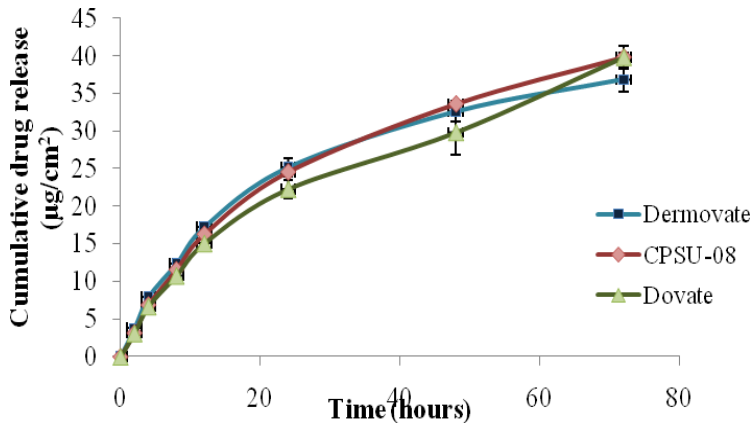
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-08
Date of Manufacture: 17/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	35
Homogenisation time (min)	60
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	51966 ± 378	0.72
Spreadability (cm^2)	20.9 ± 0.9	4.49
Centrifugation	Stable	-
pH	6.60 ± 0.00	0.09
CP content (%)	99.86 ± 0.24	0.24

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

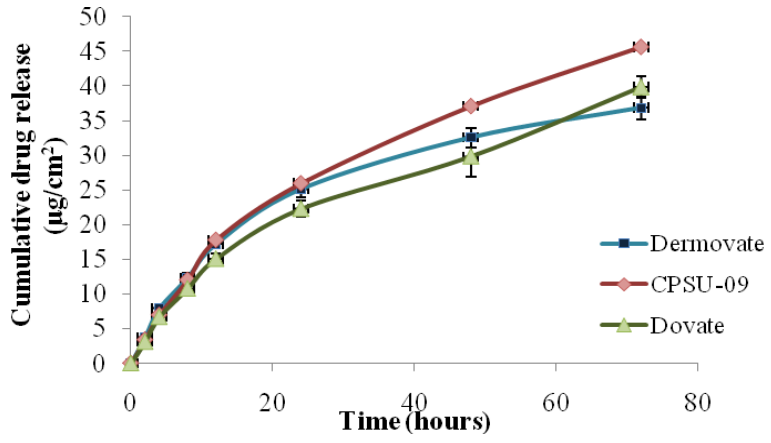
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-09
Date of Manufacture: 14/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	35
Homogenisation time (min)	100
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	29933 ± 404	1.35
Spreadability (cm^2)	30.1 ± 0.9	3.22
Centrifugation	Stable	-
pH	6.75 ± 0.03	0.44
CP content (%)	97.96 ± 0.19	0.20

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

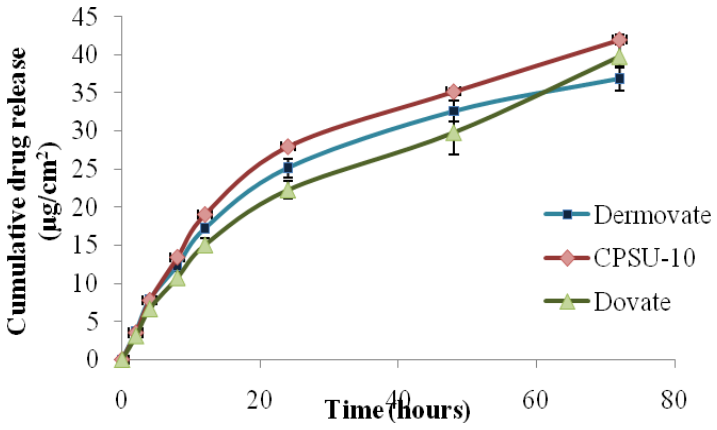
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-10
Date of Manufacture: 11/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	15
Homogenisation time (min)	60
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	42233 ± 896	2.12
Spreadability (cm^2)	24.6 ± 0.10	0.40
Centrifugation	Stable	-
pH	6.72 ± 0.05	0.76
CP content (%)	99.81 ± 0.38	0.38

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

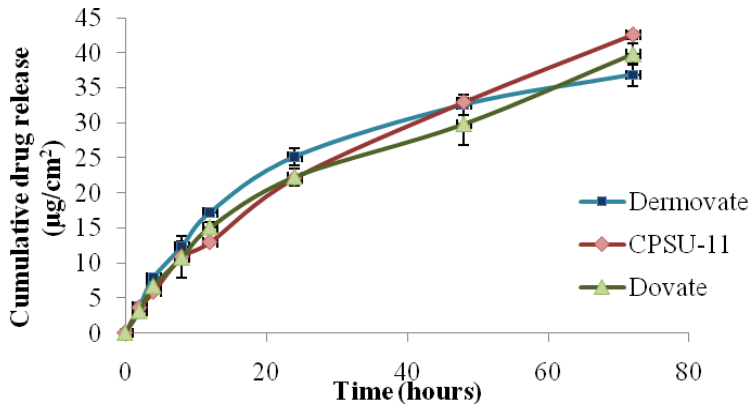
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-11
Date of Manufacture: 28/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	120
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	36316 ± 652	2.72
Spreadability (cm^2)	30.2 ± 0.5	1.62
Centrifugation	Stable	-
pH	6.51 ± 0.02	0.23
CP content (%)	98.38 ± 1.01	1.03

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

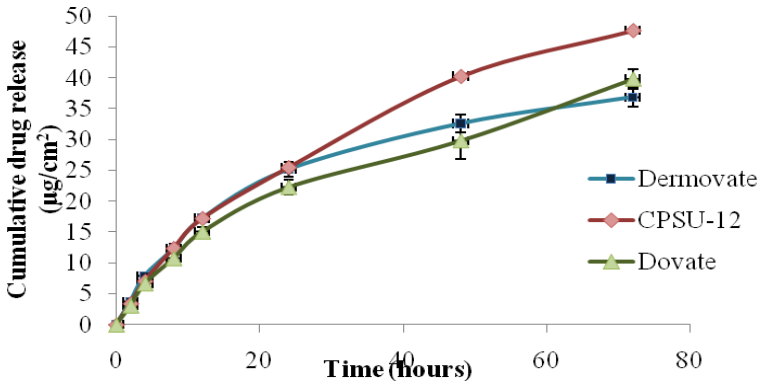
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-12
Date of Manufacture: 26/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	15
Homogenisation time (min)	100
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	22733 ± 379	1.99
Spreadability (cm^2)	30.8 ± 0.25	0.82
Centrifugation	Stable	-
pH	6.35 ± 0.03	0.55
CP content (%)	98.59 ± 0.66	0.67

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

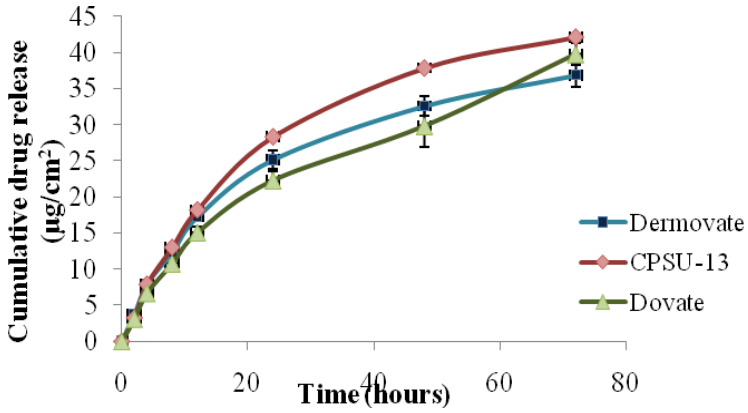
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-13
Date of Manufacture: 25/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	35
Homogenisation time (min)	60
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	50133 ± 351	0.70
Spreadability (cm^2)	21.1 ± 0.2	1.11
Centrifugation	Stable	-
pH	6.65 ± 0.03	0.45
CP content (%)	98.86 ± 0.31	0.31

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

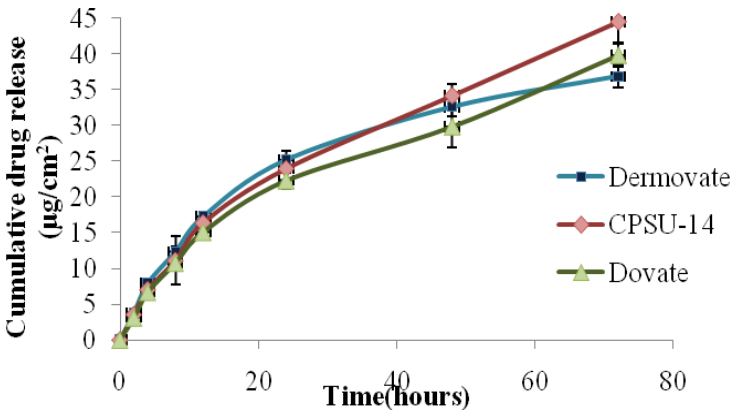
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-14
Date of Manufacture: 29/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	32800 ± 700	2.13
Spreadability (cm^2)	28.5 ± 0.5	1.92
Centrifugation	Stable	-
pH	6.65 ± 0.03	0.37
CP content (%)	99.92 ± 0.60	0.60

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

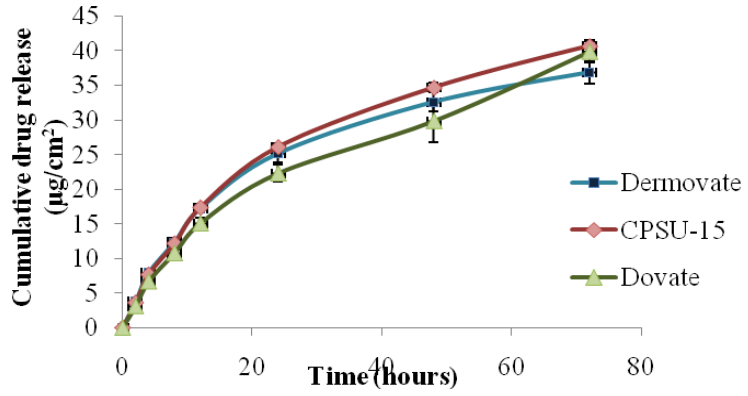
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-15
Date of Manufacture: 09/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	15
Homogenisation time (min)	100
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43933 ± 737	1.67
Spreadability (cm^2)	24.9 ± 0.5	2.05
Centrifugation	Stable	-
pH	6.89 ± 0.03	0.386
CP content (%)	98.09 ± 0.10	0.11

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

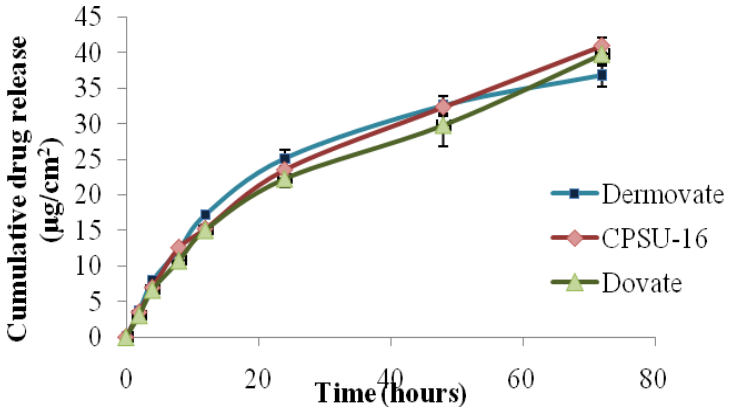
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-16
Date of Manufacture: 10/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	45
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	35833 ± 602	1.6842
Spreadability (cm^2)	26.1 ± 0.5	1.99
Centrifugation	Stable	-
pH	6.68 ± 0.05	0.79
CP content (%)	98.66 ± 1.35	1.35

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

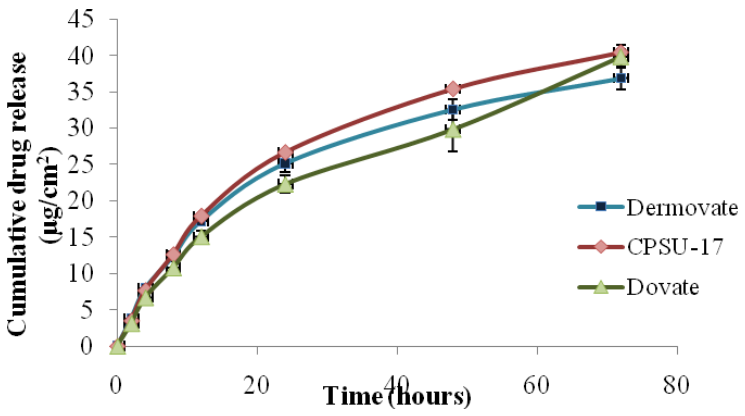
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-17
Date of Manufacture: 24/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	35
Homogenisation time (min)	60
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	43600 ± 556	1.27
Spreadability (cm^2)	25.5 ± 0.8	3.5
Centrifugation	Stable	-
pH	6.74 ± 0.03	0.51
CP content (%)	99.77 ± 0.73	0.73

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

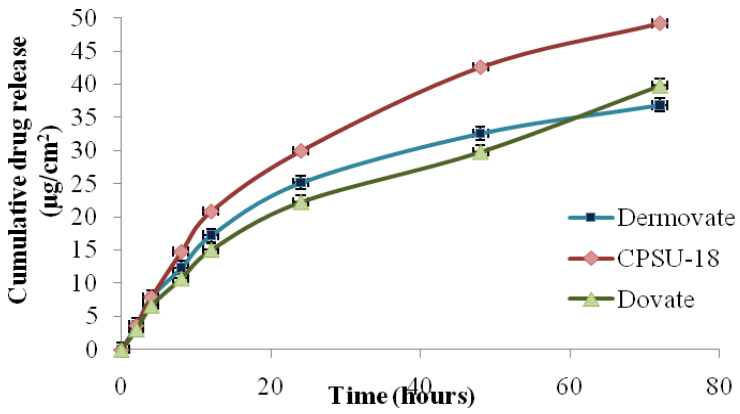
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-18
Date of Manufacture: 25/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment mixer parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	15
Homogenisation time (min)	60
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	18333 ± 832	5.67
Spreadability (cm^2)	34.9 ± 0.51	1.45
Centrifugation	Stable	-
pH	6.34 ± 0.02	0.33
CP content (%)	98.82 ± 0.47	0.48

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

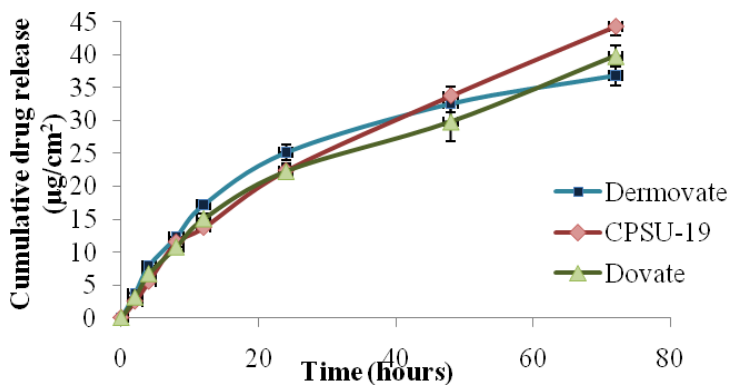
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-19
Date of Manufacture: 24/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	33566 ± 1250	5.32
Spreadability (cm^2)	29.3 ± 1.7	5.78
Centrifugation	Stable	-
pH	6.48 ± 0.05	0.79
CP content (%)	101.42 ± 0.31	0.31

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

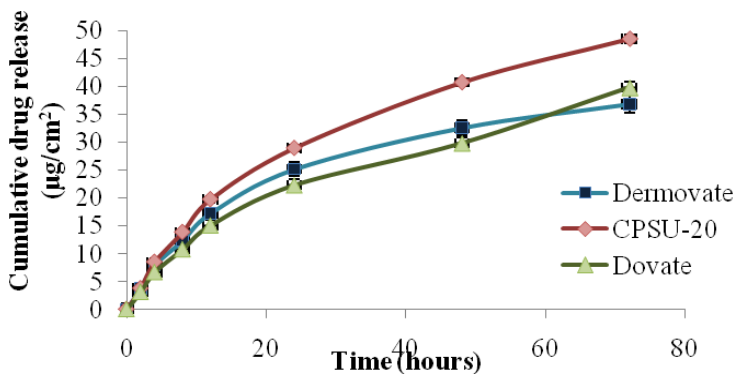
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-20
Date of Manufacture: 23/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	15
Homogenisation time (min)	100
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	20833 ± 802	4.25
Spreadability (cm^2)	32.4 ± 0.7	2.39
Centrifugation	Stable	-
pH	6.55 ± 0.04	0.62
CP content (%)	99.49 ± 0.21	0.21

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, slightly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

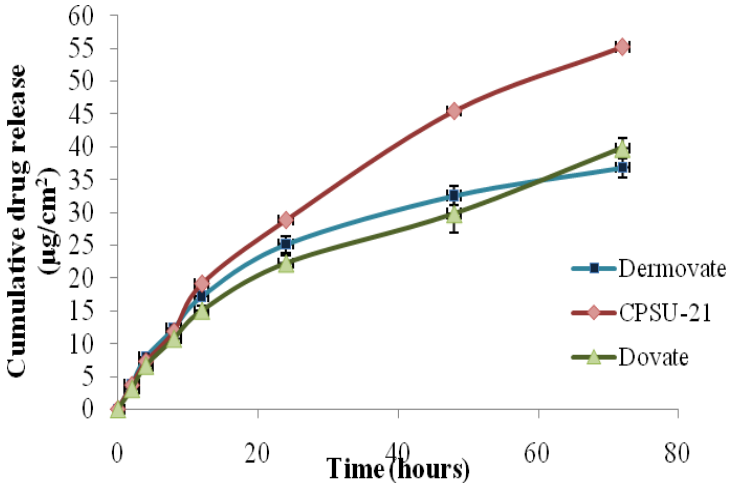
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-21
Date of Manufacture: 13/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	100
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	10800 ± 655	5.07
Spreadability (cm^2)	41.1 ± 2.86	6.97
Centrifugation	Stable	-
pH	6.94 ± 0.01	0.24
CP content (%)	98.88 ± 0.36	0.36

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, slightly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

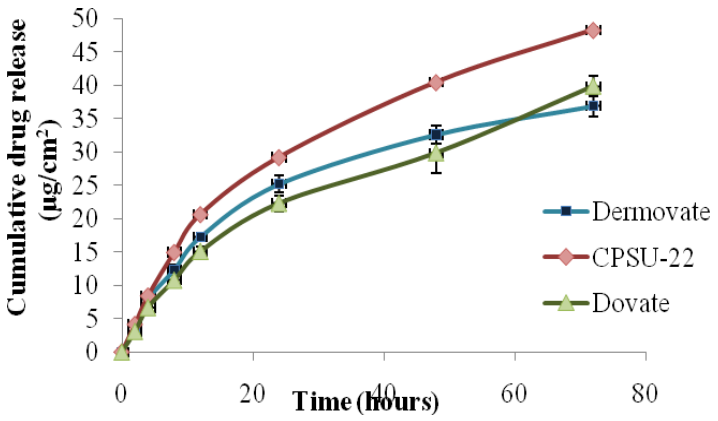
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-22
Date of Manufacture: 24/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	15
Homogenisation time (min)	60
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	20900 ± 624	4.49
Spreadability (cm^2)	33.5 ± 1.5	4.34
Centrifugation	Stable	-
pH	6.49 ± 0.11	1.63
CP content (%)	99.62 ± 2.05	2.07

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, slightly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

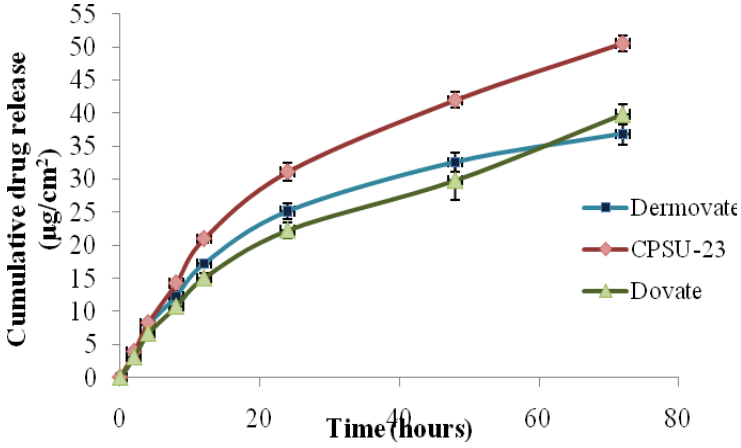
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-23
Date of Manufacture: 19/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	5
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	29633 ± 404	1.36
Spreadability (cm^2)	31.5 ± 1.5	4.83
Centrifugation	Stable	-
pH	6.62 ± 0.04	0.57
CP content (%)	99.49 ± 1.34	1.34

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, slightly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

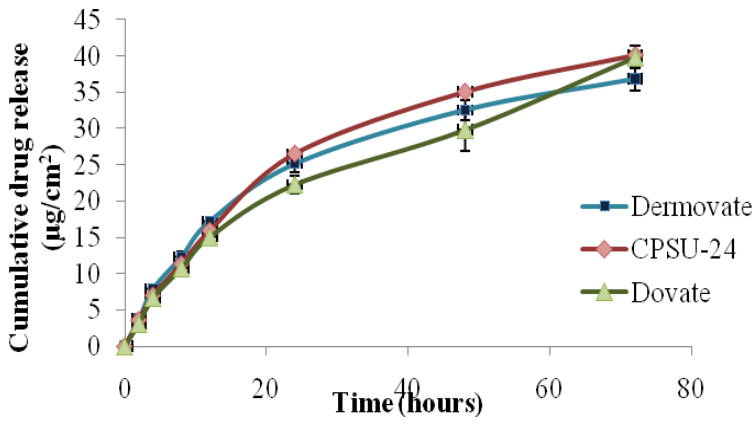
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-24
Date of Manufacture: 26/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	15
Homogenisation time (min)	100
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	48506 ± 1732	3.32
Spreadability (cm^2)	23.9 ± 0.7	2.81
Centrifugation	Stable	-
pH	6.17 ± 0.08	1.23
CP content (%)	99.39 ± 1.90	1.91

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

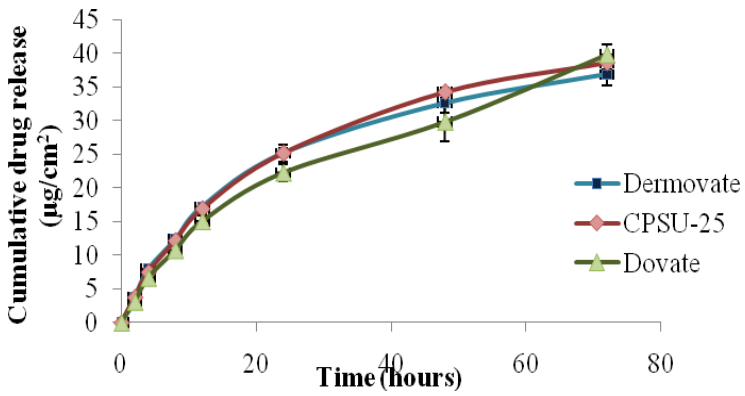
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-25
Date of Manufacture: 20/04/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1900
Anchor speed (rpm)	35
Homogenisation time (min)	100
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	52966 ± 2513	4.45
Spreadability (cm^2)	22.1 ± 2.5	2.26
Centrifugation	Stable	-
pH	6.13 ± 0.10	1.69
CP content (%)	98.79 ± 0.49	0.49

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

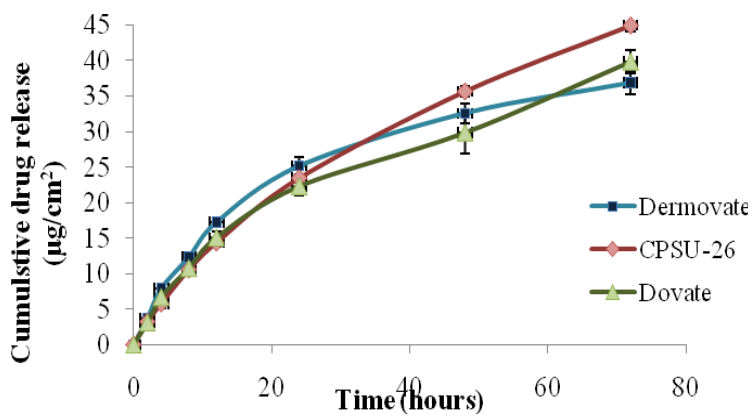
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-26
Date of Manufacture: 22/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	80

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	33766 ± 862	2.55
Spreadability (cm^2)	28.2 ± 0.1	0.35
Centrifugation	Stable	-
pH	6.47 ± 0.03	0.46
CP content (%)	98.89 ± 0.45	0.45

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

RHODES UNIVERSITY, Faculty of Pharmacy, Department of Pharmaceutics
Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

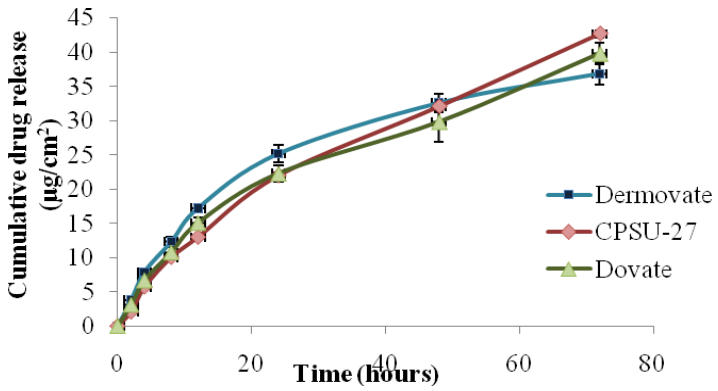
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-27
Date of Manufacture: 25/04/2010
Melting Temperature: 70°C
Batch Size : 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	1300
Anchor speed (rpm)	25
Homogenisation time (min)	80
Cooling time (min)	120

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	36166 ± 569	2.60
Spreadability (cm^2)	30.1 ± 0.7	2.18
Centrifugation	Stable	-
pH	6.31 ± 0.11	1.80
CP content (%)	98.76 ± 1.10	1.12

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

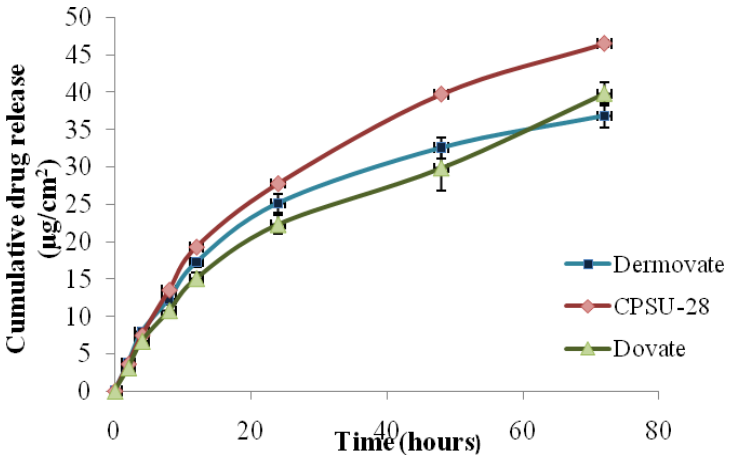
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-28
Date of Manufacture: 12/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	35
Homogenisation time (min)	60
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	25866 ± 550	2.13
Spreadability (cm^2)	33.5 ± 0.6	1.77
Centrifugation	Stable	-
pH	6.72 ± 0.05	0.81
CP content (%)	99.06 ± 0.17	0.17

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

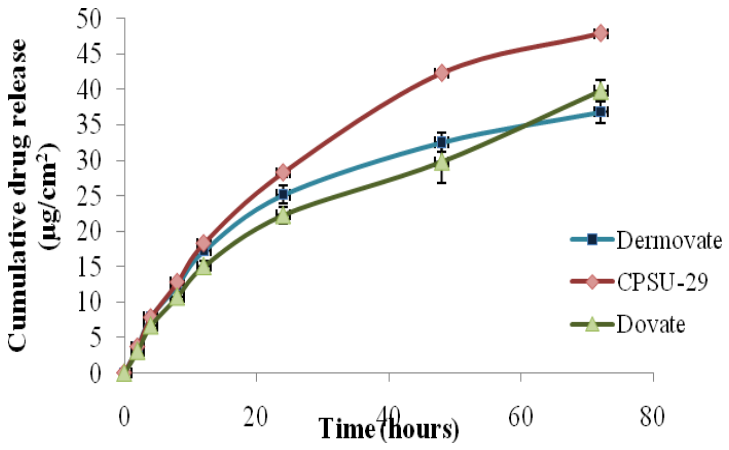
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-29
Date of Manufacture: 21/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	35
Homogenisation time (min)	100
Cooling time (min)	60

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	27600 ± 556	2.01
Spreadability (cm^2)	32.5 ± 0.5	1.80
Centrifugation	Stable	-
pH	6.56 ± 0.05	0.68
CP content (%)	98.90 ± 0.45	0.45

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, slightly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

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Grahamstown 6140, SOUTH AFRICA

Batch Summary Record

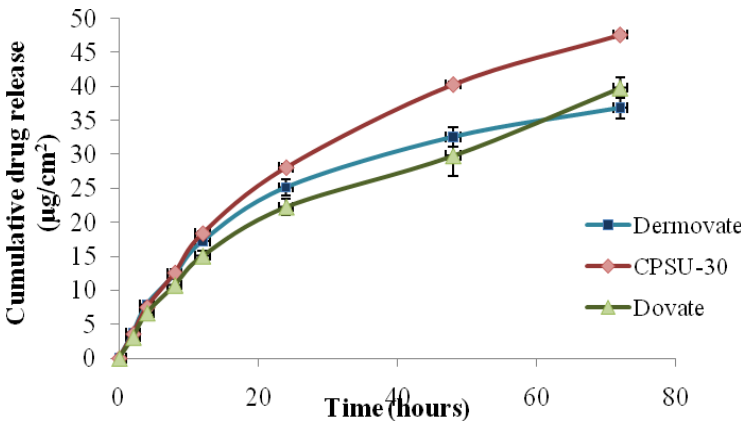
Formulator: Ayeshah Fauzee
Product: Clobetasol 17-propionate scale-up cream
Batch Number: CPSU-30
Date of Manufacture: 15/05/2010
Melting Temperature: 70°C
Batch Size: 5000 g

Excipient Name	Original Formula(% w/w)	Working Formula	Rhodes Batch Number
CP	0.05	2.50 g	RM000150
Propylene glycol	46.00	2300.00 ml	RM000181
Sodium citrate	0.05	2.50 g	RM000183
Citric acid	0.05	2.50 g	RM000185
Gelot® 64	3.00	150.00 g	RM000177
Glyceryl monostearate	11.00	550.00 g	RM000182
Ceteostearyl alcohol	8.40	420.00 g	RM000184
White beeswax	1.15	57.50 g	RM000142
Chlorocresol	0.075	3.75 g	RM000186
Distilled water	30.225	1511.25 ml	N/A

Wintech® Cream/Ointment Mixer Parameters	
Homogenisation speed (rpm)	700
Anchor speed (rpm)	35
Homogenisation time (min)	60
Cooling time (min)	100

Tests Performed	Mean ± SD (n=3)	%RSD
Viscosity (cP)	27066 ± 802	2.96
Spreadability (cm^2)	32.5 ± 0.6	1.82
Centrifugation	Stable	-
pH	6.59 ± 0.00	0.08
CP content (%)	100.31 ± 1.09	1.09

IN VITRO RELEASE PROFILE



COMMENTS/OBSERVATIONS

- A white homogenous cream was produced
- The cream was smooth, slightly viscous without any form of grittiness
- No sign of physical instability [cracking or bleeding] occurred during or after manufacturing

APPENDIX V

Table 5.13. *Summary of ANOVA analysis of in vitro release profiles for CPSU and Dermovate®*

Time (hrs)	Comparison*	Mean Difference** (R-CPLS)	95% Confidence interval for mean difference		P-value	Summary
			Lower Limit	Upper Limit		
2	**R vs 01**	0.3867	0.07684	0.6965	P < 0.01	Significant
	R vs 02	-1.473	-1.783	-1.164	P < 0.0001	Significant
	R vs 03	-1.377	-1.686	-1.067	P < 0.0001	Significant
	R vs 04	0.3233	0.01351	0.6332	P < 0.05	Significant
	R vs 05	1.557	1.247	1.866	P < 0.0001	Significant
	R vs 06	0.5867	0.2768	0.8965	P < 0.0001	Significant
	R vs 07	0.3867	0.07684	0.6965	P < 0.001	Significant
	R vs 08	0.8633	0.5535	1.173	P < 0.0001	Significant
	R vs 09	0.4800	0.1702	0.7897	P < 0.0001	Significant
	R vs 10	0.3133	0.003507	0.6232	P < 0.05	Significant
	R vs 11	0.1800	-0.1298	0.4894	P > 0.05	Not significant
	R vs 12	0.4300	0.1202	0.7398	P < 0.001	Significant
	R vs 13	0.6633	0.3535	0.9732	P < 0.0001	Significant
	R vs 14	0.2667	-0.04316	0.5765	P > 0.05	Not significant
	R vs 15	0.1767	-0.1332	0.4865	P > 0.05	Not significant
	R vs 16	0.3233	0.01351	0.6332	P < 0.05	Significant
	R vs 17	0.3367	0.02684	0.6465	P < 0.05	Significant
	R vs 18	-1.163	-1.473	-0.8535	P < 0.0001	Significant
	R vs 19	0.3433	0.03351	0.6532	P < 0.05	Significant
	R vs 20	0.003333	-0.3065	0.3132	P > 0.05	Not significant
	R vs 21	0.1167	-0.1932	0.4265	P > 0.05	Not significant
	R vs 22	-0.5800	-0.8898	-0.2702	P < 0.0001	Significant
	R vs 23	-0.2000	-0.5098	0.1098	P > 0.05	Not significant
	R vs 24	0.3033	-0.006493	0.6132	P > 0.05	Not significant
	R vs 25	-0.02000	-0.3298	0.2898	P > 0.05	Not significant
	R vs 26	0.7533	0.4435	1.063	P < 0.0001	Significant
	R vs 27	2.250	1.940	2.560	P < 0.0001	Significant
	R vs 28	0.1900	-0.1198	0.4998	P > 0.05	Not significant
	R vs 29	0.1000	-0.2098	0.4098	P > 0.05	Not significant
	R vs 30	0.2500	-0.05983	0.5598	P > 0.05	Not significant
4	**R vs 01**	3.2349	2.946	3.551	P < 0.0001	Significant
	R vs 02	-1.203	-1.506	-0.9007	P < 0.0001	Significant

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	R vs 03	-1.277	-1.579	-0.9740	P < 0.001	Significant
	R vs 04	0.1633	-0.1393	0.4660	P > 0.05	Not Significant
	R vs 05	3.093	2.791	3.396	P < 0.0001	Significant
	R vs 06	1.193	0.8907	1.496	P < 0.0001	Significant
	R vs 07	1.693	1.391	1.996	P < 0.0001	Significant
	R vs 08	1.157	0.8540	1.459	P < 0.0001	Significant
	R vs 09	1.263	0.9607	1.566	P < 0.0001	Significant
	R vs 10	0.2033	-0.09934	0.5060	P > 0.05	Not significant
	R vs 11	2.727	2.424	3.029	P < 0.0001	Significant
	R vs 12	1.103	0.8007	1.406	P < 0.0001	Significant
	R vs 13	0.0200	-0.2827	0.3227	P > 0.05	Not significant
	R vs 14	1.187	0.8840	1.489	P < 0.0001	Significant
	R vs 15	0.2933	-0.009337	0.5960	P > 0.05	Not significant
	R vs 16	1.233	0.9307	1.536	P < 0.0001	Significant
	R vs 17	0.2700	-0.03267	0.5727	P > 0.05	Not significant
	R vs 18	-0.06000	0.9407	0.2427	P > 0.05	Not significant
	R vs 19	1.243	-1.163	1.546	P < 0.0001	Significant
	R vs 20	-0.8600	0.5573	-0.5573	P < 0.0001	Significant
	R vs 21	0.8600	-0.9960	1.163	P < 0.0001	Significant
	R vs 22	-0.6933	-0.7660	-0.3907	P < 0.0001	Significant
	R vs 23	-0.4633	0.8807	-0.1607	P < 0.0001	Significant
	R vs 24	1.183	0.2573	1.486	P < 0.0001	Significant
	R vs 25	0.5600	2.654	0.8627	P < 0.0001	Significant
	R vs 26	2.957	2.731	3.259	P < 0.0001	Significant
	R vs 27	3.033	0.2673	3.336	P < 0.0001	Significant
	R vs 28	0.5700	-0.1827	0.8727	P < 0.0001	Significant
	R vs 29	0.1200	0.2307	0.4226	P > 0.05	Not significant
	R vs 30	0.5333	-4.755	0.8360	P < 0.0001	Significant
8	**R vs 01**	3.078	2.725	3.428	P < 0.0001	Significant
	R vs 02	-3.720	-4.071	-3.369	P < 0.0001	Significant
	R vs 03	-3.830	-4.181	-3.479	P < 0.0001	Significant
	R vs 04	-0.100	-0.4513	0.2513	P > 0.05	Not Significant
	R vs 05	1.113	0.7621	1.465	P < 0.0001	Significant
	R vs 06	1.483	1.132	1.835	P < 0.0001	Significant
	R vs 07	2.537	2.185	2.888	P < 0.0001	Significant
	R vs 08	1.097	0.7454	1.448	P < 0.0001	Significant
	R vs 09	0.4200	0.06874	0.7713	P < 0.01	Significant
	R vs 10	-1.516	-1.868	-1.165	P < 0.0001	Significant
	R vs 11	1.970	1.619	2.321	P < 0.0001	Significant
	R vs 12	-0.08000	-0.4313	0.2713	P > 0.05	Not significant
	R vs 13	-0.8833	-1.235	-0.5321	P < 0.0001	Significant

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	R vs 14	1.667	1.315	2.018	P < 0.0001	Significant
	R vs 15	0.2930	-0.05826	0.6443	P > 0.05	Not significant
	R vs 16	-0.4600	-0.8113	-0.1087	P < 0.001	Significant
	R vs 17	-0.3940	-0.7453	-0.04274	P < 0.01	Significant
	R vs 18	-3.270	-3.721	-3.019	P < 0.0001	Significant
	R vs 19	3.423	3.072	3.775	P < 0.0001	Significant
	R vs 20	-1.977	-2.328	-1.625	P < 0.0001	Significant
	R vs 21	0.6667	0.3154	1.018	P < 0.0001	Significant
	R vs 22	1.117	0.7654	1.468	P < 0.0001	Significant
	R vs 23	-2.717	-3.068	-2.365	P < 0.0001	Significant
	R vs 24	1.160	0.8087	1.511	P < 0.0001	Significant
	R vs 25	0.01333	-0.3379	0.3646	P > 0.05	Not significant
	R vs 26	2.390	2.039	2.741	P < 0.0001	Significant
	R vs 27	3.097	2.745	3.448	P < 0.0001	Significant
	R vs 28	-1.593	-1.945	-1.242	P < 0.0001	Significant
	R vs 29	-0.6533	-1.005	-0.3021	P < 0.0001	Significant
	R vs 30	-0.3800	-0.7313	-0.02874	P < 0.005	Significant
12	**R vs 01**	3.953	3.540	4.367	P < 0.0001	Significant
	R vs 02	-5.837	-6.250	-5.423	P < 0.0001	Significant
	R vs 03	-7.453	-7.867	-7.040	P < 0.0001	Significant
	R vs 04	-0.1567	-0.5703	0.2569	P > 0.05	Not Significant
	R vs 05	4.803	4.390	5.217	P < 0.0001	Significant
	R vs 06	2.360	1.947	2.774	P < 0.0001	Significant
	R vs 07	3.153	2.740	3.567	P < 0.0001	Significant
	R vs 08	1.240	0.8264	1.654	P < 0.0001	Significant
	R vs 09	-0.8667	-1.280	-0.4531	P < 0.0001	Significant
	R vs 10	-2.493	-2.907	-2.080	P < 0.0001	Significant
	R vs 11	5.747	5.333	6.160	P < 0.0001	Significant
	R vs 12	0.02000	-0.3936	0.4336	P > 0.05	Not significant
	R vs 13	-1.240	1.654	-0.8264	P < 0.0001	Significant
	R vs 14	1.177	0.7631	1.590	P < 0.0001	Significant
	R vs 15	-0.1033	-0.5169	0.3103	P > 0.05	Not significant
	R vs 16	2.746	2.333	3.161	P < 0.0001	Significant
	R vs 17	-0.8867	-1.300	-0.4731	P < 0.0001	Significant
	R vs 18	-4.983	-5.397	-4.570	P < 0.0001	Significant
	R vs 19	1.287	0.8731	1.700	P < 0.0001	Significant
	R vs 20	-3.427	-3.840	-3.013	P < 0.0001	Significant
	R vs 21	-2.553	-2.967	-2.140	P < 0.0001	Significant
	R vs 22	4.780	4.366	5.194	P < 0.0001	Significant
	R vs 23	-5.103	-5.517	-4.690	P < 0.0001	Significant

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	R vs 24	1.800	1.386	2.214	P < 0.0001	Significant
	R vs 25	0.7800	0.3664	1.194	P < 0.001	Significant
	R vs 26	3.893	3.480	4.307	P < 0.0001	Significant
	R vs 27	5.793	5.380	6.207	P < 0.0001	Significant
	R vs 28	-2.800	-3.314	-2.386	P < 0.0001	Significant
	R vs 29	-1.427	-1.840	-1.013	P < 0.0001	Significant
	R vs 30	-1.557	-1.970	-1.143	P < 0.0001	Significant
24	**R vs 01**	3.623	2.891	4.356	P < 0.0001	Significant
	R vs 02	-7.187	-7.919	-6.454	P < 0.0001	Significant
	R vs 03	-7.237	-7.969	-6.504	P < 0.0001	Significant
	R vs 04	0.4267	-0.3058	1.159	P > 0.05	Not significant
	R vs 05	3.787	3.054	4.519	P < 0.0001	Significant
	R vs 06	1.790	1.058	2.522	P < 0.0001	Significant
	R vs 07	6.087	5.355	6.819	P < 0.0001	Significant
	R vs 08	0.8067	0.07421	1.539	P < 0.05	Significant
	R vs 09	-1.777	-2.509	-1.044	P < 0.0001	Significant
	R vs 10	-3.843	-4.575	-3.111	P < 0.0001	Significant
	R vs 11	4.190	3.458	4.922	P < 0.0001	Significant
	R vs 12	-0.4833	-1.216	0.2491	P > 0.05	Not significant
	R vs 13	-4.353	-5.086	-3.621	P < 0.0001	Significant
	R vs 14	1.557	0.8242	2.289	P < 0.0001	Significant
	R vs 15	-1.320	-2.052	-0.5875	P < 0.0001	Significant
	R vs 16	2.146	1.414	2.879	P < 0.0001	Significant
	R vs 17	-2.008	-2.739	-1.264	P < 0.0001	Significant
	R vs 18	-6.583	-7.136	-5.851	P < 0.0001	Significant
	R vs 19	1.030	0.2975	1.762	P < 0.001	Significant
	R vs 20	-5.237	-5.969	-4.504	P < 0.0001	Significant
	R vs 21	-4.970	-5.702	-4.238	P < 0.0001	Significant
	R vs 22	3.777	3.044	4.509	P < 0.0001	Significant
	R vs 23	-8.120	-8.852	-7.388	P < 0.0001	Significant
	R vs 24	-1.868	-2.601	-1.136	P < 0.0001	Significant
	R vs 25	0.0500	-0.6825	0.7825	P > 0.05	Not significant
	R vs 26	2.430	1.698	3.1263	P < 0.0001	Significant
	R vs 27	-3.550	3.608	5.072	P < 0.0001	Significant
	R vs 28	-4.250	-4.282	-2.818	P < 0.0001	Significant
	R vs 29	-4.003	-4.982	-3.518	P < 0.0001	Significant
	R vs 30	-10.810	-4.736	-3.271	P < 0.0001	Significant
48	**R vs 01**	-1.370	-1.742	-0.9983	P < 0.0001	Significant
	R vs 02	-5.737	-5.745	-5.003	P < 0.0001	Significant
	R vs 03	-5.027	-5.398	-4.655	P < 0.0001	Significant

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	R vs 04	-0.1100	-0.4817	0.2617	P > 0.05	Not significant
	R vs 05	-1.573	-1.945	-1.202	P < 0.0001	Significant
	R vs 06	-2.160	-2.522	-1.778	P < 0.0001	Significant
	R vs 07	8.493	8.122	8.865	P < 0.0001	Significant
	R vs 08	-1.277	-1.648	-0.9049	P < 0.0001	Significant
	R vs 09	-6.140	-6.512	-5.868	P < 0.0001	Significant
	R vs 10	-3.530	-3.902	-3.158	P < 0.0001	Significant
	R vs 11	-0.4900	-0.8617	-0.1183	P < 0.001	Significant
	R vs 12	-10.45	-10.82	10.08	P < 0.0001	Significant
	R vs 13	-7.173	-7.545	-6.802	P < 0.0001	Significant
	R vs 14	-2.153	-2.525	-1.785	P < 0.0001	Significant
	R vs 15	-2.893	-3.265	-2.522	P < 0.0001	Significant
	R vs 16	0.2200	-0.1517	0.5917	P > 0.05	Not significant
	R vs 17	-3.863	-4.235	-3.492	P < 0.0001	Significant
	R vs 18	-13.63	-14.00	-13.25	P < 0.0001	Significant
	R vs 19	-2.377	-2.748	-2.005	P < 0.0001	Significant
	R vs 20	-11.12	-11.50	-10.75	P < 0.0001	Significant
	R vs 21	-17.49	-17.86	-17.12	P < 0.0001	Significant
	R vs 22	-1.410	-1.782	-1.038	P < 0.0001	Significant
	R vs 23	-12.75	-13.12	-12.38	P < 0.0001	Significant
	R vs 24	-3.277	-3.648	-2.905	P < 0.0001	Significant
	R vs 25	-2.210	-2.582	-1.838	P < 0.0001	Significant
	R vs 26	-4.113	-4.485	-3.742	P < 0.0001	Significant
	R vs 27	0.7167	0.3449	1.088	P < 0.0001	Significant
	R vs 28	-9.760	-10.13	-9.388	P < 0.0001	Significant
	R vs 29	-13.33	-13.70	-12.96	P < 0.0001	Significant
	R vs 30	-10.54	-10.91	-10.17	P < 0.0001	Significant
72	**R vs 01**	-11.03	-11.22	-10.85	P < 0.0001	Significant
	R vs 02	-12.86	-13.05	-12.67	P < 0.0001	Significant
	R vs 03	-13.18	-13.37	-12.99	P < 0.0001	Significant
	R vs 04	0.01000	-0.1777	0.1977	P > 0.005	Not significant

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R vs 05	-10.18	-10.36	-9.986	P < 0.0001	Significant
R vs 06	-10.38	-10.46	-10.19	P < 0.0001	Significant
R vs 07	8.010	7.822	8.198	P < 0.0001	Significant
R vs 08	-4.040	-4.338	-3.852	P < 0.0001	Significant
R vs 09	-12.20	-12.39	-12.01	P < 0.0001	Significant
R vs 10	-7.000	-7.188	-6.812	P < 0.0001	Significant
R vs 11	-7.790	-7.978	-7.602	P < 0.0001	Significant
R vs 12	-14.66	-14.85	-14.46	P < 0.0001	Significant
R vs 13	-7.293	-7.481	-7.106	P < 0.0001	Significant
R vs 14	-10.38	-10.57	-10.20	P < 0.0001	Significant
R vs 15	-5.287	-5.474	-5.099	P < 0.0001	Significant
R vs 16	-4.813	-5.864	-5.489	P < 0.0001	Significant
R vs 17	-16.81	-5.001	-4.626	P < 0.0001	Significant
R vs 18	-10.80	-16.99	-16.62	P < 0.0001	Significant
R vs 19	-15.96	-10.99	-10.64	P < 0.0001	Significant
R vs 20	-25.98	-16.15	-15.77	P < 0.0001	Significant
R vs 21	-15.53	-25.17	-24.79	P < 0.0001	Significant
R vs 22	-18.66	-15.72	-15.34	P < 0.0001	Significant
R vs 23	-4.417	-18.75	-18.48	P < 0.0001	Significant
R vs 24	-2.020	-4.604	-4.229	P < 0.0001	Significant
R vs 25	-11.03	-2.208	-1.832	P < 0.0001	Significant
R vs 26	-7.907	-11.22	-10.84	P < 0.0001	Significant
R vs 27	-13.14	-8.064	-7.719	P < 0.0001	Significant
R vs 28	-15.07	-13.32	-12.95	P < 0.0001	Significant
R vs 29	-14.70	-15.25	-14.88	P < 0.0001	Significant
R vs 30	-1.827	-14.88	-14.51	P < 0.0001	Significant

***Comparison = Dermovate® (R) vs CPSU (-01 to -30)**

****Mean Difference = % Drug released Reference - % Drug released CPSU**

APPENDIX V1

RESEARCH PROTOCOL

APPLICATION OF SKIN BLANCHING STUDY FOR THE ASSESSMENT OF THE *IN VIVO* BIOEQUIVALENCE OF CLOBETASOL 17-PROPIONATE (CP) 0.05% W/W CREAM FORMULATIONS WITH RESPECT TO DERMOVATE® IN HEALTHY HUMAN VOLUNTEERS

STUDY NUMBER: DMV/CP 01-2010
BROCHURE VERSION: 12th AUG 2010

Reference Product	Dermovate® 0.05% w/w cream
Generic Product	Dovate® 0.05% w/w cream
Test Products	Two scale-up Clobetasol 17-propionate (CP) 0.05% w/w creams
Site	Faculty of Pharmacy, Rhodes University, Grahamstown 6140, South Africa

1. STUDY PROTOCOL

Study Number	DMV/CP 01-2010
Study Title	To assess the skin blanching effect for the *in vivo* bioequivalence study of clobetasol 17-propionate 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers
Reference Product	Dermovate® 0.05% w/w cream
Generic Product	Dovate® 0.05% w/w cream
Test Products	Two scale-up clobetasol 17-propionate 0.05% w/w creams

Principal Investigator **Prof. Roderick Walker** Faculty of Pharmacy, Rhodes University Grahamstown 6140, South Africa *Tel No:*	**Signature:**---------- **Date:**----------
Co-investigator **Dr. Mike Skinner** Faculty of Pharmacy, Rhodes University Grahamstown 6140, South Africa *Tel No:*	**Signature:**---------- **Date:**----------
Study Investigator & Chromameter Assessor **Ms. Ayeshah Fateemah B. Fauzee** Faculty of Pharmacy, Rhodes University Grahamstown 6140, South Africa *Tel No*:	**Signature:**---------- **Date:**----------
Study Visual Observer 1 **Prof. Roderick Walker** Faculty of Pharmacy, Rhodes University Grahamstown 6140, South Africa *Tel No:*	**Signature:**---------- **Date:**----------
Study Visual Observer 2 **Mr. Leon Purdon** Faculty of Pharmacy, Rhodes University Grahamstown 6140, South Africa *Tel No:*	**Signature:**---------- **Date:**----------

2. STUDY SUMMARY

2.1 Title: To assess the skin blanching effect for the *in vivo* bioequivalence study of CP 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers using visual and chromameter assessments.

2.2 Objective: To evaluate the *in vivo* bioequivalence of CP 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers using the skin blanching effect assessed by a vasoconstrictor assay technique.

2.3 Products:-
Reference product: Dermovate ® 0.05% w/w cream
Generic product: Dovate® 0.05% w/w cream
Test product: Two scale-up Clobetasol 17-propionate 0.05% w/w cream

2.4 Facilities: The pre-screening test will be conducted in the Biopharmaceutics Research Group (BRG) facility and the research study will be conducted in the Biopharmaceutics Research Institute (BRI) clinic, situated at Rhodes University, Grahamstown 6140. The BRI clinic will be staffed by the principal investigator, co-investigator, study investigator, visual observers and chromameter assessor.

2.5 Study design: An *in vivo* bioequivalence study will be done to compare the reference and the test products. A pilot dose duration-response study has already been determined by Au *et al.* [511]. Prior to the study, a pre-screening test will be done to allow the selection of volunteers for inclusion in that study.

2.6 Date and duration of study: The study will consist of one phase which will be carried out over a period of 30 hours.

2.7 Subject number: Twenty volunteers will be chosen for the pre-screening test in which fourteen subjects will be enrolled for the study. Dropouts will not be replaced during the study.

2.8 Subject characteristics: Healthy non-smoking male/female volunteers aged 18-60 years who have not been treated with any topical corticosteroids for at least two months prior to the study trial.

2.9 Pre-screening test: Volunteers will be selected according to the stated inclusion and exclusion criteria as mentioned in § 8.3 and § 8.4. Subjects will have to carry out a pre-screening questionnaire in which they will be asked general questions regarding their health condition as well as topical drug related questions. Subjects must look healthy and will be asked whether they suffer from any chronic or skin conditions. The pre-screening test will include subjects that demonstrate adequate vasoconstriction to topical corticosteroids.

2.10 Study procedure: After the pre-screening selection is done, the chosen subjects will be asked to read the "Information for volunteers brochure". Subjects will then be asked to provide their written consent to participate in the pivotal study. The subjects will check into the research facility at 07h30 on the day of study and will remain in the facility from check in until 30 hour assessment time. Subjects will be asked a brief medical history by the principal/subject investigator at the check-in, inclusion and exclusion criteria check and study restrictions check as mentioned in § 8.3, § 8.4 and § 8.5.

2.11 Duration of application: ED_{50}: 40 minutes, D_1: 20 minutes and D_2: 80 minutes.

2.12 Skin blanching assessment methods: Visual observation will be performed by two trained observers on a multiple unit scale of 0-4 and chromameter assessment will be done using a Minolta® Model CR 400 chromameter (Minolta® Ltd, Osaka, Japan).

2.13 Skin blanching assessment times: At t =2, 4, 6, 8, 10, 12, 14, 22, 24 and 30 hours

2.14 Statistical analysis: For visual assessment, a graph of a percentage total possible score (%TPS) will be plotted against time in hours whereas for chromameter assessment, an average of *a*-scale values against time in hours will be plotted respectively for each formulation. The area under the curve (AUEC) for each formulation will be calculated using the trapezoidal rule. Statistical analysis will be carried out using a Locke's method to determine the bioequivalence of the formulations using data for all subjects.

3. BACKGROUND INFORMATION

3.1 Structure of Clobetasol 17-propionate (CP)

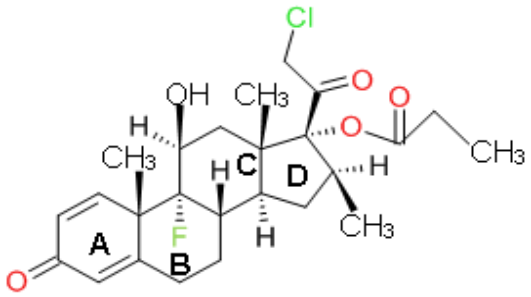

Figure 1. *Molecular Structure of Clobetasol 17-propionate*

3.2 Mode of Action

CP is a potent steroid which has high glucocorticoid and low mineralocorticoid activity [41,73]. Since CP has high glucocorticoid activity, it can bind with high affinity to glucocorticoid receptors following intravenous administration. CP has both catabolic and anti-anabolic effects on proteins in the peripheral tissues and may also cause insulin resistance and impair peripheral glucose utilization [18]. CP passes readily through cellular membranes and causes immediate vasoconstriction in the vascular tissues but however this mechanism of action is not really known. CP also inhibits the adherence of neutrophils and monocyte-macrophages to endothelial cells of capillaries at the site of inflammation and blocks the effect of macrophage migration inhibitory factor whilst decreasing the conversion of inactive plasminogen to active plasmin [7]. CP therefore has immunosuppressive anti-proliferative, anti-pruritic, vasoconstrictive and anti-inflammatory effects [12,13,15,16,18].

3.3 Uses

CP topical formulations are used for the initial control of all forms of hyperacute eczema, chronic hyperkeratotic eczema, patches of chronic lichen simplex, chronic hyperkeratotic psoriasis, severe acute photosensitivity, acute contact dermatitis, hypertrophic lichen planus, localised bullous planus, keloid scarring, pretibial myxoedema, vitiligo and for the suppression of a reaction following cryotherapy. CP cream is also indicated for use in the short-term topical treatment of inflammatory and pruritic manifestations of moderate-to-severe corticosteroid-responsive dermatoses and for short-term topical treatment of mild-to-moderate plaque-type psoriasis [18,75,76].

3.4 Dose

The action of corticosteroids usually lasts for six to eight hours only and therefore formulations are applications are best applied three times a day. Since CP is a high potency topical corticosteroid, 0.05% w/w CP cream should be applied in a thin layer to the affected areas of the skin twice daily, preferably in the morning and evening with gentle and complete rubbing. Treatment with corticosteroid formulation beyond two consecutive weeks is not recommended due to the potent nature of many of these compounds. Due to potential for CP to suppress the Hypothalamic-Pituitary-Adrenal (HPA) axis, the total dose should not exceed 50 g per week [74,79,80].

3.6 Contraindications

The use of topical corticosteroids is absolutely contraindicated in patients with primary bacterial infections and may mask bacterial infections due to their vasoconstrictive and anti-inflammatory properties [85]. Impetigo, furuncles and carbuncles, paronychia, ecthyma, erysipelas, cellulitis, lymphagitis and erythrasma are all conditions in which the use of topical steroids must be avoided. Patients suffering from cutaneous infections that are caused by viruses, bacteria or fungi should not be treated with topical corticosteroids. The use of topical CP in patients with acne vulgaris can result in suppression of the inflammatory response but that may be followed by serious rebound if the therapy is stopped. In the majority of patients, rosacea responds dramatically to topical CP preparations but breakthrough occurs rapidly [18]. Additional contraindications include the use of steroids in the treatment of candida and dermatophytic infections as the organisms thrive on macerated skin. The use of topical corticosteroids in conjunction with an appropriate anti-infective agent may occasionally be appropriate. In order to treat herpes simplex and zoster-customary, the virus itself must be subjected to aggressive treatment. High potent steroids such as CP may have a tendency to cause topical side effects such as atrophy, stria, purpura or even ulceration. Special precautions must be taken when applying topical steroids around the eye as their use may precipitate serious glaucoma. CP topical formulations are highly contraindicated for use in patients with a history of hypersensitivity to any of the components of the formulations [85,86].

3.7 Adverse Effects

Most common observed local side effects of CP are burning, itching, dryness, irritation, purpura, acne and telangietasis, hypertrichosis, hypopigmentation, glaucoma and allergic contact dermatitis. Skin atrophy and thinning of the skin, the development of stria, telangiectasis, subcutaneous haemorrhage and easy bruising and bleeding may occur as a consequence of the long-term use of topical corticosteroids [91,92]. Patients presenting with psoriasis may become prone to papulopustular flare following withdrawal of topical corticosteroid therapy if large areas are treated with high potency agent for prolonged periods of time. Systemic reactions or potentially life-threatening effects can occur which may lead to adrenal suppression, Cushing's syndrome, diabetes and hypertension. It has also been reported that the misuse of super potent topical corticosteroids has resulted in death due to Addisonian-type crises [9,91].

4. STUDY DRUGS

4.1 Description

Table 1. *The details regarding the different CP products used*

	Reference Product	**Generic Product**	**Test Products**
Name	Dermovate® 0.05% w/w	Dovate® 0.05% w/w	Scale-up 0.05% w/w CP
Active Ingredient	Clobetasol 17-propionate	Clobetasol 17- propionate	Clobetasol 17-propionate
Dosage Form	Topical cream	Topical cream	Topical creams
Manufacturer	Sekpharma Pty Ltd, Gauteng, South Africa	Aspen Pharmacare Ltd, Port-Elizabeth, South Africa	Rhodes University, Faculty of Pharmacy, Grahamstown 6140, South Africa
Date of Manufacture	-	-	One week before study
Expiry Date	04/2012	04/2013	-
Batch Number	E750008	295938	CPSU-04, CPSU-16

4.2 Manufacturing Procedure, Supply and Storage

The reference product, Dermovate® 0.05% w/w cream and the generic product, Dovate® 0.05% w/w cream will be purchased in sufficient amount from the respective pharmaceutical manufacturers and will be kept at a temperature of 22°C. The scale-up test products (5000 g) will be manufactured in a Rhodes University cream manufacturing under the principles of Good Manufacturing Practice (GMP) and stored at 22°C in an opaque container. Each product quantity, batch number and expiry dates will be recorded appropriately in a register and the quantity of cream used will also be noted. Unused product will be kept for a maximum of a year after the final report has been submitted and will then be disposed accordingly in the appropriate waste disposal container.

5. OBJECTIVE

Thirty batches of scale-up cream formulations of CP (5000 g) were manufactured using a Wintech® cream/ointment mixer (Model WLP/13/2006-2007, Wintech® Pharmachem Equipments PVT. Ltd, Mumbai, India). The two scale-up formulations that gave the most desirable *in vitro* release profile similar to Dermovate® and Dovate® were chosen to be the two test products (CPSU) for this study. Therefore it was necessary to evaluate the *in vivo* bioequivalence of CP 0.05% w/w cream formulations with respect to Dermovate® in healthy human volunteers using the skin blanching technique.

6. STUDY DESIGN

Prior to the study, a pre-screening test will be conducted to allow the selection of volunteers for inclusion in this study. One application site on the upper forearm will be demarcated using a pre-punched adhesive template exposing a 1.1 cm x 1.1 cm square for the application of the reference product, Dermovate®. Approximately 10 µl which is equivalent to 11 mg will be applied on the site of application using a 10 µl Eppendorf® micropipette and will be uniformly spread using a glass rod. The dose duration will be 40 minutes. The remaining topical corticosteroid will be gently removed from the skin by three consecutive swabbing using wet and dry cotton swabs. A visual assessment evaluation will be done six hours after the drug product removal to see if any skin blanching effect has taken place. Any volunteer who will experience a degree of blanching on a multiple unit scale of 1-4, where 1 is intermediate degree of blanching and 4 is intense blanching will be selected for the pivotal study.

The study will consist of fourteen healthy human subjects who showed positive skin blanching response in the pre-screening test. According to the FDA guidelines [522], both visual and chromameter assessment methods will be used to evaluate the degree of skin blanching on the subjects. Fourteen application sites will be used per forearm and will be demarcated using a pre-punched adhesive label template exposing a 1.1 cm x 1.1 cm square for the application of the reference, generic and two CP creams. Four cream formulations of 0.05% w/w CP, Dermovate® (reference product), Dovate® (generic product) and scale-up CP creams (test products) will be used in this study. Approximately 10 µl, equivalent to 11 mg of creams will be applied to the designated application sites on each forearm using 10 µl Eppendorf® micropipettes and will be uniformly spread using a glass rod.

The FDA Guidance [522] mentions three different dose durations (ED_{50}, D_1, and D_2) to be used. ED_{50} is the dose duration at which half of the skin blanching effect is achieved. D_1 is the dose duration equal to half of the ED_{50} whereas D_2 is the dose duration that doubles that of ED_{50}. The dose duration values for CP cream were determined by Au *et al.* [511], where a pilot study was conducted. ED_{50} chosen for both reference (R_1), generic (R_2) and two test products (T_1, T_2) will be 40 minutes and will assess the bioequivalence of the CP topical creams. Only the reference product will be applied to application sites demarcated as D_1 (20 minutes) and D_2 (80 minutes) for the determination of "detectors" among subjects. Four control sites will be randomly selected from the fourteen application sites and will be demarcated as UNT (Figure 2). The application of the dose duration to the sites on the ventral forearms will be randomly assigned and the sites will be secluded using guards. The remaining cream will be gently removed by three consecutive swabbing at each application site by using wet and dry cotton swabs. The skin blanching response will then be evaluated both visually and using a chromameter at t =2, 4, 6, 8, 10, 12, 14, 22, 24 and 30 hours after the removal of the products. The facility will be maintained at a room temperature of 22°C and relative humidity of 55 ± 3%. A standard lighting by the overhead lamp will be used for both studies.

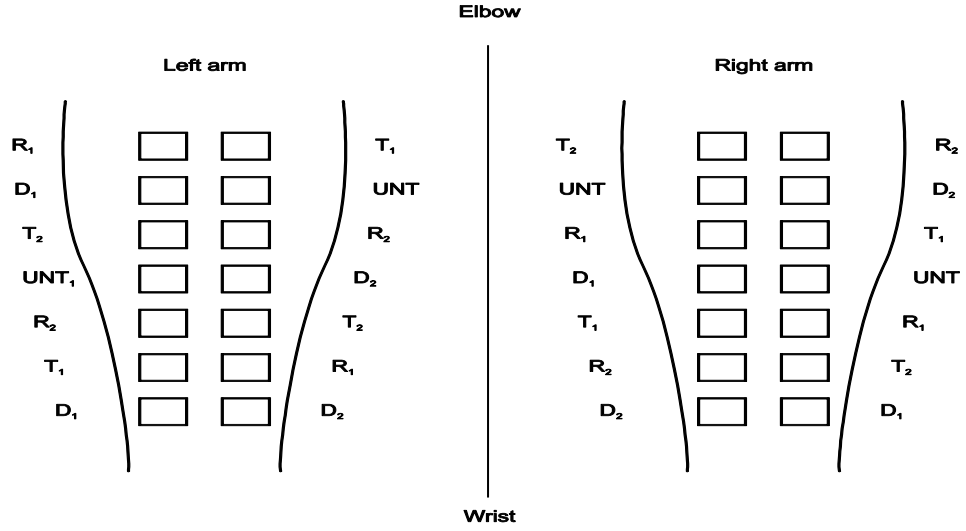

Figure 2. *A schematic diagram representing the application sites on both forearms for a human skin blanching study*

Key	Formulation	Duration	Key	Formulation	Duration
R_1	Reference	40 minutes	D_1	Reference	20 minutes
R_2	Generic	40 minutes	D_2	Reference	80 minutes
T_1	Test 1	40 minutes	UNT	Unmedicated	-
T_2	Test 2	40 minutes			

7. SKIN BLANCHING ASSESSMENTS

7.1 Visual Assessment

The degree of blanching will be measured visually by two trained observers using a multiple unit scale of 0-4 where 0 indicates no blanching, 4 indicates intense blanching and 1, 2, 3 represent intermediate grades. Each application site will be assigned a blanching score by comparing the extent of blanching of the treated skin at the site to the surrounding skin. The blanching response results will be stored as % TPS (percentage total possible score). The %TPS will be calculated for each formulation and will then be plotted against time in hours after product application to produce the blanching profiles for each individual observer. The trapezoidal rule will be used to calculate the area under the effect curve (AUEC) for each formulation.

7.2 Chromameter Assessment

The chromameter assessment will be done using a Minolta® Model CR 400 chromameter (Minolta® Ltd, Osaka, Japan) for the quantification of the intensity of blanching. The instrument will be calibrated using the white calibration plate. Baseline readings (zero time) will be taken at all times (including the untreated control sites).The chromameter provides readings based on three scales: *L*-scale, *a*-scale and *b*-scale. The FDA guidelines [522] indicate that only an *a*-scale data can be used to calculate the area under the effect curve (AUEC). The FDA Guidance [522] states that a "detector" is a "responder" whose blanching data must meet the following criterion, AUEC at D_2 /AUEC at $D_1 \geq 1.25$. At each observation, the recorded *a*-value for each application site will be corrected by subtracting the baseline (time zero) values to yield baseline-corrected values, Δa-values. The mean of Δa-values of all formulations will be calculated and plotted against time in hours. The areas under the Δa-curve (AUEC) will be calculated for each formulation using the trapezoidal rule.

7.3 Data Analysis

The AUEC values obtained from the left and right forearms will be averaged for both visual and chromameter data. Statistical analysis will be carried out using Locke's method to calculate the confidence intervals from the visual and chromameter AUEC data according to the directive of the FDA Guidance

[522]. AUEC data sets for all the formulations (reference (R_1), generic (R_2) and the two test formulations (T_1 and T_2)) will be compared to obtain an exact confidence interval.

8. STUDY POPULATION AND CRITERIA FOR PARTICIPATION

8.1 Number of Subjects

Fourteen subjects will be enrolled for the pivotal study. Dropouts will not be replaced during the study. All subjects who complete the study will be included in the statistical analysis.

8.2 Conditions for Participation in This Study

1. Volunteers must be able to complete a written consent that they will be willing to participate fully in this study.
2. Volunteers must undergo a pre-screening test to assess any possibility or sensitivity to CP and to evaluate the degree of blanching.
3. Subjects must be able to fulfil the inclusion and exclusion criteria which have been set out in § 8.3 and § 8.4 in this research protocol.
4. Subjects must be able to be fully committed in this study and abide to all the restrictions require of the subject in § 8.5.

8.3 Subject Inclusion Criteria

Only subjects that are able to meet the following criteria will be able to participate in this study.
1. Subjects must be physically and mentally healthy and must be available for the entire period of the study.
2. Subjects can be male or female, between the ages of 18-60.
3. Subjects must not suffer from any chronic or skin conditions.
4. Subjects must not be hairy on the ventral forearms.
5. Subjects must demonstrate adequate vasoconstriction to topical corticosteroids.

8.4 Subject Exclusion Criteria

Please do not participate if subjects:
1. Have any mental or physical handicap/inability.
2. Are currently pregnant or breast feeding.
3. Suffer clinically significant hypertension or circulatory disease.
4. Smoke within one week of study.
5. Caffeine intake greater than 500 mg per day (500 mg of caffeine is equivalent to three cups of instant coffee or tea) prior to or during the study.
6. Have clinically significant history of alcoholism or drug abuse.
7. Have participated in another skin blanching study within two months of the study date.
8. Have been exposed or willing to be exposed to any sun tanning during or a week prior to the study.
9. Use topical dermatologic drug therapy on ventral forearms, including prior dosing of a topical corticosteroid in a pharmacodynamic study to a particular skin site, within one month prior to the study.
10. Have any adverse reactions to topical or systemic corticosteroids.
11. Suffer from any allergic conditions (allergic rashes, dermatitis and eczema).
12. Have any current or past skin medical condition, including active dermatitis, or any other dermatologic condition, which might significantly affect the pharmacodynamic response to the administered drug.
13. Have hairy ventral forearms surfaces and/or any abrasions on the fore arms and would require shaving ventral forearms to ensure consistent dose on skin surface.
14. Would use any vasoactive (constrictor/dilator) medication, prescription or OTC that would modulate blood flow. Examples of such drugs include nitroglycerin, anti-hypertensives, anti-histamines, NSAIDs, aspirin and OTC cough/cold products containing anti-histamines, and/or either phenypropanolamine or phentolamine.
15. Any obvious difference in skin colour between arms.

8.5 Study Restrictions

Restriction	Duration of restriction	Examples of restriction	Comment
Medications	No systemically or locally applied corticosteroids, no vasoactive (dilator/ constrictor) prescription or OTC medication are allowed a week prior to the start of the study until a week after the end of the study.	Betamethasone cream/ointment/ gel, hydrocortisone cream/ointment/gel, nitroglycerine, anti-hypertensives, NSAIDS, aspirin, OTC cough/cold products containing anti-histamines and/or phenypropanolamine or phentolamine.	Some effects of CP with other high potency topical steroids have been reported such as acneiform eruptions, allergic contact dermatitis, hypertrichosis, hypopigmentation, maceration of the skin, perioral dermatitis and secondary infection.
Moisturising creams	No use of creams, emollients or similar products to forearms for 24 hours prior to and throughout the study	All skin creams for example vaseline, aqueous creams, tanning creams, sunscreen creams, medicated topical dosage forms	It can interfere with the absorption of the API from the appropriate cream through the stratum corneum.
Bathing/Showering	No bathing or showering during the periods of drug application and assessment of skin blanching	Water	If water gets in contact with the application sites during the dose duration period, negative results will be obtained
Smoking	No smoking will be allowed one day prior to the study and throughout the study	Cigarettes, pipe smoking, cigars	Tobacco can cause interactions with the drug, preventing the drug from getting absorbed
Alcohol	No alcohol may be taken by subjects two days prior to the study and throughout the study	All alcoholic drinks for example whiskey, rum, beer and alcohol containing food products	Alcohol may affect the absorption effect of clobetasol 17-propionate
Exercise	No exercise with both arm and no strenuous exercise, overall for study duration	Badminton, squash, rugby, volley ball, soccer, tennis/tennis table, rowing, gym	Walking only will be permitted
Caffeine	No caffeine intake greater than 500 mg per day will be allowed	More than three cups of instant coffee or tea	A lot of caffeine can affect the action of the corticosteroid

8.6 Criteria for Removal from the Study

Subjects may withdraw from the study if they:

1. Suffer from any adverse reaction or sign of toxicity during the study period.

2. Have not been able to abide to the study restrictions.

3. Feel that they are not able to continue participating due to any reason.

4. Suffer from any illness or injury during the study if regarded as clinically significant by the principal investigator/co-investigator.

9. STUDY PROCEDURE

9.1 Pre-Screening Test

The study will consist of a single phase of a 30 hour period. However volunteers will conduct their pre-screening test at least one week prior to the study.

9.2 Facility Check-in

On the morning of the study, subjects will check in the Biopharmaceutical Research Institute (BRI) clinic (Rhodes University, Grahamstown 6140) at 07h30. Subjects will be asked a brief medical history by the principal/subject investigator at the check-in, inclusion and exclusion criteria check and study restrictions check as mentioned in § 8.3, § 8.4 and § 8.5. The study will start at 08h00 where the application of the creams on the sites will take place. Blanching assessments will be made on subjects in the same order in which the applications will be performed, thus ensuring equal time differences. Subjects accepted in this study will remain in the clinic from check in until 30 hour assessment time. Subjects will then be allowed to leave the clinic provided that there are no significant symptoms or adverse effects present.

9.3 Posture and Physical Activity

At the time of application, subjects will be seated exposing their forearms on the table where pre-punched adhesive templates, exposing 1.1 cm x 1.1 cm squares will be placed on both of the ventral forearms respectively. Subjects will have to remain seated until the removal of the remaining cream after the dose duration time. Subjects will be allowed to lie down after the removal of the cream, however at each blanching assessment, subjects will be requested to be seated. Subjects will be required to adhere to restrictions on physical activity during the study.

9.4 Refreshments

Any food or non alcoholic drink will be allowed during the study period. Subjects will be allowed to bring their own meals but however snacks such as finger food snacks and non-alcoholic drinks such as water and juice will be provided during the study.

9.5 Subject Monitoring

The study investigator as well as the principal investigator will be in the clinic from the time of check-in till the time the subjects will be allowed to leave. Subjects will be asked open-ended questions about their health and if they are comfortable with the study.

9.6 Adverse Effects

Subjects will be monitored for any adverse events during the study. If any adverse effects are reported, whether or not they are thought to be related to the investigational products or procedures, the principal investigator/co-investigator will monitor the adverse effect and will initiate appropriate treatment if required. All adverse events will be treated accordingly until a satisfactory result is attained. However if the adverse event persists, a decision will be made by the principal investigator as to whether or not to withdraw the subject from the study.

10. ETHICAL CONSIDERATIONS AND PRACTICE

10.1 Ethical Review

Approval by The Faculty of Pharmacy's Ethics Committee will be obtained prior to the skin blanching study. The study will be conducted in accordance with the recommendation of the Food and Drug Administration guidelines (1995) [522].Several topical CP formulations have been used in the past for treating skin conditions such as atopic dermatitis, eczema, lichen planus and psoriasis. A similar bioequivalence study of CP including a pilot study was conducted by Wai Ling Au [511] at Rhodes University.

10.2 Written Consent Form

Preceding the study, the nature, purpose and the risk in participating in the study will be explained to all volunteers. If the volunteers desire, they will be given time overnight to consider the information and any questions that they might have will be answered. If they decide to participate in the study, they must be

fully committed in completing the study. However they will also be informed that they may withdraw from the study at any time without penalty, other than a reduced remuneration. They will sign a consent form in the presence of a witness and they are encouraged to consult their parents or a personal medical doctor for approval in this study.

10.3 Confidentiality

All medical histories and physical examination records and any other information regarding volunteers in this study will be kept confidential. However volunteers must agree that all documentation or data can be released for any lawful purpose and for publication purposes in scientific journals and/or presentation in a thesis submitted to Rhodes University in fulfilment of the requirement for a degree of Masters of Science (Pharmacy) after the completion of the study. In those cases, subject's name will be removed from all documentation to ensure adequate anonymity. In signing the consent form for this study, subjects must agree to the granting of access to their medical data. The medical data will be provided to them upon request and they will be informed of significant abnormalities identified during the study.

11. REMUNERATION

Subjects will be remunerated R 500.00 for full participation in the study. The payment following withdrawal from the study will be calculated on a pro-rated basis from the beginning of the study to the end of the study.

STUDY PARTICIPATION INFORMED CONSENT
STUDY NUMBER: DMV/CP 01-2010

I.....Born on.....
Residing at.....
hereby give permission to participate in the skin blanching study described to me by the study investigator.....and to apply the following cream formulations stated below onto my skin during the course of the study.

The Reference Product is **Dermovate®** **(**Sekpharma Pty Ltd, Gauteng South Africa**)**
The Generic Product is **Dovate®** **(**Aspen Pharmacare Ltd, Port-Elizabeth, South Africa**)**
The Test Products are **0.05% w/w cream CP formulations** (Cream manufacturing facility, Faculty of Pharmacy, Rhodes University)

I consent voluntarily to participate as a participant in this skin blanching study and I realise that I may withdraw/may be withdrawn at any time from the study and I understand that I do not give up any of my legal rights by signing this consent form. I have been fully informed by the study investigator, regarding the adverse effects and contraindications that are involved in the active pharmaceutical ingredient (Clobetasol 17-propionate). A "Information for Volunteers Brochure" has been given to me which entails all the procedures and risks involved in this skin blanching study. I have read the information brochure before signing the consent form and a copy or the information brochure and a signed consent form will be given to me for my own personal record before the study takes place.

I assume to fulfil all the important conditions that are found in the "Information to Volunteers Brochure" and confirm that I understand that it is important not to withhold or misrepresent any information asked of me. I undertake to inform the study investigator immediately of any symptoms or adverse effects which can happen. In case of an adverse effect, I agree to allow the principal investigator (Prof. Roderick Walker) to initiate an appropriate treatment if necessary. I also agree that my medical records can be reviewed in case of an enquiry, monitoring and/or inspection on the understanding that my anonymity will be kept.

If I fail to abide to the conditions detailed in the research protocol, I may be excluded from the study and the payment following withdrawal from the study will be calculated on a pro-rated basis from the beginning of the study to the end of the study. Both oral and written information has clearly been given to me by the study investigator and I understand them. I have had an opportunity to ask questions and any questions that I have asked have been answered to my satisfaction. I agree that a policy to cover volunteers in clinical studies against death or disablement due to a direct result of participation in such clinical studies

has been taken out by the Biopharmaceutics Research Group. I accept the conditions of the policy as set out in the insurance policies.

I acknowledge that I will receive a remuneration of **R 500.00** for full participation in this skin blanching study and if I withdraw from the study before it has been completed, I will receive a pro-rated amount.

.. **..**

Volunteer (Full name) ***Volunteer (Signature)***

I...have accurately read or witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Ayeshah Fateemah B. Fauzee

.. **..**

Study investigator (Full name) ***Study investigator (Signature)***

I...have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

.. **..**

Witness (Full name) ***Witness (Signature)***

.. **..**

Date ***Place***

BRG Advertisement Expiry date: 5th Oct 2010

The **B**iopharmaceutics **R**esearch **G**roup is looking for

Healthy Male/Female Volunteers

to participate in a **SKIN BLANCHING STUDY** which forms part of a Masters degree

If You Are:

1. 18 years or older and a non-smoker
2. Healthy without any skin condition or long-term illnesses
3. Not applying any topical pharmaceutical agents to your skin

Then You May Be Eligible to Participate

YOU WILL BE REMUNERATED FOR PARTICIPATING

If you are interested, please contact **Ayeshah Fauzee**

as soon as possible for more information

By E-mail (preferably) at**: g05f3068@campus.ru.ac.za**

Or by Phone on: **076 899 2810**

Principal Investigator*:* Prof. Roderick Walker

Co-investigator*:* Dr. Mike Skinner

Please take a tear-off slip below

BRG Skin Blanching Study
Contact: Ayeshah Fauzee
E-mail: g05f3068@campus.ru.ac.za
Cell: 0768992810

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