APPLICATION OF QUALITY BY DESIGN TO THE MANUFACTURE OF A MULTIPARTICULATE PREDNISONE DOSAGE FORM

By

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ABSTRACT

For many years, quality by testing was the only approach to guarantee quality of drug products before the Food and Drug Administration launched the concept of current Good Manufacturing Practice. In order to gain more knowledge of the manufacturing process, a new system known as Quality by Design was introduced into the pharmaceutical industry. Quality by Design is based on thorough understanding of how materials, process parameters and interaction thereof impact final product quality. Quality by Design is a systematic approach to product development which ensures that quality is built into a product during product development and not just tested into it. The aim of Quality by Design is to achieve optimum product quality with consistent dosage form performance and minimal risk of failure in patients.

The objective of these studies was to implement a Quality by Design approach to establish a design space for the development and manufacture of a safe, effective and stable multi-partite solid oral dosage form for prednisone as an alternative to currently marketed prednisone formulations. Multi-particulate dosage forms offer significant advantages over conventional technologies. In addition to lowering the incidence of gastrointestinal irritation they exhibit a reduced risk of dose dumping and a large surface area which favours dissolution. Furthermore, their free flowing nature facilitates reproducible capsule filling and consequently uniformity of dosing. Different multi-particulate dosage forms exist however a multiple-unit pellet system was investigated during these studies.

Quality by Design principles were used to develop and establish a reversed-phase high performance liquid chromatographic method for quantifying prednisone from solid oral dosage forms. A Central Composite Design was used to generate multivariate experiments and to investigate the impact of input variables on the quality and performance of the analytical method. The optimized method was validated according to International Council for Harmonization guidelines and was found to be linear, precise, accurate and specific for the quantitation of prednisone.

Pre-formulation studies were conducted and included the assessment of particle size, particle shape, powder flow properties and compatibility studies. Carr's index, Hausner ratio and the Angle of Repose were used to evaluate powder flow properties and results generated from all studies

suggest the need for adding a glidant and lubricant to improve pellet flow. The images generated from Scanning Electron Microscopy were used to analyze particle shape and size. Differential Scanning Calorimetry and Fourier Transform Infrared Spectroscopy were used to evaluate API-excipient compatibility. All excipients investigated were found to be compatible with prednisone and suitable for formulation development studies.

Extrusion-spheronization was used to manufacture prednisone pellets. Extrusion-spheronization is a multi-step process involving many factors. Quality risk management tools particularly an Ishikawa Fishbone (cause and effect) diagram and failure mode and effects analysis were used to narrow down potentially significant factors to a reasonable number that could be investigated experimentally. Risk priority numbers were used to quantify risk and factors above a set threshold value were considered to be of high risk. A total of eleven risk factors were identified as high. A Plackett-Burman study was conducted to narrow down the eleven high risk factors to identify the most impactful factors *viz.*, microcrystalline cellulose content, sodium starch glycolate content, extrusion speed and spheronization time. Evaluation of four factors was carried over to optimization studies using a Box-Behnken Design and following identifaction of the optimum process settings and excipient content a design space for the manufacture of a multi-partite dosage form containing prednisone was established.

This thesis is dedicated to:

My parents Mrs A and Mr C Manda and my sisters Ophilia and Teclar.

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STUDY OBJECTIVES

Even though prednisone has multiple therapeutic benefits, its oral delivery is associated with many challenges. Prednisone is a borderline BCS (I) compound therefore poor solubility and a low dissolution rate in gastrointestinal fluids result in low bioavailability. The low tolerance level which depends on dosage strength results in fluctuations in overall absorption. Furthermore prednisone exhibits harsh effects on the gastrointestinal tract and has an extremely bitter taste which needs to be masked to achieve better palatability. Attempts to improve the efficiency of delivering prednisone and its derivatives include the use of micro-emulsions, polymeric micelles, polymeric implants and microspheres. Approaches such as amorphilization, nanocrystallization, complexation, salt formation and polymorph transformation have been attempted to improve the dissolution rate of poorly soluble compounds such as prednisone.

To our knowledge, the use of a multiple unit pellet system to address the challenges associated with oral delivery of prednisone has not been reported. The overall aim of this study was to establish a design space for the manufacture of an immediate release multiple-unit pellet system containing prednisone. A multi-particulate dosage form is a viable and rational formulation technology as an alternative to currently marketed prednisone formulations. Specifically the objectives of the study were:

(i) Application of the principles of Quality by Design to identify relationships between formulation and process variables and the impact on manufacturing processes and pellet quality.
(ii) Application of Quality by Design to develop an analytical method for the quantitation of prednisone in solid dosage forms.
(iii) Determination of an efficient dissolution method to to investigate the release kinetics of prednisone from formulations.
(iv) Determination of a suitable method to evaluate shape and morphology of each formulation.
(v) Investigation of compatibility of all excipients prior to formulation.
(vi) Optimization of pellet quality using Response Surface Methodology and establish limits for the design space.

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ABBREVIATIONS

ACN	Acetonitrile
AIDS	Acquired Immunodeficiency Syndrome
ANOVA	Analysis of variance
AoR	Angle of Repose
API	Active pharmaceutical ingredient
AQbD	Analytical Quality by Design
ATP	Analytical target profile
BCS	Biopharmaceutics Classification System
BP	British Pharmacopoeia
CAPA	Corrective action and preventive action
CCD	Central Composite Design
cGMP	CurrentGood Manufacturing Practice
CI	Carr's index
CMA	Critical material attributes
CPP	Critical process parameters
CQA	Critical quality attributes
CV	Coefficient of variation
DoE	Design of experiments
DSC	Differential Scanning Calorimetry
DS	Design space
DS-LL	Design space lower limit
DS-UL	Design space upper limit
FDA	Food and drug administration
FMEA	Failure mode and effects analysis
FMECA	Failure mode effects and criticality analysis
FTA	Fault tree analysis
FT-IR	Fourier Transform Infrared Spectroscopy
GIT	Gastrointestinal tract
HAZOP	Hazard operability analysis

HPLC	High Performance Liquid Chromatography
HR	Hausner ratio
ICH	International Council for Harmonization
IS	Internal standard
LOD	Limit of detection
LOQ	Limit of quantitation
MCC	Microcrystalline cellulose
Mg	Magnesium
MUPS	Multiple unit pellet system
NSAID	Non-steroidal anti-inflammatory drug
PAT	Process analytical technologies
PHA	Preliminary hazard analysis
PTF	Peak tailing factor
QbD	Quality by Design
QbT	Quality by testing
QRM	Quality risk management
QTPP	Quality target product profile
RP-HPLC	Reversed-Phase High Performance Liquid Chromatography
RPN	Risk priority number
RSD	Relative standard deviation
RSM	Response Surface Methodology
SAA	Surface active agents
SEM	Scanning Electron Microscopy
SSG	Sodium starch glycolate
USP	United States Pharmacopoeia
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1
PREDNISONE

1.1 INTRODUCTION TO CORTICOSTEROIDS

Corticosteroids are a class of steroid hormones that are produced in the adrenal cortex of vertebrates [1, 2]. Synthetic analogues are also classified as corticosteroids and their functions mimic naturally occurring compounds [1]. Endogenous corticosteroids play pivotal roles in the maintenance of homeostasis. They particularly aid physiological processes such as immune response, stress response, growth and electrolyte balance. If need be, exogenous corticosteroids are provided to patients and these are widely used for treating asthma, rheumatoid arthritis, systemic lupus, eczema, and Crohn's disease amongst others [3, 4].

Two main classes of corticosteroids have been identified *viz*., glucocorticoids and mineralocorticoids [5]. Glucocorticoids are steroid hormones produced by the adrenal gland and regulated by the hypothalamic-pituitary-adrenal axis. Glucocorticoids play a pivotal role in glucose metabolism via up and down regulation of carbohydrate, protein and fat metabolism. In addition, they have anti-inflammatory and immune suppressive actions [6]. The name glucocorticoid is derived from glucose, cortex and steroid because of their primary function, source and structure [7]. Examples of glucocorticoids include prednisone, prednisolone and dexamethasone. Mineralocorticoids are primarily involved in regulating electrolyte and water balance by modulating ion transport in renal tubules [8]. The name mineralocorticoid is derived from mineral and steroid due to their structure and primary function of up or down regulating sodium and potassium absorption [7, 8]. Aldosterone is the main mineralocorticoid hormone which is produced by the zona glomerulosa of the adrenal cortex in the adrenal gland [8].

In this study a multi-particulate drug delivery system for the glucocorticoid prednisone was designed and formulated. The oral route of administration was selected because of its convenience, economical, and user-friendly nature [9].

Prednisone is a pro-drug which is converted to the active metabolite, prednisolone by the liver [10]. Prednisone is used to treat symptoms of low corticosteroid levels but it may also be prescribed for patients with normal corticosteroid levels to treat different conditions. These conditions include certain types of arthritis, severe allergic reactions, multiple sclerosis, lupus, and certain pathologies

that affect lungs, skin, eyes, kidneys, blood, thyroid, stomach, and intestine [11-13]. Prednisone is also prescribed for serious pathologies such as some types of cancer, or as adjunct to pneumonia therapy in patients with Acquired Immunodeficiency Syndrome (AIDS). HIV positive patients may also use prednisone if need be, but only under close monitoring because the immunosuppressive properties of prednisone may exacerbate the risk of opportunistic infections [14-16].

Prednisone has multiple therapeutic benefits, but oral delivery is associated with many challenges. The poor solubility and low dissolution rate in gastrointestinal (GIT) fluids often causes low bioavailability [17]. The low tolerance level which depends on dosage strength may lead to fluctuations in overall absorption [10], furthermore it has harsh effects on the GIT [9, 18] and an extremely bitter taste which needs to be masked to achieve better palatability [16].

Many attempts have been made to improve the efficiency of delivering prednisone or its derivatives. These methods involve encapsulation in microspheres, micro-emulsions, polymeric micelles, and polymeric implants [19-24]. In addition, other approaches to improve the dissolution of poorly soluble drugs have been reported. These include amorphilization [25], nanocrystal [26, 27], complexation [28], salt formation [29], and polymorph transformation [30].

In this study, we investigated the use of a multiple unit pellet system (MUPS) as a drug delivery system for prednisone. Compared to traditional single unit dosage forms MUPS have lower incidences of gastrointestinal irritation due to a decrease in the local concentration of API in the GIT following oral administration [31]. Furthermore, lower individual variability in plasma concentrations is observed compared to tablets since there is a reduced risk of dose dumping [32, 33]. In addition, the presence of many individual units increases surface area leading to improved solubility and ultimately bioavailability. The use of discrete units also offers a simple solution to minimizing potential API-API or API-excipient interactions in some cases [34] and the free flowing nature of pellets facilitates reproducible capsule filling and content uniformity of doses [35]. Individual pellets may also be filled into gelatin capsules as a means to mask the taste [36].

1.2 PHYSICOCHEMICAL PROPERTIES

1.2.1 Description

Prednisone is a white or almost white odourless crystalline powder which exhibits polymorphism [37, 38]. It contains 97.0 % to 103.0 % of prednisone when determined with reference to dried substance [37, 39]. Its chemical structure is depicted in Figure 1.1 and it is known as 17,21-Dihydroxypregna-1,4-diene-3,11,20-trione [39] or Pregna-1,4-diene-3,11,20-trione [40].

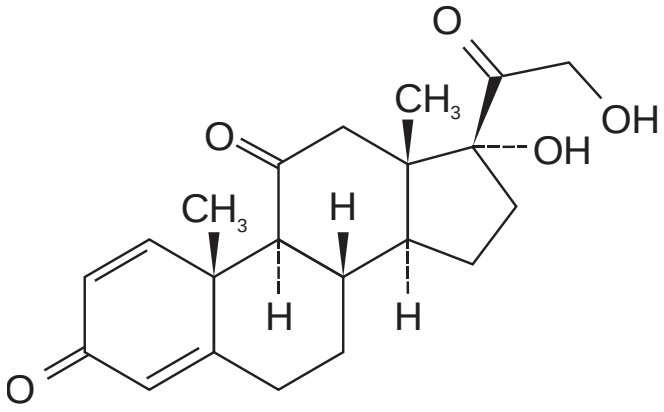


Figure 1.1 Chemical structure of prednisone [Adapted from 39]

Prednisone is either anhydrous or contains one water of hydration. The chemical formulas is $C_{21}H_{26}O_5$ or $C_{21}H_{26}O_5.H_2O$. The anhydrous form has a molecular weight (MW) of 358.44 g/mol and the monohydrate has a MW of 376.46 g/mol [37, 39]. Commercially prednisone is available as an anhydrous, monohydrate or prednisone acetate salt.

1.2.2 Polymorphism

Prednisone exhibits pseudo-polymorphism. In this phenomenon different crystal forms of the same compound result from hydration or solvation [37, 41, 42].

1.2.3 BCS classification

The Biopharmaceutics Classification System (BCS) considers two factors to be the most impactful towards the rate and extent of drug absorption *viz.*, solubility and permeability. Following assessment of solubility and intestinal permeability the results are used to categorize the compound as either Class, 1, 2, 3 or 4 [43]. Even though data generated from assessing prednisone is not totally conclusive, some reports suggest that prednisone is a border line class 1 compound [44, 52].

1.2.4 Solubility

Prednisone is practically insoluble in water, and more than 10000 mL of water are needed to dissolve 1 g. It is slightly soluble in 96 % ethanol at a 1 in 150 ratio. A summary of solubility data available and the corresponding drug/solubility ratios is listed in Table 1.1. All assessments were performed at 25 °C and three dosage strengths from the lower extreme to the upper extremes were analyzed [45-48].

Table 1.1 Solubility and drug/solubility ratios of prednisone in water

Medium	Solubility mg/mL	Dose/Solubility 1 mg	Dose/Solubility 10 mg	Dose/Solubility 50 mg
Water	0.133	7.5	75	376

1.2.5 Partition coefficient

Prednisone has a log P value of 1.46 [49].

1.2.6 pKa

Prednisone is a neutral molecule with a pKa of 12.58 [52].

1.2.7 Melting point

The reported melting point of prednisone is 243 °C [52] and this is in agreement with our experimentally determined melting point of 242.49 °C.

1.2.8 Specific optical rotation

The specific optical rotation of dried prednisone is between + 183 and + 191 when measured in a 96 % v/v ethanol solvent [39].

1.2.9 Dosage form strength

Immediate release solid oral dosage forms of prednisone are available in 1, 5, 20, and 50 mg strengths. Be-Tab® prednisone (5 mg) tablets are registered in South Africa, and were used as a reference for this study.

1.3 SYNTHESIS OF PREDNISONE

Prednisone and other steroids may be synthesized from any of these three starting materials: hecogenin, tigogenin, and diosgenin [50, 53]. All of them are sapogenins, however in addition to producing higher yields, the extraction and modification of diosgenin is more efficient and economic therefore presently most industries only use diosgenin. Diosgenin **(I)** (Figure 1.2) is extracted from roots of different yams [53]. The roots contain disogenin a gluco-derivative, which

after extraction is fermented to produce diosgenin as a pure white solid. Diosgenin is subjected to a three step process called the Marker degradation to yield pseudodiosgenin diacetate **(II)**, diosone **(III)** and finally 16-dehydropregnenolone **(IV)**. 16-dehydropregnenolone **(IV)** is further modified to produce 16-dehydro pregnenolone acetate (16-DPA) **(V)**. Figure 1.2 depicts Marker's three step process.

16-DPA **(V)** is reacted with H_2O_2/NaOH/MeOH to produce the 16, 17-epoxy group; the same reaction results in saponification of the acetoxy group to produce compound **(VI).** The Oppenauer protocol is employed to oxidize the 3-hydroxy group into a 3-keto to produce 16-epoxyprogesterone **(VII)** as shown in Figure 1.3. *Rhizopus nigricans* is used to ferment 16-epoxyprogesterone **(VII)** to produce 16-epoxy-11α-hydroxy progesterone **(VIII)**. Compound **(VIII)** is a precursor to the synthesis of different corticosteroids, however for prednisone synthesis the oxidation of the hydroxyl group at carbon-11 produces compound **(IX)**. Then hydrobromic acid is used to open the epoxide **(X)** and subsequent Ni-Ra reduction of bromine yields **(XI)**.

Next, cortisone-21-acetate **(XII)** is formed via transformation of the 21-methyl group using the Ringold-Stork protocol. To introduce a double bond at the 1,2 position of the steroid skeleton Corynebacterium simplex (ATCC 6946) is used as a source of enzymes to perform 1,2-dehydrogenation. The result is prednisone acetate **(XIII)** which is later transformed into prednisone **(XIV)** with a basic treatment [54]. The final conversion from compound **(VII)** to prednisone is depicted in Figure 1.4.

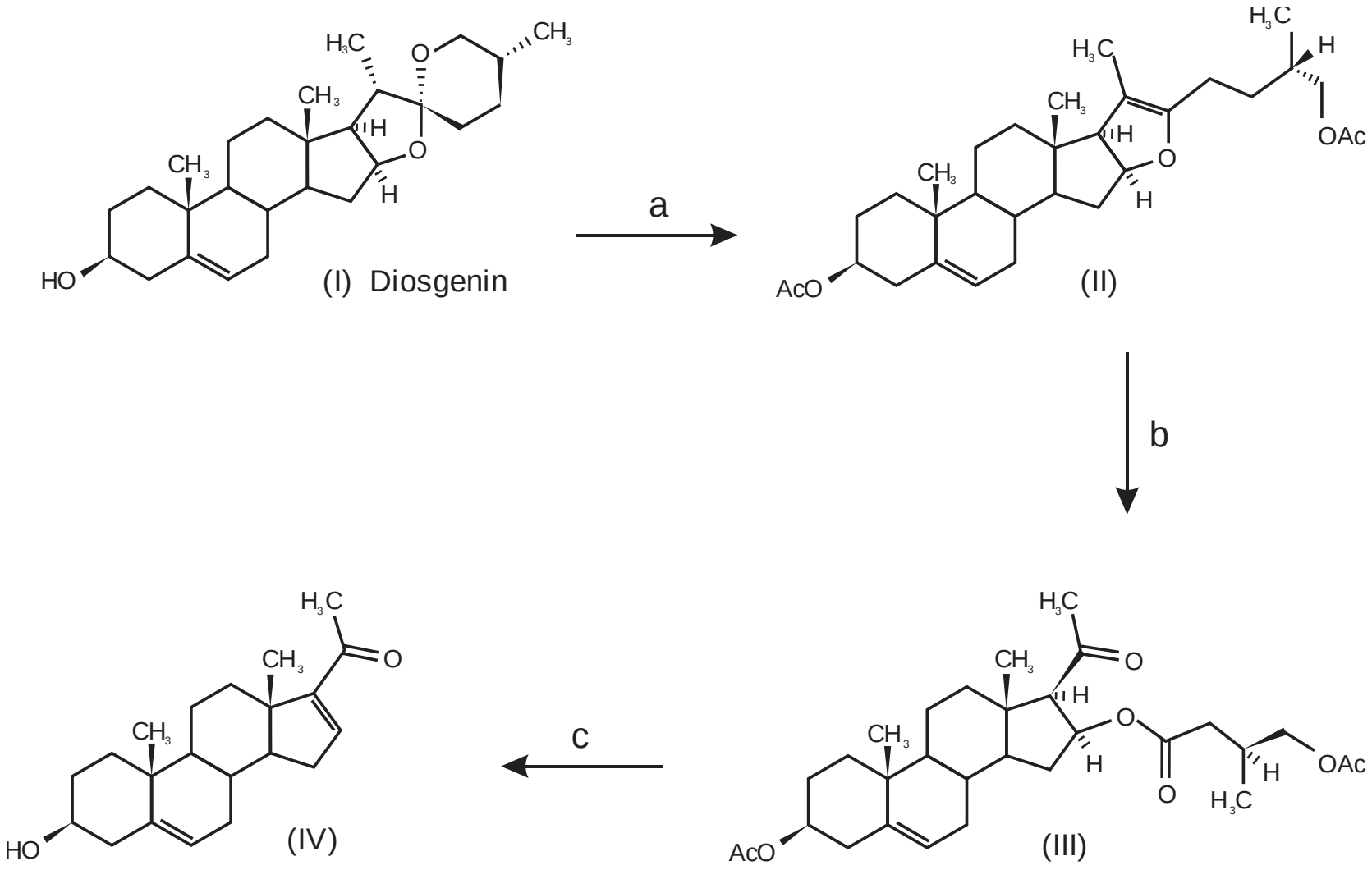


Figure 1.2 Marker's degradation (a) Ac_2O, 200 ˚C (b) CrO_3, AcOH (c) NaOH, EtOH [Adapted from 54]

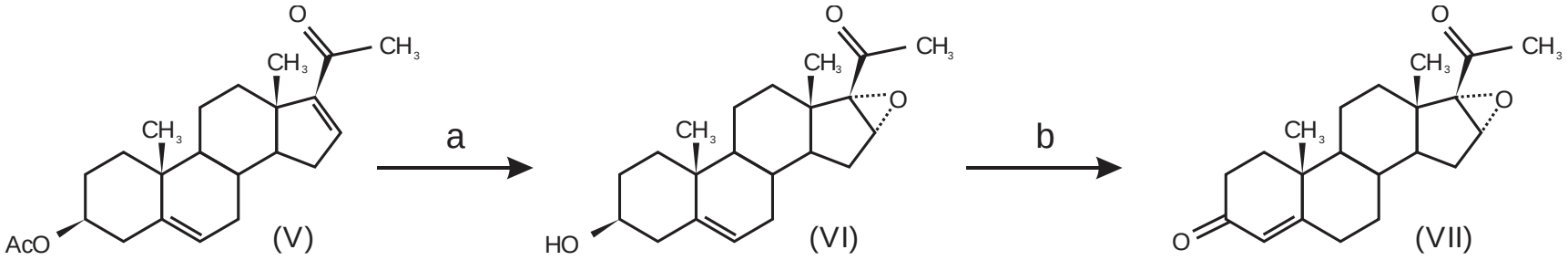


Figure 1.3 (a) H_2O_2, NaOH, MeOH (b) $Al(iPrO)_3$, cyclohexanone, toluene [Adapted from 54]

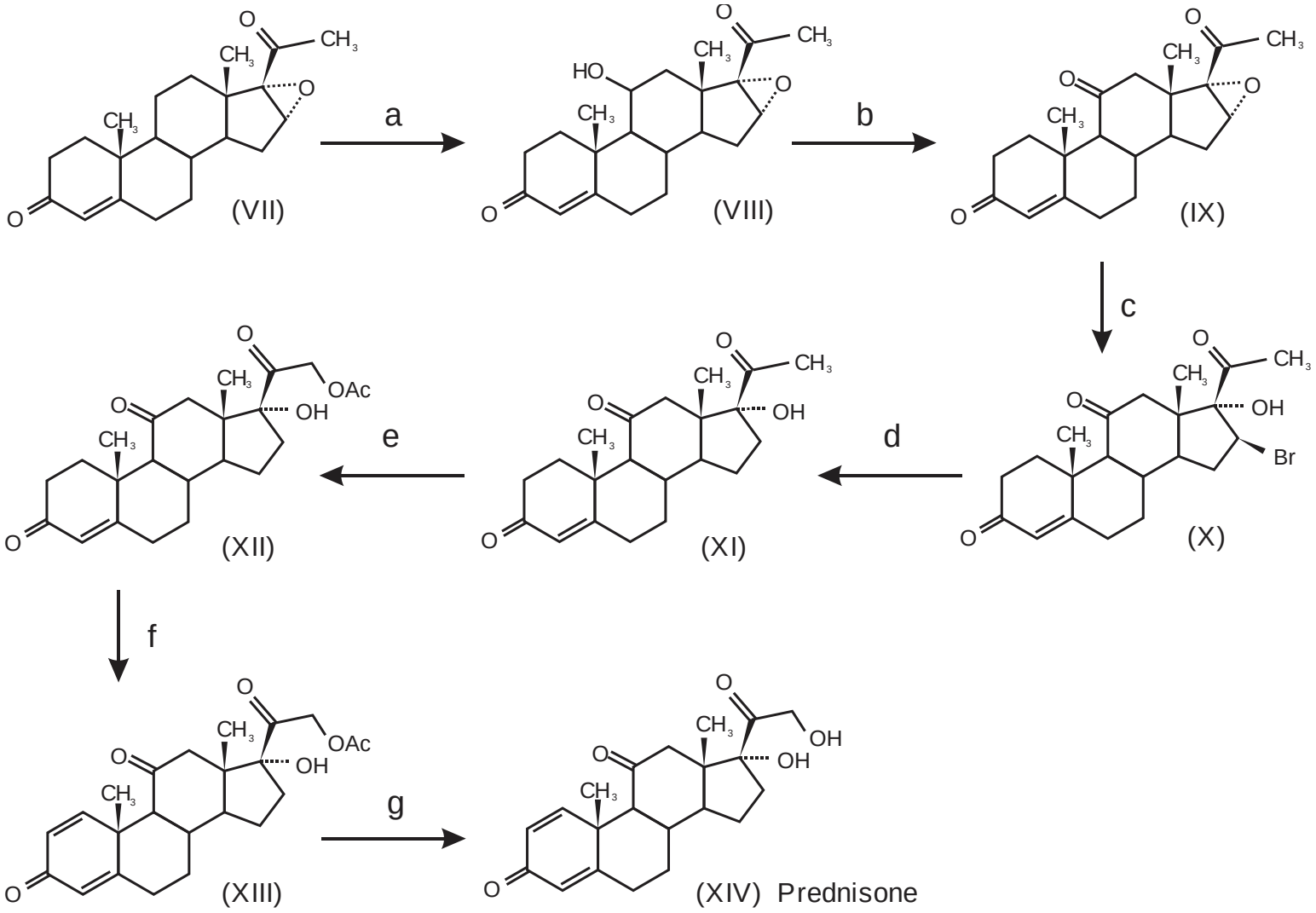


Figure 1.4 Prednisone synthesis (a) *Rhizopus nigricans* fermentation (b) CrO_3, AcOH (c) HBr (d) H_2, Ni-Ra (e) *(i)* I_2, $CaCl_2$, CaO, MeOH *(ii)* AcOK, DMF (f) *Corynebacterium simplex* (ATCC 6946) (g) KOH, MeOH [Adapted from 54]

1.4 MECHANISM OF ACTION

The mechanism of action of glucocorticoids is broadly classified into two categories, genomic effects and non-genomic effects [55-57]. Prednisone exerts its clinical effects *via* similar mechanisms to any other glucocorticoid [55].

The genomic mechanism occurs mainly *via* trans-activation or trans-repression. Due to their lipophilic nature, glucocorticoids easily permeate the plasma membrane. Once inside the cell, they bind to the cytosolic glucocorticoid receptor (cGCR) to form a glucocorticoid-cytosolic glucocorticoid receptor (GC-cGCR) complex. The complex is translocated to the nucleus where it binds as a homodimer to specific DNA binding-sites called glucocorticoid responsive elements (GREs) [58]. The binding of the ligand-activated glucocorticoid receptor to positive GREs induces synthesis of anti-inflammatory proteins such as lipocortin 1 and IκB. The same mechanism mediates synthesis of regulator proteins that exert their effect on metabolic processes. This process is called transactivation and is mediated by positive GREs [55]. Trans-repression is mediated by negative GREs (nGREs) [59]. During trans-repression, nGREs bind to the GC-cGCR complex and inhibit the transcription of inflammatory genes such as interleukin (IL)-1 and IL-2 [59-61]. Part of prednisone's anti-inflammatory properties result from trans-repression. The genomic mechanism is slow and takes anything from hours to days before changes on a cellular, tissue or organism level are observed [55].

The rapid anti-inflammatory and immunosuppressive effects of prednisone are attributed to non-genomic mechanisms [59, 62, 63]. Non-genomic mechanisms occur in three different ways: non-specific interactions of glucocorticoids with cellular membranes, non-genomic effects which are mediated by the cGCR and specific interactions with membrane-bound GCRs (mGCR) [62, 64]. Non-specific interactions with cell membranes occur when glucocorticoids are present at high concentrations. Under these conditions, GCs intercalate into membranes and change their physicochemical properties as well as activities of membrane associated proteins [62, 65]. This phenomenon is mostly observed in mitochondria where intercalation of GCs causes mitochondrial proton leaking, leading to impaired adenosine triphosphate (ATP) production. This effect contributes to immune suppression by diminishing cytokine synthesis, migration and phagocytosis [62, 65 66]. Non-genomic GC-cGCR complexation leads to the release of signaling molecules from the receptor. These signaling molecules are thought to be responsible for rapid GC effects. One such example is the inhibition of arachidonic acid release through a cGCR mediated process.

Arachidonic acid mediates inflammatory reactions and several metabolic reactions including growth. Therefore even though arachidonic acid inhibition may alleviate inflammation it may also diminish growth and cause metabolic side effects [67]. The third non-genomic mechanism is mediated by membrane bound glucocorticoid receptors [68]. The rapid occurrence of immune suppression is due to this mechanism. The process is complex and involves transduction of many signaling proteins [55, 69, 70].

1.5 PHARMACOKINETIC PROPERTIES

1.5.1 Absorption and bioavailability

Once ingested prednisone is rapidly absorbed and metabolized to active prednisolone. Prednisone is a pro-drug so to exert its effect it must be converted to the biologically active form, prednisolone. Following oral administration of prednisone, bioavailability normally ranges between 80 - 100 % [71-73], except for very high prednisone doses (> 50 mg) where lower bioavailability values between 62 % and 74 % have been reported [74-76].

Following administration of a single dose, maximum serum concentrations are reached within 1 to 3 hours (hrs) [77-79]. Food intake does not affect the extent of absorption but only prolongs the time taken to reach peak concentration [80-82]. No indications for the existence of an absorption window have been reported [78, 83].

1.5.2 Permeability

A permeability coefficient of 0.3×10^{6} cm/s is documented for prednisone following tests conducted on artificial phospholipid membranes [51].

1.5.3 Distribution and protein binding

The volume of distribution of prednisone ranges between 0.4 to 1.0 L/kg [84]. Prednisone exhibits low binding (< 50 %) to plasma proteins but the active metabolite prednisolone exhibits concentration-dependent binding to plasma proteins, mainly transcortin and albumin [85]. As a result prednisone exhibits dose-dependent pharmacokinetics. Transcortin has high affinity and low capacity to prednisolone binding while albumin has low affinity and a high capacity to prednisolone binding. Both are saturable [85].

The fraction bound varies and decreases nonlinearly with increasing concentrations as a result non-linear pharmacokinetics are observed. Saturable binding is the root cause of this nonlinear pharmacokinetics. Unbound drug concentrations are directly proportional or linearly related to

dose. An increase in dose results in an increase of unbound drug concentration. The clearance and volume of distribution are also directly proportional to dose [86, 87]. Prednisone crosses the placenta but is generally considered safe with regard to breast-feeding [79, 88].

1.5.4 Metabolism and excretion

Prednisone is biologically inert hence to exert its effect it is metabolized first, mainly to its active form prednisolone. In the liver, the enzyme 11 β-hydroxysteroid dehydrogenase catalyses the reduction of the 11-oxo group in prednisone to produce the active moiety, prednisolone [89-91]. The two are inter-convertible but the equilibrium strongly favors prednisolone formation with prednisolone reaching plasma concentrations 4 to 10 times greater than the parent prednisone [78, 86]. A representation of the inter-conversion is provided in Figure 1.5.

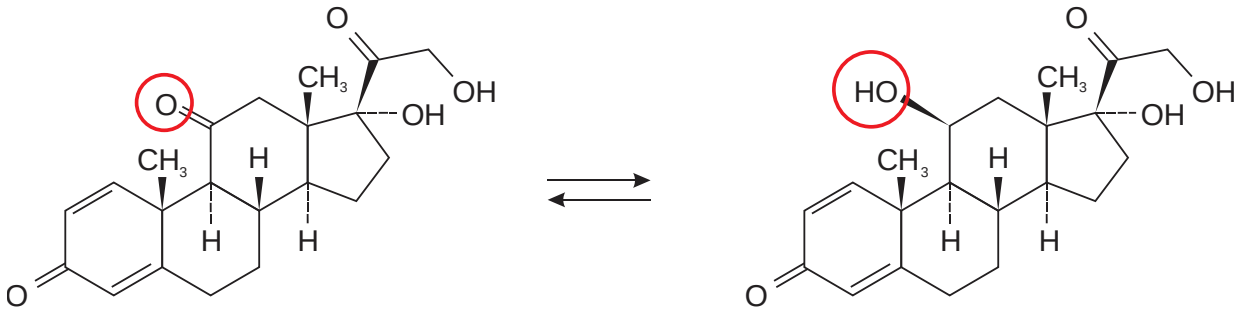


Figure 1.5 11 β-hydroxysteroid dehydrogenase catalyzed reduction of the 11-oxo group in prednisone (left) to produce prednisolone (right) [Adapted from 89]

The chemical relationship between prednisone and prednisolone is similar to the one that the endogenous glucocorticoid cortisone has with hydrocortisone. The indications and dosage of prednisone for oral use are exactly the same as those for prednisolone [86].

The inter-conversion is not gradual and proportional therefore it is regarded as non-linear. Time and dose significantly affect the non-linear inter-conversion between prednisone and prednisolone [73, 78, 86]. The serum half-life of prednisone is between 2 and 4 hours, and is subject to variability based on the time of day, age, sex, physical exercise, pregnancy, concurrent morbidities and drug use [77, 78, 92]. Prednisone is almost completely metabolized. Only a small percentage (2 - 5 %) of the initial dose is excreted unchanged in urine. Other metabolites are excreted freely or as conjugates [78]. The mean oral plasma clearances of prednisone are dose-dependent. A 5 mg dose is reported to have a plasma clearance of 572 mL/min/1.73m^2 whereas a 50 mg dose was observed to have a plasma clearance of 2271 mL/min/1.73m^2 [86].

The pharmacokinetics of prednisone and consequently prednisolone are recognized as dose-dependent and non-linear. They are not affected by the route of administration since similar profiles are observed whether the oral or intravenous route is used [86, 89]. Both prednisone and prednisolone exhibit similar extents of absorption [90]. The inter-conversion is not a limiting factor. When similar doses of prednisone and prednisolone were taken orally, the concentration-time profiles for both were very similar because the bioavailability of prednisone is almost complete [89, 90].

Even though some prescribers prefer prednisolone for patients with impaired liver function, studies have shown that the extent of absorption is still similar for both prednisone and prednisolone in these patients too [85, 90].

Prednisone and prednisolone are therapeutically equivalent, however administration of prednisone instead of prednisolone prevents high local concentrations of the biologically active prednisolone therefore minimizing gastrointestinal side effects [85, 89, 90].

1.5.5 Therapeutic index

Prednisone is not considered a narrow therapeutic index drug so when prescribed in low doses, there is no need to constantly monitor blood levels. However, when high doses are prescribed, it is advisable to monitor blood levels [44].

1.6 CLINICAL PHARMACOLOGY

Prednisone is indicated for the following conditions:

1.6.1 Endocrine disorders

Even though hydrocortisone and cortisone are the preferred choice for treating primary and secondary adrenal insufficiency, prednisone in combination with mineralocorticoids are also effective treatment choices for primary and secondary adrenal insufficiency [44]. In his case study, Salvatori confirmed the effectiveness of prednisone in treating adrenal insufficiency [93]. Concurrent mineralocorticoid supplementation is particularly important when treating infants. Prednisone may also be used to treat congenital adrenal hyperplasia, hypercalcemia associated with cancer and nonsuppurative thyroiditis [44, 94, 95].

1.6.2 Rheumatic disorders

Prednisone may be used in combination with other drugs for short term treatment of psoriatic arthritis, rheumatoid arthritis, juvenile rheumatoid arthritis, ankylosing spondylitis, acute and sub-acute bursitis, acute nonspecific tenosynovitis, acute gouty arthritis, post-traumatic osteoarthritis, synovitis of osteoarthritis and epicondylitis [44, 95, 96]. In his findings, Boland confirmed that prednisone is effective and efficient in treating rheumatoid arthritis [97]. In some cases low doses may be used for maintenance therapy [95].

1.6.3 Collagen diseases

Prednisone is sometimes prescribed for acute and maintenance therapy of systemic lupus erythematosus and acute rheumatic carditis [44, 94, 95]. Anyanwu et.al found prednisone to be effective in treating systemic dermatomyositis (polymyositis) [98]. The duration of treatment was found to be even shorter when prednisone was used with other adjunctive therapies [98].

1.6.4 Dermatologic diseases

Prednisone alleviates symptoms of pemphigus, bullous dermatitis herpetiformis; severe erythema multiforme commonly referred to as Stevens-Johnson syndrome, exfoliative dermatitis, atopic dermatitis, mycosis fungoides, severe psoriasis and severe seborrheic dermatitis [44, 94, 99]. Thompson et.al found a two to three week course of prednisone to be effective in treating severe forms of contact dermatitis [100].

1.6.5 Allergic states

Prednisone is used to control severe or incapacitating allergic conditions that do not respond to conventional treatment regimens. These may include bronchial asthma, atopic dermatitis, serum sickness and drug hypersensitivity reactions [44, 94, 101]. In their study, Brown et.al confirmed that prednisone is effective in treating seasonal and perennial allergic rhinitis [102].

1.6.6 Ophthalmic diseases

The following conditions affecting the eyes may be alleviated by prednisone therapy: sympathetic ophthalmia, allergic conjunctivitis, keratitis, chorioretinitis, optic neuritis, corneal marginal ulcers, herpes zoster ophthalmicus, anterior segment inflammation, iritis, iridocyclitis, diffuse posterior uveitis and choroiditis [103-105]. Prednisone may be used in both acute and chronic cases. Thorne et.al found prednisone to be an effective second line treatment for episcleritis [106].

1.6.7 Respiratory diseases

Prednisone may be used as adjuvant therapy to the usual anti-tuberculosis regimen in most cases of fulminant tuberculosis [107]. Alzeer et.al confirmed these findings in their study [108]. In their investigation, they found a four to six weeks course of prednisone to be an effective adjuvant to tuberculosis treatment. Prednisone is also used to treat aspiration pneumonitis, symptomatic sarcoidosis, severe Loeffler's syndrome and berylliosis [104, 107, 109].

1.6.8 Hematologic disorders

In adults prednisone may be used to treat idiopathic cases of thrombocytopenic purpura. A case study conducted by Radwi et.al reported success in using prednisone to treat severe thrombocytopenia [110]. Prednisone is also a key part of treatment of some anaemic conditions particularly acquired (autoimmune) hemolytic anemia, erythroblastopenia (red blood cell anemia) and congenital (erythroid) hypoplastic anemia [44, 105, 111].

1.6.9 Neoplastic diseases

In adults prednisone may be used for palliative management of some lymphomas. It may also be used as an adjunct to leukemia treatment in both adults and children [44, 112, 113]. In a study conducted by Ranney et.al, the use of high dose prednisone to treat acute leukemia resulted in either complete or partial remission during the treatment period [112]. However in the same study, some patients experienced serious side effects associated with high doses of prednisone therefore this treatment must be used with close monitoring and caution. Prednisone may also be used to treat edema resulting from nephrotic syndrome or lupus erythematosus [113].

1.6.10 Gastro-intestinal diseases

Prednisone is used to stop symptoms of moderate to severe ulcerative colitis and regional enteritis [114]. In their study, Teixeira et.al found prednisone to be effective as a second line treatment for colitis particularly in cases of pancolitis, extensive colitis and left sided colitis [114].

1.6.11 Miscellaneous

Inflammatory myopathies associated with trichinosis are treated with prednisone. Furthermore prednisone is used as an adjunct to treat tuberculous meningitis with subarachnoid block or impending block when used in combination with appropriate antituberculous chemotherapy [103, 104].

1.7 CONTRA-INDICATIONS

Prednisone use is contraindicated in liver disease, presence of peptic ulcers, osteoporosis, psychosis and severe psychoneurosis. Prednisone is also contraindicated in the presence of acute fungal, bacterial and viral infections because its immune suppression properties may interfere with antibody formation [103-105].

1.8 WARNINGS AND PRECAUTIONS

1.8.1 Cardio-renal

Large doses of prednisone increase salt and water retention leading to elevated blood pressure [104]. The risk of hypokalemia also increases when prednisone is used long-term in high doses hence there is need for dietary supplementation and salt restriction [105]. All corticosteroids increase calcium excretion [103, 104]. Extreme caution must be taken in myocardial infarction patients. In their case study, Silverman et.al confirmed that prednisone and other anti-inflammatory agents may lead to left ventricular wall rupture or infarct expansion when used in myocardial infarction patients [115]. Their findings suggest that the cause may be due to corticosteroids interfering with healing by impairing connective tissue regeneration, inhibiting fibroblast proliferation, hindering new vessel formation and reducing wound tensile strength [115].

1.8.2 Endocrine

Prednisone may temporarily suppress the hypothalamic-pituitary adrenal (HPA) axis potentially leading to corticosteroid insufficiency due to rapid withdrawal of treatment. This condition is called adrenocortical insufficiency [93, 116]. Current recommendations suggest dose tapering to minimize incidences of adrenal in sufficiency, however, a case study conducted by Joseph et.al demonstrated that adrenocortical insufficiency may occur at all levels of glucocorticoid exposure, even low dose and after tapering [117]. Therefore clinicians are encouraged to exercise caution when prescribing prednisone. Frequent tests for adrenal insufficiency are encouraged, and prednisone therapy should be initiated only if absolutely necessary.

Hypothyroid patients have decreased metabolic clearance of corticosteroids whereas hyperthyroid patients have increased clearance and therefore doses must be adjusted accordingly [104, 105].

1.8.3 Infection

Prednisone has immunosuppressive properties therefore patients currently on prednisone treatment are more susceptible to infection [118]. The rate of occurrence of infection increases with increasing doses [119, 120]. Corticosteroids may also mask some signs of current infection such as pain and fever [120]. Prednisone must not be used in the presence of a systemic fungal infection unless needed to control life-threatening drug reactions. Some cases have been reported where concurrent use of amphotericin B and hydrocortisone led to cardiac enlargement and congestive heart failure [104].

Latent diseases due to amoeba, candida, cryptococcus, mycobacterium, nocardia, pneumocystis and toxoplasma may be activated or exacerbated due to prednisone use. Therefore these plus presence of unexplained diarrhea must be ruled out before initiating prednisone therapy [105]. Caution must also be taken in patients with suspected strongyloid worms. Prednisone induced immunosuppression may lead to a strongyloid hyperinfection often accompanied by severe enterocolitis and potentially fatal gram-negative septicemia [103, 104].

Prednisone or any other corticosteroids should not be used in cerebral malaria [121]. HIV positive patients may also use prednisone if need be, but only under close monitoring because the immunosuppressive properties of prednisone may exacerbate the risk of opportunistic infections [14-16].

Only killed or inactivated vaccines must be administered during corticosteroid use. However, the response may be diminished due to immunosuppression caused by corticosteroids. Administration of live or live attenuated vaccines is contraindicated in patients receiving immunosuppressive doses of corticosteroids but to those receiving non-immunosuppressive doses of corticosteroids as replacement therapy for example in Addison’s disease immunization procedures may be performed as in any other patient [105, 121].

For viral infections such as chickenpox and measles, concurrent corticosteroid use may lead to serious consequences or even fatality both for pediatrics and adults. The best option is to avoid exposure, however if exposed to chickenpox, prophylaxis with varicella zoster immune globulin may be indicated. If exposed to measles, prophylaxis with pooled intramuscular immunoglobulin may be indicated and if chickenpox develops, treatment with antiviral agents may be considered [105].

1.8.4 Ophthalmic

Ophthalmic use of corticosteroids may produce posterior sub-capsular cataracts, glaucoma with possible damage to the optic nerves, and may enhance the establishment of secondary ocular infections due to bacteria, fungi or viruses [122]. The use of oral corticosteroids is not recommended in the treatment of optic neuritis and may increase the risk of new episodes. Corticosteroids should not be used in active ocular herpes simplex because of possible corneal perforation [104]. If therapy is for more than 6 weeks, intraocular pressure should be monitored. Some individuals suffer from elevated intraocular pressure as a result of corticosteroid therapy [123].

1.8.5 Musculoskeletal

Prednisone decreases osteoblast function, calcium absorption and increases calcium excretion leading to a reduction in bone formation and an increase in bone resorption [124]. Prednisone induced protein catabolism contributes to the erosion of the protein matrix in the bone. This, together with reduced sex hormone production, may lead to inhibition of bone growth in pediatric patients and the development of osteoporosis at any age [104].

Therefore pediatrics and patients at high risk of osteoporosis (for example postmenopausal women) must be monitored closely before and during treatment. Prophylactic osteoporosis therapy is advisable to minimize prednisone-induced bone loss. Lifestyle changes for example smoking cessation, reduced alcohol intake, weight-bearing exercises and use of supplements (calcium and vitamin D supplementation, bisphosphonate) help prevent the development of osteoporosis [103-105].

According to current recommendations any patient who is anticipated to take at least 5 mg of prednisone for at least 3 months should be advised towards the interventions mentioned above [104]. In addition, hypogonadal patients are advised to be on sex hormone replacement therapy (combined estrogen and progestin in women; testosterone in men) and patients with bone mineral density of the spine and hip below normal should be placed on biphosphonate therapy [104].

1.8.6 Neuro-psychiatric

High doses of prednisone are associated with acute myopathy in patients with neuromuscular transmission disorders such as myasthenia gravis. Concurrent use of prednisone and neuromuscular blocking drugs such as pancuronium has also been reported to cause acute

myopathy [125]. The myopathy is generalized and typically involves ocular and respiratory muscles and may result in quadriparesis [103]. Psychiatric changes such as euphoria, insomnia, mood swings, personality changes, and severe depression are common with prednisone therapy and clinical improvement or recovery after termination of therapy may require weeks to years [125].

1.8.7 Miscellaneous

Generally steroids may increase or decrease motility and number of spermatozoa in some patients. In addition they may suppress reactions to skin tests. Rare cases of anaphylaxis have been reported [125]. Some reports have linked the use of corticosteroids in chronic conditions to Kaposi's sarcoma. In such cases discontinuation of corticosteroids may result in clinical improvement [104].

1.9 DRUG INTERACTIONS

1.9.1 Amphotericin B injection and potassium-depleting agents

Concurrent use of prednisone and potassium depleting drugs such as amphotericin B and some diuretics may lead to hypokalemia [123, 126].

1.9.2 Antibiotics

Macrolide antibiotics such as azithromycin, clarithromycin and erythromycin decrease prednisone clearance [123].

1.9.3 Anticholinesterases

Concurrent use of prednisone and anticholinesterase agents such as neostigmine and pyridostigmine typically results in severe weakness in patients suffering from myasthenia gravis [105]. In these patients, it is recommended to withdraw anticholinesterase agents at least 24 hours before initiating prednisone therapy, otherwise if concurrent use is the only option then close monitoring is advised at all times and the need for respiratory support should be anticipated [104].

1.9.4 Oral anticoagulants

There are mixed reviews regarding the effect of prednisone on warfarin. In cases where prednisone diminishes the effect of warfarin, concurrent use must be accompanied by frequent coagulation index tests and doses must be adjusted accordingly [126].

1.9.5 Anti-diabetics

Prednisone may increase blood glucose concentrations therefore diabetic treatment doses must be adjusted accordingly [103, 125].

1.9.6 Anti-tubercular drugs

Prednisone increases plasma clearance of isoniazid [123, 128], and rifampicin increases plasma clearance of prednisone [129]. The effects of concurrent intake of other anti-tubercular drugs with prednisone have not been reported in literature.

1.9.7 Bupropion

Both prednisone and bupropion lower the seizure threshold therefore concurrent administration should be closely monitored. Low doses must be used to initiate treatment followed by gradual increases [104].

1.9.8 Cholestyramine

Cholestyramine may increase the clearance of prednisone [123].

1.9.9 Cyclosporine

Prednisone and cyclosporine stimulate each other's activity. Concurrent use has been accompanied with convulsions in previously reported cases [103].

1.9.10 Digitalis glycosides

Prednisone induced hypokalemia together with digitalis glycosides may increase the risk of arrhythmias [103]

1.9.11 Estrogens, including oral contraceptives

Estrogens may decrease the hepatic metabolism of prednisone and its derivatives thereby increasing their effect [104].

1.9.12 Fluoroquinolones

The risk of tendon rupture increases in elderly patients taking any corticosteroids including prednisone concurrently with fluoroquinolones such as ciprofloxacin and levofloxacin. Tendon rupture can occur during or after treatment with quinolones [105, 123].

1.9.13 Hepatic enzyme inducers, inhibitors and substrates

Barbiturates, phenytoin, carbamazepine and rifampicin induce the enzyme cytochrome P450 3A4. As a result, the metabolism and clearance of prednisone is increased, therefore dosage adjustments are needed during concomitant use [123]. Ketoconazole, itraconazole, ritonavir, indinavir and erythromycin inhibit the enzyme cytochrome P450 3A4 and may potentially increase plasma concentrations of prednisone [104].

Prednisone moderately induces the enzyme cytochrome P450 3A4. Concomitant administration of prednisone with drugs that are metabolized by cytochrome P450 3A4 may increase their metabolism and clearance leading to decreased plasma concentrations [104].

1.9.14 Ketoconazole

Some reports suggest that ketoconazole decreases corticosteroid metabolism and exacerbates the side effects associated with corticosteroid use. In addition ketoconazole alone may cause adrenal insufficiency during corticosteroid withdrawal since it inhibits adrenal corticosteroid synthesis [123].

1.9.15 Non-steroidal anti-inflammatory agents

Gastro-intestinal side effects are exacerbated by concurrent intake of prednisone and non-steroidal anti-inflammatory agents (NSAIDS). In such cases, antacids may help alleviate symptoms. Aspirin clearance is particularly increased during prednisone therapy leading to reduced plasma concentrations [123].

1.9.16 Phenytoin

Phenytoin stimulates prednisone metabolism and clearance resulting in diminished effects of prednisone [103].

1.9.17 Vaccines

Where possible routine vaccinations should be deferred until prednisone therapy is terminated. Prednisone causes immunosuppression so in cases where live vaccines are used there is a risk of reactivation and when inactivated vaccines are used patients exhibit diminished responses [105, 123].

1.9.18 Carcinogenesis, mutagenesis

Studies have not conclusively determined whether prednisone may potentially cause carcinogenesis or mutagenesis [104].

1.10 USE IN HIGH RISK GROUPS

1.10.1 Pregnancy

Prednisone is classified as a category C drug in relation to teratogenicity. Even though well controlled studies in pregnant women were not found to support this fact, when animals were given doses equivalent to the human dose teratogenic effects were observed. Cleft palate was the most common birth defect in the offspring [104]. As a result prednisone use in pregnancy should be avoided unless the potential benefit justifies the potential risk to the fetus and once born, the infants should be observed for any signs of hypo-adrenalism [105, 130].

1.10.2 Nursing Mothers

Corticosteroids are passed to the infant through breast feeding where they potentially suppress growth or interfere with endogenous corticosteroid production. Due to the potential for serious adverse effects in the infant a decision has to be made whether to discontinue breast feeding or to discontinue the drug after considering the risks versus the benefits [123, 130].

1.10.3 Pediatric use

Prednisone is indicated for the treatment of nephrotic syndrome in patients that are 2 years or older as well as aggressive lymphomas and leukemias in patients who are 1 month or older [104, 130]. It may also be used in cases of severe asthma and wheezing. The adverse effects in pediatric patients are similar to those in adults, so frequent tests are conducted to monitor blood pressure, weight, height, intra-ocular pressure, presence of infection, psychosocial disturbances, thromboembolism, peptic ulcers, cataracts and osteoporosis [130]. A decrease in growth velocity is typically observed in pediatric patients on prednisone therapy. Therefore the risks versus benefits of treatment should be weighed out and only if treatment is necessary, the lowest effective dose must be used. Growth should be monitored at all times [103, 130].

1.10.4 Geriatric use

Even though clinical studies did not show significant differences in response between younger patients and elderly patients, generally lower doses are advised for elderly patients owing to their decreased hepatic, cardial and renal function. Special considerations should be taken particularly for diabetic and hypertensive patients [123, 130].

1.11 ADVERSE REACTIONS

The following adverse reactions have been reported:

1.11.1 Allergic reactions

Prednisone may at times cause anaphylactoid reactions, hypersensitivity reactions, anaphylaxis and angioedema [126, 123].

1.11.2 Cardiovascular system

Prednisone may cause serious adverse reactions on the cardiovascular system such as bradycardia, cardiac arrest, cardiac arrhythmias, cardiac enlargement, circulatory collapse, congestive heart failure, ECG changes caused by potassium deficiency, edema, fat embolism, hypertension or aggravation of hypertension, hypertrophic cardiomyopathy in premature infants, myocardial rupture following recent myocardial infarction, necrotizing angiitis, pulmonary edema, syncope, tachycardia, thromboembolism, thrombophlebitis and vasculitis [105, 123].

1.11.3 Dermatologic

Prednisone may cause serious reactions affecting the skin, such as acne, acneiform eruptions, allergic dermatitis, alopecia, angioedema, angioneurotic edema, atrophy and thinning of skin, dry scaly skin, ecchymoses and petechiae (bruising), erythema, facial edema, hirsutism, impaired wound healing, increased sweating, Karposi's sarcoma, lupus erythematosus-like lesions, perineal irritation, purpura, rash, striae, subcutaneous fat atrophy, suppression of reactions to skin tests, striae, telangiectasis, thin fragile skin, thinning scalp hair and urticaria [104, 126, 130].

1.11.4 Endocrine

Prednisone may cause endocrine associated adverse effects such as amenorrhea, postmenopausal bleeding, menstrual irregularities, decreased carbohydrate and glucose tolerance, development of cushingoid state, diabetes mellitus (new onset or manifestations of latent), glycosuria, hyperglycemia, hypertrichosis, hyperthyroidism, hypothyroidism, increased requirements for insulin or oral hypoglycemic agents in diabetics, moon face, negative nitrogen balance caused by protein catabolism, secondary adrenocortical and pituitary unresponsiveness (particularly in times of stress, as in trauma, surgery or illness), and suppression of growth in pediatric patients [104, 126, 130].

1.11.5 Fluid and electrolyte disturbances

The use of prednisone may sometimes result in congestive heart failure especially in susceptible patients, fluid retention, hypokalemia, hypokalemic alkalosis, metabolic alkalosis, hypotension or shock-like reaction, potassium loss, sodium retention with resulting edema [105].

1.11.6 Gastrointestinal

The prolonged use of prednisone may sometimes result in abdominal distention, abdominal pain, anorexia which may result in weight loss, constipation, diarrhea, elevation in serum liver enzyme levels (usually reversible upon discontinuation), gastric irritation, hepatomegaly, increased appetite and weight gain. In addition prednisone may also cause nausea, oropharyngeal candidiasis, pancreatitis, peptic ulcer with possible perforation and hemorrhage, perforation of the small and large intestine (particularly in patients with inflammatory bowel disease), ulcerative esophagitis, and vomiting especially when a high dose is used [103, 123].

1.11.7 Hematologic

The use of prednisone may result in anemia and neutropenia (including febrile neutropenia) [105].

1.11.8 Metabolic

Protein catabolism induced by prednisone may result in negative nitrogen balance [131].

1.11.9 Musculoskeletal

Prolonged use of prednisone especially at high doses may cause reactions that affect the muscular and skeletal system. These adverse reactions often lead to arthralgias, aseptic necrosis of femoral and humeral heads, increased risk of fracture, loss of muscle mass, muscle weakness, myalgias, osteopenia, osteoporosis, pathologic fracture of long bones, steroid myopathy, tendon rupture (particularly of the Achilles tendon), and vertebral compression fractures [103, 123].

1.11.10 Neurological/Psychiatric

Following prolonged prednisone intake, some patients experience amnesia, anxiety, benign intracranial hypertension, convulsions, delirium, dementia (characterized by deficits in memory retention, attention, concentration, mental speed and efficiency, and occupational performance), depression, dizziness, emotional instability and irritability, euphoria, hallucinations, headache and impaired cognition. Incidences of severe psychiatric symptoms, increased intracranial pressure with papilledema (pseudotumor cerebri) usually following discontinuation of treatment, increased motor activity, insomnia, ischemic neuropathy, long-term memory loss, mania, mood swings, neuritis, neuropathy, paresthesia, personality changes, psychiatric disorders including steroid

psychoses or aggravation of pre-existing psychiatric conditions, restlessness, schizophrenia, verbal memory loss, vertigo and withdrawn behavior have also been reported [103-105].

1.11.11 Ophthalmic

The use of prednisone may at times result in blurred vision, cataracts (including posterior subcapsular cataracts), central serous chorioretinopathy, establishment of secondary bacterial, fungal and viral infections, exophthalmos, glaucoma, increased intraocular pressure, optic nerve damage, and papilledema [122, 123].

1.11.12 Other

Prolonged prednisone use sometimes results in abnormal fat deposition, aggravation or masking of infections, decreased resistance to infection, hiccups, and immunosuppression. Prednisone has also been reported to either increase the motility or decrease the number of spermatozoa. General malaise, insomnia, moon face, and pyrexia are also commonly associated with prednisone use [104, 123].

1.12 DOSAGE AND ADMINISTRATION

It is recommended to take prednisone with or immediately after meals or milk to reduce gastric irritation [126, 127].

The adrenal cortex is highly active between 2 am and 8 am, and has minimal activity from 4 pm to midnight. Therefore prednisone should preferably be administered before 9 am since it suppresses adrenocorticoid activity the least during this time, this is particularly important for single dose therapy [104]. When large doses are taken, concurrent intake of antacids may help prevent the development of peptic ulcers. When multiple doses are prescribed, they should be evenly spaced throughout the day. Dietary adjustments such as salt restriction are also encouraged. Withdrawal of doses should be done gradually [123].

Doses normally range between 5 mg to 60 mg depending on age and the disease under treatment but the lowest possible effective dose should be used. Due to multiple side effects associated with prednisone use, frequent monitoring should be undertaken and doses adjusted accordingly if need be [105]. In the treatment of acute exacerbations of multiple sclerosis daily doses of 200 mg of prednisone for a week followed by 80 mg every other day for 1 month have been shown to be effective. The same range applies for prednisolone [126].

For long durations of treatment another way to minimize adverse effects is use to utilize the alternate day therapy method. With this particular regimen twice the usual daily dose of corticoid is administered every other morning [126].

The goal is to provide the clinical benefits of prednisone whilst minimizing pituitary-adrenal suppression, the cushingoid state, corticoid withdrawal symptoms, and growth suppression in children [105, 123]. The rationale for this method is that alternate administration of the corticosteroid every other morning allows for re-establishment of more nearly normal hypothalamic-pituitary-adrenal (HPA) activity on the off-steroid day, and secondly the clinical effects of corticoids persists longer than their physical presence [104, 126].

1.13 CONCLUSIONS

Prednisone is one of the most widely used corticosteroids due to its effectiveness when treating a broad spectrum of conditions. It is commonly used to treat rheumatic arthritis, severe allergic reactions, dermatitis, some forms of anemia and lupus among other conditions. Regardless of many therapeutic benefits, oral delivery of prednisone is associated with many challenges. Poor solubility and low dissolution rate in gastrointestinal fluids often result in low bioavailability. Additionally, prednisone has harsh effects on the GIT and has an extremely bitter taste. In this study, an attempt was made to address the challenges associated with oral delivery of prednisone by formulating it as an immediate release multiple unit pellet system for inclusion in capsules. Multi-particulate dosage forms offer significant advantages over conventional technologies in many aspects particularly as they exhibit a lower incidence of gastrointestinal irritation due to decreased local concentration of the API in the GIT following oral administration. Furthermore, lower individual variability in plasma concentrations is observed when compared to tablets since there is a reduced risk of dose dumping. In addition, the presence of many individual units increases the surface area leading to improved solubility and bioavailability. The use of discrete units also offers a simple solution to minimizing potential API-excipient interactions and the free flowing nature of pellets facilitates reproducible capsule filling, content uniformity and dosing. Moreover, loading the dosage form into gelatin capsules presents a simple way to mask the bitter taste of prednisone. Prednisone use is associated with many side effects, both during and after treatment. Clinicians are encouraged to advise patients accordingly and limit prednisone use to cases where it is absolutely necessary.

CHAPTER 2
USE OF QUALITY BY DESIGN TO DEVELOP AND VALIDATE A LIQUID CHROMATOGRAPHIC METHOD FOR ANALYSIS OF PREDNISONE

2.1 INTRODUCTION

Liquid chromatography (LC) is an analytical technique that is useful for separating compounds that are dissolved in solution. LC is comprised of two phases, a stationary and a mobile phase. During the process, the mobile phase percolates through the stationary phase carrying the analyte and separation occurs due to a difference in affinity between the analyte and the stationary or mobile phase [132-135]. Traditional versions of LC - thin layer chromatography and paper chromatography, have now, due to numerous advantages been modified to High Speed LC, High Efficiency LC, High Performance LC (HPLC) and Ultra-Performance LC (UPLC) [136-140]. The modified methods still use the same basic principle derived from LC.

HPLC is one of the most widely used analytical techniques in the pharmaceutical, food, cosmetic, environmental, forensic, industrial and chemical industries [141-143]. There are two types of HPLC: Normal-Phase HPLC (NP-HPLC) and Reversed-Phase HPLC (RP-HPLC). In both cases separation is dependent on the nature of interactions that exist between the stationary and the mobile phase [144-146]. In NP-HPLC the stationary phase is polar and the mobile phase is nonpolar. Polar analytes have a high affinity for a polar stationary phase hence they are easily adsorbed and move slowly through the column [145]. Non-polar analytes have a strong affinity for a non-polar mobile phase hence they exit the column together with the mobile phase. It follows therefore, that increasing the polarity of a mobile phase in NP-HPLC subsequently decreases adsorption of polar analytes and *vice versa* [145].

In contrast, RP-HPLC makes use of a polar mobile phase and a non-polar or hydrophobic stationary phase. In RP-HPLC non-polar analytes are easily adsorbed to the stationary phase due to their strong affinity for hydrophobic surfaces. In order for elution to occur faster, organic solvents are typically added to the mobile phase to reduce polarity. Addition of an organic solvent decreases the hydrophobic interactions that exist between the analyte and stationary phase leading to desorption [145, 146]. RP-HPLC is more widely used due to its numerous advantages and broad

applications. The results generated from RP-HPLC are reproducible and the technique is robust. Furthermore, mobile phase composition, pH and ratio can be precisely controlled [147-149].

The objective of this study was to develop and optimize a RP-HPLC method capable of quantifying prednisone from solid dosage forms. Principles of analytical Quality by Design (AQbD) were used to produce a good quality method. The method was intended for use both during developmental stages and throughout the product's life cycle for quality assurance purposes.

2.2 LITERATURE REVIEW

A summary of RP-HPLC methods that have been developed and used for the quantitative analysis of prednisone in pharmaceutical dosage forms is presented in Table 2.1. These data were used to identify and set preliminary conditions for the development of a suitable HPLC method for the analysis of prednisone.

Table 2.1 Summary of RP-HPLC methods reported for the analysis of prednisone

Column	Mobile Phase	Flow rate	Detector	Retention time (min)	Reference
Luna® C18, 150 x 4.6 mm, 5 µm	17 mM monobasic potassium phosphate: acetonitrile (60: 40 v/v)	1.5 mL/min	UV 254 nm	19.5	190
Kinetex® Biphenyl, 50 x 3.0 mm, 2.6 µm	10 mM ammonium acetate in water: 10 mM ammonium acetate in MeOH (gradient elution)	0.4 mL/min	Tandem Mass Spectrometer	3.31	191
Lichrosphere® C8, 250 × 4.0 mm, 5 µm	methanol: tetrahydrofuran: water (25: 25: 50 v/v/v)	1.0 mL/min	UV 240 nm	6.20	192
Spherisorb® C6, 250 x 4.6 mm	methanol: water (40: 60 v/v)	1.4 mL/min	UV 254 nm	11.8	193
Symmetry® C18, 30 x 2.1 mm, 3.5 µm	methanol: 5 mM acetate buffer, pH 3.25 (70: 10 v/v)	400 µL/min	API 3000 Mass spectrometer	2.80	194
Brownlee Spheri® RP-18, 250 x 4.6 mm, 5 µm	methanol: water (57.5: 62.5 v/v)	1.2 mL/min	UV 242 nm	13.0	195
Zorbax-SIL®, 250 x 4.6 mm	dichloromethane: ethanol (92.5: 7.5 v/v)	1.0 mL/min	UV 254 nm	4.70	196
Whatman Partisil®, 250 x 4.5 mm	dichloromethane: ethanol: water (95: 4: 1 v/v)	1.5 mL/min	UV 239 nm	8.90	197
LichrospherSi® 60, 250 x 4.6 mm, 5 µm	methanol: glacial acetic acid: dichloromethane (1.5: 8.0: 90.5 v/v/v)	1.8 mL/min	UV 254 nm	8.00	198
Supelcosil® LC-18-DB, 150 x 4.6 mm, 5 µm	16 % isopropanol in water containing 0.1 % trifluoroacetic acid	1.2 mL/min	UV 254 nm	11.8	199
Zorbax Eclipse® XBD C18, 150 x 4.6 mm, 3.5 µm	10 mM dihydrogen potassium phosphate: acetonitrile pH 4.6 (70: 30 v/v)	1.0 mL/min	PDA 241 nm	4.90	200

2.3 EXPERIMENTAL

2.3.1 Chemicals and reagents

Prednisone was purchased from Skyrun (Taizhou, China) and Be-Tab Prednisone® tablets from a local pharmacy. The internal standard, hydrochlorothiazide (HCTZ) was donated by MSD (Johannesburg, South Africa). HPLC far UV grade acetonitrile (ACN) was purchased from ROMIL-SpS™ (Port Elizabeth, South Africa). HPLC grade water used for analyses was prepared using a Milli-Q® Academic A10 water purification system (Millipore®, Bedford, MA, USA) consisting of an Ionex® Ion Exchange cartridge, and a quantum Ex-ultrapore Organex® cartridge and filtered through a 0.22 µm Millipak® stack filter (Millipore®, Bedford, MA, USA). Sample solutions were filtered through a 0.45 µm HVLP Durapore® membrane filters (Millipore®, Bedford, MA, USA). Sodium hydroxide (NaOH) 0.1 M was prepared by dissolving 0.4 g of NaOH pellets (Rochelle Ltd, Johannesburg, South Africa) and hydrochloric acid (HCl) 0.1M was prepared by accurately pipetting 3.14 mL of 32 % v/v HCl (Merck chemicals Ltd, Wadeville, South Africa) into a 1000 mL conical flask and making up to volume with HPLC grade water.

2.3.2 Instrumentation

The HPLC system was a Waters® Alliance Model e2695 Separations Module equipped with an auto-sampler, degasser, a solvent delivery module; and a Model 2489 UV/Vis detector. Data processing was achieved using Waters Empower® 3 software (Waters®, Milford, MA, USA). The stationary phase was a Phenomenex Synergi™ Polar-RP 80Å 250 mm x 4.6 mm x 4 µm column (Separations®, Randburg, South Africa). Samples were placed in a Colora® ultra-thermostat water bath (Colora®, Lorch, Germany) to maintain temperature during degradation studies.

2.3.3 Chromatographic conditions

Separation was achieved by using a Waters Alliance system with a UV/Vis detector set at 254 nm on a Phenomenex Synergi™ Polar-RP 80Å 250 mm x 4.6 mm x 4 µm stationary phase. The mobile phase consisted of water and acetonitrile at a 65:35 % v/v composition. For all assessments, 1 mL of prednisone or prednisone containing formulation (100 µg/mL) plus 0.5 mL of HCTZ (100 µg/mL) solution was pipetted into the same vial then analyzed. Samples were injected into the system with a constant flow rate of 1 mL/min and a column temperature of 25 °C was usedused after optimization for this isocratic separation.

2.3.4 Preparation of stock solution

Approximately 10 mg prednisone and HCTZ were accurately weighed into two 100 mL A-grade volumetric flasks using a Mettler Toledo model AG135 balance (Mettler, Zurich, Switzerland) and dissolved in 35 mL acetonitrile and made up to volume with HPLC grade water to producte a stock solution with a final concentration of 100 µg/mL. Aliquots of the stock solution were serially diluted to produce working standards of 1, 5, 30, 40, 50 and 80 µg/mL. The internal standard used was maintained at 100 µg/mL for every experiment. For limit of detection experiments, the 1.0 mL working standard was further diluted to produce solutions of 0.2, 0.4, 0.6 and 0.8 µg/mL.

2.3.5 Preparation of mobile phase

Milli-Q® water was degassed under vacuum with the aid of a Model A-2S Eyela Aspirator degasser (Rikakikai Co., Ltd, Tokyo, Japan) and filtered through a 0.45 µm HVLP Durapore® membrane filter (Millipore® Corporation, Bedford, MA, USA). The water and HPLC-grade ACN were placed in separate 1000 mL Schott® Duran bottles (Schott® Duran GmbH, Wertheim, Germany) and mixed online to produce the desired mobile phase composition.

2.3.6 Column selection

The success of RP-HPLC as an analytical method largely depends on column choice. Separation is achieved due to interactive forces that exist between the analyte and stationary phase, therefore the physicochemical properties of the analyte largely influence suitable column type [150-153]. Different materials are commercially used as stationary phases in columns, however due to superior physical characteristics, silica is the most commonly used [154]. Silica based stationary phases have high mechanical strength, are stable and can withstand high operating pressures [152, 155]. Furthermore, silica is compatible with water and most organic solvents and does not swell when the solvent is changed [152, 156, 157]. In addition, the silica surface can be easily manipulated via covalent bonding to produce stationary phases with different functionalities.

Porous microspheres are preferred over irregularly shaped particles because of their easy packing. Different particle sizes are available, each with different pore size and surface area [152, 158]. Irregularly shaped particles fracture easily producing fines which accumulate in the column and this often results in high operating back pressures and distorted results [152, 159, 160].

Even though silica-based stationary phases possess many beneficial attributes, they also possess some undesirable properties. The most common one being their instability at extreme pH. At high pH (> 8) silica dissolves and the silica back-bone collapses. This is accompanied by a rapid decline in column efficiency and increased peak asymmetry [161]. In order to avoid these unwanted effects operating at a pH between 2 and 8 is generally recommended.

The surface area of a stationary phase has an impact on retention time. Stationary phases that have a large surface area tend to produce long retention times [162]. When separation is sorely due to hydrophobic interactions, retention times increase with increasing carbon content as follows: C18 > C8 > C3 > C1 [152, 163]. However when different mechanisms are involved and separation is due to multiple interactions then the number of bonded carbons has no significance on retention time [164, 165].

2.3.6.1 Column efficiency

Column efficiency is determined by calculating the theoretical plate number (*N*). A column that has ≥ 2000 theoretical plates is regarded as efficient [166]. The higher the *N* value, the more resolved and more defined the resulting peaks are. The theoretical plate number can be calculated by either Equation 2.1 or Equation 2.2.

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

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

Where,

N = Number of theoretical plates of a column
t_r = Retention time of the probe molecule
w_b = Width of the peak at the baseline
$w_{½}$ = Width of the peak at one half the maximum height [167]

A mixture of uracil, naphthalene, acetophenone, benzene and toluene in ACN was used to assess column suitability. A mobile phase of ACN-water in the ratio 65:35 v/v, ambient temperature of 22 ˚C, injection volume of 20 µL, flow rate of 1 mL/min and a wavelength of 254 nm were used for testing. Two columns were compared during method development. The average theoretical plate numbers obtained were 4122 and 2850 for a Phenomenex Synergi™ Polar-RP, 4 µm particle size column (250 mm x 4.6 mm) and Phenomenex Prodigy™, 5 µm particle size column (150 mm x 4.6 mm) respectively. Both columns were suitable as per FDA guidelines, therefore they could be used for analysis.

2.3.6.2 Resolution factor

The resolution factor (*Rs*) indicates the degree and quality of a separation between adjacent peaks [156]. *Rs* is a function of the column, operating conditions and instrument variables and is calculated using Equation 2.3.

$$Rs = \frac{t_2 - t_1}{0.5(w_1 - w_2)} \qquad \textit{Equation 2.3}$$

Where,

Rs = Peak resolution
t_2 = Retention time for second eluting peak
t_1 = Retention time for first eluting peak
w_1 = Width of first eluting peak at the base
w_2 = Width of second eluting peak at the base

A *Rs* > 1.5 is desirable as it produces enough separation for accurate integration and quantitation of individual peaks. *Rs* < 1.5 indicates poor resolution and is unacceptable since inaccurate results may be produced [156, 168]. For the Phenomenex Synergi™ Polar-RP 250 mm x 4.6 mm x 4 µm column the *Rs* was 3.1 and for Phenomenex Prodigy™ 150 mm x 4.6 mm x 5 µm column was 2.4 indicating that the peaks were well resolved and the columns were suitable for these studies.

2.3.6.3 Asymmetry factor

The level of peak tailing can be determined by calculating the asymmetry factor (*As*) [156]. *As* is important to consider because peak tailing may result in poor resolution, inaccurate quantitation, and varying retention times. Equation 2.4 and Equation 2.5 are used to calculate the asymmetry factor, also known as the peak tailing factor (*PTF*) [156, 168].

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

$$A_s = \frac{B}{A} \qquad \textit{Equation 2.5}$$

Where,

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

The *As* is established at 10 % of the full peak height, whereas the *PTF* is calculated at 5 % of the full peak height. Perfectly spherical peaks have an *As* = 1, however *As* values between 0.95 and 1.5 are considered acceptable. Values closest to 1 are associated with good column efficiency. The asymmetry factor of prednisone was 1.08 for the Phenomenex Synergi™ Polar-RP column.

2.3.6.4 Capacity factor

The capacity factor (*K'*) provides an indication of the length of time that a compound is retained on a column relative to the peaks observed for the void volume [152]. *K'* is often used to determine the rate of analyte migration through a column. The age of the column, temperature of operating system and mobile phase composition have an influence on *K'* [152, 165]. Equation 2.6 is used to calculate *K'*.

$$K' = \frac{V_1 - V_0}{V_0} \qquad \textit{Equation 2.6}$$

Where,

V_0 = Void volume of the column
V_1 = Retention volume of the analyte (retention time x flow rate)

Uracil was used to establish the void volume. *K'* values > 2 produce adequate separation [152, 165]. Low *K'* values are associated with low resolution so they are not acceptable. The capacity factor of prednisone was 2.66.

2.3.7 Method of detection

Establishing a highly sensitive method of detection that is selective for the analyte is of paramount importance [169, 170]. In addition to mobile phase composition, the physicochemical properties of the analyte, concentration of analyte and composition of dosage form, detection is highly dependent on the type and sensitivity of detector used [171]. For HPLC analysis, UV/VIS spectrophotometric detection is commonly used. Ease of use, relatively inexpensive maintenance, linearity, versatility, reliability and sensitivity warrant the increasing use of UV/VIS detectors [172, 173]. The majority of organic compounds absorb light in the UV region (190-400 nm) and a few in the visible region (400-750 nm) [266]. Moreover most HPLC grade solvents are transparent in the UV/VIS region, further enhancing sensitivity. However one major disadvantage of using a UV/VIS detector is that it can only be used for analytes that contain UV-absorbing chromophores [172, 173]. According to Beer-Lambert law, the light intensity transmitted from the detector is directly proportional to analyte concentration as highlighted in Equation 2.7 [174]. A wavelength of 254 nm was used for the quantitation of prednisone in subsequent studies.

$$I_T = I_0 e^{-kLc} \qquad \textit{Equation 2.7}$$

Where,

I_T = Intensity of the transmitted light
I_0 = Intensity of light entering the cell
L = Optical path length
c = Solute concentration
k = Constant

2.3.8 Selection of internal standard

To minimize quantitation errors during HPLC analysis, the peak area of the analyte is measured against that of a reference standard [175, 176]. The use of a reference standard, also known as an internal standard enhances the accuracy and quality of results. In this study all quantitative analysis involved the use of an internal standard.

To identify an internal standard, several compounds were assessed for peak quality and retention time using the same chromatographic conditions as those for prednisone. Mestranol, beclomethasone and hydrochlorothiazide (HCTZ) were assessed and the results for these studies are summarized in Table 2.2. HCTZ was selected as the internal standard and was used for all subsequent studies.

Table 2.2 Selection of internal standard

Internal standard	Retention time (min)	Comments
Mestranol	2.7	Too close to the solvent front. Peak tailing was observed.
Beclomethasone	2.7	Too close to the solvent front. Peak tailing was observed.
Hydrochlorothiazide	4.9	Well resolved from prednisone. Sharp peaks were observed. Ideal internal standard.

2.4 QUALITY BY DESIGN APPROACH TO RP-HPLC METHOD DEVELOPMENT

Recently, the pharmaceutical industry has made use of Quality by Design (QbD) for analytical method development. When used for this objective, QbD is more specifically referred to as analytical QbD (AQbD). To our knowledge, application of AQbD in this regard has not been applied to any of the currently documented analytical methods used for the analysis of prednisone.

AQbD is a systematic, knowledge based approach to understanding the impact of critical method parameters (CMP) on critical analytical attributes (CAAs) [177]. AQbD uses quality risk management principles and Design of Experiments to identify potential risks and interactions that may exist between variables. By so doing, the number of experiments required is significantly reduced, saving a time, effort and reducing costs [178].

AQbD facilitates the establishment of a multi-dimensional analytical design space, which permits regulatory flexibility should method transfer be required [179, 180]. Furthermore, some studies have reported success in using AQbD principles to develop analytical methods capable of separating active ingredients from degradation products, and to establish cost effective efficient chromatographic separations [181-183]. We attempted to develop a robust, accurate, reliable, sensitive and selective analytical method for quantitation of prednisone by implementing the principles of AQbD to gain aholistic understanding of method performance through AQbD.

2.4.1 Analytical target profile

For systematic development of the analytical method for analysis of prednisone, the analytical target profile (ATP) was defined, setting the objectives and purpose of the method required to meet the desired quality characteristics in accordance to prescribed guidelines [184]. The ATP set the foundation upon which the method was designed and developed. The ultimate goal was to develop a selective analytical method capable of quantifying prednisone without any interference from excipients or degradation products. A short run time was necessary for rapid quantitation and to avoid unnecessary solvent wastage. The main aim was to achieve a robust, accurate, reliable, sensitive and selective analytical method for use during quality control and assay. The elements of the defined ATP are listed in Table 2.3.

Any newly developed HPLC method must be validated in accordance with the International Council for Harmonization (ICH), Food and Drug Administration (FDA) or United States Pharmacopoeia (USP) guidelines before it is deemed appropriate for use. Validation entails use of a series of tests conducted to establish the specificity, selectivity, linearity, range, accuracy, precision, sensitivity and robustness of an analytical method [185, 186] and are discussed in detail in subsequent sections of this thesis.

Table 2.3 Analytical target profile for the RP-HPLC method for analysis of prednisone

Method performance characteristics	Target profile
Accuracy and precision	Comply with ICH, FDA and USP standards.
Linearity, limit of quantitation and detection	Comply with ICH, FDA and USP standards.
System suitability	Comply with ICH standards.
Run time	< 10 minutes
Specificity	Method should be selective for prednisone and peaks should be well resolved.
Stability-indicating properties	Prednisone peaks should be well resolved from impurities.
Quantitation	Sharp, well resolved peaks for prednisone assay and dissolution studies.

2.4.2 Critical method parameters

As a prelude to developing an effective and efficient analytical method, a prioritization exercise has to be conducted, using quality risk management principles [187]. This exercise enables the identification of a few prominent parameters, among many, which have the most significant effect on desired method performance [188]. These few yet prominent input variables are termed critical method parameters (CMP). A list of potential method variables was gathered from literature and preliminary experimentation. An Ishikawa fishbone diagram was used to establish potential cause and effect relationships between input method variables and analytical method performance [189]. The Ishikawa fishbone diagram generated for this study is depicted in Figure 2.1. Following analysis of the Ishikawa fishbone diagram and data generated from preliminary experimentation, critical factors were identified and noted.

Column type, mobile phase ratio and column temperature were identified as critical factors affecting critical analytical attributes (CAA) hence were selected for optimization studies. The remaining factors were regarded as controllable, hence factor screening was not conducted. The non-critical factors were not evaluated further to save time and to reduce the number of experiments. A Central Composite Design (CCD) approach was used to facilitate systematic evaluation of all three factors in an efficient and comprehensive manner.

Several studies have reported success in quantifying prednisone using a wavelength of 254 nm [190, 193, 196, 198, 199]. Sharp peaks were observed with our system settings and a wavelength of 254 nm during preliminary experimentation and therefore this wavelength was used for the duration of the study. An injection volume of 20 µL and flow rate of 1 mL/min were used for all analyses. Prednisone is a neutral molecule, and separations are unaffected by changes in pH, consequently a buffer was not required for this analysis. Water and acetonitrile were used in combination as the mobile phase.

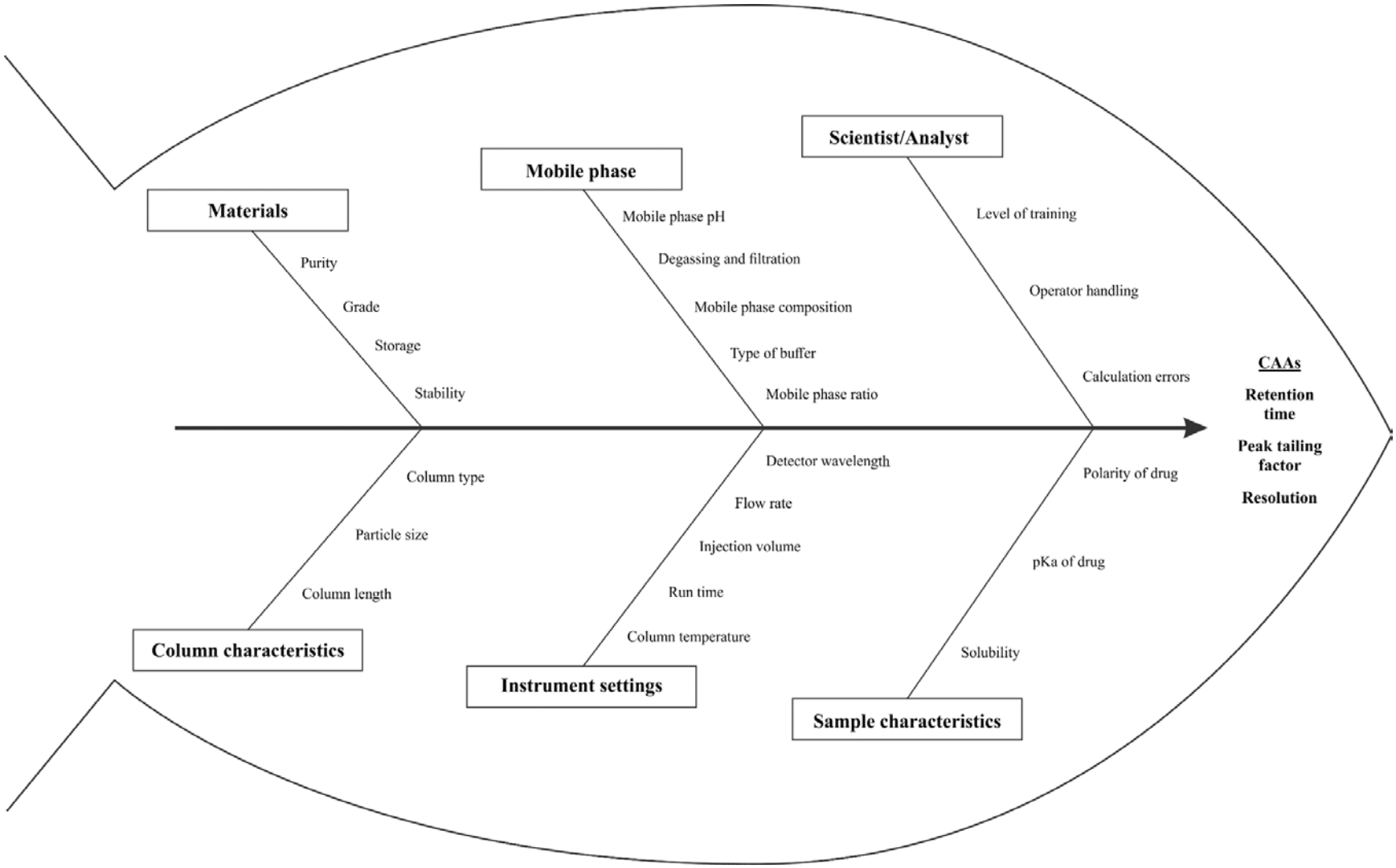


Figure 2.1 Ishikawa fishbone diagram illustrating factors that may affect the performace of a RP-HPLC method for determination of prednisone

2.4.3 Critical analytical attributes (CAA)

Different Critical Analytical Attributes (CAA) were identified in order to meet the ATP. CAA are performance attributes by which the impact of critical method parameters are measured. The retention time of prednisone, retention time of HCTZ, peak tailing and resolution were selected as CAA as tetention time and resolution determine separation ability, whereas the level of peak tailing determines efficiency of quantification with minimum error [184].

2.4.4 Design of Experiments (DoE)

Different input factors and process settings interact to achieve separation during an HPLC analysis. Instead of using the traditional 'one factor at a time' approach to assess the impact of each input factor, Response Surface Methodology (RSM) was used for the study. RSM facilitates simultaneous and systemic evaluation of multiple factors with the aid of statistical and mathematical tools [201-203]. The relationships are computed as mathematical and statistical models [202].

RSM broadly encompasses a multiplicity of designs, however the Central Composite Design (CCD) is the most commonly used [201, 202]. CCD is a combination of a two-level full or fractional factorial design with additional axial or star points and at least one point at the center of the experimental region. It allows the determination of both linear and quadratic models [205, 206]. CCD is commonly used because it is efficient at investigating relationships with fewer experiments [204, 207]. In 1957 Box and Hunter derived conditions required to make a CCD rotatable. When k-number of factors are investigated the design should contain a center point, 2k factorial runs and 2k axial runs spaced at $\pm \alpha$ for each variable [208]. Each of these three parts need to be specified when building a CCD. In addition, the number of factorial (cubic) points, their location and the value of α need to be specified [208].

The value for α either ranges between 1 and $\sqrt{k}$ or is equal to $\sqrt{k}$. The points investigated in the study, are located at a distance proportional to α and the design is near rotatable [208, 209]. The experimental runs for a CCD model can be calculated using Equation 2.8 [210-213].

$$N = 2^k + 2k + n_c \qquad \textit{Equation 2.8}$$

Where,

k = Number of factors
n_c = Number of center points

Design-Expert® software (Version 8.0.7.1, Stat-Ease Inc., Minneapolis, MN, USA) was used to generate CCD experiments. The input factors were acetonitrile content (X_1), column temperature (X_2) and column type (X_3). The output responses monigored include were retention time of prednisone (Y_1), retention time of hydrochlorothiazide (Y_2), resolution (Y_3), peak tailing factor of prednisone (Y_4) and peak tailing factor of hydrochlorothiazide (Y_5). The actual and coded levels used are summarized in Table 2.4. The design had two numerical factors and one categorical factor. The multivariate experiments generated for the study are summarized in Table 2.5.

Table 2.4 Actual and coded levels used for CCD experiments

Variable	Type	Level		
		-1	**0**	**+1**
Acetonitrile (%)	Numeric	25	35	45
Temperature (°C)	Numeric	20	25	30
Column type	Categorical	Column A		Column B

*Column A - Phenomenex Synergi™ Polar-RP 4 µm 250 mm x 4.6 mm i.d. column.
*Column B - Phenomenex Prodigy™ 5 µm 150 mm x 4.6 mm i.d. column.

Table 2.5 CCD for the development of a RP-HPLC method for the analysis of prednisone

Run	Acetonitrile (X_1) %	Column temperature (X_2) °C	Column type (X_3)
1	25.0	30.0	A
2	25.0	20.0	A
3	35.0	25.0	A
4	35.0	25.0	A
5	35.0	25.0	B
6	35.0	25.0	B
7	45.0	25.0	B
8	45.0	20.0	A
9	35.0	25.0	B
10	35.0	25.0	A
11	35.0	30.0	B
12	35.0	25.0	A
13	25.0	30.0	B
14	25.0	25.0	B
15	45.0	20.0	B
16	35.0	25.0	A
17	35.0	20.0	A
18	35.0	20.0	B
19	25.0	25.0	A
20	35.0	30.0	A
21	25.0	20.0	B
22	45.0	30.0	A
23	35.0	25.0	B
24	35.0	25.0	B
25	45.0	25.0	A
26	45.0	30.0	B

2.5 RESULTS

The responses observed from CCD studies are summarized in Table 2.6.

Table 2.6 Experimental responses from CCD studies

Run	Rt Prednisone (Y_1) min	Rt Hydrochlorothiazide (Y_2) min	Resolution (Y_3)	*PTF* Prednisone (Y_4)	*PTF* Hydrochlorothiazide (Y_5)
1	23.22	6.99	2.94	1.04	1.06
2	23.05	7.75	2.69	3.99	1.03
3	8.06	4.84	2.07	1.14	1.12
4	7.98	4.88	2.16	1.04	1.51
5	3.87	2.48	5.03	2.01	1.85
6	3.90	2.50	4.97	2.02	1.87
7	2.67	2.23	2.03	2.03	2.01
8	4.95	4.04	4.47	1.04	1.04
9	3.91	2.51	4.93	1.95	1.83
10	8.04	4.94	2.10	1.06	1.07
11	3.75	2.33	5.38	1.98	1.72
12	8.07	4.98	2.06	1.06	1.07
13	9.91	2.95	1.19	6.61	1.85
14	10.4	3.15	1.63	7.20	1.78
15	2.52	2.10	1.73	1.73	1.79
16	8.56	5.13	2.19	1.06	1.07
17	8.08	5.22	2.07	1.02	1.04
18	3.79	2.48	4.00	2.15	1.82
19	25.05	7.73	2.99	1.03	1.06
20	7.90	4.72	2.26	1.06	1.08
21	10.28	3.21	1.09	1.46	2.23
22	5.09	3.88	6.03	1.02	1.04
23	3.90	2.50	4.91	1.90	1.78
24	3.91	2.51	4.89	1.88	1.77
25	5.09	4.01	5.76	1.07	1.07
26	2.50	2.01	2.16	1.75	1.87

The retention time of prednisone ranged between 2.50 min and 25.05 min, retention time of hydrochlorothiazide between 2.01 min and 7.75 min, resolution between 1.09 and 6.03, peak tailing factor of prednisone between 1.02 and 7.20, and peak tailing factor of hydrochlorothiazide between 1.03 and 2.23.

2.5.1 Model fitting and statistical analysis

Following experimentation, a number of statistical tools were used to assess the significance of each factor investigated. Data generated from ANOVA were used to evaluate model adequacy and fitness. The probability (p) value was used to establish if the result was statistically significant. The p-value is used to determine whether to accept or reject the null hypothesis, where a p-value < 0.05 implies that the model used is significant, and that the evidence favors the alternative hypothesis instead of the null hypothesis [214]. The model F-value determines the utility of the model and establishes if a model best fits the data set. The F-value is a ratio of explained variability to unexplained variability. Explained variability is based on the value for R^2 and unexplained variability is based on $(1 - R^2)$, each divided by corresponding degrees of freedom. The larger the F-value, the more useful the model is [215, 216]. R^2 values and standard deviation (SD) for the responses indicate the quality of the model. R^2 values close to 1 indicate good correlation between the observed experimental and predicted response. Therefore all models that had R^2 values close to 1 were of good quality. Adjusted (Adj) R^2 values in close agreement with predicted R^2 values indicate reliability of the model [217, 218]. Adequate (Adeq) precision is a comparison of the range of predicted values at the design points and the average prediction error. Adequate precision is also defined as the signal-to-noise ratio, where a ratio greater than 4 is desirable, and indicates that there is adequate model discrimination [216, 219, 220]. The coefficient of variation (CV) quantifies the variability associated with repeated measurements [221]. Low CV values indicate better precision and reliability of the experiments performed [222]. A summary of ANOVA data generated from CCD experiments is listed in Table 2.7.

Table 2.7 ANOVA results from CCD experiments

Response	R^2	Adj R^2	Predicted R^2	Adeq Precision	CV %	F-value	P-value	SD
Y_1	0.9747	0.9628	0.9202	28.382	15.20	81.91	< 0.0001	1.22
Y_2	0.9890	0.9839	0.9655	42.725	5.52	191.81	< 0.0001	0.22
Y_3	0.1702	0.0571	-0.2098	4.546	59.30	0.73	0.6608	1.75
Y_4	0.8335	0.7551	0.1953	8.696	32.64	10.63	< 0.0001	0.18
Y_5	0.9133	0.9015	0.8776	17.595	8.65	77.25	< 0.0001	0.13

*Data written in purple indicates significant factors.

The retention time of prednisone (Y_1), retention time of hydrochlorothiazide (Y_2), peak tailing factor of prednisone (Y_4) and peak tailing factor of hydrochlorothiazide (Y_5) exhibited p-values < 0.05 implying that the models for these terms were significant. In addition they exhibited R^2 values close to 1 which were in close agreement with the Adj R^2 value implying good model fitness and adequacy. The model for resolution exhibited a p-value > 0.05 implying that it was insignificant. Even though models for the retention time and peak tailing of hydrochlorothiazide were also generated, they will not be discussed in detail since hydrochlorothiazide was only used as an internal standard.

2.5.1.1 Retention time (Prednisone)

Rt is a measure of the time taken for an analyte (prednisone) to pass through a column from injection to detection. At the point of detection the chromophores in the analyte absorb UV radiation, and the result is computed as a peak, where maximum peak height represents the Rt. The Rt of an analyte is not inherently fixed, but is dependent on column temperature, mobile phase composition, column type (stationary phase, length, particle size) and the pressure used to generate flow [223, 224]. The goal of the study was to find a balance between these independent factors, and establish factor combinations and settings that yield reliable and reproducible results for use in all subsequent studies. A summary of ANOVA data for the retention time of prednisone is presented in Table 2.8. The model had an F-value of 81.91 and a p-value < 0.0001 indicating that the model was significant and adequate for establishing the impact of independent variables on the retention time of prednisone.

Table 2.8 ANOVA data for the retention time of prednisone

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F
Model	973.47	8	121.68	81.91	< 0.0001
X_1	521.56	1	521.56	351.07	< 0.0001
X_2	$7.701e^{-003}$	1	$7.701e^{-003}$	$5.184e^{-003}$	0.9434
X_3	232.82	1	232.82	156.72	< 0.0001
X_1X_2	0.013	1	0.013	$8.562e^{-003}$	0.9274
X_1X_3	92.19	1	92.19	62.05	< 0.0001
X_2X_3	0.027	1	0.027	0.018	0.8938
X_1^2	114.82	1	114.82	77.29	< 0.0001
X_2^2	0.76	1	0.76	0.51	0.4832
Residual	25.26	17	1.49		
Lack of Fit significant	25.03	9	2.78	98.83	< 0.0001
Pure Error	0.23	8	0.028		
Cor Total	998.73	25			
R^2					0.9747
Adj-R^2					0.9628
Pred-R^2					0.9202
Adeq-Precision					28.382
CV					15.20
SD					1.22

*Data written in purple indicates significant factors.

The relationship between input factors and retention time is described mathematically using Equation 2.9.

$$Y_1 = 6.09 - 6.59X_1 - 0.025X_2 - 2.99X_3 + 0.040X_1X_2 + 2.77X_1X_3 - 0.048X_2X_3 + 4.56X_1^2 - 0.37X_2^2 \quad \textit{Equation 2.9}$$

Individually, the ACN content, temperature and column type all had an antagonistic effect on retention time as highlighted by the negative signs in Equation 2.9 Interactions between ACN-temperature (AB) and ACN-column type (AC) resulted in agonistic effects on retention time as highlighted by the positive signs.

During HPLC method development, the retention time is the most critical response because it directly influences the run time of the analytical method. A short run time was set as one of the desired ATPs, to save time and to minimize solvent wastage. For the analytical method to be considered rapid, the run time should lie between 5 to 10 minutes [152].

ANOVA data revealed ACN content to be the most significant variable that affected retention time. ACN concentration had the largest F-value of 351.07 and a p-value that was < 0.0001. A visual representation of the impact of changes in ACN concentration on retention time is presented in the 3D response surface and contour plots depicted in Figures 2.2 and 2.3 respectively. A water-ACN mixture was used as the mobile phase for analysis. An increase in concentration of ACN from 25 % v/v to 45 % v/v resulted in a decrease in retention time of prednisone. This is confirmed by the negative sign in the quadratic Equation 2.9. The faster elution is attributed to a decrease in hydrophobic interactions between prednisone and the stationary phase of the column [152, 225]. Increasing the concentration of ACN led to a decrease in polarity of the mobile phase, causing preferential partitioning of prednisone into the mobile phase, and hence rapid elution since prednisone is hydrophobic [226]. Retention times < 10 minutes were of interest in this study.

The effect of column temperature on retention time was insignificant as highlighted by a very low F-value of $5.184e^{-003}$ and a p-value of 0.9434 which is > 0.05. The negative sign on the coefficient of temperature in Equation 2.9 may be because an increase in temperature reduces the viscosity of the mobile phase leading to a small reduction of Rt [152, 227]. Contour and 3D response surface plots confirm how insignificant the change in Rt was with an increase in column temperature.

The other significant factors were column type and the ACN-Column type interactions with F-values of 156.72 and 62.05 respectively and p-values < 0.0001 for both. Column A had a length of 250 mm and had a larger surface area for chromatography than column B which had a length of 150 mm. In addition, both columns had different silica back-bones for comparison, so even when similar instrument settings and same mobile phase compositions were used, the results were different. Column A produced sharper peaks with adequate separation therefore it was selected as the column of choice for all subsequent studies.

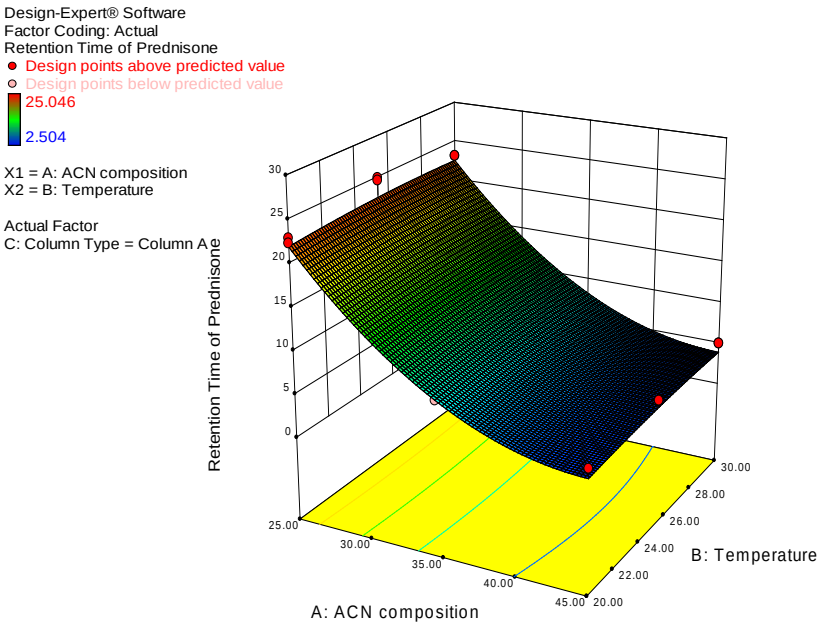


Figure 2.2 3D response surface plot depicting the impact of ACN content and temperature on retention time

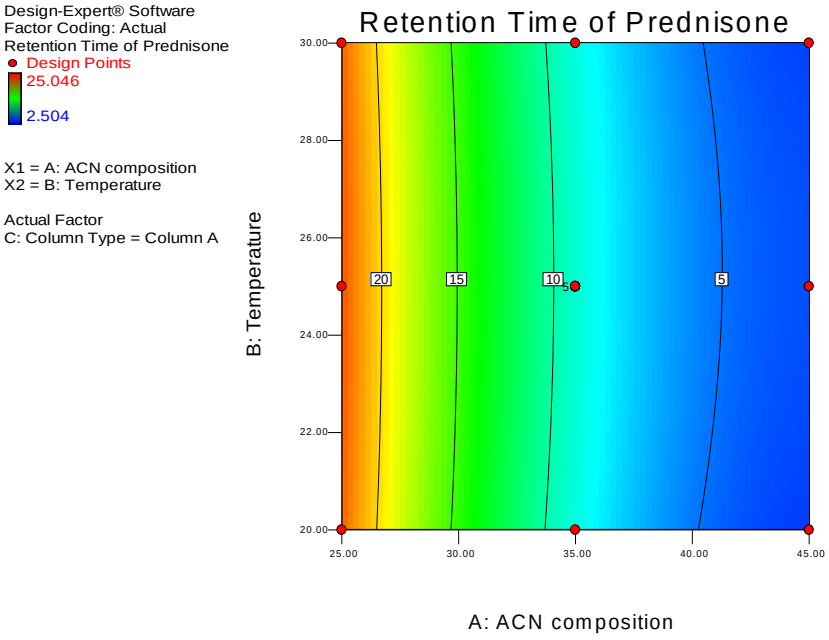


Figure 2.3 Contour plot depicting the impact of ACN content and temperature on retention time

The model for Rt produced an R^2 value of 0.9747, indicative of good quality. The predicted R^2 value of 0.9202 was in reasonable agreement with the adjusted R^2 value of 0.9628. Adequate precision produced a ratio of 28.382 indicating good signal. Residuals were examined to further evaluate model adequacy. Studentized residuals are used to measure the number of standard deviations that separate the actual and predicted responses [220]. Analysis of residuals is conducted by either evaluation of normal probability plots of residuals or alternatively by evaluation of the plot of residuals versus predicted responses. An adequate model is accompanied by points which fall on a straight line on the normal probability plot, indicative of equal distribution of error across each individual point. In contrast, points on the plot of residuals versus predicted responses should show random scatter and not follow a specific pattern, indicating that the variance from original observations is constant for all responses [215, 216, 220]. Figure 2.4 which depicts the normal plot of residuals for the retention time of prednisone and Figure 2.5 which depicts the plot of residuals versus predicted responses for the retention time of prednisone clearly indicate that the model satisfied these criteria. Therefore the model was deemed suitable and adequate for use in evaluating the impact of input variables on Rt of prednisone.

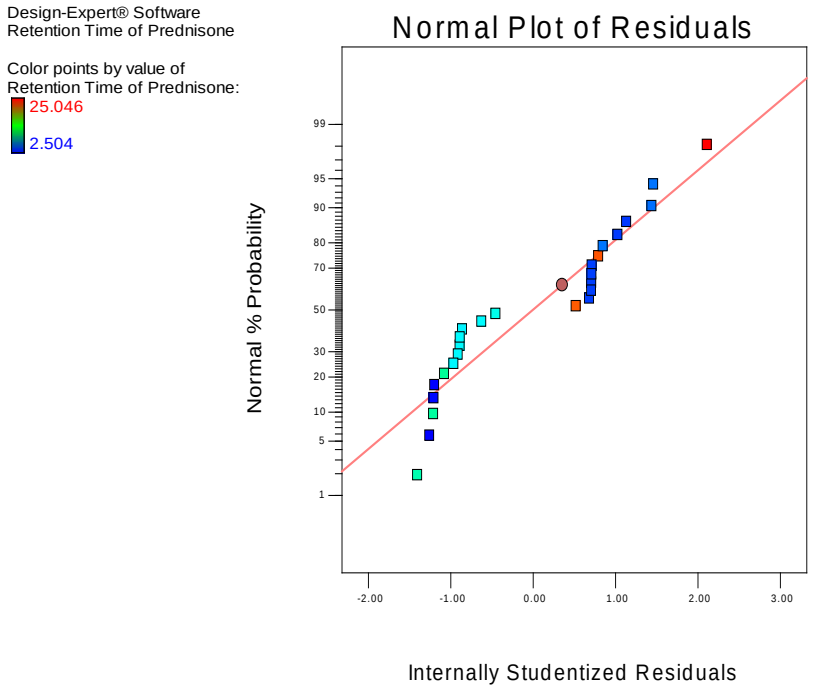


Figure 2.4 Normal plot of residuals for retention time of prednisone

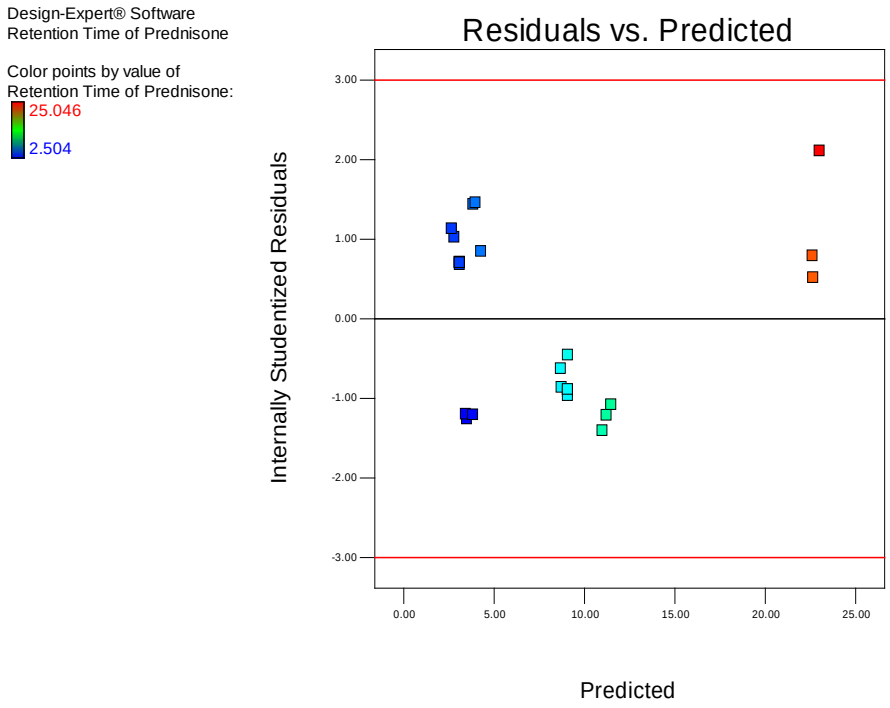


Figure 2.5 Plot of Residuals vs. Predicted responses for retention time of prednisone

2.5.1.2 Peak tailing (Prednisone)

Peak tailing is an important parameter for consideration due to its ability to directly affect resolution, quantitation, and the reproducibility of retention times. Following ANOVA, the model for peak tailing was found to have an R^2 value of 0.6666, indicative of poor quality. Data generated for the model are listed in Table 2.9. Most statistical analyses assume normal distribution of data for easy computation [215, 216]. However most data sets do not in fact follow a normal distribution. Better results are obtained when these data sets are transformed to approximate normality [228].

Table 2.9 ANOVA data for peak tailing of prednisone before Box-Cox transformations

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F
Model	42.34	8	5.29	4.25	0.0059
X_1	13.33	1	13.33	10.70	0.0045
X_2	0.34	1	0.34	0.27	0.6072
X_3	12.57	1	12.57	10.09	0.0055
X_1X_2	0.63	1	0.63	0.51	0.4865
X_1X_3	3.85	1	3.85	3.09	0.0969
X_2X_3	5.18	1	5.18	4.16	0.0572
X_1^2	6.21	1	6.21	4.98	0.0393
X_2^2	0.26	1	0.26	0.21	0.6553
Residual	21.18	17			1.25
Lack of Fit significant	21.16	9	2.35	842.30	< 0.0001
Pure Error	0.022	8			$2.791e^{-003}$
Cor Total	63.52	25			
R^2					0.6666
Adj-R^2					0.5097
Pred-R^2					-0.4584
Adeq-Precision					8.6290
CV %					56.510
SD					1.1200

*Data written in purple indicates significant factors.

Box-Cox power transformations were used to improve the applicability and quality of the model for peak tailing. Analysis of the Box-Cox plot for peak tailing depicted in Figure 2.6 revealed that transformation to the power $\lambda = -2.03$ was recommended, since the current $\lambda = 1$ was not located within the confidence interval. Data located within the confidence interval have the best approximation of normality.

Box-Cox plots prior to and following transformation of data sets are depicted in Figure 2.6 and Figure 2.7 respectively. The blue line reflects the current position of λ and the red lines indicate the 95 % confidence interval region. The lower confidence interval limit was -3.32 and the higher confidence interval limit was -0.93. Prior to transformation, $\lambda = 1$ was located outside the confidence interval region as indicated by the blue line in Figure 2.6. Following data transformation, the new $\lambda = -2.03$ indicated by the blue line in Figure 2.7 was located in the confidence interval region indicating the best approximation of normality [229-231].

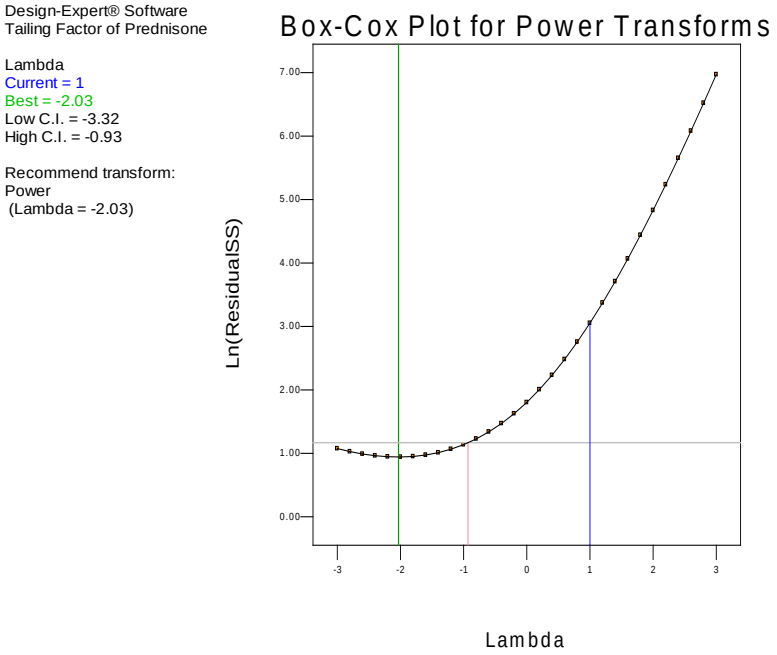


Figure 2.6 Box-Cox plot for peak tailing before transformation

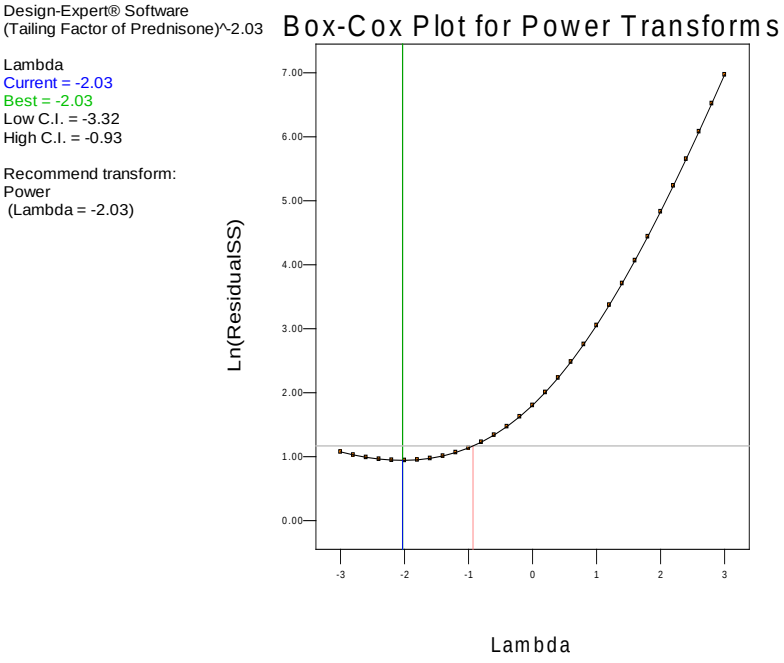


Figure 2.7 Box-Cox plot for peak tailing after transformation

Following Box-Cox power transformations, the R^2 value of the model improved from 0.6666 to 0.8335 and the adjusted R^2 value from 0.5097 to 0.7551 indicative of improved quality. In addition the predicted R^2 value improved from -0.4584 to 0.1953. The predicted R^2 value of 0.1953 was still not as close to the adjusted R^2 as normally expected possibly due to a large block effect. However the adequate precision was 8.696 which is > 4 therefore adequate signal was generated, enough for the model to be deemed fit for use in navigating the design space in explaining the results of peak tailing. ANOVA data generated following Box-Cox transformations are presented inTable 2.10.

Table 2.10 ANOVA data for peak tailing of prednisone after Box-Cox transformations

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F
Model	2.63	8	0.33	10.63	< 0.0001
X_1	0.12	1	0.12	3.78	0.0687
X_2	0.015	1	0.015	0.50	0.4890
X_3	2.31	1	2.31	74.76	< 0.0001
X_1X_2	0.019	1	0.019	0.61	0.4440
X_1X_3	0.017	1	0.017	0.56	0.4644
X_2X_3	0.12	1	0.12	4.02	0.0612
X_1^2	0.022	1	0.022	0.70	0.4137
X_2^2	$4.658e^{-005}$	1	$4.658e^{-005}$	$1.507e^{-003}$	0.9695
Residual	0.53	17	0.031		
Lack of Fit significant	0.51	9	0.057	29.70	< 0.0001
Pure Error	0.015	8	$1.909e^{-003}$		
Cor Total	3.16	25			
R^2					0.8335
Adj-R^2					0.7551
Pred-R^2					0.1953
Adeq-Precision					8.6960
CV %					32.640
SD					0.1800

*Data written in purple indicates significant factors.

The relationship between input variables and peak tailing is mathematically depicted by Equation 2.10.

$$Y_4^{-2.03} = 0.57 + 0.099X_1 + 0.036X_2 - 0.30X_3 - 0.049X_1X_2 - 0.038X_1X_3 - 0.10X_2X_3 - 0.063X_1^2 - 2.904e^{-003}X_2^2$$

Equation 2.10

Positive coefficients of ACN content and column temperature indicate agonistic effects whereas negative coefficients on column type and all factor interactions indicate antagonistic effects. A summary of ANOVA data generated for peak tailing after Box-Cox power transformations is presented in Table 2.9.

The model had an F-value of 10.63 and a p-value < 0.0001 therefore the model was significant. Column type was the only significant factor, accompanied with an F-value of 74.76 and p-value < 0.0001. Column A (Phenomenex Synergi™ Polar-RP, 250 mm x 4.6 mm, 4 µm) produced sharper peaks with reproducible retention times compared to column B. Therefore column A was selected for use in all subsequent analyses.

Visual representations of the effect of ACN concentration and column temperature on peak tailing (column A) are presented in the 3D response surface and contour plots in Figure 2.8 and Figure 2.9 respectively. Both variables had minute effects on peak tailing but the concentration of ACN was slightly more significant. Increasing the concentration of ACN resulted in tailing factors closer to 1. Likewise, the higher the column temperature, the closer the tailing factor was to 1.

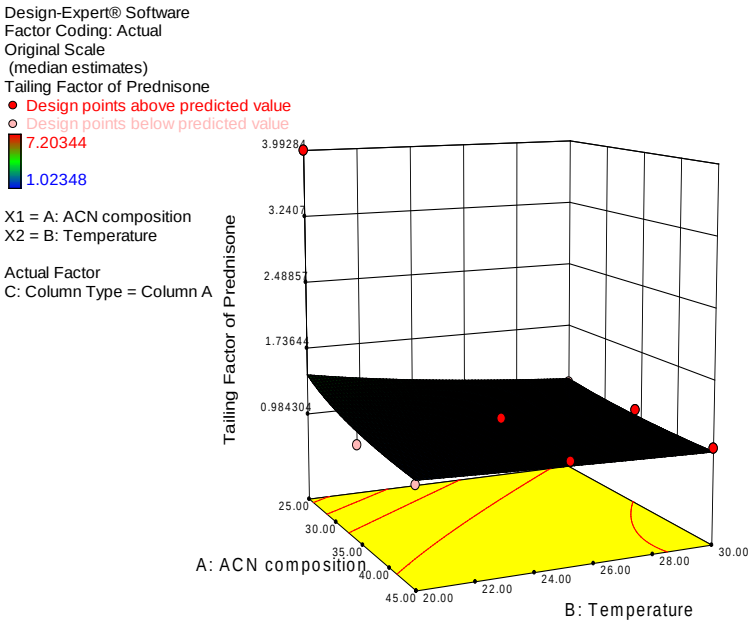


Figure 2.8 3D response surface plot depicting the impact of ACN content and temperature on peak tailing

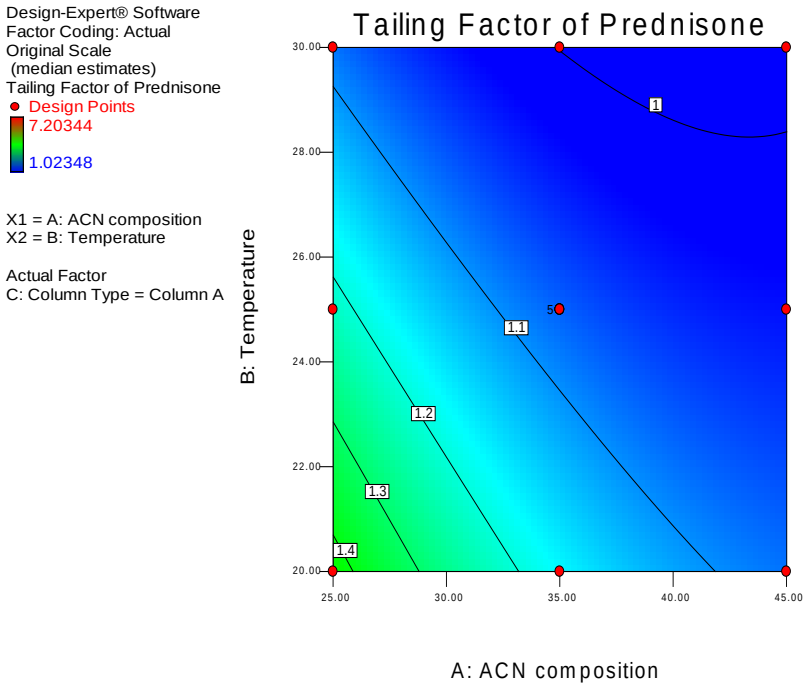


Figure 2.9 Contour plot depicting the impact of ACN content and temperature on peak tailing

As previously mentioned, the model for peak tailing produced an R^2 value of 0.8335 which was not very close to unity. Furthermore the adjusted R^2 value of 0.7551 was not close to the predicted R^2 value of 0.1953, so even though the model was significant, to further validate model adequacy, residuals were examined. When residuals are analyzed, an adequate model is accompanied by points which fall on a straight line on the normal probability plot to show that error was equally distributed across each individual point, whilst points on the plot of residuals versus predicted responses should be scattered and not follow a specific pattern [215, 216, 220]. The plots in Figures 2.10 and 2.11 clearly indicate that the model satisfied the criteria therefore the model was deemed suitable and adequate.

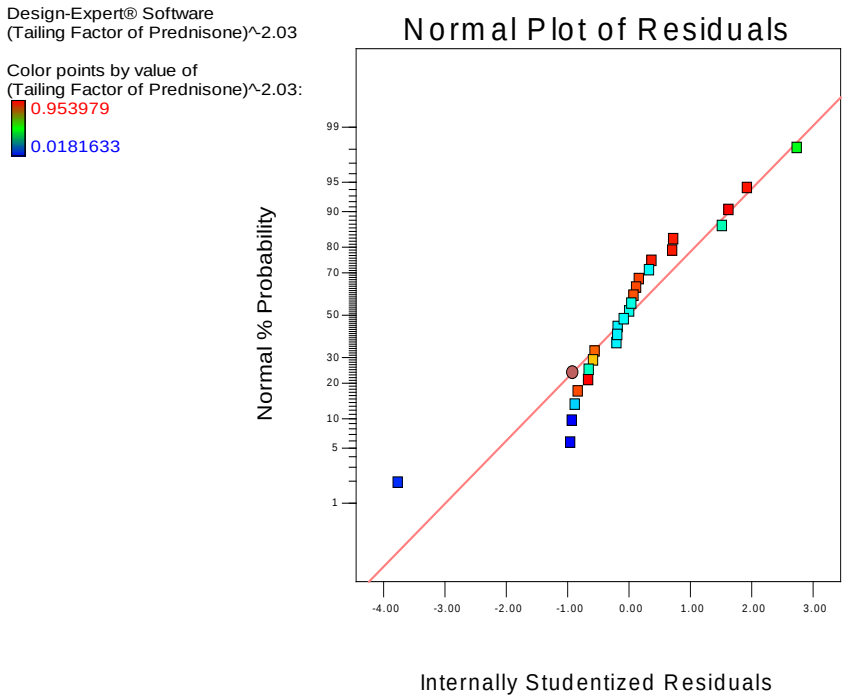


Figure 2.10 Normal plot of residuals for peak tailing

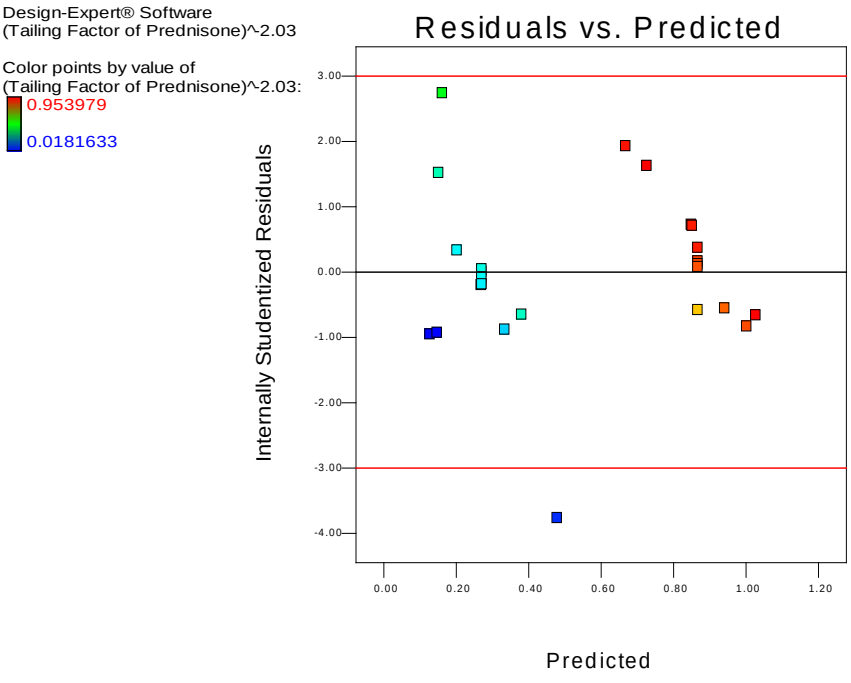


Figure 2.11 Plot of residuals *versus* predicted for peak tailing

2.6 OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Final chromatographic conditions for the analysis of prednisone were established by optimizing the regression models using Design Expert® statistical software. These indicate the control region in the design space concept. Table 2.11 presents the optimal RP-HPLC conditions for the analysis of prednisone.

Table 2.11 Optimized RP-HPLC conditions for the analysis of prednisone

Parameter	Optimized conditions
HPLC system	Waters® Alliance solvent delivery module
Detector	Model 2489 UV/Vis detector
Column	Phenomenex Synergi™ Polar-RP 80Å, 250 x 4.6 mm, 4 µm
Mobile phase	Acetonitrile : water (35 : 65 v/v)
Mode	Isocratic
Flow rate	1 mL/min
Column temperature	25 °C
Wavelength	254 nm
Injection volume	20 µL
Internal standard	Hydrochlorothiazide
Ratio	Hydrochlorothiazide : prednisone (1:2)
Retention time	Prednisone 8.0 min and hydrochlorothiazide 4.9 min

Sharp, symmetrical and well resolved peaks for both prednisone and HCTZ were observed when optimized chromatographic conditions were used. A typical chromatogram generated from optimized conditions is presented in Figure 2.12. Responses predicted by Design-Expert® software were found to be in close agreement with the experimental responses indicating that the method was robust. In addition, the efficiency of RSM as a modelling tool was highlighted.

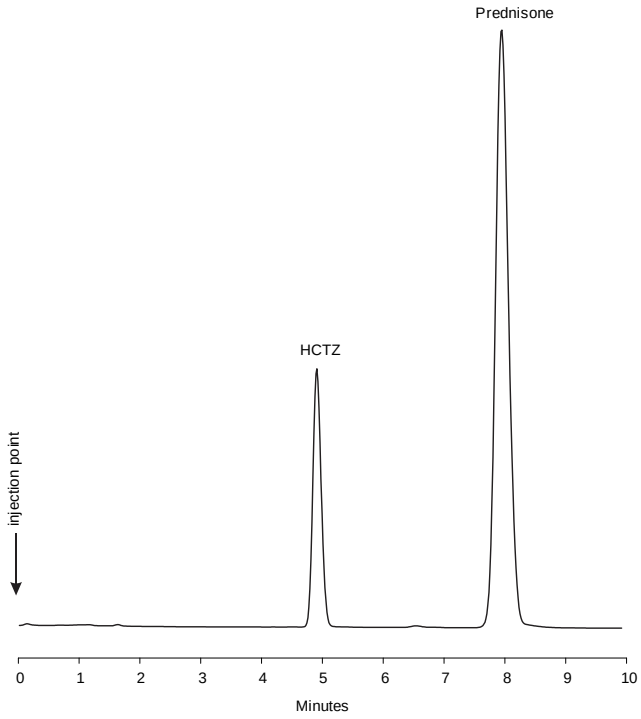


Figure 2.12 Typical chromatogram for the separation of prednisone (100 µg/mL) and hydrochlorothiazide (100 µg/mL) generated using the optimized chromatographic conditions

2.7 METHOD VALIDATION

To ensure that a new or amended analytical method performs within acceptable limits of its intended use, it has to be validated [232]. The goal is to identify method accuracy, reliability, precision and robustness when used by the same or different operators on different days, in the same or different locations using similar or equivalent instrumentation [233, 234]. Furthermore, validation is a key component in QbD systems. It plays a pivotal role in quality assurance [235].

The ICH Q2 (R1), FDA and USP have prescribed guidelines for analytical method validation [236-238]. To establish if the method meets regulatory requirements, the guidelines recommend tests for precision, accuracy, specificity, limit of detection, limit of quantitation, linearity and range. The HPLC method developed for the analysis of prednisone was validated in accordance with ICH Q2 (R1) guidelines.

2.7.1 Linearity and range

Linearity studies are conducted to establish if an analytical method produces results that are directly proportional to the concentration of analyte over a specific range [239, 240]. The range being the interval between the lower and upper concentrations used for the study [239, 240]. Linearity is determined by analyzing samples of increasing concentration using the proposed RP-HPLC method. The resulting plot of peak area against concentration is evaluated by determining the regression coefficient (R^2), where $R^2 \geq 0.99$ is considered to be of acceptable linearity [242, 243]. Furthermore the regression plot should have a y-intercept < 2 %, with intercepts closest to zero being more acceptable [239, 243]. Prescribed guidelines stipulate the use of 3 to 6 injections of at least 5 standards, with concentrations between 80 % and 120 % of the anticipated test samples [244].

Seven standards of 1, 5, 30, 40, 50, 80 and 100 µg/mL were analyzed to determine linearity. Six replicates (n = 6) of each standard were sampled and the peak to height ratios of prednisone to HCTZ were calculated and plotted against concentration. A calibration curve was constructed from the results and the line of best fit determined. The calibration curve presented in Figure 2.13 was obtained.

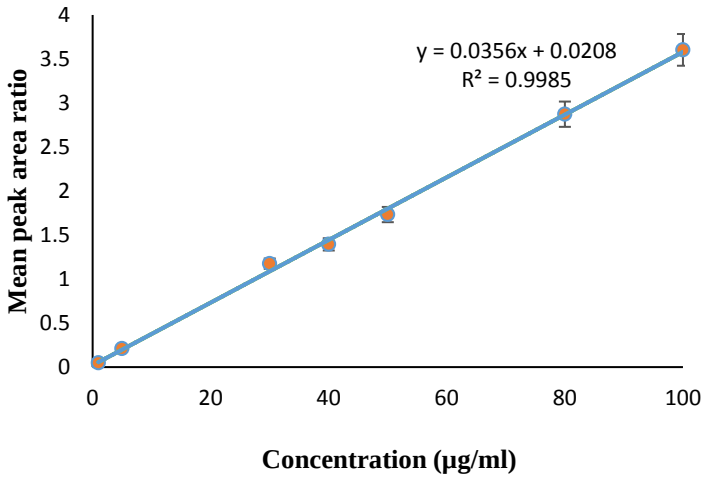


Figure 2.13 Calibration curve for prednisone over the concentration range 1-100 µg/mL

The equation for the best-fit linear regression line was $y = 0.0356x + 0.0208$ and $R^2 = 0.9985$ was obtained. These data indicate that the method satisfied the criteria set for linearity over the concentration range studied. Therefore the method was deemed acceptable.

2.7.2 Precision

Precision studies indicate the degree of random error within an analytical method. They provide a measurement of how scattered data is following the conduction of the same experiment multiple times. Prescribed guidelines recommend conduction of precision studies at three levels: repeatability (intra-day precision), intermediate precision (inter-day precision) and reproducibility (testing in different labs or by different analysts) [245, 246]. Following experimentation, data is expressed as standard deviation (SD) and relative standard deviation (RSD) or through a coefficient of variance with an associated confidence interval [246].

2.7.2.1 Intra-day precision (Repeatability)

Intra-day precision or repeatability studies are conducted to establish if an analytical method yields accurate and precise results when the same operating conditions are used over a short period of time [241]. According to ICH guidelines, at least 9 analyses are necessary for precision studies, for example n = 3 experiments for 3 different concentrations [244]. For this study, repeatability was determined by analyzing the % RSD of three different concentrations 5, 50 and 80 µg/mL, representing low, medium and high concentration respectively. Experiments were conducted on the same day and a summary of the results is presented in Table 2.12. In all cases the % RSD fell within acceptable limits of ≤ 5 % indicating that the method was repeatable and the intra-day precision of the method was adequate.

Table 2.12 Intra-day precision data for analysis of prednisone

Prednisone level	Concentration µg/mL	Mean peak area ratio (n = 3)	SD	RSD %
Low	5	0.177	0.002	0.889
Medium	50	1.717	0.001	0.040
High	80	2.857	0.001	0.034

2.7.2.2 Intermediate precision (Inter-day)

Intermediate precision studies are conducted to establish if the analytical method will produce reliable results in a different environment after completion of method development [247, 248]. These studies entail analysis on different days, use of the method by a different analyst or use of different but equivalent instruments [215, 249]. The evaluation of intermediate precision was conducted by analyzing three different concentrations on three consecutive days using the same HPLC instrument. Three different concentrations 5, 50 and 80 µg/mL, representing low, medium and high concentration respectively were assessed and the results are presented in Table 2.13. In all cases the % RSD fell within the acceptable limit of ≤ 5 % indicating that the method conforms to specifications for intermediate precision.

Table 2.13 Intermediate precision data for the analysis of prednisone

Day	Concentration µg/mL	Mean peak area ratio (n = 3)	SD	RSD %
1	5	0.177	0.002	0.889
	50	1.717	0.001	0.040
	80	2.857	0.001	0.034
2	5	0.186	0.001	0.541
	50	1.872	0.002	0.105
	80	3.000	0.001	0.041
3	5	0.167	0.003	1.788
	50	1.676	0.001	0.079
	80	2.870	0.004	0.130

2.7.2.3 Reproducibility

Studies to establish reproducibility are conducted to evaluate if the method produces statistically equivalent results when used in different laboratories by different analysts [215, 250, 251]. This is of particular importance for joint research purposes or compendial publications. The mean results from reproducibility studies must be within 2 % of the results generated in the primary laboratory. The reproducibility of this method was not evaluated because the analytical method was intended for use in the same laboratory, by the same analyst with the same instrumentation.

2.7.3 Accuracy

The accuracy of an analytical method refers to the closeness of measured experimental values to an accepted reference [232, 241]. Prescribed ICH guidelines recommend at least n = 3 experiments for three different concentration levels within the calibration range [244]. For this study, accuracy was established by using the optimized RP-HPLC method to assess a prednisone standard of known purity at low, medium and high concentrations. The analytical method was considered accurate if a % recovery close to 100 % and a % bias < 2 % was obtained [240]. Bias is the extent of deviation of an experimental data set from the true value and is calculated using Equation 2.11. Data presented in Table 2.14 indicates that the method met the criteria set for accuracy, therefore the method was deemed accurate.

$$\% \, Bias = \frac{Actual\ value - Theoretical\ value}{Actual\ value} \, x \, 100 \qquad \textit{Equation 2.11}$$

Table 2.14 Accuracy results for RP-HPLC analysis of prednisone (n = 4)

Theoretical concentration µg/mL	Actual concentration mean ± SD µg/mL	Recovery %	RSD %	Bias %
5	4.98 ± 0.013	99.66	0.264	-0.402
50	49.98 ± 0.034	99.96	0.069	-0.040
100	100.04 ± 0.127	100.04	0.127	+0.040

2.7.4 Limits of detection (LOD) and quantitation (LOQ)

The LOD and LOQ indicate the sensitivity of an analytical method [252, 253]. The LOQ is the lowest concentration that can be quantified with adequate accuracy and precision [253]. The LOD refers to the concentration that can be detected but not necessarily quantified to an exact numeric value [253]. The ICH guidelines describe three methods for establishing LOD and LOQ. (*i*) Analysis of samples of known concentration followed by establishing the minimum level at which the analyte can be reliably detected, (*ii*) use of the signal-to-noise ratio based on the generalization that LOD can characteristically be detected three times above the noise level of a system, and (*iii*) calculations based on the standard deviation of a response and the slope of the calibration curve [253-255]. The first method was used for this study.

The following concentrations were assessed using the optimized RP-HPLC method: 0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL. Prednisone peaks were only observed from concentration 0.6 µg/mL upwards hence it was considered to be the LOD. From the remaining standards, the 1 µg/mL analyte was found to have a relative standard deviation of 0.7 % which is < 5 % hence it was regarded to be the LOQ. Table 2.15 presents a summary of the results obtained.

Table 2.15 Limits of quantitation and detection for RP-HPLC analysis of prednisone

Concentration µg/mL	Mean Peak Area Ratio (n = 6)	SD	RSD %
0.2	-	-	-
0.4	-	-	-
0.6	0.0191	0.0019	9.7422
0.8	0.0293	0.0018	6.0407
1.0	0.0353	0.0002	0.7068

2.7.5 Specificity

The ability of an analytical method to elute desired peaks even in the presence of excipients, impurities or degradation compounds is known as its specificity [240]. The goal is to ensure suitability of the method to assess and quantify final dosage forms. Following the assessment of commercially available Be-Tab® Prednisone tablets, a distinct well resolved peak with the same retention time as pure prednisone was observed. The excipients present in the commercially available formulation did not interfere with detection. Hence the method was deemed specific for prednisone. Figure 2.14 depicts the chromatogram generated from analyzing Be-Tab® Prednisone 5 mg tablets, which may be compared to the chromatogram generated from pure prednisone depicted in Figure 2.12.

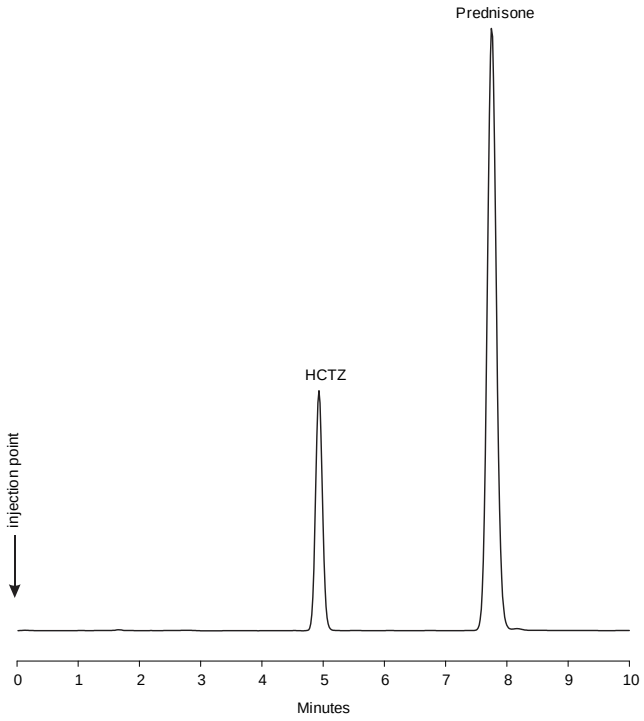


Figure 2.14 Typical chromatogram generated following analysis of commercially available Be-Tab® Prednisone tablets

2.7.6 Assay

Be-Tab® Prednisone tablets (5 mg) were used for assay. Ten tablets were weighed using a Metler Toledo balance (Mettler Instruments, Zurich, Switzerland) and crushed using a mortar and pestle. An amount equivalent to 10 mg of prednisone was weighed (124.4 mg) and dissolved in 100 mL of an ACN-water mixture (35: 65) in a 100 mL volumetric flask. The resulting solution was filtered twice using 0.2 µm filters (Acrodisc® PSF) and analyzed using optimized RP-HPLC conditions. A summary of the results is presented in Table 2.16.

Table 2.16 Assay results for a commercially available product (Be-Tab® Prednisone)

Product	Prednisone added	Prednisone found mg ± SD	Recovery %	RSD %
Be-Tab® Prednisone (5 mg)	10.0 mg	9.82 ± 0.106	98.2	1.078

2.8 FORCED DEGRADATION STUDIES

An effective analytical method should also be stability indicating. A stability indicating method is capable of accurately quantifying the analyte without any interference from degradation products and impurities [256, 257]. The ICH guideline Q1A (R2) recommends conduction of forced degradation or stress studies to establish possible interference of degradation products towards API quantitation [258, 259]. In addition, knowledge generated from these studies may be used to elucidate degradation pathways, and to determine optimal handling and storage conditions [256].

Forced degradation studies were conducted by exposing prednisone to acidic, alkaline, neutral hydrolytic and dry heat conditions, followed by analysis with the validated RP-HPLC method. Generally degradation of API up to 10 % from its original state is set as the limit of stability on the label claim, so tolerance levels between 10 to 15 % degradation were set for this study [259, 260]. The chromatogram of untreated prednisone in Figure 2.15 was used as a reference.

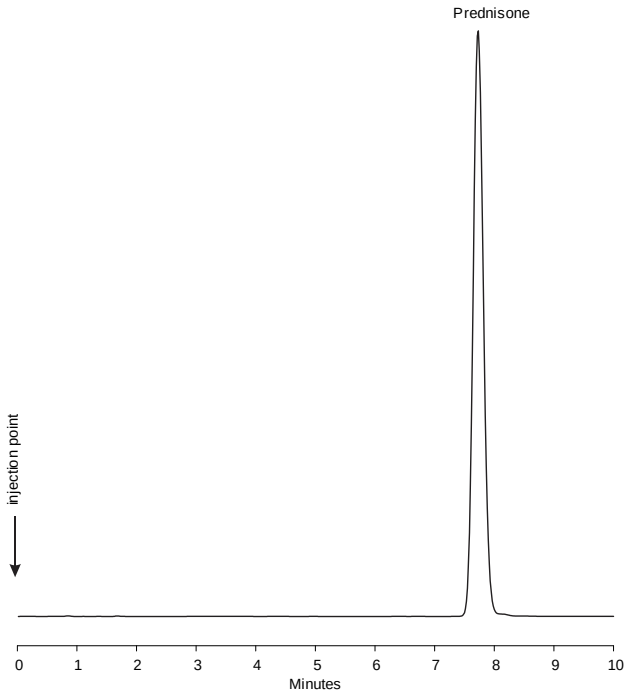


Figure 2.15 Typical chromatogram of untreated prednisone

2.8.1 Acid degradation

Prednisone was refluxed in 0.1 M HCl at 90 °C for 8 hours (hrs) as per ICH Q1A (R2) recommendations [259]. The solution was allowed to cool to room temperature (22 °C) prior to HPLC analysis. Degradation peaks observed on the chromatogram (Figure 2.16) indicate that prednisone is susceptible to acid hydrolysis. Hydrolysis started immediately after exposure, indicated by a recovery of 67.9 % at t = 0 hrs. The extent of degradation was analyzed again at t = 8 hrs, and only 40.7 % of prednisone was recovered.

2.8.2 Alkali degradation

Prednisone was refluxed in 0.1 M NaOH at 90 °C for 8 hrs as per previous recommendations [257]. The solution was allowed to cool to room temperature (22 °C) prior to HPLC analysis. Degradation peaks observed on the chromatogram indicate that prednisone is susceptible to alkali hydrolysis. Hydrolysis was rapid and the extent of degradation was more pronounced. At t = 0 hrs only 8.3 % prednisone was recovered, and at t = 1.5 hrs only 0.29 % of prednisone was recovered. No peaks were observed after 2 hrs of refluxing indicative of full degradation. The chromatogram obtained from alkali degradation at t = 1.5 hrs is presented in Figure 2.16, t = 1.5 hrs was the last sample time with quantifiable results.

2.8.3 Neutral hydrolysis

Neutral hydrolytic studies were conducted by refluxing prednisone in HPLC grade water at 90 °C for 8 hrs [257]. The solution was allowed to cool to room temperature (22 °C) prior to HPLC analysis. Degradation peaks observed on the chromatogram (Figure 2.16) indicate that prednisone is susceptible to neutral hydrolysis. The extent of degradation was evaluated at t = 8 hrs, and 74.6 % of prednisone was recovered.

2.8.4 Dry heat degradation

Prednisone was placed in an oven (Gallenkamp®, Loughborough UK) set at 90 °C for 8 hrs to evaluate if it was susceptible to thermal degradation. Upon completion of the experiment, a 100 µg/mL stock solution was prepared from the powder and analyzed using the validated HPLC method. The resultant chromatogram depicted in Figure 2.16 indicates that prednisone did not show any degradation.

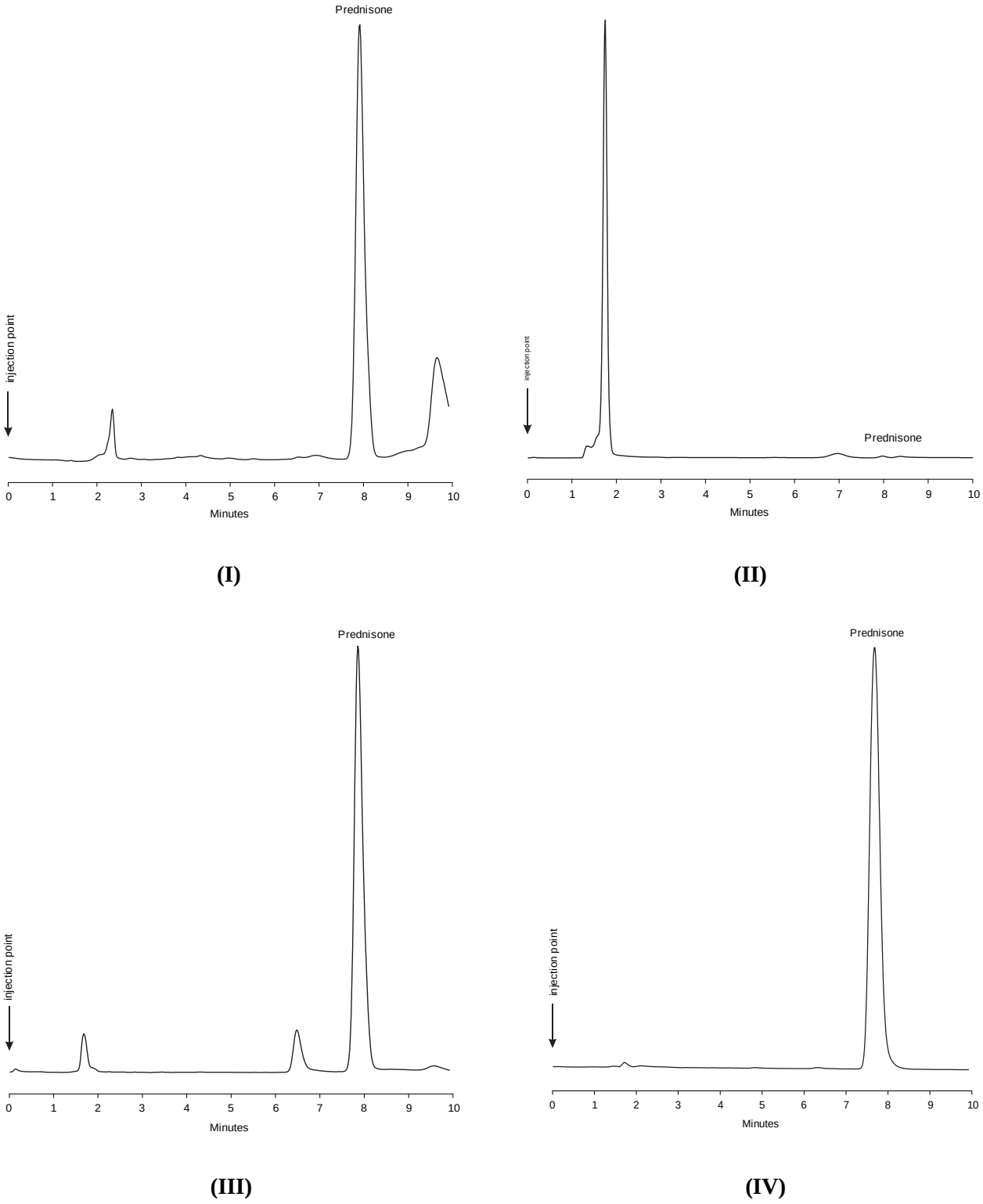


Figure 2.16 Chromatograms generated following (I) acidic degradation of prednisone for 8 hrs, (II) alkali degradation of prednisone for 1.5 hrs, (III) neutral hydrolysis of prednisone for 8 hrs and (IV) dry heat degradation of prednisone for 8 hrs

2.9 CONCLUSIONS

AQbD principles were successfully used to develop, optimize and validate a rapid, simple, precise, accurate, selective, reproducible and stability-indicating RP-HPLC method for the quantitation of prednisone in pharmaceutical dosage forms. The DoE approach combined with CCD was used to facilitate simultaneous evaluation of all input variables, with particular emphasis on their impact on CAA. Input variables including column temperature, column type and mobile phase composition were evaluated and following ANOVA and analysis of mathematical models in the design space, mobile phase composition and column type were found to have the most impact on the quality of the RP-HPLC process. The use of the optimized conditions produced sharp, well resolved peaks with short retention times as per the desired analytical target profile. The use of a simple mobile phase *viz*., ACN-water and isocratic flow provided an easy alternative to use of complex gradient-based elution and methods which often use buffers and are time consuming to implement. Analysis conducted to evaluate commercially available tablets proved that the method was selective and suitable for assessing the dissolution profiles of other solid dosage forms containing prednisone. Following validation, the newly developed method was found to meet all the criteria set in prescribed guidelines, proving that the method was robust, adequate and was capable of accurately quantifying prednisone from solid dosage forms. Forced degradation of prednisone was conducted under acidic, basic, neutral and thermal conditions. The mass balance data varied between 0.29 % and 100 %. This work was successful in establishing a RP-HPLC method capable of quantifying prednisone in the presence of its degradation products. Future studies will include the isolation and characterization of major degradation products from acidic, basic and neutral conditions by ^{1}HNMR, ^{13}C-NMS and mass spectral studies. The optimized RP-HPLC method developed in these studies was applied in formulation and product development studies in addition to assessing *in vitro* release behaviour of prednisone from experimental pellet formulations. To our knowledge, there are no reports in currently published literature for an HPLC method developed for the quantitation of prednisone using principles of AQbD. An AQbD-based method is guaranteed to meet desired performance attributes since in addition to end-process testing AQbD principles ensure that quality is built into the method during development *versus* currently published methods which rely sorely on end-process testing to confirm method quality.

CHAPTER 3
PRE-FORMULATION STUDIES FOR A PREDNISONE MULTIPARTICULATE SYSTEM

3.1 INTRODUCTION

Pharmaceutical dosage forms usually consist of an active pharmaceutical ingredient in addition to one or more excipients. Each excipient plays a specific role and in combination they contribute to the production of a high quality product [261]. Some excipients function to enhance the manufacturing process, whilst some contribute to the *in vivo* performance of the dosage form. The choice of excipients is largely influenced by the target dosage form and physicochemical properties of the active pharmaceutical ingredient (API) [261]. To elucidate the suitability for use of excipients an extensive literature review is conducted to gather information pertaining to the function and properties of excipients after which a series of tests are performed on each excipient alone and in combination with the API to gather more data. The tests used to characterize the physiochemical and mechanical properties of the API and excipients are collectively known as pre-formulation studies [262]. Ultimately, the goal of pre-formulation is to select the correct form of API and excipients to use, evaluation of their physical properties and generation of a thorough understanding of the stability of materials under conditions that will lead to development of an optimum drug delivery system [263-266].

The ICH Q6A guidelines provide an indication of the test procedures required for new dosage forms. These guidelines channel the researcher to prepare for compliance with process analytical technology (PAT), an initiative by the FDA to encourage formulation scientists to build quality into their development processes and product [267]. In practice PAT is not compulsory, however it is specifically designed to improve process understanding in order to minimize potential failure and reduce the levels of testing required at the end of the formulation and manufacturing process [267]. Furthermore the traditional trial and error approach used during drug product development is tedious, time consuming and costly. Pre-formulation studies support PAT by providing more information regarding the characteristics of the API and excipients [268]. The goal of PAT is to improve knowledge and control of the manufacturing process in consonance with the main principle of QbD that quality cannot be tested into products but must rather be built in or should be by design [267]. Consequently pre-formulation studies can be used not only to improve product

and process understanding, but also to meet regulatory requirements for validating and controlling a manufacturing process [267, 269].

3.2 EXCIPIENTS

The successful manufacture of a dosage form is largely dependent on the choice of excipients. Typically excipients must be inert, non-toxic and must not interact with the API and or other excipients that form part of the dosage form [270]. Excipients bulk up formulation, enhance palatability, aesthetic appeal and stability amongst perfoming other functions including facilitating the manufacturing process [270, 271]. Excipients are classified according to their primary role in the formulation. Binders, diluents, disintegrants, lubricants are some of the classes of excipient used in the manufacture of a multiple unit pellet system for prednisone and discussed in this section.

3.2.1 Binders

Binding agents facilitate agglomeration during the granulation phase of manufacture resulting in the formation of a wet mass that is further processed into pellets. The amount and choice of binding agent may impact the rate of API release [272]. Most binders are polymeric in nature and common examples include starch, gelatin, polyvinylpyrrolidone (PVP) and microcrystalline cellulose (MCC) [272].

3.2.2 Diluents

Generally API are dosed in very low quantities to exert therapeutic effects. However these amounts are often too small to handle and therefore diluents are often used to bulk up the dosage form making it a suitable size to handle during processing and for patient convenience [261, 273]. In some formulations inert diluents are more suitable to prevent any unwanted interactions with the API however for some formulations diluents play active roles in the stability and performance of the dosage form [274]. Therefore compatibility with the API and other excipients is important [275]. Common examples of diluents include MCC, dibasic calcium phosphate and lactose [276].

3.2.3 Disintegrants

Disintegrants are incorporated into solid dosage forms to facilitate their breakdown into smaller particles. Smaller particles have a larger surface area to volume ratio, which significantly improves the rate of dissolution. The choice and concentration of disintegrants is therefore very important since it indirectly affects bioavailability [277]. Disintegrants are conventionally categorized into two classes, traditional and superdisintegrants. Traditional disintegrants were discovered first and

the most popular ones are starch and alginic acid. Superdisintegrants were named as such because of their superior effects compared to traditional disintegrants. They are highly effective even when available at low concentrations. Sodium starch glycolate and croscamellose sodium are examples of superdisintegrants [278].

Disintegrants exert their effect via swelling, wicking, elastic deformation and repulsion. The disintegrant effect may be due to a single mechanism or a combination. Disintegrants which work via swelling have a high affinity for water. Once exposed to aqueous environments, their particles swell until they overcome the adhesiveness of other excipients in the dosage form, causing the solid dosage form to break apart [279]. Wicking utilizes the pores on the surface of the dosage form. During the process, aqueous fluid is drawn or wicked into these pores and spreads throughout the dosage form via capillary action. As a result inter-particulate bonds that hold the dosage form together are disrupted thereby facilitating disintegration [279]. Elastic deformation is typical when an elastic material such as starch is part of the formulation. Upon exposure to aqueous media, the energy potential of compressed starch is released. The expansion of starch overcomes the adhesive forces holding the formulation together resulting in disintegration [279, 280]. The repulsion mechanism was proposed to explain the swelling of dosage forms that were formulated with non-swelling disintegrants. Once exposed to aqueous environments these disintegrants introduce repulsive forces which stimulate disintegration [279].

3.2.4 Anti-frictional agents

3.2.4.1 Lubricants

Lubricants are used to minimize friction during processing. In the pelletization context when lubricants are not incorporated, it is common for extrudates to stick to spheronizer walls leading to agglomeration, low pellet yield and poor pellet quality [281]. Lubricants are classified according to their aqueous solubility *viz*., insoluble and soluble [277]. Insoluble lubricants include magnesium stearate, stearic acid and glyceryl behenate [277] that are generally added to the powder blend with other excipients in low qunaitites due to their deleterious effect on disintegration [277]. Soluble lubricants include polyethylene glycol and lauryl sulphate salts that have little impact on disintegration [277, 282].

3.2.4.2 Glidants

Glidants are incorporated to minimize inter-particular friction and improve powder flow properties [283]. Several mechanisms have been proposed via which glidants improve flow properties. Some studies proposed that they reduce roughness by filling surface irregularities and by physically separating powders thereby reducing attractive forces. Other hypotheses suggest that glidants modify electrostatic charges and adsorb moisture therefore diminishing cohesive forces [284]. Talc and colloidal silicon are common examples of glidants [285].

3.2.4.3 Anti-adherents

Anti-adherents prevent the powder blend from sticking to machine surfaces (homogenizer, extruder, spheronizer) during the pelletization process. Sticking is commonly due to a high moisture content of the powder blend possibly resulting from the addition of too much granulating fluid or a highly humid manufacturing environment [286]. Talc and starch are commonly used anti-adherents however many lubricants such as magnesium stearate also possess anti-adherent properties so they perform both functions in the formulation [276].

3.3 EXCIPIENT SELECTION

The following excipients were used in the manufacture of a multiple unit pellet system for prednisone. These materials are all generally regarded as safe (GRAS) and possess GRAS status.

3.3.1 Microcrystalline cellulose (MCC)

MCC is purified, partially depolymerised cellulose that occurs as a white crystalline powder [287]. MCC consists of porous particles of different grades depending on particle size and moisture content [276]. The larger particle sized grades exhibit good flow properties. MCC is crystalline and amorphous depending on the orientation of cellulose chains in the material [288]. MCC-based pellets are generally spherical and exhibit excellent flow properties [289].

Comprecel® M102 D + (Mingtai Chemical Company Ltd, Taiwan) was used as a diluent and spheronization aid due to its good flow and binding properties [290, 291]. The highly porous nature of MCC permits it to adsorb and retain large quantities of granulating fluid thereby imparting the necessary plasticity and cohesiveness to the wet mass for extrusion and spheronisation [292-294]. MCC is hygroscopic in nature therefore bulk material should be stored in a well closed container in a cool dry place [276].

Two different models have been proposed to explain the mechanism by which MCC aids the extrusion spheronization process. The first model characterizes MCC as a molecular sponge which retains granulating fluid [293]. During the extrusion phase some of the adsorbed granulating fluid is squeezed out of the wet mass and acts as a lubricant. The resulting extrudates are slightly dry and brittle so they break easily during spheronization. Mass transfer facilitates final densification of the sponge until pellets are formed [293]. The second model suggests that when MCC is exposed to granulating fluid, typically water, it breaks down to smaller crystalline units [295]. The porous crystalline units form a gel-like network that immobilizes and traps the granulation liquid. At a specific water content which corresponds to a particular gel strength, extrusion and spheronisation becomes possible [295].

3.3.2 Methacrylic acid copolymers

The methacrylic acid copolymer is a fully polymerized copolymer of methacrylic acid and an acrylic acid or methacrylic ester. Three types exist *viz*., Type A, B and C based on different methacrylic acid content. All have different viscosities and typically have molecular weights in excess of 100000 mass units. The different types impart different properties to dosage form but they are most commonly used as binders or film-forming agentsin oral dosage forms [296].

Type A *viz*., Eudragit® L, RL and Type B *viz*., Eudragit® S, RS that contain ethyl acrylate, methyl methacrylate and trimethylammonioethyl methacrylate chloride in 1:2:0.2 and 1:2:0.1 ratios respectively whereas Type C *viz*., Eudragit® L 30, D-55 is an aqueous dispersion of ethyl acrylate copolymer and methyl methacrylate that may contain surface active agents (SAA) [297].

Eudragit® RS and RL are the most widely used methacrylic acid copolymers. Eudragit® RS and RL differ in molecular structure due to different degrees of quaternary ammonium substitution. Eudragit® RS exhibits a lower degree of substitution at 5 % w/w compared to Eudragit® RL at 10 % w/w. The ammonium groups exist as salts and their function is to facilitate pH-independent permeability of the polymers [298].

Eudragit® RL has superior hydrophilicity than Eudragit® RS therefore, water readily permeates through this polymer, a property that makes it particularly useful both as a spheronization aid and enhancer of dissolution *in vivo* [298].

Eudragit® NE 30D is a neutral aqueous ester dispersion. It is a milky white dispersion that consists of polymethacrylic acid esters. It has low viscosity and a weak aromatic odour. It is commonly used to aid the granulation process when manufacturing sustained release matrix tablets [299]. Eudragit® RL 30D and RS 30D are 30% w/v aqueous dispersion copolymers of acrylic and methacrylic acid esters with a low content of quaternary ammonium functional groups [299].

Eudragit® RL 30 D (Rohm® Pharma, GmbH, Darmstadt, Germany) a type A copolymer was used as a binder, granulating agent and dissolution enhancer in this study. Eudragit® RL 30 D is a milky white liquid of low viscosity with a characteristic chocking odour. The quaternary ammonium group in the structure facilitates pH-independent permeability of the polymer. In this study, an immediate release multiple unit pellet system of prednisone was manufactured. To exploit the hydrophilic properties of Eudragit® RL 30 D without delaying prednisone release, a 50 % v/v dilution with water was used. The final dispersion was an excellent spheronization aid contributing some binding properties, acting as a granulating fluid and imparting tensile strength to the pellets [276].

3.3.3 Sodium starch glycolate

Sodium starch glycolate (SSG) (Aspen Pharmacare, Port Elizabeth, South Africa) is the sodium salt of a carboxymethyl ether of starch [354]. It is a very fine white to off white powder that is free flowing, odourless, hygroscopic and practically insoluble in water [276] with it's a molecular weight between 50000 to 100000 mass units. Three types exist *viz*., type A, B and C that exhibit different pH, sodium and sodium chloride content [276].

When exposed to an aqueous environment, sodium starch glycolate absorbs water and swells. The swelling disrupts inter-particulate adhesive forces within the dosage form resulting in disintegration. In addition, it is spherical in shape and therefore improves flow. Usually between 2 % and 8 % w/w is used when making solid oral dosage forms [276, 279].

3.3.4 Tween® 80

Tween® 80 (Aspen® Pharmacare Port Elizabeth, South Africa) is chemically known as polysorbate 80. It is a viscous, yellow, oily liquid with a characteristic odor and bitter taste [300]. Tween® 80 is a hydrophilic non-ionic surfactant that is soluble mostly in ethanol and water. It is stable in the presence of electrolytes and weak acids or bases [300]. The structure is composed of a polyethoxylated ether of anhydrous sorbitol that is esterified with oleic acid [301]. It is used as a

solubilizing and wetting agent in pharmaceutical manufacturing [300]. Generally Tween® 80 is regarded as non-toxic when the daily intake is limited to ≤ 2.5 g/kg body weight [300, 302, 303].

3.3.5 Talc

Talc (Aspen® Pharmacare Port Elizabeth, South Africa) is hydrated magnesium silicate that occasionally contains trace amounts of aluminium silicate and iron [274]. The composition is variable based on geographical source of the material [276]. Talc is a very fine, white to greyish-white, odourless, impalpable, crystalline powder that is used as a glidant and lubricant during the manufacture of solid dosage forms [304, 305]. Talc exhibits very low water permeability [276]. Generally talc is used at levels between 1.0 % and 10.0 % w/w [305]. The hydrophobic nature of talc is a major limitation, and when used at high concentrations it minimizes wetting which consequently reduces the rate of dissolution [276].

3.3.6 Magnesium stearate

Magnesium (Mg) stearate (Aspen® Pharmacare Port Elizabeth, South Africa) is a mixture of magnesium and a variety of organic acids that primarily consist of variable proportions of magnesium stearate and magnesium palmitate obtained from plant or animal origin [306]. It is a fine, white powder with a faint odour and a characteristic taste [306, 307]. In addition, it has low bulk density and is insoluble in water [274]. Mg stearate is used as a lubricant in solid dosage forms and one major limitation is that it is hydrophobic. When used at levels > 5.0 % w/w it retards the rate of dissolution therefore low levels typically between 0.25 and 5.0 % w/w are recommended [308, 309]. Some studies have also established a link between Mg stearate retarding dissolution and decreasing the crushing strength of the final product when long blending times are used [276].

3.3.7 Polyethylene glycol 400

Polyethylene glycol (PEG 400) (Merck, South Africa) also known as macrogol 400 is an addition polymer of ethylene oxide and water. PEG 400 is a clear, colourless, viscous liquid, with a slight odour and characteristic bitter taste. Due to its highly hydrophilic nature, PEG 400 is used in formulations to improve solubility and to enhance dissolution. PEG 400 should be stored in a cool, dry place, preferably in well-closed containers [276].

3.4 PHYSICOCHEMICAL PROPERTIES

The fundamental principle governing QbD is that quality must be built into the product during development. To minimize risk a thorough understanding of each of the raw materials, is imperative. Successful pelletization and subsequent performance of the dosage form require selection of excipients that contribute to the achievement of the desired quality target product profile (QTPP). Physicochemical properties of excipients affect bioavailability so prior to initiating the manufacturing process, characterization of the physical and chemical properties of the API and excipients is particularly important [310, 311]. Typically particle size, shape, flow properties, density and potential interactions are assessed [312].

3.4.1 Particle size and shape

Assessing the particle size of solid excipients is a key element of pre-formulation studies. Flow properties, formulation characteristics, porosity, homogeneity of powder blend, dissolution and bioavailability may all be potentially affected by particle size distribution [276, 313, 314]. Particle sizes of between 10 μm and 150 μm are generally ideal for wet granulation processes [314].

Good powder flow properties are required for successful pelletization [315]. To achieve pellets with acceptable content uniformity, a homogeneous mixture must be formed during dry powder blending and wet, prior to extrusion and spheronization [314, 316-318]. Generally spherical particles exhibit excellent flow properties whereas irregular shaped particles that are cuboidal, flake-like, pyramidal, flaky, granular, rod or needle-like, block-shaped, sponge-like and fibrous in nature exhibit poor flow [319-321]. A narrow particle size distribution contributes to achieving an acceptable QTTP as wider particle size distributions may lead to segregation and consequently non-uniformity of dosage forms [321, 322]. Therefore knowledge of particle size distribution and shape of solid excipients is important in a quality system. In general, an increase in particle size is proportional to an increase in flow up to a maximum. Powders that are < 50 μm or > 1200 μm typically exhibit irregular or no flow due to agglomeration induced by Van der Waal's forces [314]. Several techniques may be used to determine particle size distribution including microscopy, sieve analysis, Coulter Vounter light scattering among others [323]. Scanning Electron Microscopy followed by image size analysis was used in this study.

Particle size and shape were determined using a Vega® Scanning Electron Microscope (Tescan, Vega LMU, Czechoslovakia Republic). Small amounts of prednisone and excipients were separately dusted onto graphite plates and sputter coated with gold for 20 minutes in a Hitachi vacuum coating unit. Images were captured by SEM, followed by particle size analysis using analySIS docu® software (Olympus, Hamburg, Germany).

3.4.2 Powder rheology

3.4.2.1 Angle of Repose

The Angle of Repose (AOR) provides an indication of inter-particulate cohesion, therefore it may be used used to establish the level of powder flow with only small quantities of sample material. The powder is poured through a funnel onto a horizontal surface to form a cone. Particles of the powder flow down the surface of the cone until inter-particulate and frictional forces balance out gravitational forces at which point powder flow ceases [319, 320, 324]. The powder rests as a conical shape and the angle between the side of the cone and the horizontal surface is known as the AOR [319, 324]. Free flowing powders form cones with shallow sides whereas cohesive and poorly flowing powders made up of rough and irregular shaped particles form steep sided cones [325]. AOR $\leq 30^{\circ}$ typically indicate good flow properties whereas AOR $\geq 40^{\circ}$ are generally associated with poorly flowing powders and may require the addition of a glidant [314, 326]. The interpretation for AOR is summarized in Table 3.1 [327].

Table 3.1 Interpretation of AOR

AOR °	Flow
< 25	Excellent
25 - 30	Good
30 - 40	Satisfactory
> 40	Very poor

The funnel method was used to measure AOR. Prednisone, microcrystalline cellulose, sodium starch glycolate and magnesium stearate were evauated by allowing 10 g of powder to flow under the force of gravity through a funnel with an orifice and base diameter of 10 mm and 50 mm, respectively. The funnel was maintained at a height of 30 cm above the horizontal plane for all experiments. The height of the resultant cone (h) and the radius of the base (r) of the powder were measured and the AOR calculated using Equation 3.1.

$$Tan\ \theta = \frac{h}{r}$$ *Equation 3.1*

Where,

θ = Angle of Repose
h = Height of the powder cone
r = Diameter of the base of the cone [327]

3.4.2.2 Bulk density

The bulk density of a powder is the ratio of mass to volume and is variable depending on the packing arrangement of the powder and the level of aeration of bulk materials [328, 329]. Generally a minimum bulk density is achieved when a maximum volume is occupied [314, 326] and the bulk density can be calculated using Equation 3.2.

$$\rho_{(bulk)} = \frac{Weight\ of\ powder\ mass\ (g)}{Volume\ occupied\ by\ powder\ mass\ (cm^3)}$$ *Equation 3.2*

Some studies have suggested a relationship between bulk density and powder flow properties and report that the same inter-particulate forces that influence bulk density are those that impact flow properties and therefore bulk density can, to a degree, be used to assess powder flowability [330]. Furthermore a knowledge of bulk density may be used to determine the ideal blender size for the manufacture of pellets [322, 325] and used to establish Carr's Index (CI).

A 10 g amount of prednisone, microcrystalline cellulose, sodium starch glycolate, talc and Mg stearate were separately weighed and transferred into 100 mL measuring cylinders. The volume occupied by each powder was determined by direct measurement.

3.4.2.3 Tapped density

The tapped density of a powder is essentially the bulk density of a powder established after tapping or vibrating the powder for a specific time period [322]. Different instruments exist for the determination of tapped density, but all use e the same principle of a fixed weight of powder being tapped from a set distance at a specific and constant frequency for a set period of time untilan equilibrium volume of powder is reached [328]. The tapped density is then calculated using Equation 3.3.

$$\rho_{(tapped)} = \frac{Weight\ of\ powder\ mass\ (g)}{Volume\ occupied\ by\ tapped\ powder\ (cm^3)}$$ *Equation 3.3*

Tapping the powder removes air voids that exist between particles of the powder. Cohesive powders that exhibit poor flow tend to collapse on tapping whereas free flowing powders already occupy most of the space and therefore have little room for further compression [314, 317]. In summary materials that exhibit a high compressibility index exhibit poor flow properties and *vice versa* [331, 332]. Tapped density is therefore an important parameter to consider when analyzing flow properties and is used to calculate the Hausner ratio (HR) and CI [326]. A 10 g amount of prednisone, microcrystalline cellulose, sodium starch glycolate and magnesium stearate were tapped for two minutes after which the volume of the tapped powder was noted and the tapped density calculated using Equation 3.3.

3.4.2.4 Carr's index

The Carr's index (CI) is a measure of powder compressibility. Highly cohesive powders exhibit high CI values and have poor flow properties [325]. For excipients that exhibit CI > 40 % the extremely poor flow properties can be ameliorated by addition of a glidant and or lubricant [320, 333]. CI is calculated using Equation 3.4 and the interpretation of Carr's index values is reported in Table 3.2.

$$CI = \frac{\rho_{(tapped)} - \rho_{(bulk)}}{\rho_{(tapped)}} \text{ x } 100 \qquad \textit{Equation 3.4}$$

Where,

CI = Carr's index
$\rho_{(tapped)}$ = Tapped density
$\rho_{(bulk)}$ = Bulk density

Table 3.2 Interpretation of Carr's index

Carr's index (%)	Type of flow
5 - 12	Excellent
12 - 18	Good
18 - 23	Satisfactory
23 - 35	Poor
35 - 38	Very poor
> 40	Extremely poor

3.4.2.5 Hausner ratio

The Hausner ratio (HR) is also used to analyze flow properties. Both powder and pellet flow properties can be analyzed using HR that can be calculated using Equation 3.5.

$$HR = \frac{\rho_{(tapped)}}{\rho_{(bulk)}} \qquad \textit{Equation 3.5}$$

Where,

HR = Hausner ratio
$\rho_{(tapped)}$ = Tapped density
$\rho_{(bulk)}$ = Bulk density

A summary of the relationship between HR and flow properties is presented in Table 3.3. Addition of glidants to powders with HR ≥ 1.35 may improve flow properties [320, 333].

Table 3.3 Interpretation of Hausner ratio

Hausner ratio	Flow
1.00 - 1.11	Excellent
1.12 - 1.18	Good
1.19 - 1.25	Fair
1.26 - 1.34	Satisfactory
1.35 - 1.45	Poor
> 1.46	Very poor

3.5 API-EXCIPIENT COMPATIBILITY

Chemical interactions due to properties of each component may potentially precipitate instability due to excipient-excipient or API-excipient interactions [334]. In accordance to the priciples of QbD, active measures must be taken throughout the design process to ensure high quality [314]. Therefore API-excipient compatibility tests were conducted prior to accepting any excipients for manufacture.

Different methods exist for studying API-excipient compatibility, however for this study Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared Spectroscopy (FT-IR) were used as they facilitate early detection of incompatibilities in the development process.

Generally, excipients are pharmacologically inert, however they may indirectly influence efficacy due to their physicochemical properties [335]. Physical interactions do not involve any chemical changes but when they occur they typically impact the manufacturing process. Examples of

physical interactions include complexation, adsorption and formation of solid dispersions [336, 337]. Physical interactions may be used strategically to aid the manufacturing process, however they may also result in detrimental effects to the formulation [338, 339]. During pelletization, small particles interact with larger particles to form a homogeneous powder blend and this is a beneficial physical interaction. In addition the effects of Mg stearate and talc as lubricants and glidants are a result of physical interaction with other particles to improve flow [322, 339]. Chemical interactions are less desirable and the rate of causing instability is higher than that due to physical interactions. Chemical interactions sometimes result in the formation of degradation products some of which may be toxic to the end user [335]. Even though well-known molecules in most cases have already been screened, researchers are encouraged to perform compatibility studies even for these molecules since differences in environmental conditions and or synthetic pathway may result in different performance. Compatibility studies aid decision making prior to formulation. DSC and FT-IR were used for this study since the equipment was readily available and knowledge generated from them was deemed suitable for preliminary investigation of compatibility [314].

3.5.1 Differential Scanning Calorimetry (DSC)

Differential Scanning Calorimetry (DSC) is a thermo-analytical method that measures changes in physical properties of a sample along with temperature for a specified time [340, 341]. The temperature changes and heat flow are regulated such that during a change in temperature, the heat which is radiated or absorbed excessively by the sample on the basis of a temperature difference between the sample and the reference material is measured [342].

In principle the temperature of two ovens is allowed to increase at the same rate. The sample in a sealed pan is placed on one oven and an empty pan (with the lid) is placed on the other oven as a reference. Aluminium pans are typically used due to their inertness and ability to withstand elevated temperatures [342, 343]. When a change such as melting of the sample occurs, energy is consumed and this is called an endothermic reaction [344]. However when the sample crystallizes, energy is released due to an exothermic reaction. The temperature of the reference material remains constant since no energy changes occur. A thermogram displaying the thermodynamic events that the sample undergoes is produced, showing release or uptake of energy during the experiment [345, 346].

In addition to establishing API-excipient compatibility, DSC may also be used to characterize the melting point, glass transition temperature, purity of compounds and presence of polymorphism [346-348]. DSC was used to determine predisone compatibility with excipients in this study.

DSC studies were performed using a Model Q100 TA instrument DSC fitted with a RCS 90 refrigerated cooling system (New Castle, DE, USA). Small quantities (2.5 mg - 5.0 mg) of each sample wasweighed directly into aluminium pans and thermograms were generated by heating the sample from 30 °C to 440 °C at a rate of 10 °C/min whilst purging the system with liquid nitrogen at a rate of 20 mL/min. Pyris 6000 for windows software (Perkin Elmer®, Johannesburg, South Africa) was used to analyze the data generated.

3.5.2 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FT-IR) Spectroscopy is one of the most widely used methods for infrared (IR) spectroscopy. When using IR spectroscopy, IR light is passed through a sample that absorbs someand transmits some radiation. The result is a raw signal or interferogram that represents the intensity of the light as a function of the position of the mirror used to distribute IR light. A mathematical technique or Fourier Transformation is then used to transform the raw data into a spectrum that is characteristic of the sample [349, 350]. The spectrum represents the optical signal as a function of wavelength in the infrared region of the electromagnetic spectrum and is a depiction of the absorption and transmission that occurred. A molecular fingerprint of the sample is created as no two unique molecular structures will produce the same IR spectrum [350-352]. Functional groups in a molecule exhibit characteristic vibrations relative to specific structural attributes. Reaction of an API with excipients and presence of polymorphs or solvatomorphs results in structural changes that typically alter the energy associated with a particular molecular conformation [353, 354].

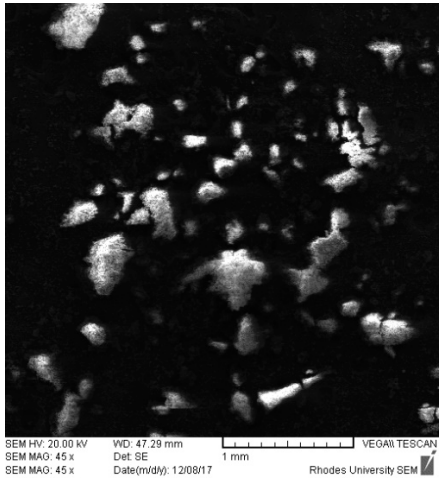
In addition to establishing API-excipient compatibility, FT-IR may also be used during quality assurance to identify or profile unknown compounds, to determine the quality or consistency of samples and to determine the amount of components in a mixture [265, 355].

A Spectrum 100 FT-IR ATR Spectrophotometer (Perkin Elmer® Ltd Beaconsfield, England) was used to generate the spectra. Initially prednisone was analyzed alone to generate the molecular fingerprint after which 1:1 binary mixtures of prednisone and each excipient were analysed to identify potential interactions. The mixtures were prepared by physically mixing the components after which a small amount of each sample was placed on a diamond crystal and a force ≥ 100 N applied. Analysis was conducted over the wavelength range of 4000 - 650 cm^{-1} at a resolution of 4 cm^{-1}.

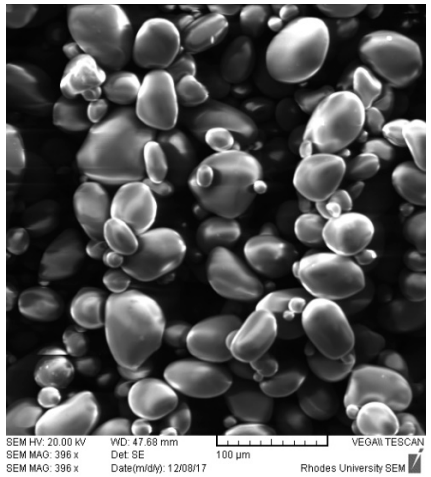
3.6 RESULTS AND DISCUSSION

3.6.1 Particle shape and size distribution

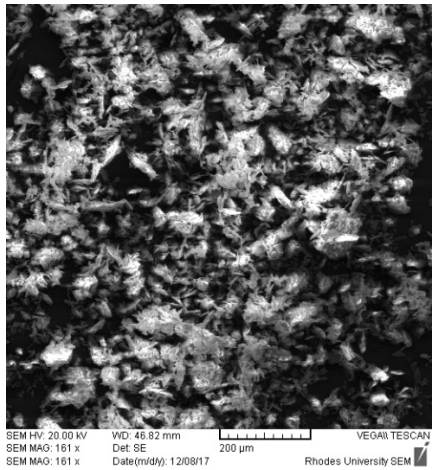
SEM was used to determine the particle shape of all powdered excipients in this study. The images generated are depicted in Figure 3.1. Prednisone particles (I) were observed to be small and irregular with very low sphericity. The images indicate formation of irregular aggregates suggesting that prednisone may exhibit poor bulk flow properties. Followinng collection of measurements, the particle size distribution of prednisone was found to be between 16.3 µm and 28.1 µm. Images obtained for sodium starch glycolate (II) revealed the particles to be very smooth and nearly spherical indicative of good flow properties. The particle size range of sodium starch glycolate was between 16.2 µm and 22.2 µm. Flake-like, irregular particles were observed for talc (III), with a particle size range between 30.4 µm and 46.1 µm. Microcrystalline cellulose exhibited irregular elongated crystals with a particle size distribution between 42.0 µm and 62.7 µm. Flake-like particles with a tendency to aggregate were observed for magnesium stearate. Magnesium stearate had a particle size distribution of 4.5 µm to 20.7 µm. Sample images depicting how particle size distribution was acquired are presented in Figure 3.2. With the exception of sodium starch glycolate, all other powdered excipients had irregular shapes and very low sphericity suggesting poor flow properties. It is imperative to note however that a definitive judgment regarding flow properties cannot be made by simply analyzing particle shape. Further tests have to be performed for more definitive results.



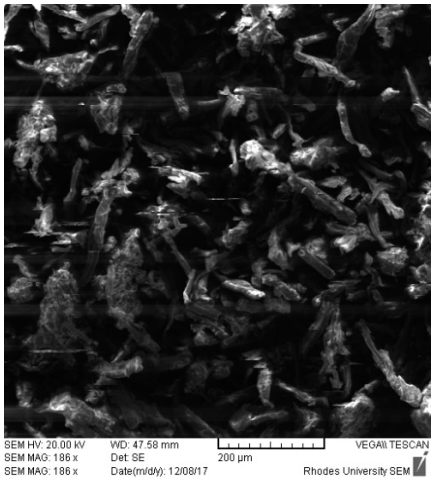
(I)



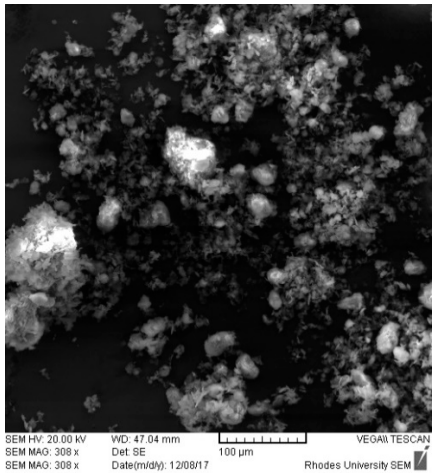
(II)



(III)

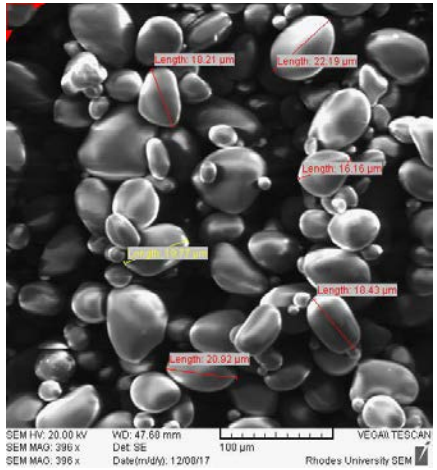


(IV)



(V)

Figure 3.1 SEM images of (I) prednisone, (II) SSG, (III) talc, (IV) MCC and (V) Mg stearate



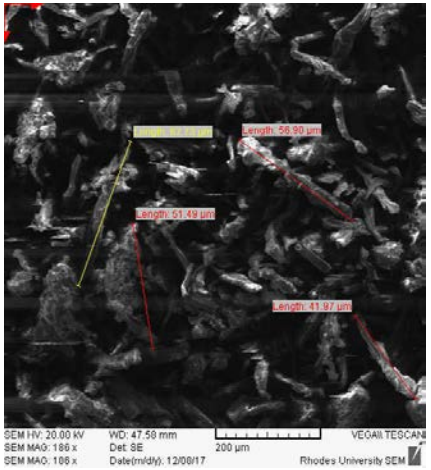


Figure 3.2 SEM images depicting particle size analysis was conducted

3.6.2 Powder rheology

3.6.2.1 Bulk and tapped density

A summary of powder flow property analysis is listed in Table 3.4. The bulk and tapped densities were used to calculate CI and HR using Equations 3.4 and 3.5 respectively, and the results were used to assess flow properties of powder blends. The bulk density ranged between 0.16 g/cm^3 and 0.74 g/cm^3 whereas tapped density ranged between 0.27 g/cm^3 and 0.96 g/cm^3.

3.6.2.2 Carr's index (CI) and Hausner ratio (HR)

A summary of the CI and HR values is listed in Table 3.4. CI values ranged between 22.92 % and 51.28 % indicating a high degree of variability in powder flow properties. Only sodium starch glycolateexhibited adequate flow, indicated by a CI value of 22.92 %. The results generated for the remaining excipients are indicative of poor to extremely poor flow properties, commonly observed in highly cohesive powders. Prednisoneexhibited the highest CI of 51.28 % implying extremely poor flow. However prednisone content (5 mg) was very low relative to the final dosage form and such a small quantity is unlikely to make a significant difference in flow once incorporated with other powdered excipients. Nonetheless, the addition of a lubricant and/or glidant was recommended to improve powder flow.

HR values ranged between 1.30 and 2.05 indicative of satisfactory to poor flow. Only talc and sodium starch glycolate produced HR values that indicate satisfactory flow. Prednisone and all the other excipients exhibited poor flow properties. With the addition of a glidant and lubricant, acceptable flow was generated for efficient pelletization.

3.6.2.3 Angle of Repose (AOR)

Angle of Repose (AOR) values generated from analyzing powdered excipients are summarized in Table 3.4. AOR ranged between 31° and 41° indicative of satisfactory to very poor flow, further validating the need for incorporating a glidant and or lubricant. Incorporation of a glidant or lubricant would ensure adequate flow properties that lead to the production of a homogeneous dosage form.

Table 3.4 Summary of powder flow properties

Compound	BD g/cm^3	TD g/cm^3	CI %	HR	AR °
Prednisone	0.19	0.39	51.28	2.05	41
Sodium starch glycolate	0.74	0.96	22.92	1.30	31
Talc	0.51	0.67	23.88	1.31	34
Magnesium stearate	0.16	0.27	40.74	1.69	37
Microcrystalline cellulose	0.31	0.45	31.11	1.45	32

3.7 DIFFERENTIAL SCANNING CALORIMETRY

3.7.1 Prednisone

The DSC thermogram for prednisone generated at a heating rate of 10 °C/min revealed a melting endotherm at 242 °C with a ΔH = 109.51 J/g which was slightly higher than the previously reported range of 233 - 235 °C [356, 357], probably indicating the presence of polymorphs in the sample [357, 358]. Prednisone was observed to undergo decomposition at about 248 °C, as shown on the DSC thermogram (Figure 3.3).

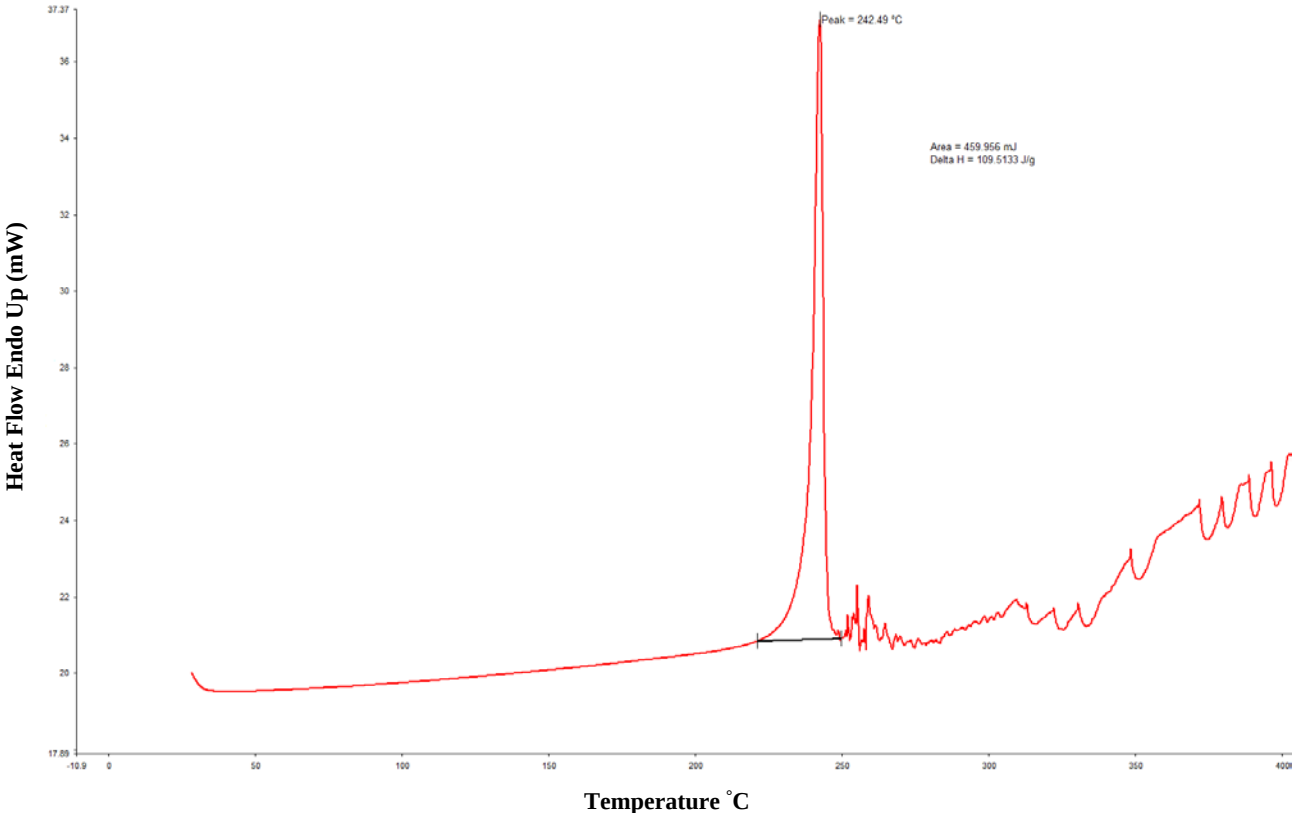


Figure 3.3 DSC thermogram for prednisone generated at a heating rate of 10 °C/min

3.7.2 Prednisone and Mg stearate

DSC thermograms for Mg stearate are variable depending on the source of material. In addition to variable free fatty acid composition, Mg stearate exists in several polymorphic forms, therefore different DSC fingerprints exist for the compound [359, 360]. In our study, three endothermic peaks were observed. The major ones were twin peaks at 116.83 °C and 126.72 °C with ΔH = 71.54 J/g and ΔH = 16.47 J/g respectively. The melting endotherms were in agreement with the previously reported melting point range of 117 °C to 150 °C [276]. Peaks for both prednisone and Mg stearate were observed in the DSC thermogram generated for the binary mixture indicating that the two compounds are likely to be compatible. The melting endotherm of prednisone at 205.85 °C occurs at a lower temperature to that of prednisone alone possibly due to presence of Mg stearate as an impurity [361]. The DSC thermogram for Mg stearate alone is presented in Figure 3.4 and the DSC thermogram for a binary mixture of prednisone and Mg stearate is presented in Figure 3.5.

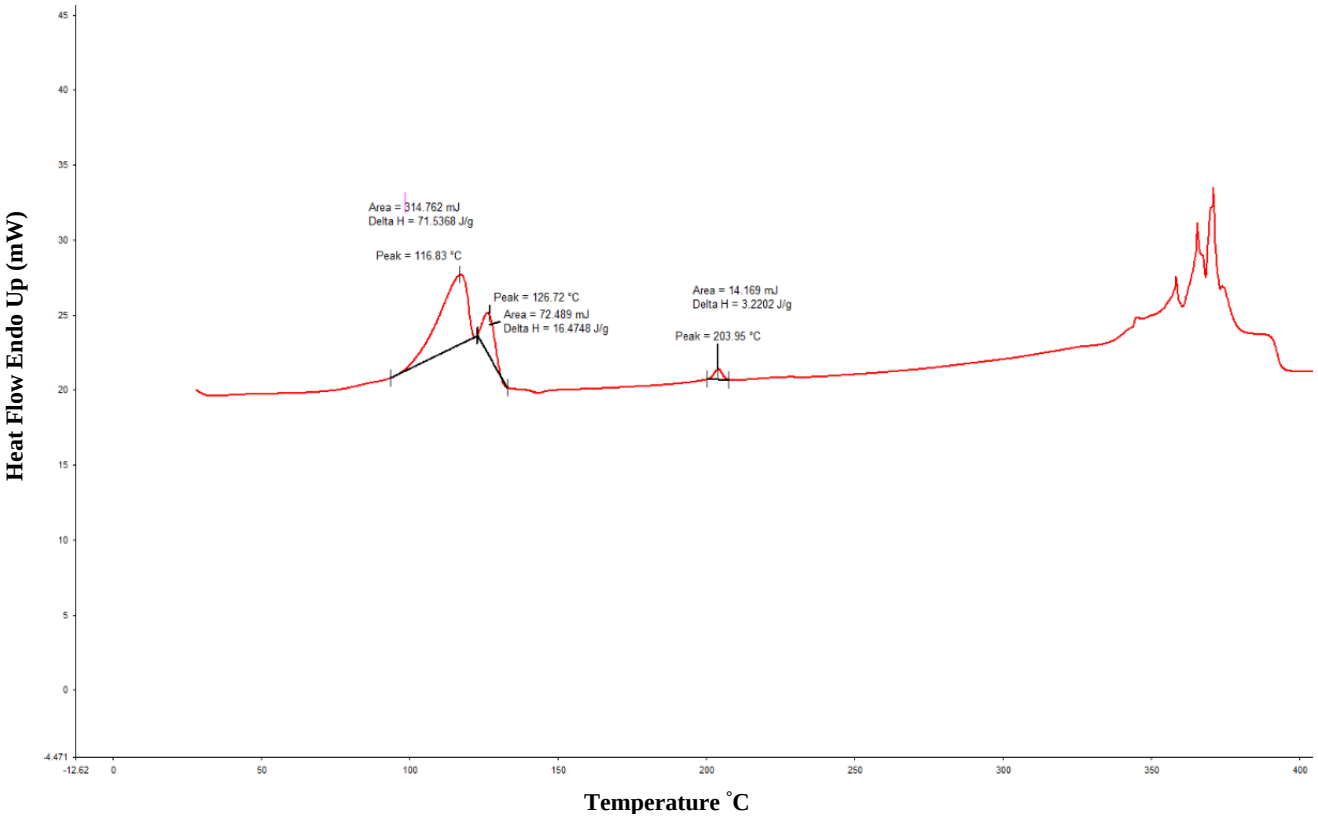


Figure 3.4 DSC thermogram for Mg stearate generated at a heating rate of 10 °C/min

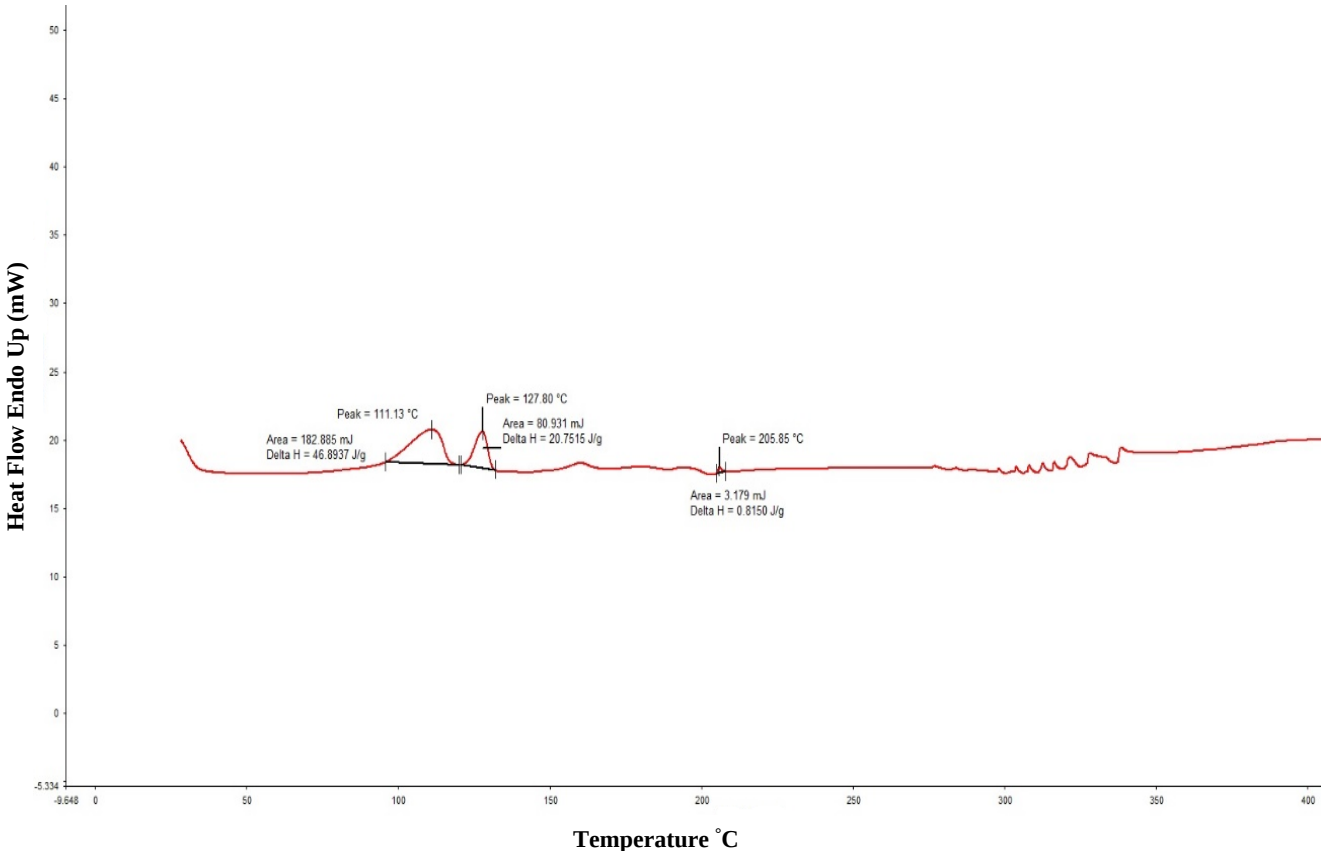


Figure 3.5 DSC thermogram of a 1:1 binary mixture of prednisone and Mg stearate generated at a heating rate of 10 °C/min

3.7.3 Prednisone and sodium starch glycolate

The DSC thermogram for sodium starch glycolate revealed the presence of two peaks, an endothermic peak at 57.92 °C and an exothermic peak at 289.35 °C with ΔH = 78.90 J/g and ΔH = -114.03 J/g respectively. The endotherm at 57.92 °C is most likely due to loss of water. Sodium starch glycolate does not melt, it burns and chars at temperatures ≥ 200 as indicated by the exotherm at 289.35 °C in Figure 3.6 [276]. The DSC thermogram generated for a binary mixture of prednisone and sodium starch glycolate is depicted in Figure 3.7. Both the melting endotherm of prednisone and the exotherm from sodium starch glycolate were present indicating that the two compounds are compatible. The melting endotherm of prednisone in the binary mixture was observed at 228.48 °C which is slightly lower than that of prednisone alone due to presence of sodium starch glycolate as an impurity [361].

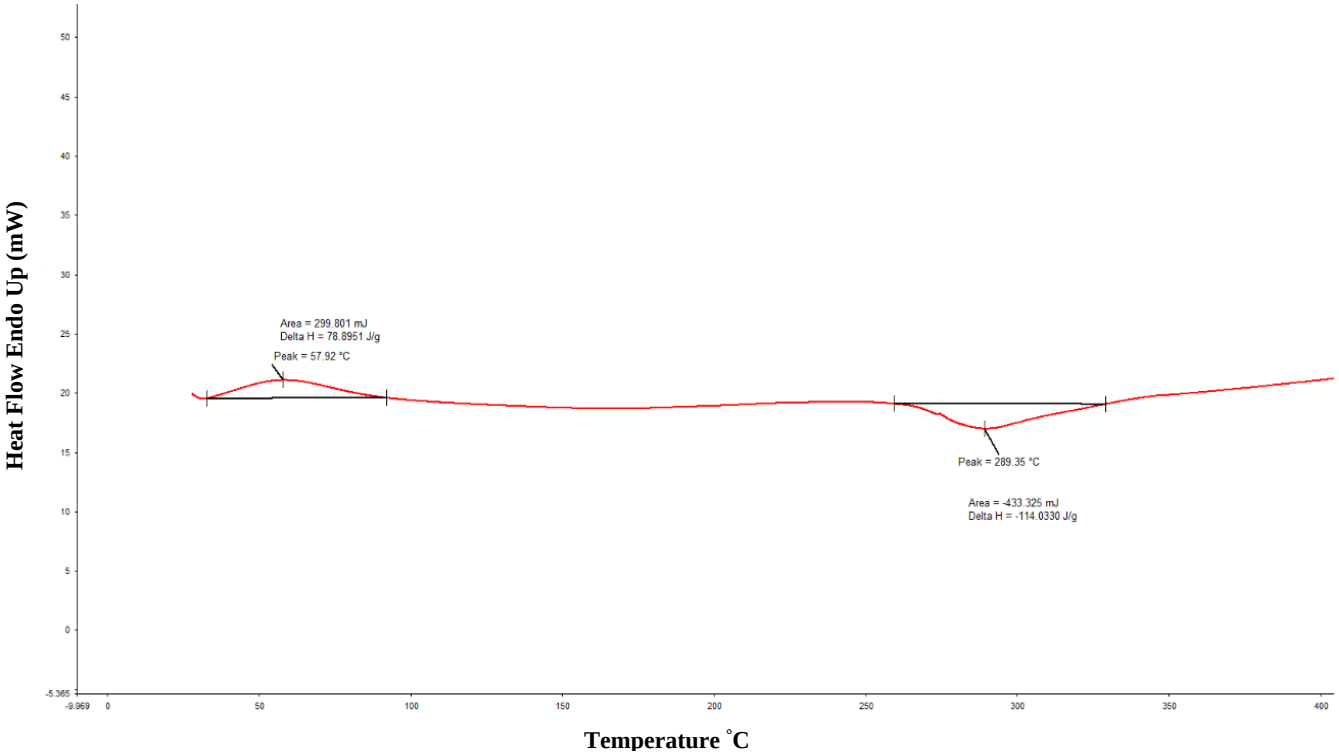


Figure 3.6 DSC thermogram for sodium starch glycolate generated at a heating rate of 10 °C/min

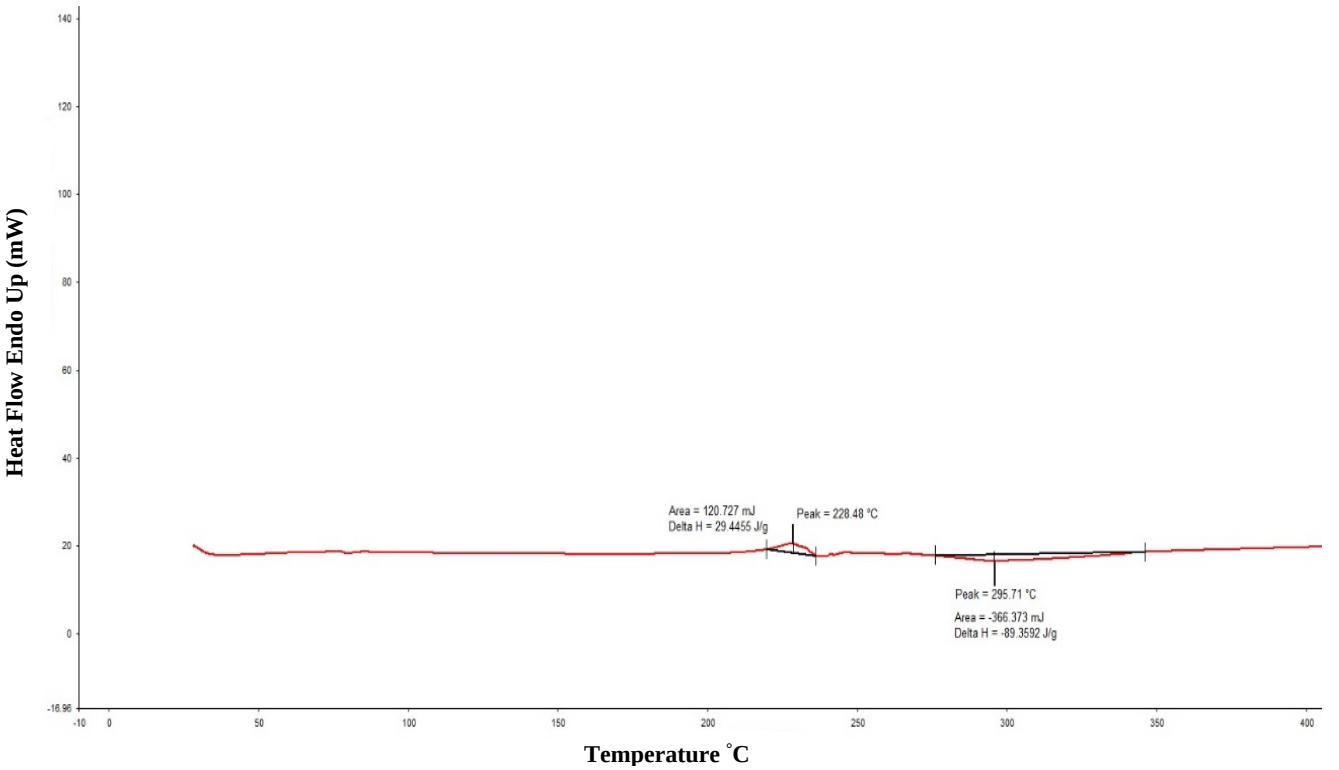


Figure 3.7 DSC thermogram for a 1:1 binary mixture of prednisone and sodium starch glycolate generated at a heating rate of 10 °C/min

3.7.4 Prednisone and talc

The DSC thermogram generated for talc is presented in Figure 3.8 and did not reveal any significant thermal events. A binary mixture of prednisone and talc however produced a peak at 236.64 °C with a ΔH = 33.47 J/g corresponding to the melting endotherm of prednisone, therefore talc and prednisone were found to be compatible. Melting occurred at a temperature slightly lower than that of prednisone alone, indicating that impurities lower the melting point of a substance. The DSC thermogram generated for a binary mixture of talc and prednisone is presented in Figure 3.9.

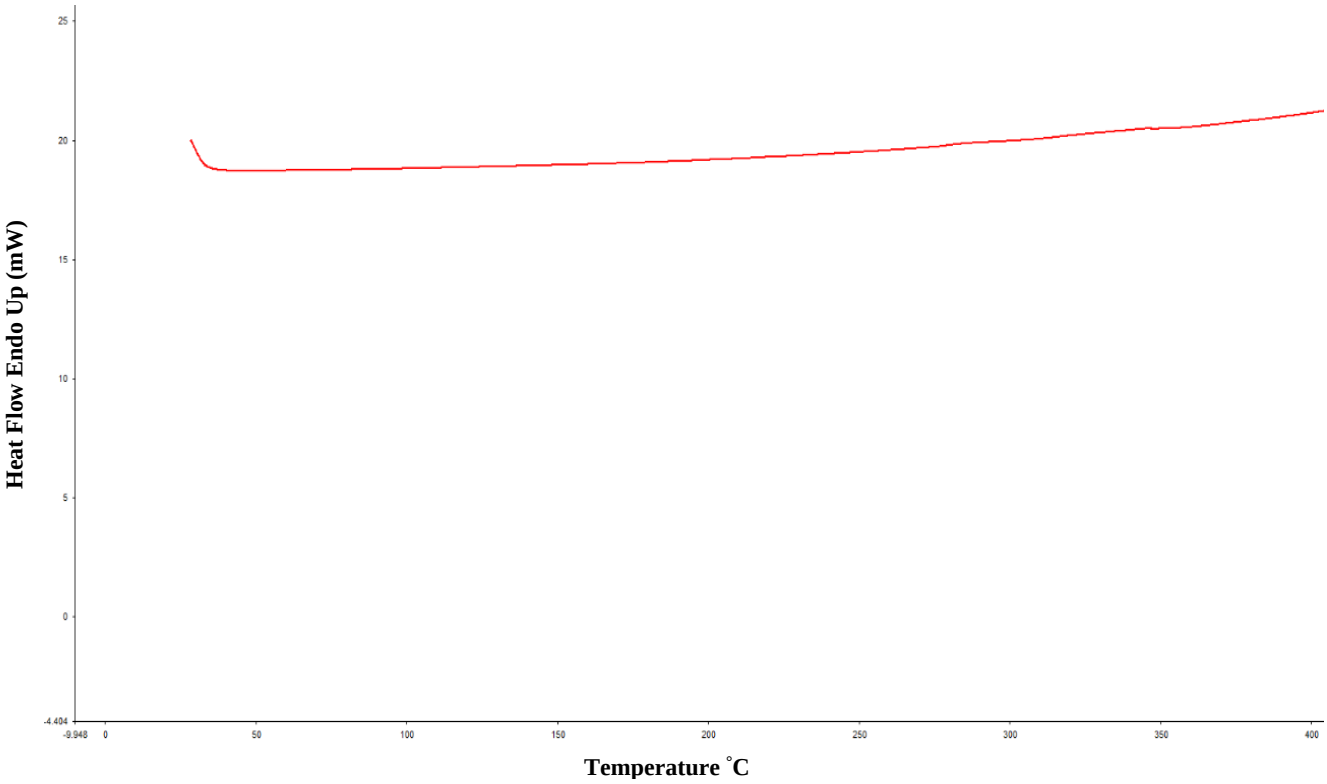


Figure 3.8 DSC thermogram for talc generated at a heating rate of 10 °C/min

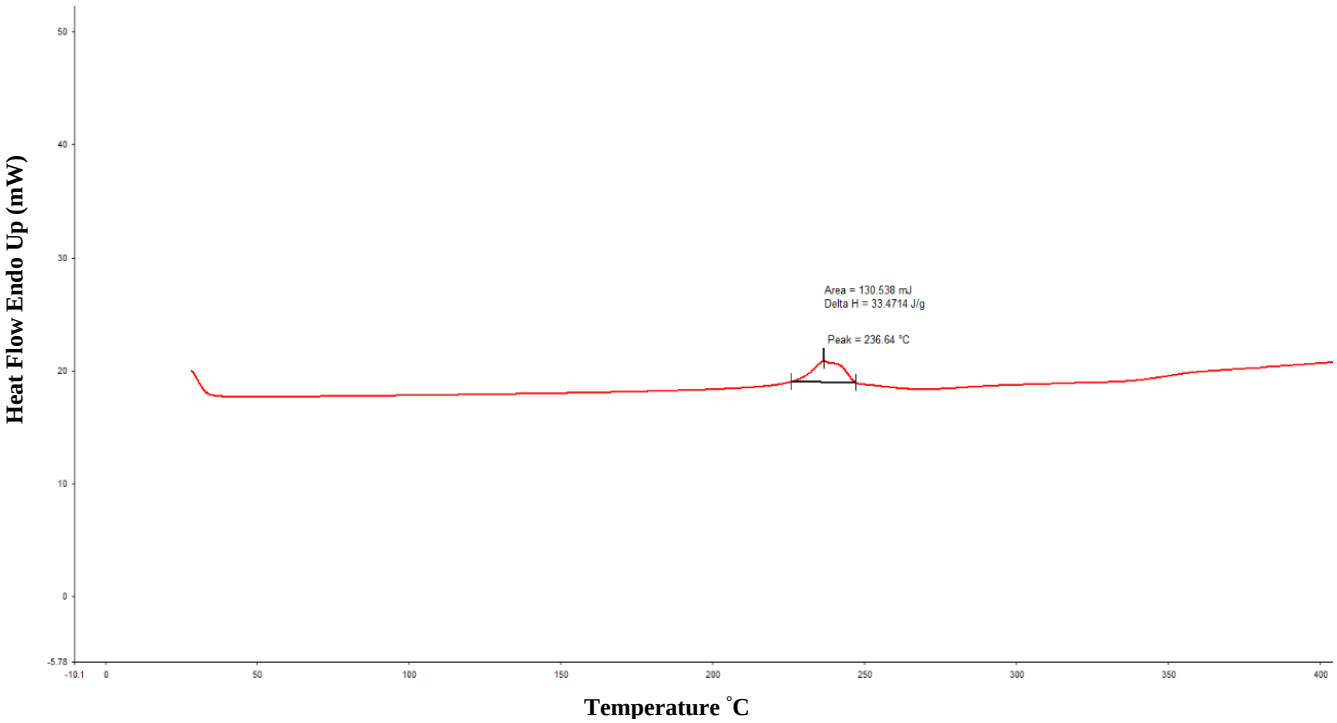


Figure 3.9 DSC thermogram for a 1:1 binary mixture of prednisone and talc generated at a heating rate of 10 °C/min

3.7.5 Prednisone and Tween® 80

The DSC thermogram generated for Tween® 80 revealed an endothermic peak at 139.89 °C with a ΔH = 20.66 J/g as shown in Figure 4.10. The thermogram generated for a binary mixture of prednisone and Tween® 80 revealed presence of two peaks, one at 135.99 °C and a second one at 219.61 °C which were attributed to Tween® 80 and prednisone respectively. Peaks from both compounds were presented on the DSC thermogram (Figure 4.11) therefore Tween® 80 and prednisone were found to be compatible.

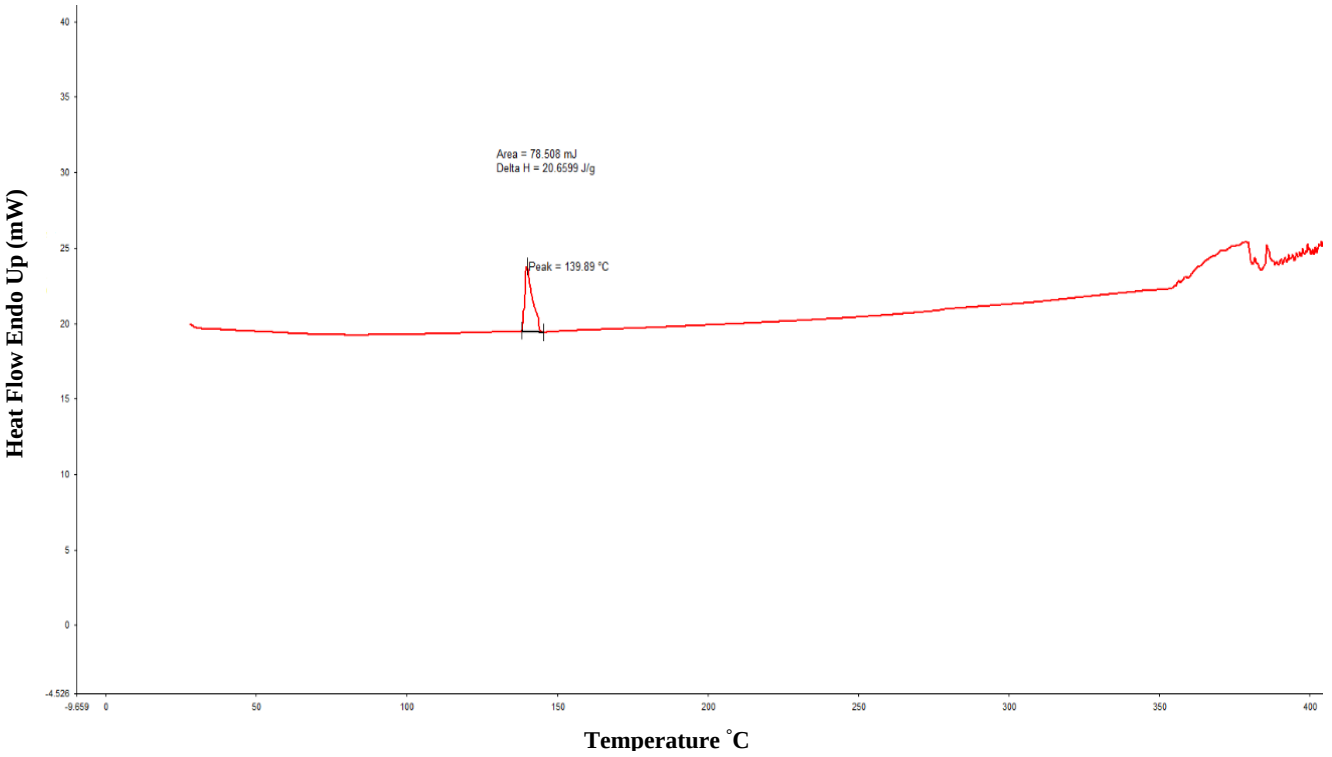


Figure 3.10 DSC thermogram for Tween® 80 generated at a heating rate of 10 °C/min

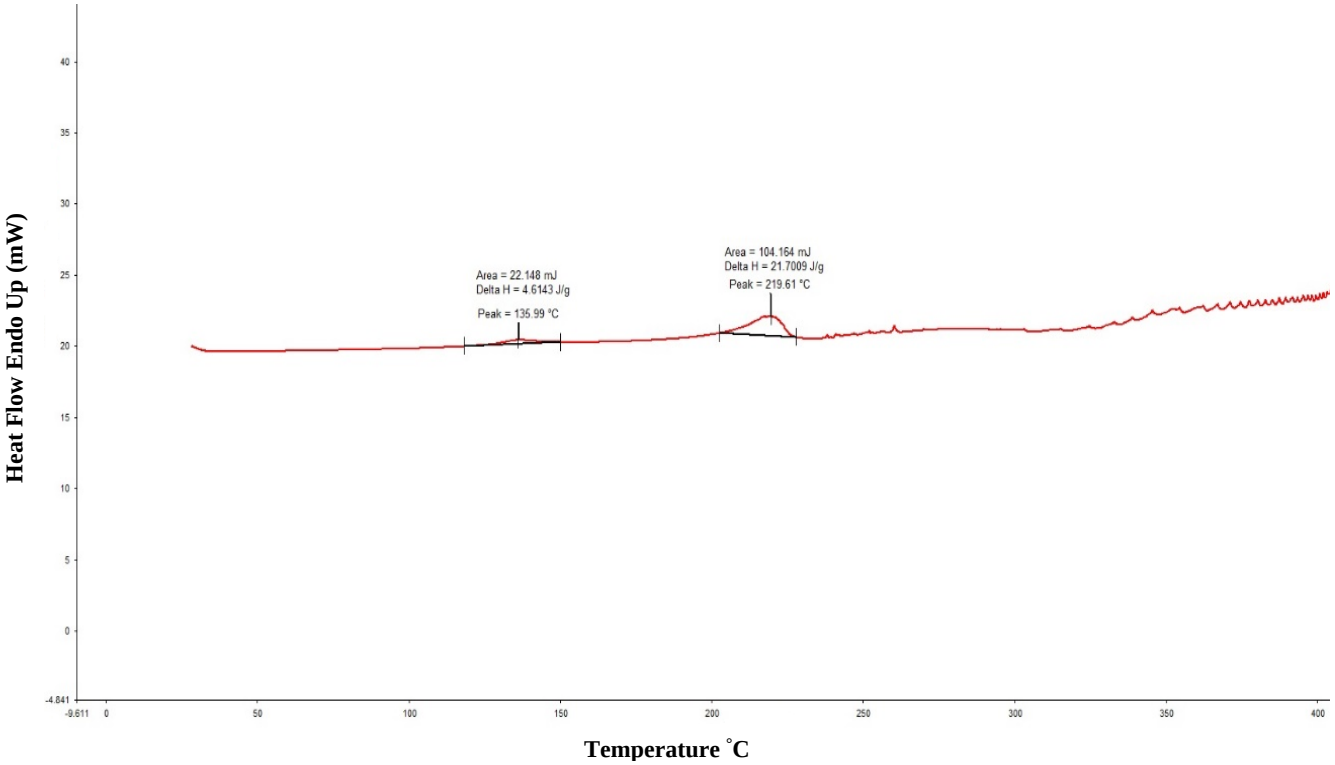


Figure 3.11 DSC thermogram for a 1:1 binary mixture of prednisone and Tween® 80 generated at a heating rate of 10 °C/min

3.7.6 Prednisone and microcrystalline cellulose

Microcrystalline cellulose exhibited an endothermic peak at 346.43 °C with a ΔH = 388.04 J/g as shown in the DSC thermogram presented in Figure 3.12. The thermogram generated for a binary mixture of prednisone and microcrystalline cellulose revealed peaks at 239.78 °C and 356.96 °C which were attributed to prednisone and microcrystalline cellulose respectively. Peaks from both compounds were present as shown in Figure 3.13 therefore microcrystalline cellulose and prednisone were found to be compatible.

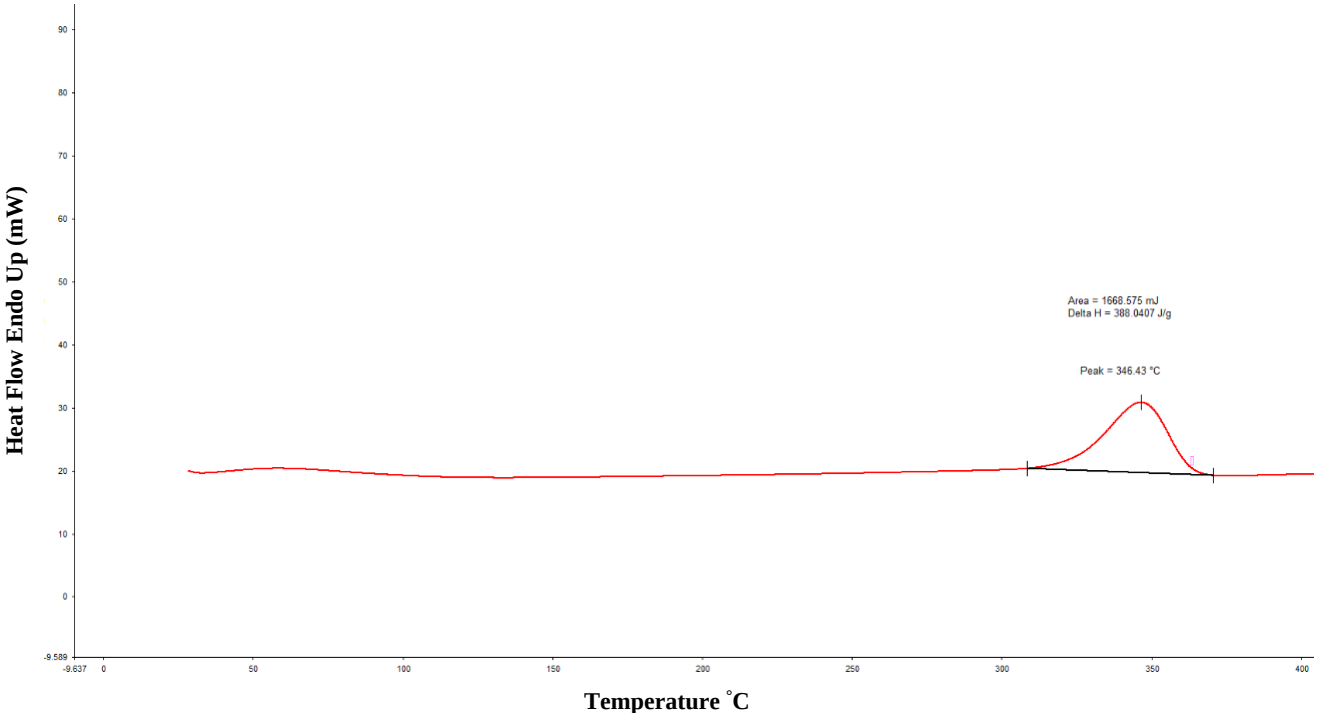


Figure 3.12 DSC thermogram for microcrystalline cellulose generated at a heating rate of 10 °C/min

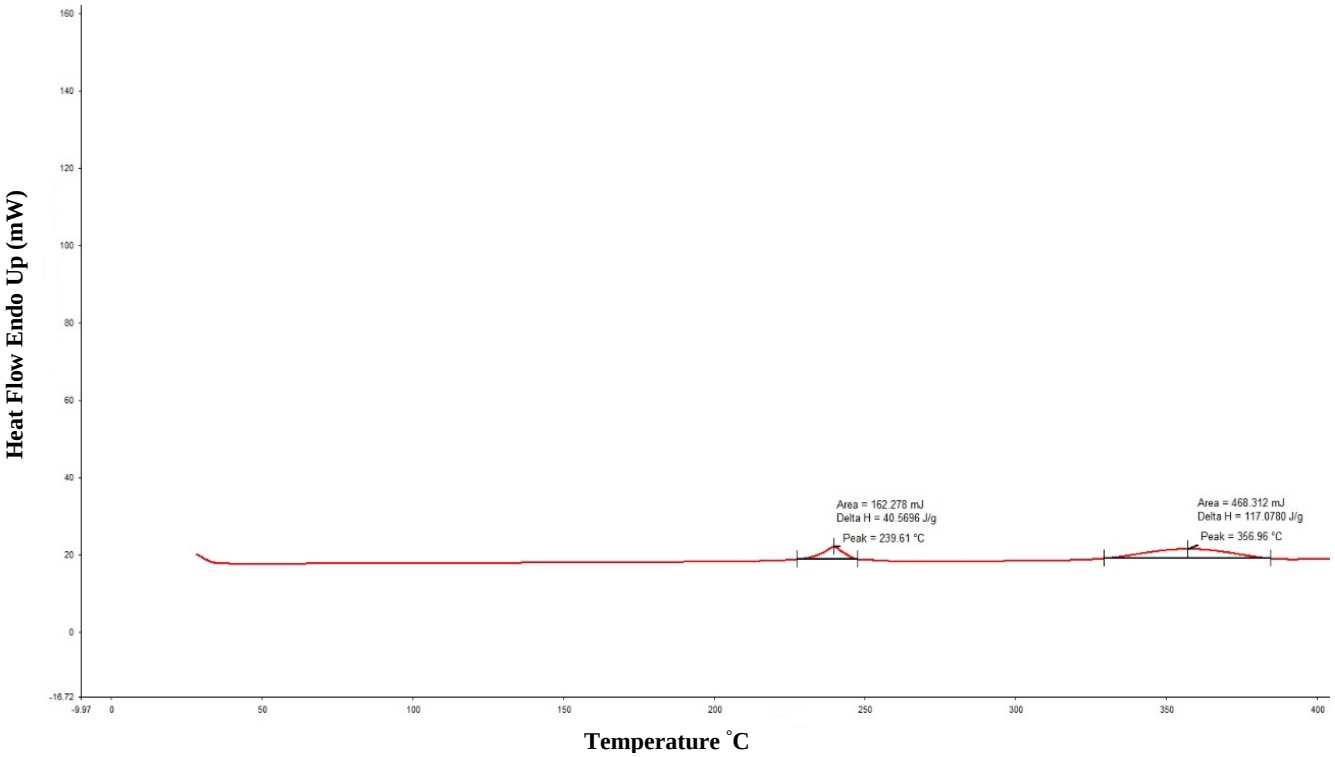


Figure 3.13 DSC thermogram for a 1:1 binary mixture of microcrystalline cellulose and prednisone generated at a heating rate of 10 °C/min

3.7.7 Prednisone and PEG 400

The DSC thermogram for PEG 400 revealed a melting endotherm at 130.80 °C with a $\Delta H = 8.95$ J/g. The presence of an exotherm was also noted at temperatures ≥ 350 °C which was possibly due to the degradation of PEG 400. The DSC thermogram obtained for PEG 400 is presented in Figure 3.14. Analysis of a binary mixture of prednisone and PEG 400 revealed presence of both compounds indicating that they were compatible. Figure 3.15 presents the DSC thermogram generated for the binary mixture of prednisone and PEG 400.

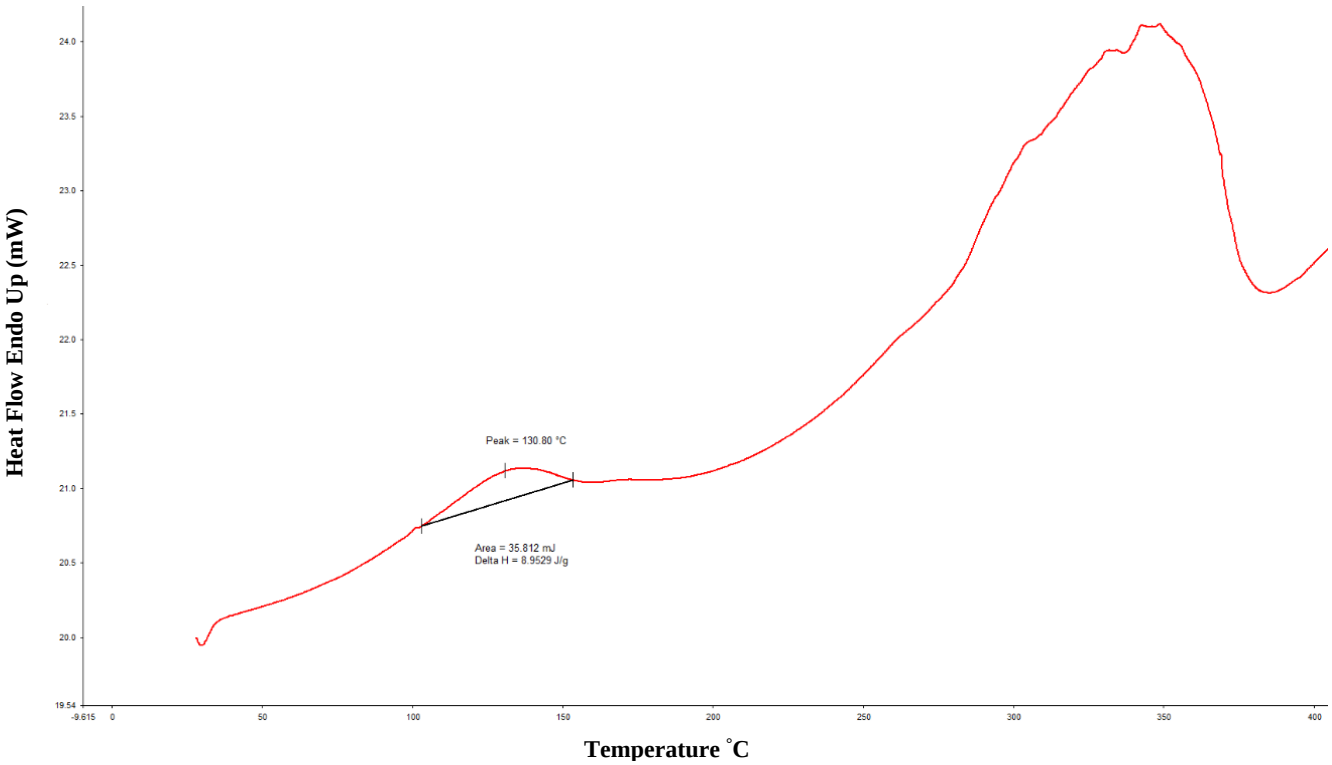


Figure 3.14 DSC thermogram for PEG 400 generated at a heating rate of 10 ˚C/min

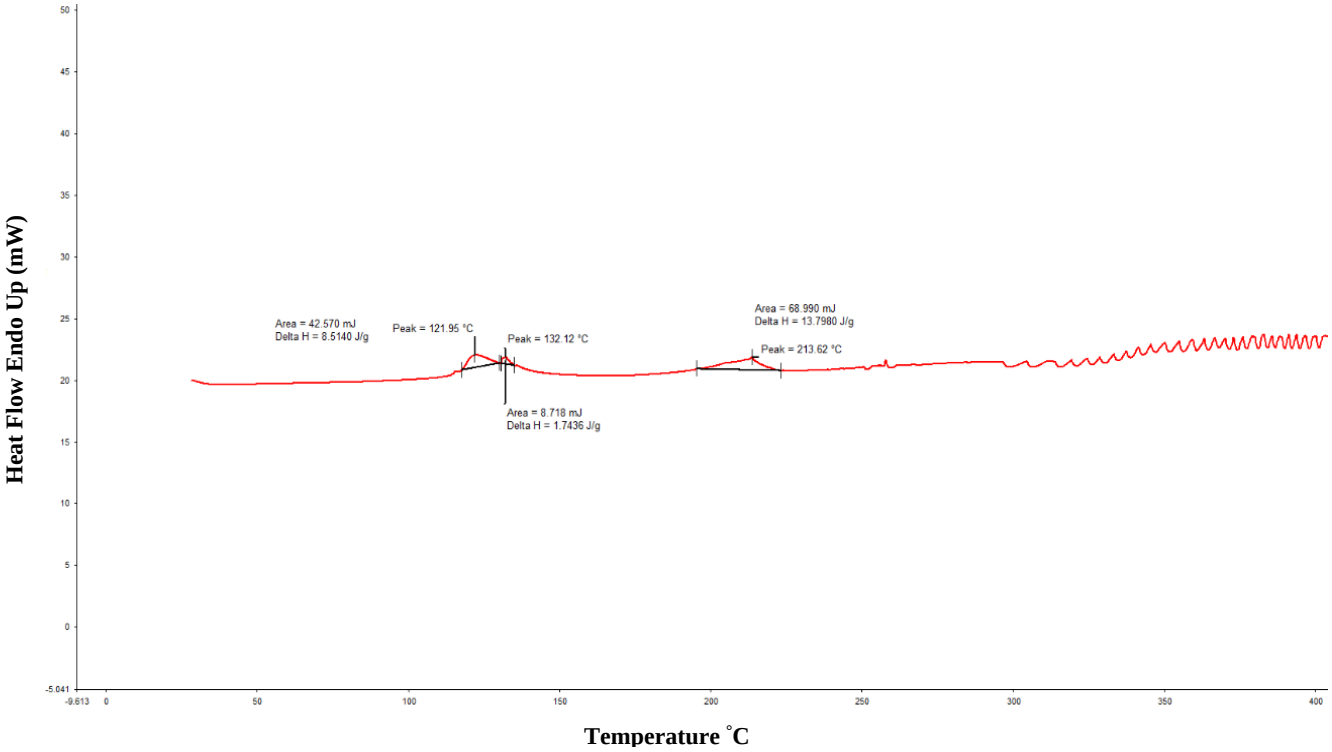


Figure 3.15 DSC thermogram for a binary mixture of prednisone and PEG 400 generated at a heating rate of 10 ˚C/min

3.7.8 Prednisone and Eudragit® RL 30 D

The DSC thermogram generated for Eudragit® RL 30 D reveals two thermal transitions indicated by twin peaks at 105.23 and 107. 89 in Figure 3.16. The peaks had ΔH = 88.15 J/g and ΔH = 29.73 J/g respectively. The binary mixture of prednisone and Eudragit® RL 30 D however resulted in the disappearance of peaks from both compounds, suggesting an incompatibility at extreme temperatures. As confirmation, FT-IR spectroscopy was conducted and spectra for the same binary mixture at room temperature revealed the presence of characteristic peaks from both compounds therefore the possibility of an incompatibility was ruled out. However long term stability studies of the final product were recommended to elucidate the impact of the interaction on shelf life. The DSC thermogram for Eudragit® RL 30 D alone is presented in Figure 3.16, and the DSC thermogram for a binary mixture of prednisone and Eudragit® RL 30 D is presented in Figure 3.17.

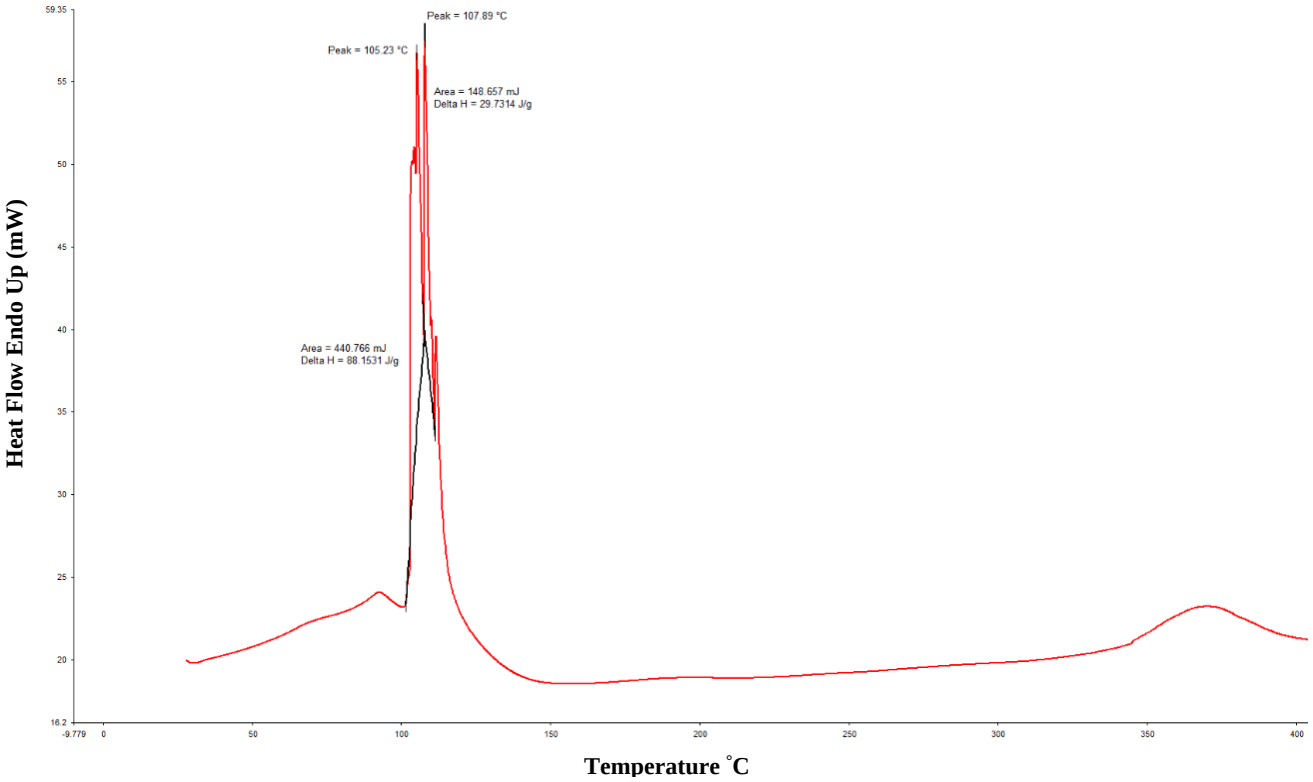


Figure 3.16 DSC thermogram for Eudragit® RL 30 D generated at a heating rate of 10 °C/min

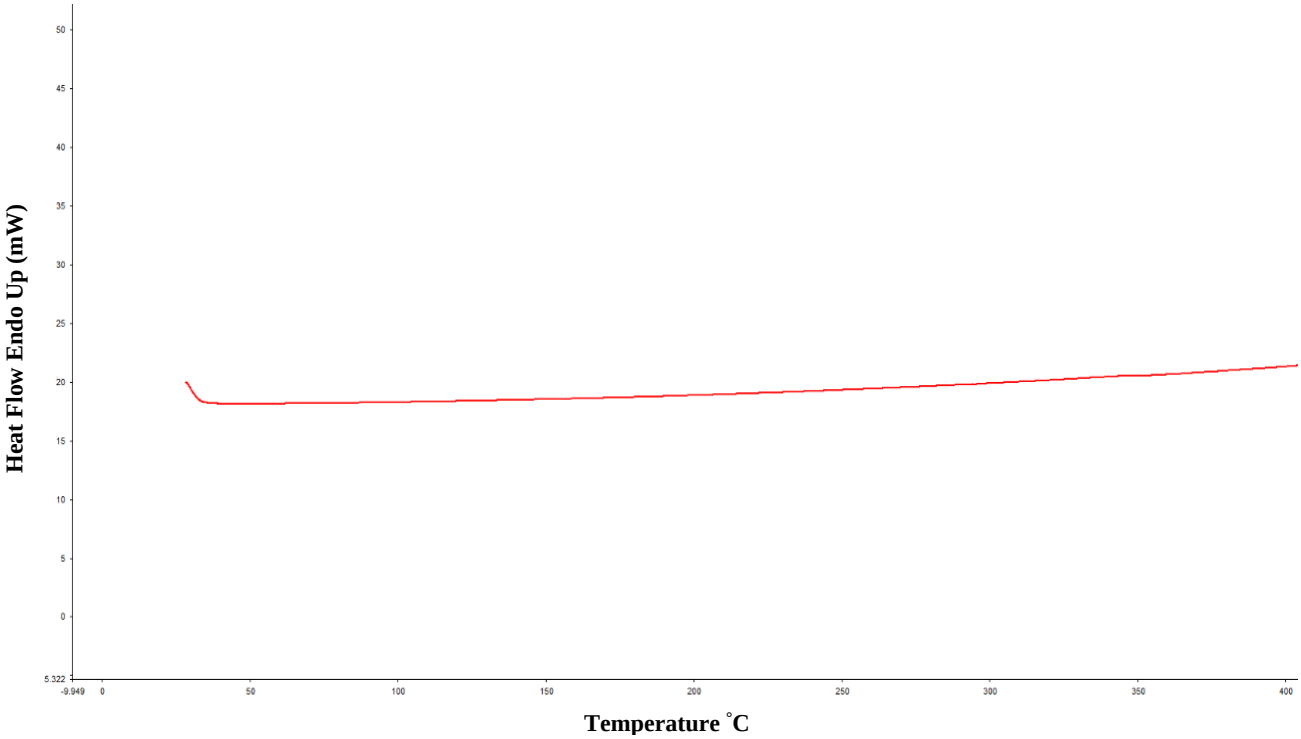


Figure 3.17 DSC thermogram for a1:1 binary mixture of prednisone and Eudragit® RL 30 D generated at a heating rate of 10 ˚C/min

3.8 FOURIER TRANSFORM INFRARED SPECTROSCOPY

Spectra generated from FT-IR spectroscopy are unique for each and every compound. It follows therefore, that each spectrum may be regarded as a molecular fingerprint, through which the quality of a compound can be established [362]. Any shift or deviation in peak bands may be used to elucidate potential chemical interactions [362]. FT-IR spectra generated for pure prednisone *versus* binary mixtures of prednisone and excipients were analyzed.

3.8.1 Prednisone

The FT-IR spectrum of pure prednisone exhibited a broad peak at 3290 cm^{-1} which can be attributed to the -OH vibration. The set of peaks between 2872 cm^{-1} and 2956 cm^{-1} are assigned to aliphatic C-H stretching modes. Strong absorption bands at 1707 cm^{-1} (cyclic) and 1666 cm^{-1} (aliphatic) are characteristic ketone C=O stretching modes. The band at 1621 cm^{-1} is characteristic to α-β-unsaturated cyclohexadiene C=C vibrations. Peaks below 1000 cm^{-1} were attributed to C-C-H angular deformation modes [363-365].

The FT-IR spectrum generated for prednisone is depicted in Figure 3.18. The main absorption peaks and their assignments are summarized in Table 3.5 [363-365].

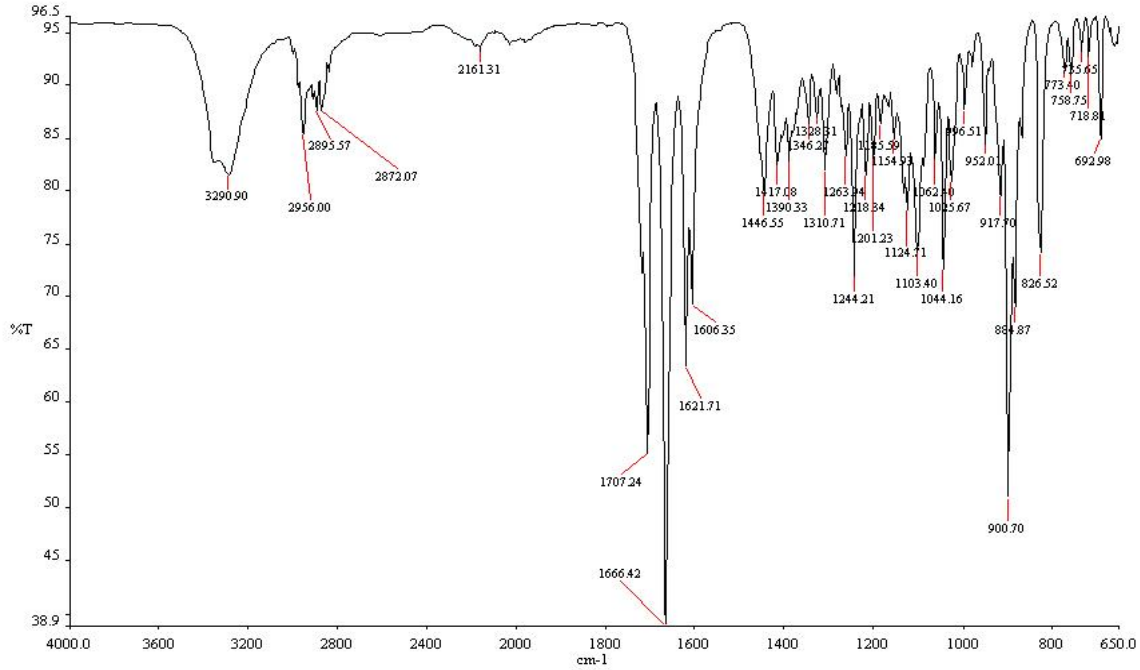


Figure 3.18 FT-IR spectrum for prednisone

Table 3.5 FT-IR band assignment for prednisone

Wave number cm^{-1}	Functional group
3290	OH
2956 - 2872	C-H stretching
1707	C=O cyclic
1666	C=O aliphatic
1621	C=C cyclohexadiene
1000 - 650	C-C-H angular deformation

3.8.2 Prednisone and 1:1 binary mixtures

Following analysis of spectra generated for 1:1 binary mixtures, the cyclic C=O peak of prednisone with an absorption band at 1707 cm^{-1} was absent or microcrystalline cellulose, sodium starch glycolate, talc and Mg stearate. The absence of the peak was not regarded to be an incompatibility since it was most likely due to peak overlapping of the C=O groups present in microcrystalline cellulose, sodium starch glycolate and talc and COO^- groups present in sodium starch glycolate and Mg stearate which exhibited strong absorption bands at a wavelength of 1738 cm^{-1} [366]. The C-H stretching mode of prednisone, typically present between 2956 cm^{-1} and 2872 cm^{-1} was absent in a binary mixture with Eudragit® RL 30 D due to an overlap of peaks generated by strong OH vibrations from Eudragit® RL 30 D [367]. All other main peaks were present for all binary mixtures indicating a lack of significant interactions.

The data presented in Tables 3.6 to 3.12 summarize the shifts observed in main absorption bands of prednisone following FT-IR analysis of binary mixtures. The FT-IR spectra generated for the studies are presented in Figures 3.19 to 3.25.

3.8.2.1 Prednisone and microcrystalline cellulose

Table 3.6 Absorption bands of pure prednisone vs. prednisone: MCC binary mixture

Functional group	Absorption bands cm^{-1} Prednisone	Absorption bands cm^{-1} Prednisone:MCC
OH	3291	3291
C-H stretching	2956 - 2872	2971 - 2872
C=O cyclic	1707	Absent
C=O aliphatic	1666	1667
C=C cyclohexadiene	1622	1622

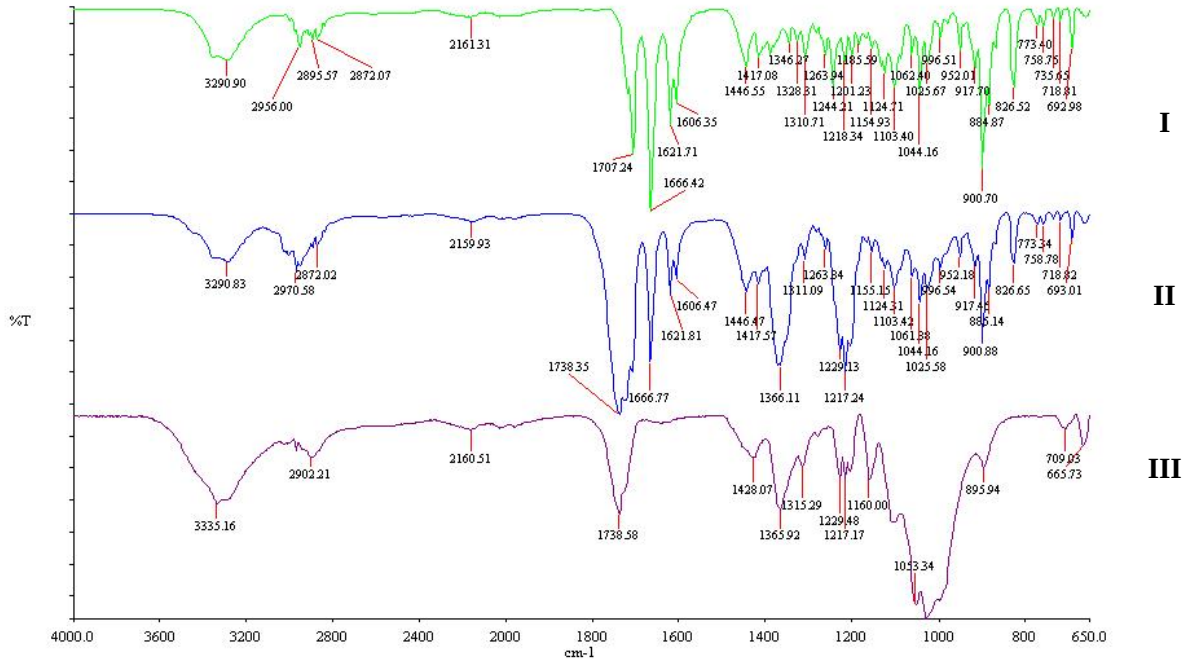


Figure 3.19 FT-IR spectra generated for (I) prednisone, (II) 1:1 prednisone:MCC binary mixture and (III) MCC

3.8.2.2 Prednisone and sodium starch glycolate

Table 3.7 Absorption bands of pure prednisone vs. prednisone: SSG binary mixture

Functional group	Absorption bands cm^{-1} Prednisone	Absorption bands cm^{-1} Prednisone : SSG
OH	3291	3290
C-H stretching	2956 - 2872	2970 - 2872
C=O cyclic	1707	Absent
C=O aliphatic	1666	1666
C=C cyclohexadiene	1622	1622

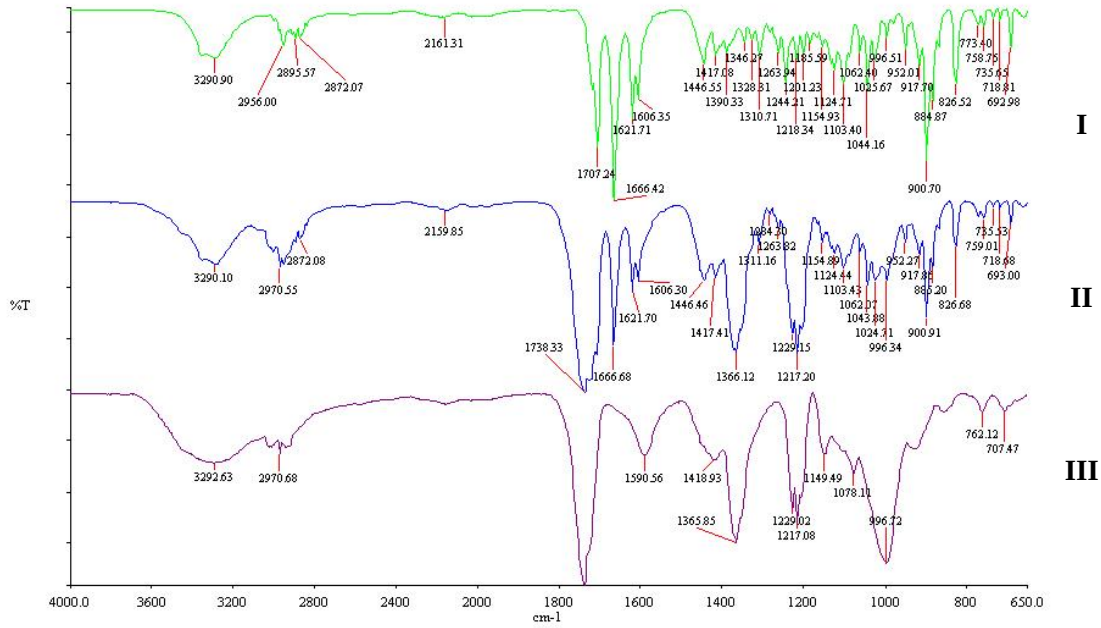


Figure 3.20 FT-IR spectra generated for (I) prednisone, (II) 1:1 prednisone:SSG binary mixture and (III) SSG

3.8.2.3 Prednisone and talc

Table 3.8 Absorption bands of pure prednisone vs. prednisone: talc binary mixture

Functional group	Absorption bands cm^{-1} Prednisone	Absorption bands cm^{-1} Prednisone : talc
OH	3291	3291
C-H stretching	2956 - 2872	2970
C=O cyclic	1707	Absent
C=O aliphatic	1666	1667
C=C cyclohexadiene	1622	1622

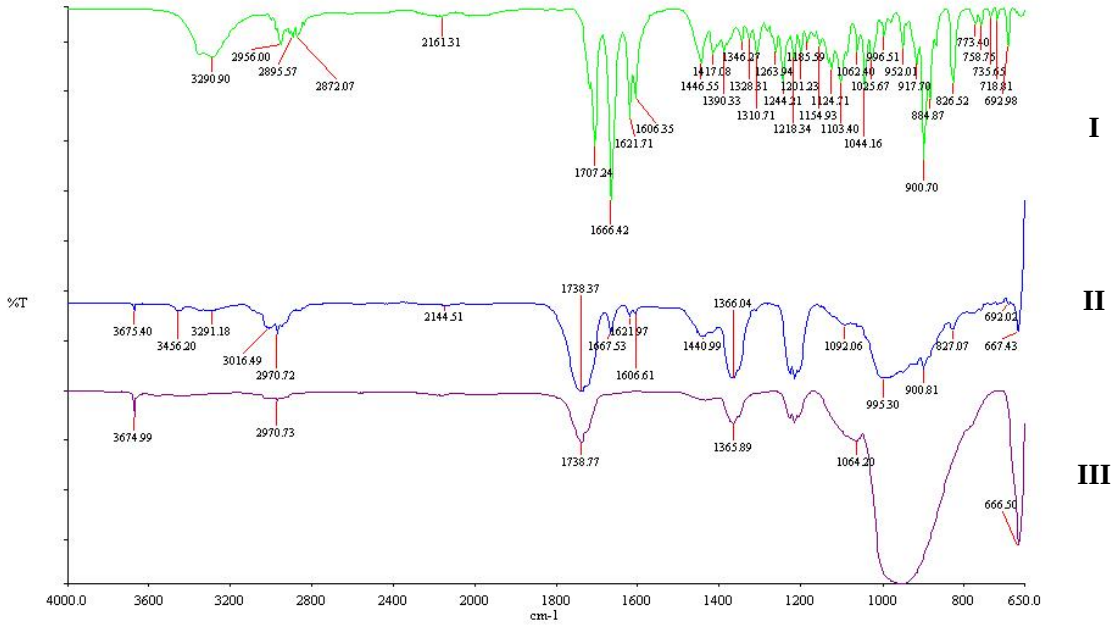


Figure 3.21 FT-IR spectra generated for (I) prednisone, (II) 1:1 prednisone:talc binary mixture and (III) talc

3.8.2.4 Prednisone and Mg Stearate

Table 3.9 Absorption bands of pure prednisone vs. prednisone: Mg Stearate binary mixture

Functional group	Absorption bands cm^{-1} Prednisone	Absorption bands cm^{-1} Prednisone : Mg Stearate
OH	3291	3456
C-H stretching	2956 - 2872	2850 - 2970
C=O cyclic	1707	Absent
C=O aliphatic	1666	1668
C=C cyclohexadiene	1622	Absent

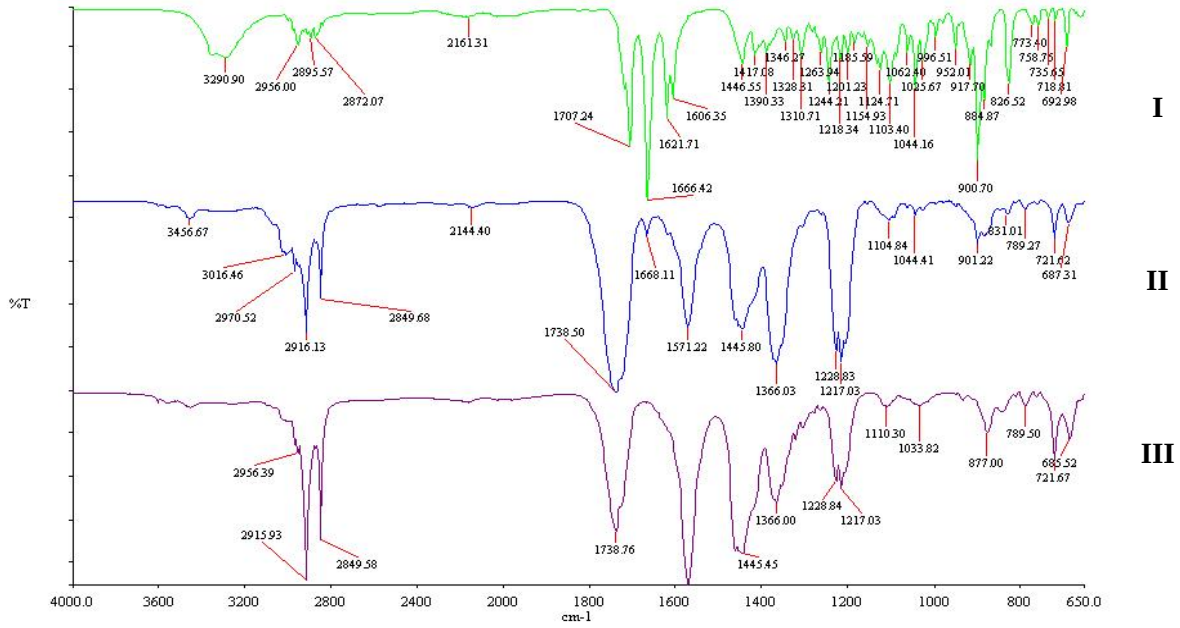


Figure 3.22 FT-IR spectra generated for (I) prednisone, (II) 1:1 prednisone:Mg Stearate binary mixture and (III) Mg Stearate

3.8.2.5 Prednisone and Tween® 80

Table 3.10 Absorption bands of pure prednisone vs. prednisone: Tween® 80 binary mixture

Functional group	Absorption bands cm^{-1} Prednisone	Absorption bands cm^{-1} Prednisone : Tween® 80
OH	3291	3437
C-H stretching	2956 - 2872	2923 - 2857
C=O cyclic	1707	1708
C=O aliphatic	1666	1667
C=C cyclohexadiene	1622	1622

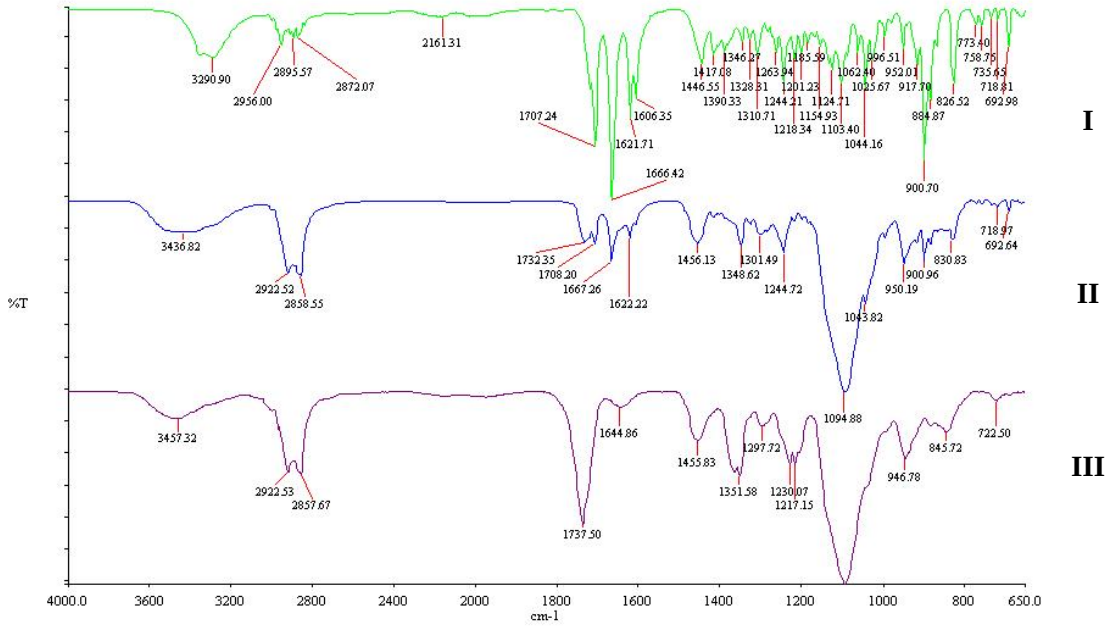


Figure 3.23 FT-IR spectra generated for (I) prednisone, (II) 1:1 prednisone:Tween® 80 binary mixture and (III) Tween® 80

3.8.2.6 Prednisone and PEG 400

Table 3.11 Absorption bands of pure prednisone vs. prednisone: PEG 400 binary mixture

Functional group	Absorption bands cm^{-1} Prednisone	Absorption bands cm^{-1} Prednisone : PEG 400
OH	3291	3366
C-H stretching	2956 - 2872	2871
C=O cyclic	1707	1708
C=O aliphatic	1666	1667
C=C cyclohexadiene	1622	1622

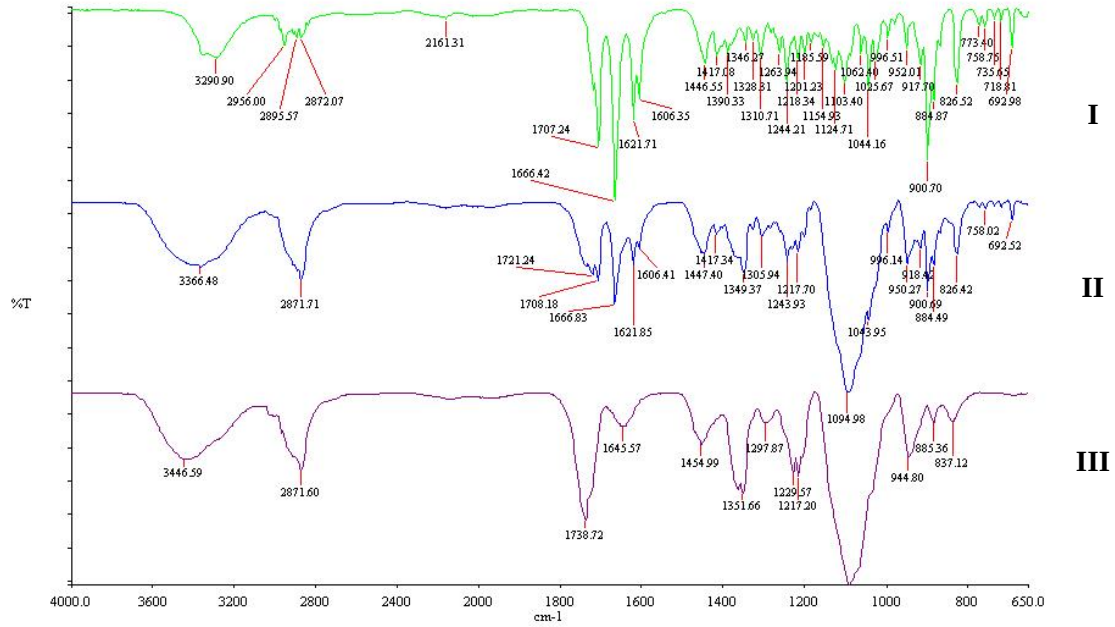


Figure 3.24 FT-IR spectra generated for (I) prednisone, (II) 1:1 prednisone:PEG 400 binary mixture and (III) PEG 400

3.8.2.7 Prednisone and Eudragit® RL 30 D

Table 3.12 Absorption bands of pure prednisone vs. prednisone: Eudragit® RL 30 D binary mixture

Functional group	Absorption bands cm^{-1} Prednisone	Absorption bands cm^{-1} Prednisone : Eudragit® RL 30 D
OH	3291	3351
C-H stretching	2956 - 2872	Absent
C=O cyclic	1707	1707
C=O aliphatic	1666	1666
C=C cyclohexadiene	1621	1622

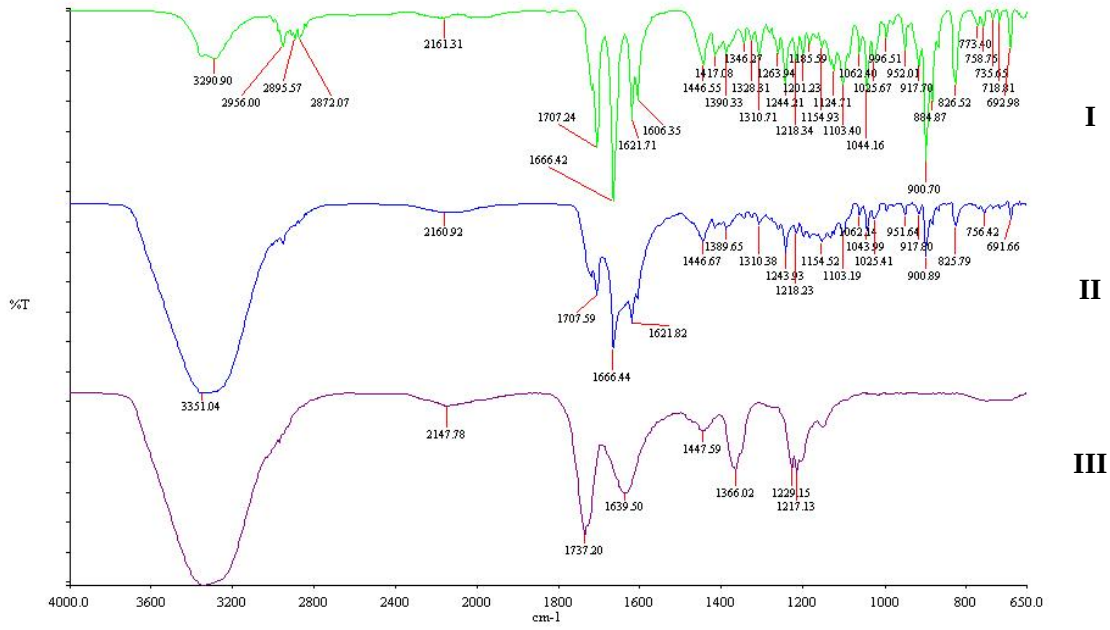


Figure 3.25 FT-IR spectra generated for (I) prednisone, (II) 1:1 prednisone:Eudragit® RL 30 D binary mixture and (III) Eudragit® RL 30 D

3.9 CONCLUSIONS

Pre-formulation studies are an essential step during the development of a pharmaceutical dosage form. Data generated from these studies provide insight into the potential behaviour of the API and excipients during the manufacturing process.

The success of a dosage form relies heavily on the correct choice of excipients. It follows therefore that excipients must be chosen carefully to fit particular purposes. In this study microcrystalline cellulose had a dual functionality. It was used as a bulking agent and as a spheronization aid due to its excellent plasticity and binding properties. When compared to other disintegrants tested during preliminary investigation, sodium starch glycolate had a superior effect therefore it was selected for further evaluation. Talc and Mg stearate were incorporated as anti-frictional agents. The objective of this study was to develop a rapidly disintegrating multiple-unit pellet system to enhance the dissolution rate of poorly soluble prednisone. Previous studies have revealed that incorporating surfactants alone or in combination with glycerides can improve the aqueous solubility of hydrophobic compounds [368-370], therefore Tween® 80, a hydrophilic non-ionic surfactant was added to the formulation to aid the solubilization of prednisone. PEG 400 was added as a pore-forming agent to allow easy penetration of aqueous fluid into pellets and to improve solubility and increase the rate of disintegration due to its hydrophilic nature [370]. Eudragit® RL 30 D was used as a granulation aid, it imparted tensile strength to the pellets and aided solubility due to its hydrophilic nature.

Investigation of rheological properties, suggested a poorly flowing powder blend. CI, HR and AOR produced near-similar conclusions, therefore the addition of talc and Mg stearate as lubricants and glidants was essential.

DSC and FT-IR was used to investigate prednisone-excipient compatibility. The DSC thermogram for a binary mixture of prednisone and Eudragit® RL 30 D revealed absence of a thermal event from both compounds, however characteristic peaks from both compounds were present in the FT-IR spectrum generated for the same binary mixture. FT-IR spectroscopy is conducted at room temperature therefore the absence of peaks during DSC suggests the probability of an interaction only at elevated temperatures. All other excipients were found to be compatible with prednisone, and were deemed suitable for formulation development studies.

CHAPTER 4
EXTRUSION-SPHERONIZATION

4.1 INTRODUCTION

The use of multi-particulate dosage forms has increased in recent years due to their important pharmacological as well as technological advantages over conventional single unit dosage forms [371]. Pellets are one of the most widely used multi-particulate dosage forms. Pellets are agglomerates of powder ncluding API and excipients that are small, free-flowing, spherical or semi-spherical solid units [372, 373] ranging in in size between 0.5 mm and 1.5 mm [374].

Some of the pharmacological benefits associated with use of a multiple unit pellet system are reduced risk of dose dumping, flexible release patterns, less inter and intra-subject variability, less irritation of the gastro-intestinal tract (GIT) and increased bioavailability [375-377]. Whereas technological advantages include better flow properties, narrow particle size distribution, reduced friability, ease of coating and reproducible capsule filling [378, 379].

Pelletization is an agglomeration process that converts powders (API and excipients) into pellets [380]. The processes leading to pellet formation are discussed in this chapter.

4.2 PELLET FORMATION

The events leading to pellet formation are classified as nucleation, coalescence, layering, abrasion transfer and size reduction [374, 378]. The first four processes lead to growth of the pellet. During nucleation, particles are drawn together to form an air-water-solid tri-phase nuclei. When multiple nuclei collide to form larger particles the process is referred to as coalescence. Addition of material onto pre-existing nuclei is referred to as layering, whereas transfer of material from one particle to another is known as abrasion transfer. Size reduction of well-formed particles generally occurs via three mechanisms, attrition, breakage or shattering [374, 378].

4.3 PELLETIZATION TECHNIQUES

The exact mechanism of pellet formation depends on the type of equipment used. Generally, the most widely used techniques include drug layering, direct pelletization, spray drying or spray congealing, balling and extrusion-spheronization [381].

4.3.1 Drug layering

Drug layering involves deposition of successive layers of the components of a formulation namely the API and excipients from a solution, suspension or dry powder on to preformed nuclei which may be granules or crystals of the same material or inert starting material [381, 382].

4.3.1.1 Powder layering

In powder layering, a binder solution is typically sprayed onto preformed nuclei, followed by the addition of powder [380]. Moist nuclei are allowed to tumble in a rotating pan or disc where they pick up more powder particles. Capillary forces developed in the liquid phase facilitate adherence of powder particles to each other and to the nuclei. When more binding liquid is sprayed, more powder particles adhere to the nuclei. The process is continued until desired pellet size is achieved. Upon drying, the binding solution and any other dissolved substances crystallize out and previously formed liquid bridges are partially replaced by solid bridges maintaining pellet state [383].

4.3.1.2 Solution and suspension layering

In solution and suspension layering, the active ingredient and excipients are either dissolved (solution layering) or suspended (suspension layering) in the binding liquid. The liquid and its contents are then sprayed on to preformed nuclei typically on a product bed, spread out evenly and allowed to dry [374]. During drying, the liquid phase and other dissolved substances crystallize out. In the process liquid bridges are replaced by solid bridges hence the layers remain attached. The size of pellets increases gradually with each addition. The process is repeated until the desired concentration of active ingredient has been added [381].

4.3.2 Direct pelletization

In direct pelletization, all powdered excipients are first blended, followed by addition of liquid excipients and/or the granulating fluid. The resulting blend is subjected to centrifugal motion. Agglomerates form due to centrifugal forces and eventually round up into pellets. The pellets are dried on a fluid bed drier. Properties of the resulting pellets such as size, density and shape are largely influenced by the rotation speed [381, 384, 385].

4.3.3 Spray drying or spray congealing

4.3.3.1 Spray drying

Spray drying is a technique where the active ingredient in a solution or suspension alone or in combination with excipients are sprayed into a stream of hot air. Upon contact with the hot air, evaporation of liquid is initiated. In the process, heat and mass transfer occur resulting in the formation of dry and more spherical particles [380, 381, 383]. The design and process settings of the spray drier may influence pellet characteristics such as friability, flow properties, moisture content, porosity, particle size and distribution [380].

4.3.3.2 Spray congealing

The technique used in spray congealing is almost similar to the one employed during spray drying. Spray congealing is a process where a drug is dissolved, melted or dispersed in hot melts of gums, waxes or fatty acids [380]. The dispersion is then sprayed into a stream of hot air and other gases. With appropriate formulation composition and process settings, spherical congealed pellets are obtained [381]. The processing temperature should however be kept lower than the melting points of all formulation components during the entire process [381, 386].

4.3.4 Balling

During balling, pellets are formed as a result of a continuous rolling, tumbling and thumbing motion of particles in pans, discs or mixers. Balling is predominantly an agitation process. Spherical pellets are only formed upon addition of appropriate amounts of granulation fluid. The fluid may be added to the powder blend before or during agitation [381, 386].

4.3.5 Extrusion-spheronization

The extrusion-spheronization method was used for this study since the equipment was readily available.

Extrusion-spheronization is the most widely used method for pellet manufacture The method was first reported by Conine and Hardley in 1970 who outlined the steps involved in the extrusion-spheronization process [387]. Later a clearer description of the actual mechanisms that lead to spherical pellet formation following spheronization was reported [388]. Consequently the method started gaining more traction as more researchers realized the potential of the method as a viable means of enhancing drug delivery.

Extrusion-spheronization offers advantages over other methods of manufacturing solid oral dosage forms. Generally, high concentrations of API may be incorporated into a formulation without producing excessively large particles and more than one API may be incorporated into a formulation in addition to modification of thephysical characteristics of formulation components [389].

Extrusion-spheronization involves four key steps *viz*., (*i*) granulation - the preparation of a wet mass by adding granulating fluid to a powder blend, (*ii*) extrusion - shaping the wet mass into cylinders, (*iii*) spheronization - rounding the extrudate into spherical pellets, and (*iv*) and drying the pellets [390].

4.3.5.1 Granulation

Prior to granulation, all powdered excipients must be thoroughly mixed to achieve a homogeneous powder blend. Granulation is then performed for preparation of a wet plastic mass through addition of granulation liquid to the powder blend [383]. The process is generally performed under continuous agitation. Both dry mixing and granulation are commonly peformed using a planetary, high shear or sigma blade mixers [390] sometimes referred to as granulators.

4.3.5.2 Extrusion

Extrusion is a process where the plastic mass is subjected to pressure, forcing it to flow through die orifices to produce rod shaped particles of uniform diameter known as extrudates [383, 381]. The length of extrudates may vary depending on the physical characteristics of the wet mass, extrusion method and how particles are manipulated following extrusion [383]. An ideal extrudate must be non-adhesive to itself and must be rigid enough to retain the shape imposed by the die, yet brittle enough to be broken down to short lengths in the spheronizer, but not so friable that it disintegrates completely [391]. Four main classes of extruders are used for extrusion andare screw, sieve or basket, roll and ram extruders [374, 378].

Screw extruders use a screw to generate the necessary pressure to force the plastic mass through an axial or radial screen. In an axial design, the screen is placed at the end of the screw whereas in a radial design, the screw is placed around the screw and discharges extrudate perpendicularly to the axis of the screw [383]. Sieve extruders utilize an oscillating arm to press the wet mass through a sieve, causing the extrudates to fall vertically from the sieve plate. Basket extruders utilize a similar concept to that of sieve extruders except that the sieve or screen is part of a vertical

cylindrical wall and extrudates are formed in a horizontal plane [374]. In roll extruders, the wet mass is fed between a roller and a perforated plate called a ring die. Three types of roll extruders exist, all with different positions of the roller relative to the die [378]. Ram extruders are equipped with a piston that compresses and forces the wet mass through a die. Extrusion forces generated by ram extruders are always greater when compared to the force generated by other extruders. These are the oldest type of extruders [383].

4.3.5.3 Spheronization

During spheronization, extrudate rods are broken into nearly uniform lengths and then gradually rounded into spheres. The rounding is a result of plastic deformation. Pellets of nearly uniform diameter are produced since extrudate are initially broken into nearly uniform lengths [374].

A number of models have been proposed to explain spheronization. Rowe [392] proposed that during spheronization, extrudates break into short lengths which collide with each other, the friction plate and spheronizer walls. The extrudate undergoes plastic deformation and in the process go through a dumb-bell shape, an ellipsoid or egg-shape and finally a sphere. The model proposed by Rowe is depicted as model A in Figure 4.1. Baert and Remon [393] proposed that in addition to breakage, extrudates are rounded by collisions with spheronizer walls and other pellets. In the process they twist and eventually break into sub-pellets with rounded and fractured sides. The fractured side is folded together by the rotating and frictional forces on the friction plate to form a spherical or near spherical pellet. In this model, the folding action was claimed to explain why some pellets contain a cavity. The model proposed by Baert and Remon is depicted as model B in Figure 4.1. The third model is referred to as the combined deformation and agglomeration mechanism. This model suggests that pellets pass through a dumb-bell stage and eventually become more round by attachment of fines in the mid-plane region [394]. A depiction of the third model is presented as model C in Figure 4.1. All three models emphasize the significance of collisions between pellets, the friction plate and spheronizer walls. Fines generated by attrition are particularly important in model C hence this model maybe specific for formulations which tend to form fines.

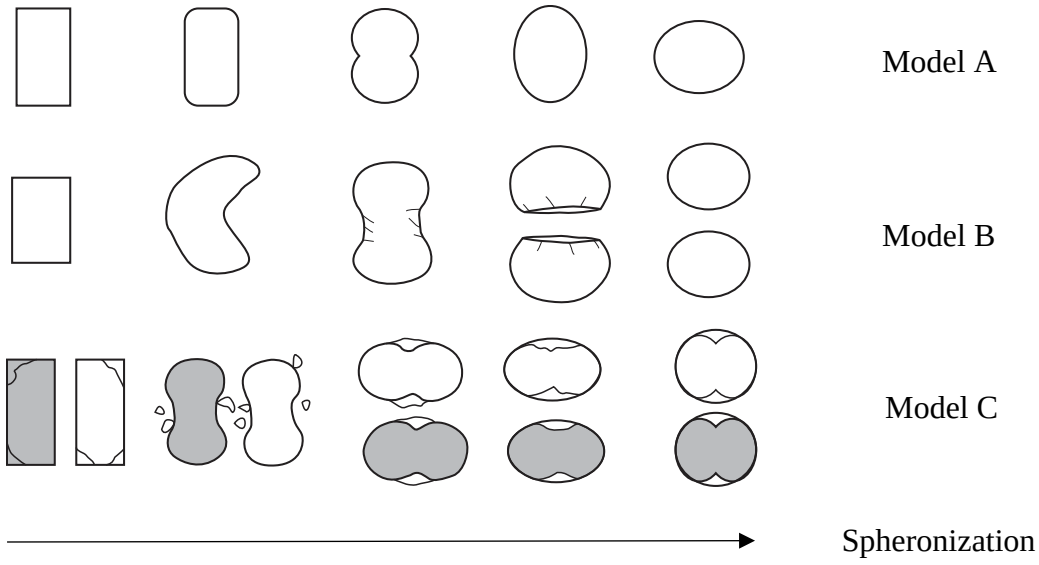


Figure 4.1 Mechanisms of spheronization according to (*i*) Rowe - Model A (*ii*) Baert and Remon - Model B (*iii*) ombined deformation and agglomeration mechanism - Model C [Adapted from 395]

The spheronizer is a cylindrical instrument equipped with a horizontal rotating disc at the bottom of the cylinder. The rotating disc is known as a friction plate. When extrudates are loaded into the spheronizer, contact with the friction plate in combination with inter-particulate collisions and collisions with the spheronizer wall cause the extrudates to break [374]. They deform gradually into a spherical shape. Kinetic energy induced by the rotating friction plate facilitates continuous movement of particles until processing is terminated [383].

Grooves on the surface of the rotating disc increase frictional forces. The grooves are generally in one of two geometric patterns, a cross-hatched pattern with grooves running at right angles to one another and a radial pattern with grooves running radially from the center of the disc [374, 381]. The rotation speed of the friction plate varies between instruments but it is generally between 100 to 2000 rpm. Spheronization is conducted until satisfactory spherical pellets are produced. Normally spherical pellets are obtained within minutes.

4.3.5.4 Drying

The final step of the process is drying the pellets. Pellets may be dried at room temperature, in a dessicator or at elevated temperature in an oven or fluidized bed drier [390]. During drying granulating liquid evaporates leaving solid spherical pellets.

4.4 PARAMETERS INFLUENCING FINAL PELLET QUALITY

4.4.1 Equipment parameters

4.4.1.1 Mixer

The type of mixer used influences paste properties. Generally either planetary mixers or screw based mixers are used. When compared, it has been reported that pellets formulated using material from a screw based mixer are stronger, smaller, have a narrower size distribution and show higher yield compared to material from a planetary mixer [396].

4.4.1.2 Extruder specifications

Axial screw extruders have been shown to produce denser material when compared to radial screw extruders. Even though radial extruders tend to have higher yields, they also show greater heat production during processing. Final pellet quality is particularly influenced by the screen and diameter of perforations [397-399]. Thinner screens tend to produce rough and loosely bound extrudate, whereas thicker screens produce smooth and well-bound extrudate due to higher densification of the wet mass. The diameter of perforations on the screen determines the size of pellets. Small diameter screens produce pellets with small diameters and *vice versa* [400, 401].

4.4.1.3 Spheronizer specifications

The friction plate is the most important component of the spheronizer. Different geometric patterns exist and they impact pellet yield and shape. Loss of yield due to formation of fines is particularly noticeable in cross-hatched friction plates especially the ones with large studs [402, 403]

4.4.2 Processing parameters

4.4.2.1 Dry blending speed and time

Caution must be exercised during dry blending. If inadequate time is apportioned, the risk of producing a non-uniform powder blend increases leading to an inconsistent paste and ultimately non-uniformity of doses. Similarly, extremely low or extremely high speeds lead to non-uniform mixing therefore optimum settings for both speed and time need to be selected [404].

4.4.2.2 Extrusion speed

Total extrudate yield is mainly dependent on extrusion speed. In addition, extrusion speed has an impact on surface texture of extrudates. For example, high extrusion speeds are associated with surface defects such as roughness and shark-skinning which often result in formation of poor quality pellets. Generally, extrudates with surface defects break up unevenly during early stages of spheronization leading to formation of fines and wider particle size distribution [400, 401].

Extrusion speed must be selected carefully to ensure the highest output for economic reasons whilst maintaining extrudate quality [390].

4.4.2.3 Spheronizer load

According to some studies, low load produces poor particle-particle interaction whereas high load produces poor plate-particle interaction [405]. Pellet diameter has also observed to increase with spheronizer load [390]. In some cases, low pellet yields were observed when high spheronization speed was used for a low spheronizer load. In such cases, the yield was observed to increase when higher spheronizer load was used for extended spheronization time [406].

4.4.2.4 Spheronization time

For microcrystalline cellulose based formulations, extended spheronization times tend to be associated with higher sphericity, narrower particle size distribution, increase in diameter, change in bulk and tapped density and changes in yield [390, 406, 407]. An ideal spheronization time maximizes yield without compromising pellet quality.

4.4.2.5 Spheronization speed

Extremely low spheronization speeds do not generate enough shear to cause a significant shape change in the extrudate whereas higher speeds produce smaller and more spherical pellets [405, 408]. Other factors such as hardness, porosity, friability, bulk and tapped density, aspect ratio and surface structure are also influenced by changes in spheronization speed [407, 409]. In some cases however, yield decreases with increasing spheronization speed, hence an ideal speed has to be used, one that maximizes yield without compromising pellet quality.

4.4.2.6 Drying method

Drying method has an influence on pellet quality particularly in terms of porosity. The most commonly used drying methods include freeze drying, fluid-bed drying, oven drying and desiccation with silica-gel [383]. Freeze dried pellets tend to be more porous and have a higher surface area than pellets dried by other means. Pellets dried by desiccation are the least porous. The difference in final pellet porosity is attributed to different levels of densification during drying [410]. Drying method also impacts pellet shape and morphology. Oven dried pellets tend to be more rough due to uneven shrinkage of wet pellets, whereas freeze dried pellets retain their shape and size [411]. These factors need to be considered when selecting a drying method.

4.4.3 Formulation parameters

4.4.3.1 Moisture content

Presence of moisture in a wet mass brings cohesiveness and plasticity to a powder blend allowing it to be extruded and spheronized. The amount of water added to a powder blend largely influences the quality of pellets obtained. When moisture content is less than the lower limit, the wet mass produced is weakly cohesive resulting in poor quality extrudates and large formation of fines during spheronization [380]. Exceeding the ideal range of moisture content leads to over wetting and agglomeration of pellets during spheronization due to presence of excess water on the surface of pellets [390]. Identifying the wetting optimum is key to successful extrusion and spheronization. Water content additionally influences the density of pellets and surface morphology [409].

4.4.3.2 Granulating liquid

The type of granulating fluid used contributes to mechanical and structural properties of pellets [412]. This is due to differences in contraction driving and contraction counteracting forces during drying [412]. Water is the most commonly used granulating liquid. However, use of alcohol-water mixtures particularly ethanol-water has been reported [390]. Pellets made from an ethanol-water granulating fluid tend to have less tensile strength in comparison to those made from water alone [413]. Liquid excipients may also aid granulation in addition to their main purpose.

4.4.3.3 Excipients

The overall performance of a dosage form depends on choice of excipients. These excipients must interact and work cohesively to produce the required drug release. Generally, formulation of pellets requires a binder, disintegrant, anti-adherent, bulking agent and plasticizer [381, 383]. In addition, some formulations require a surfactant, solubilizer, flavouring agent, release modifier and pH adjusters [381, 383]. The aim of this study was to produce an immediate release dosage form. Hence excipients were selected accordingly.

4.5 CONCLUSIONS

To our knowledge, the use of a multiple unit pellet system to address the challenges associated with oral delivery of prednisone has not been reported. Multi-particulate dosage forms offer significant advantages over conventional technologies in many aspects particularly as they exhibit a lower incidence of gastrointestinal irritation due to decreased local concentration of the API in the GIT following oral administration. Generally, lower individual variability in plasma concentrations is observed in multiparticulate dosage forms when compared to tablets since there is a reduced risk of dose dumping. In addition, the presence of many individual units increases the surface area leading to improved solubility and bioavailability. The use of discrete units also offers a simple solution to minimizing potential API-excipient interactions and the free flowing nature of pellets facilitates reproducible capsule filling, content uniformity and dosing. Moreover, loading the dosage form into gelatin capsules presents a simple way to mask the bitter taste of the API. In this study, the extrusion spheronization method was used to manufacture a multiple unit pellet system for prednisone. The method is quick, cost effective and relatively easy to use. Moreover, the equipment was readily available in our laboratories.

CHAPTER 5
QUALITY BY DESIGN AND RISK ASSESSMENT

5.1 INTRODUCTION

For many years, quality by testing (QbT) was the only way to guarantee quality of drug products before the Food and Drug Administration (FDA) launched current Good Manufacturing Practice (cGMP) [414]. In order to gain more knowledge of the manufacturing process, a new system known as Quality by Design (QbD) was introduced into the pharmaceutical industry. QbD is based on thorough understanding of how materials, process parameters and their interaction thereof impacts final product quality [415]. The application of QbD is largely dependent on good understanding of the sources of variability and the manufacturing process. In this chapter, principles of QbD, steps for implementing QbD, design of experiments (DoE) and the design space concept in the pharmaceutical industry are discussed. The risk assessment conducted for our studies is also presented.

5.2 QUALITY BY TESTING (QbT)

In a QbT framework, product quality is ensured by raw material testing, API manufacture, fixed product manufacturing process, in-process material testing and end product testing [416]. The quality of raw materials such as active ingredients and excipients is monitored by testing. If they meet United States Pharmacopoeia (USP), FDA and the manufacture's guidelines and specifications they are considered safe and can be used to manufacture products. Due to uncertainties as to whether API specifications alone are sufficient to ensure quality, the API manufacturing process is tightly controlled [417]. Should there be any changes to the API manufacturing process, supplements have to be filed with the FDA [416, 417].

In a QbT system, the quality of a finished drug product is tested by assessing how well it meets FDA approved specifications and manufacturer's proposed specifications [416]. If the product fails to meet these specifications, the batch is discarded. Root causes of failure are usually not well understood hence developers risk ongoing losses until root causes of failure are understood and addressed or until the FDA approves a request to widen the acceptance criteria in order to pass previously failed batches. Stringent specifications by the FDA have often resulted in recalls and drug shortages [418]. Since only a small percentage of the final dosage form is tested, manufacturers are required to conduct extensive in-process tests and the outcomes are supposed to

meet FDA approved in-process testing specifications. Whenever manufacturers make changes to operating parameters they are required to file for supplements with the FDA, otherwise the parameters already approved by the FDA have to be maintained [419, 420]. This combination of fixed manufacturing steps and extensive in-process and end process testing is what ensures quality in a traditional QbT system [421]. Acceptance criteria are very rigid and specifications are not flexible often prohibiting the release of products that otherwise have acceptable clinical performance [421].

Simply put, final product quality is in the traditional QbT framework achieved by predominantly restricting flexibility in the manufacturing process and by end product testing. Very little focus is placed on how designing an effective manufacturing process contributes to product quality. Often, product specifications are set using test data from small batches and challenges may arise when production scale batches are produced. Finally regulatory requirements imposed on manufacturers for executing minor changes inhibit continuous improvement [421].

5.3 QUALITY BY DESIGN (QbD)

The International Council for Harmonization (ICH) Q8 guidelines define QbD as a systematic, scientific, risk-based, holistic and proactive approach to pharmaceutical development which begins with predefined objectives and emphasizes product and processes understanding and process control [422]. This system addresses that quality cannot be tested into products but should be built-in or should be by design [423]. In a QbD system, final product quality is assured by understanding and controlling formulation and manufacturing variables. End product testing is conducted to confirm performance and quality, but these tests do not necessarily form part of process control. It is important to note that during both QbT and QbD, the end product is reviewed against a drug product specification. However the role of this review is completely different in QbT *versus* QbD. In a QbT framework, each batch is tested against a drug product specification to ensure quality and manufacturing consistency [414]. In a QbD framework, testing against a drug product specification is conducted sorely for confirming product quality and not necessarily to confirm manufacturing consistency or process control [414]. In a QbD framework, the end product may in fact not be tested against a specification since process understanding and control are sufficient to produce a final product that meets desired specifications [414].

QbD consists of several elements [422, 424]. The quality target product profile (QTPP) is a prospective summary of quality characteristics of a drug product to be achieved. It includes the dosage form, dosage strength, intended clinical use, route of administration, drug delivery system and drug release criteria among other attributes [425]. The QTPP is the predefined objective mentioned in the definition of QbD. Critical quality attributes (CQAs) are physical, chemical, biological or microbiological properties or characteristics that should be within an appropriate limit or range in order to achieve desired final product quality. For solid oral dosage forms, CQAs may include particle size distribution, hardness, sphericity and drug release among other attributes. Selection of CQAs is based on prior knowledge derived from literature and on the desired product profile [425]. CQAs must be maintained within predefined limits to ensure that the product meets its intended safety, efficacy, stability and performance. Hence all factors which affect final product quality and safety should be controlled [426]. Simply put, CQAs are the output responses monitored. Critical process parameters (CPPs) are process input factors that have an influence on CQAs when they are varied within regular operation range. Critical material attributes (CMAs) are raw material based input factors that have an influence on CQAs when they are varied within regular operation range. CPPs and CMAs together impact appearance, yield, sphericity, hardness, friability, drug release and the overall performance of a product [427].

5.3.1 Steps for implementing Quality by Design

Generally, QbD is implemented *via* a stepwise approach that includes definition of the QTPP, identification of CQA, CPP and CMA. The set up and performance of a design of experiments (DoE) study is used to identify the impact of CPP and CMA on CQA and the knowledge generated is used to evaluate the impact of CPP and CMA on QTPP to define a process design space. Identification and control of sources of variability of raw materials and the manufacturing process is undertaken and the manufacturing process is monitored and improved to ensure consistent product quality [428, 429].

5.4 QUALITY BY DESIGN TOOLS

5.4.1 Quality risk management

Quality risk management (QRM) is comprised of three key processes *viz.*, risk assessment (A), risk control (B) and risk review (C) (Figure 5.1). Risk assessment is the first step in a risk management process. Risk assessment is a systematic process of gathering and organizing information to support a risk decision. It consists of identifying potential hazards (risk identification), analysis of the potential hazards thereof (risk analysis) and evaluation of the risk associated with exposure to those hazards (risk evaluation) [414, 425].

Risk control involves decision making with regard to reducing, rejecting and/or accepting risk. The main objective of risk control is to reduce risk to an acceptable level. Risk review is the assessment of results generated from the risk management process taking into consideration new knowledge and experience gained throughout the process [414]. During the QRM process, communication between regulators, developers, industries and clinicians should be ongoing. The different stakeholders may exchange information pertaining to the existence, nature, form, probability, control, treatment and other aspects of risks to quality [430].

Risk assessment is comprised of three components *viz.*, risk identification, risk analysis and risk evaluation. Risk identification is the systematic use of information to detect potential hazards or sources of harm to the problem description. The information used may include literature reviews, historical data, informed opinions and the concerns of stakeholders. Risk analysis involves estimation of risk associated with the potential hazards identified. Risk evaluation involves a comparison of the estimated risk against a qualitative or quantitative criteria in order to determine the significance of the risk [414, 425].

A schematic representation of how QRM processes are integrated together is presented in Figure 5.1. It is important to note that decision points are not mentioned in the schematic diagram. This is because decisions can be made any time during the process.

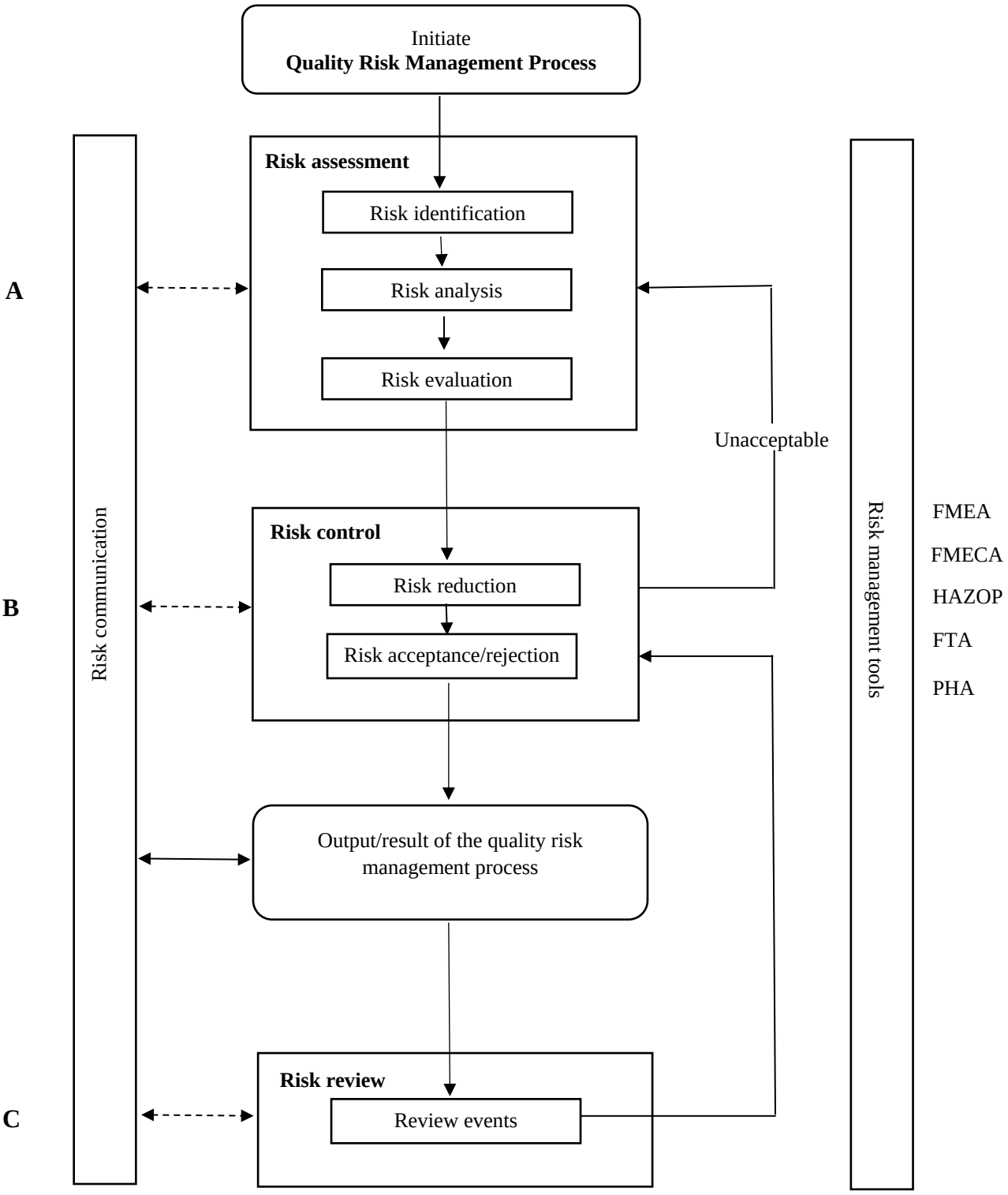


Figure 5.1 Quality risk management process, adapted from [430]

The goal of QRM is to answer the following questions prior to manufacturing. What might go wrong? What is the likelihood (probability) that it will go wrong? What are the consequences (severity) [430]?

The ICH Q9 guidelines provide a list of common risk management tools (Figure 5.1). These are: failure mode and effects analysis (FMEA), failure mode effects and criticality analysis (FMECA), hazard operability analysis (HAZOP), basic risk management facilitation methods (such as Ishikawa fishbone diagrams, flowcharts and check sheets), fault tree analysis (FTA), risk ranking and filtering, hazard analysis and critical control points, preliminary hazard analysis (PHA), and supporting statistical tools [430].

Among these tools, the Ishikawa fishbone diagram and FMEA are the more widely used approaches [431-433]. They can be used separately or in combination [434, 435]. Examples of an integrated Ishikawa and FMEA approach include the successful manufacture of ciprofloxacin hydrochloride tablets [432], ibuprofen granules [431] and lamivudine multiple-lipid nanoparticles [433]. An Ishikawa fishbone diagram is used to classify risk factors in broad categories, then FMEA is used to quantitatively rank factors according to their potential to cause product failure. Failure modes represent any defects caused by the process, design, equipment, materials, environmental factors and manufacturing personnel. The outcome of an FMEA is presented as a risk priority number (RPN) for each potential risk factor [432]. RPN is calculated according to Equation 5.1.

$$RPN = Severity \times Probability \times Detectability \qquad \textit{Equation 5.1}$$

Where,

Severity = Measure of a factor's impact on product safety and efficacy
Probability = Chance of a factor to cause failure or chance of occurring
Detectability = Ability to identify existence of a failure mode before it reaches the end user

A RPN threshold is set before conducting FMEA. Factors below the threshold do not have a significant impact on final product quality and are eliminated from the study. Factors above the threshold have high potential to impact final product quality hence they are carried over for further investigation [436, 437]. A linear scale was used for this study, and the ranks for risk quantification are presented in Table 5.1.

Table 5.1 Ranks for risk quantification, adapted from [436]

	Score	Definition
Severity	1	No impact. No influence on product quality.
	2	Low impact. Little influence on product quality.
	3	Moderate impact. Moderate influence on product quality.
	4	High impact. Severe influence on product quality.
Probability	1	Rare. Failure is rare.
	2	Occasional. Failure occurs sometimes.
	3	Frequent. Failure is common.
Detectability	1	Failure can be detected every time.
	2	Failure can be detected sometimes.
	3	Failure cannot be detected.

During the study, the scores assigned to severity, probability and detectability are based on prior knowledge and preliminary experimentation. We set the RPN threshold for this study at 15 [436]. All factors with a RPN $\leq$ 15 were insignificant and were eliminated. All factors with a RPN > 15 were carried over for further investigation. Risk levels were also qualitatively presented as low, medium or high. A description of the qualitative risk levels is presented in Table 5.2.

Table 5.2 Qualitative risk levels

Risk level	Description
Low (RPN < 10)	Factors which do not have significant effects on product quality. Risks posed by these factors can be easily corrected.
Medium (10 $\leq$ RPN $\leq$ 15)	If not monitored closely these factors may affect product quality. Good manufactures practices must be observed at all times to minimize risk.
High (RPN > 15)	Factors which have a significant effect on product quality, safety and efficacy. These should be carefully monitored and failure due to these factors should be prevented at all costs.

Despite using RPN scores to present a simple and effective way to prioritize risk, the use of RPN scores alone for assessment is not recommended. Since RPN scores are a product of three different components, it is possible for different circumstances to produce identical RPN scores. As a result making decisions based on RPN scores alone is inefficient and may even increase risk. Therefore, formulators are advised to use sound, well researched scientific data and principles to make knowledge based decisions [438].

5.4.2 Design of Experiments (DoE)

A design of experiments (DoE) is a systematic and structured method for determining the relationship between input factors and output responses [416]. In a DoE, raw material attributes (excipient content) and process settings (extrusion speed, spheronization time, *etc.*) are the input factors, while critical quality attributes (sphericity, drug release, *etc.*) are the output factors. Each stage of processing has many input and output variables and it is impossible to experimentally investigate all of them within a reasonable time frame. Therefore developers and scientists use prior knowledge and risk assessment to identify key input and output variables to investigate during DoE. DoE results can be used to identify optimum conditions and to determine significant and insignificant factors. In addition, details such as the existence of interactions and synergistic effects between factors can be gathered from assessing DoE results [416].

Different statistical methods are available for performing DoE. Some common examples include Central Composite Design (CCD), Plackett-Burman, Box-Behnken, Taguchi, full and factorial designs [439]. The choice is largely influenced by suitability to the study. For example Plackett-Burman and Taguchi designs are more useful for screening studies whereas CCD and Box-Behnken designs are more useful for optimization studies [425].

5.4.3 Design space

Once optimum conditions are determined and the acceptance range for CQA set, the design space can be established. ICH Q8 guidelines define the design space as a multidimensional combination and interaction of input variables that have been demonstrated to provide assurance of quality [422]. Once a design space is set, operation within the design space will produce products that meet desired performance characteristics. The design space is subject to regulatory assessment and regulatory approval [428, 429].

5.4.4 Control strategy

A control strategy is required to ensure that input factors are maintained within expected lower and upper limits. Exercising control assures reproducibility. Generally, the control space is smaller than and located within the design space to ensure robustness [440]. The control strategy involves control on excipients, drug substances, specifications, in-process testing and end product testing. Control may also be exercised on other aspects depending on the product and processes involved [440, 441].

5.4.5 Product lifecycle management

Product quality can be improved throughout a product's lifecycle as more knowledge and experience is gained through routine manufacture. ICH Q10 guidelines list four elements that are required for effective product lifecycle management *viz*., establishing a process performance and product quality monitoring system, establishing a corrective action and preventive action (CAPA) system, establishing a change management system and lastly review of process performance and product quality [442].

If used properly, knowledge gained from conducting these activities can result in new and innovative approaches to improve quality. If these changes are implemented within the approved design space there is no need to file for supplements with the FDA. This level of flexibility presents an enormous advantage to products manufactured under a QbD system *versus* the conventional QbT approach. However, changes or improvements that deviate from the initial design space require approval from regulatory authorities [442].

Many successful pharmaceutical companies use product lifecycle management to their advantage. In order to extend their patents they use strategic techniques such as reformulation, new delivery systems for the same drug and fixed dose combinations among other ways to maintain market share for the same drug product [443, 444]. These legal loop holes can be of benefit to the manufacturer. Therefore following the launch of a new drug product, efforts must be made to introduce modified formulations, new drug delivery systems and combinational therapy as part of lifecycle management [445].

5.5 EXPERIMENTAL

5.5.1 Quality target product profile

The desired QTPP set at the beginning of the study is presented in Table 5.3.

Table 5.3 Desired quality target product profile for a multiple unit pellet system for prednisone

Quality element	Target profile	Justification
Dosage form	Multiple-unit pellet system	Easily reproducible, improves dissolution, less side effects.
Design of dosage form	Immediate release	For use in acute conditions.
Dosage strength	5 mg	Dose may be easily titrated up or down to suit patient needs.
Route of administration	Oral	Convenient and acceptable to patients.
Appearance	White to off-white spheres enclosed in a capsule	Visually appealing. Acceptable to patients.
Shape	Spherical	Increased surface area for dissolution. Improved flow properties.
Dissolution	80 % in 30 minutes	Pharmacopoeial specifications for immediate release solid oral dosage forms.
Container closure system	Tightly closed amber container with appropriate labelling	Prednisone is hygroscopic and sensitive to light.
Stability	24 months at room temperature	To comply with regulatory standards.

5.5.2 Critical quality attributes (CQA)

The critical quality attributes investigated in the study were aspect ratio, yield and prednisone release. The aspect ratio was used to assess sphericity of the pellets. A spherical shape was desired for this product because spherical particles have a larger surface area to volume ratio which favours dissolution. The yield was used to assess efficiency of the manufacturing process and pednisone release to assess if products performed according to pharmacopoeial specifications for such dosage forms.

5.5.3 Risk assessment

The extrusion-spheronization method (§4.3.5) was used to manufacture prednisone pellets. Extrusion-spheronization is a multi-step process involving many factors and each stage of the manufacturing process is associated with potential risks. The schematic diagram presented in Figure 5.2 is a representation of the steps involved during manufacture and the potential risk involved.

An Ishikawa fishbone (cause and effect) diagram was used to scope potential risk factors [414]. In an Ishikawa fishbone diagram, potential risk factors are classified under broad categories and for this study *viz.*, manufacturing process variables, formulation variables, environmental and operator based variables. The Ishikawa fishbone diagram generated for this study is presented in Figure 5.3.

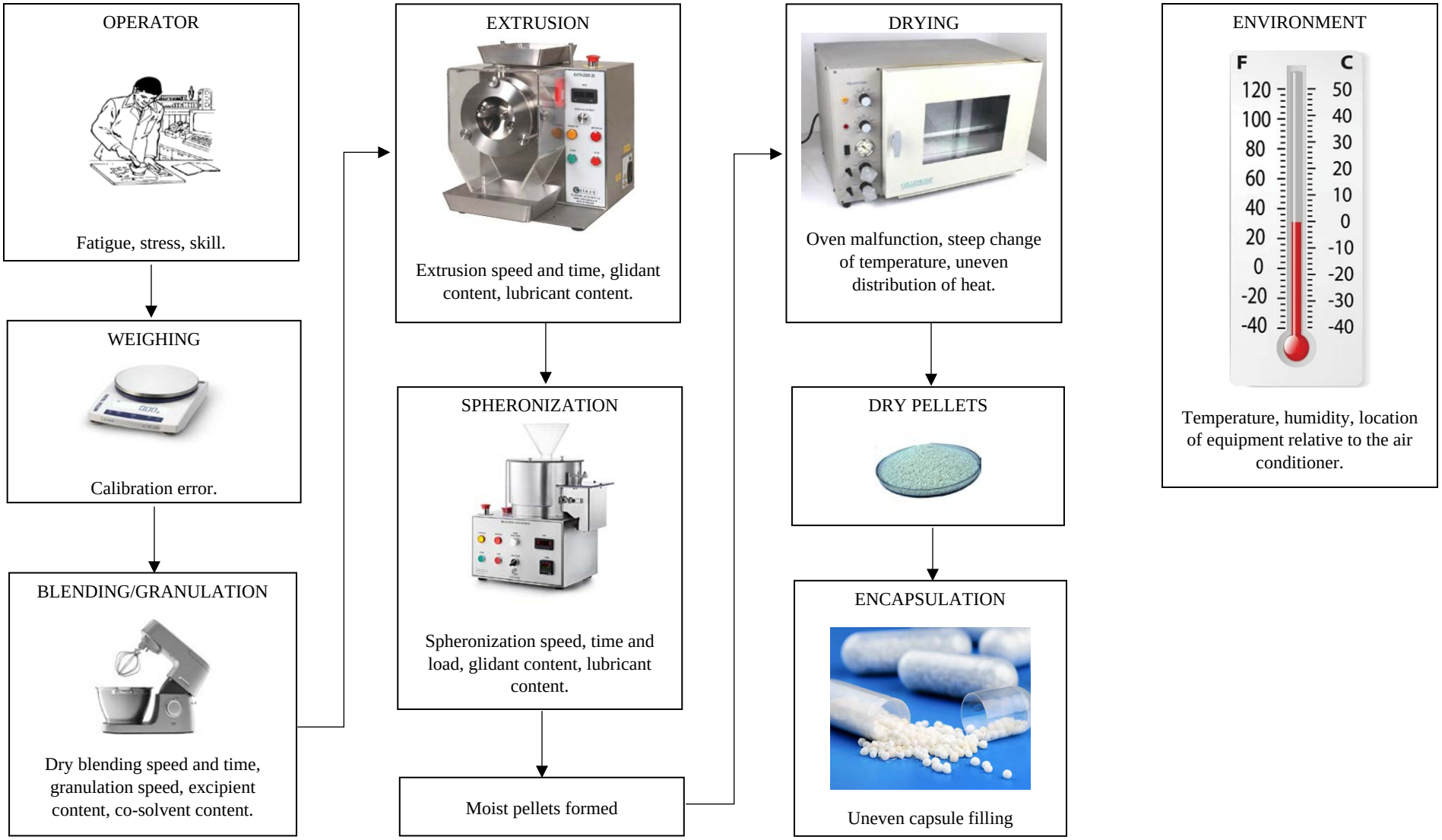


Figure 5.2 Schematic representation of the production process of prednisone pellets and the risk associated with each stage

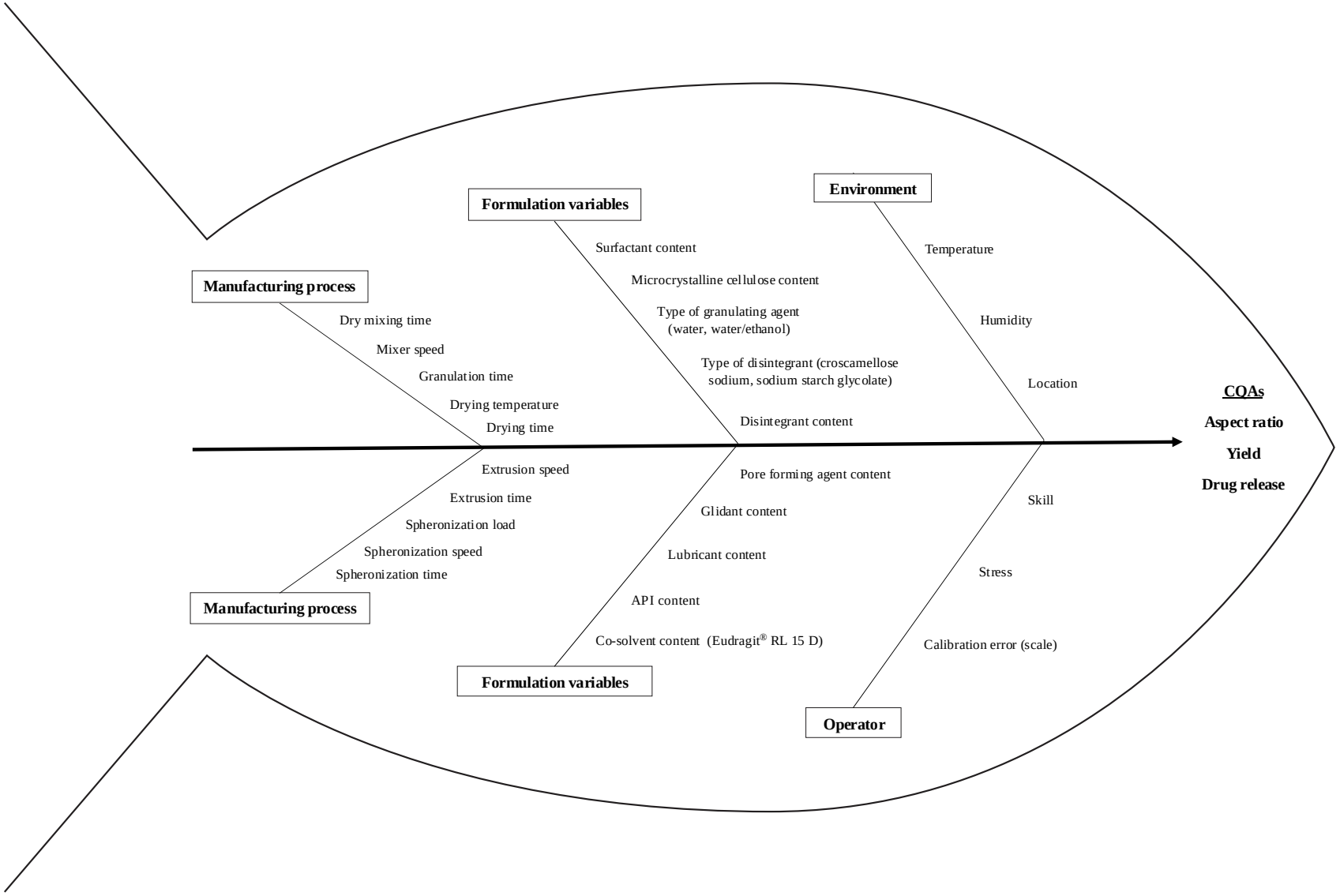


Figure 5.3 Ishikawa fishbone diagram for potential risk factors for the manufacture of prednisone pellets

The potential risk factors listed in the Ishikawa fishbone diagram were further analyzed using FMEA. Data from preliminary experimentation and literature review were used to provide reasoning. The results are summarized in Tables 5.4 - 5.11.

Table 5.4 Failure mode and effect analysis of manufacturing process parameters

Manufacturing Process	Risk factor	Potential failure	Severity	Potential causes	Probability	Current controls	Detectability	RPN
1	Dry blending time	Non-uniformity of doses.	4	Insufficient time. Errors in recording time.	3	Adhere to the set production method. Record data in real time.	2	24
2	Dry blending speed	Incomplete mixing, segregation of powders, non-uniformity of doses.	4	Wrong speed settings. Instrument failure.	2	Double check speed settings.	2	16
3	Granulation time	Non-uniform wet mass. Non-uniformity of doses.	4	Insufficient time. Errors in recording time.	3	Adhere to the set production method. Record data in real time.	1	12
4	Extrusion speed	Poor quality extrudates. Large formation of fines.	4	Wrong speed.	3	Double check speed settings.	2	24
5	Extrusion time	Low extrudate yield.	3	Insufficient time.	2	Allow sufficient time to harvest maximum yields.	1	6
6	Spheronization speed	Poor pellet rounding, large formation of fines, globulation.	4	Wrong speed settings. Instrument failure.	2	Double check speed settings.	2	16
7	Spheronization time	Poor pellet rounding, large formation of fines, globulation.	4	Insufficient or too much time. Errors in recording time.	3	Adhere to the set production method. Record data in real time.	2	24
8	Spheronizer load	Low pellet yield.	3	Lack of knowledge.	2	Adhere to set production method.	2	12
9	Drying temperature	Insufficient drying. Degradation.	4	Inconclusive pre-formulation studies.	2	Conduct pre-formulation studies prior to manufacturing.	1	8
10	Drying time	Moist pellets, that are more susceptible to microbial contamination.	4	Insufficient time. Errors in recording time.	3	Adhere to the set production method. Record data in real time.	2	24

Table 5.5 Failure mode and effect analysis for formulation variables

Formulation variables	Risk factor	Potential failure	Severity	Potential causes	Probability	Current controls	Detectability	RPN
1	Microcrystalline cellulose content	Pellet yield. Retardation of dissolution.	4	Insufficient pelletization aid. Extensive binding.	3	Adhere to set production method.	2	24
2	Surfactant content	Poor solubilization. Rate of dissolution.	4	Insufficient content.	2	Adhere to set production method.	2	16
3	Type of granulating agent	Poor pellet tensile strength.	4	Different types and level of bonding.	3	Conduct preliminary experiments to identify a suitable granulating agent.	1	12
4	Type of disintegrant	Low rate of dissolution.	4	Different mechanism of action. Incompatibility with other excipients.	3	Conduct dissolution and/or disintegration tests.	1	12
5	Disintegrant content	Low rate of dissolution.	4	Insufficient or too much disintegrant.	3	Conduct dissolution and/or disintegration tests.	2	16
6	Co-solvent content	Poor wet mass plasticity.	4	Insufficient co-solvent. Incompatible co-solvent.	2	Visual observation during processing.	2	16
7	Pore forming agent content	Poor pellet porosity.	4	Insufficient pore-forming agent.	2	Conduct SEM of transverse sections of pellets.	2	16
8	Glidant content	Inter-particular friction. Sticking of the wet mass to equipment.	4	Absence of or insufficient glidant.	3	Visual observation during the manufacturing process.	1	12
9	Lubricant content	Friction between equipment and particles. Sticking of the wet mass to equipment.	4	Absence or insufficient lubricant.	3	Visual observation during the manufacturing process.	1	12
10	API content	Poor drug loading. Unacceptable size of the final dosage form.	4	Incorporation of too much or too little API.	3	Conduct dissolution and HPLC tests. Perform assay.	1	12

Table 5.6 Failure mode and effect analysis of environmental factors

Environmental	Risk factor	Potential effect	Severity	Potential causes	Probability	Current controls	Detectability	RPN
1	Humidity	Static charge build up. Inter-particular sticking.	3	Poor air conditioning.	2	Maintain humidity within specified levels according to GMP.	2	12
2	Temperature	Varied moisture content.	3	Poor air conditioning.	2	Maintain temperature within specified levels according to GMP.	2	12
3	Location	Varied moisture content.	3	Equipment position relative to the air conditioner.	2	Maintain temperature and humidity within specified levels according to GMP.	2	12

Table 5.7 Failure mode and effect analysis for operator based factors

Operator	Risk factor	Potential effect	Severity	Potential causes	Probability	Current controls	Detectability	RPN
1	Skill	Influences final product quality.	4	Different levels of training.	3	Operators should have adequate training prior to formulation.	1	12
2	Calibration error	Non-uniformity of doses.	4	Machine error.	1	Check if equipment is performin up to standard.	2	8
3	Stress	May affect concentration levels leading to mistakes.	2	Personal reasons.	2	Postpone laboratory work until conditions permit.	3	12

A visual representation of the risk levels for each factor was presented in the form of a bar graph (Figure 5.4), where column height indicates the RPN score. Orange bars represent factors above the RPN threshold (RPN > 15) implying that they were of potentially high risk. Green bars represent factors that exhibit moderate impact ($10 \leq RPN \leq 15$) and red bars represent factors that exhibit very little impact (RPN < 10).

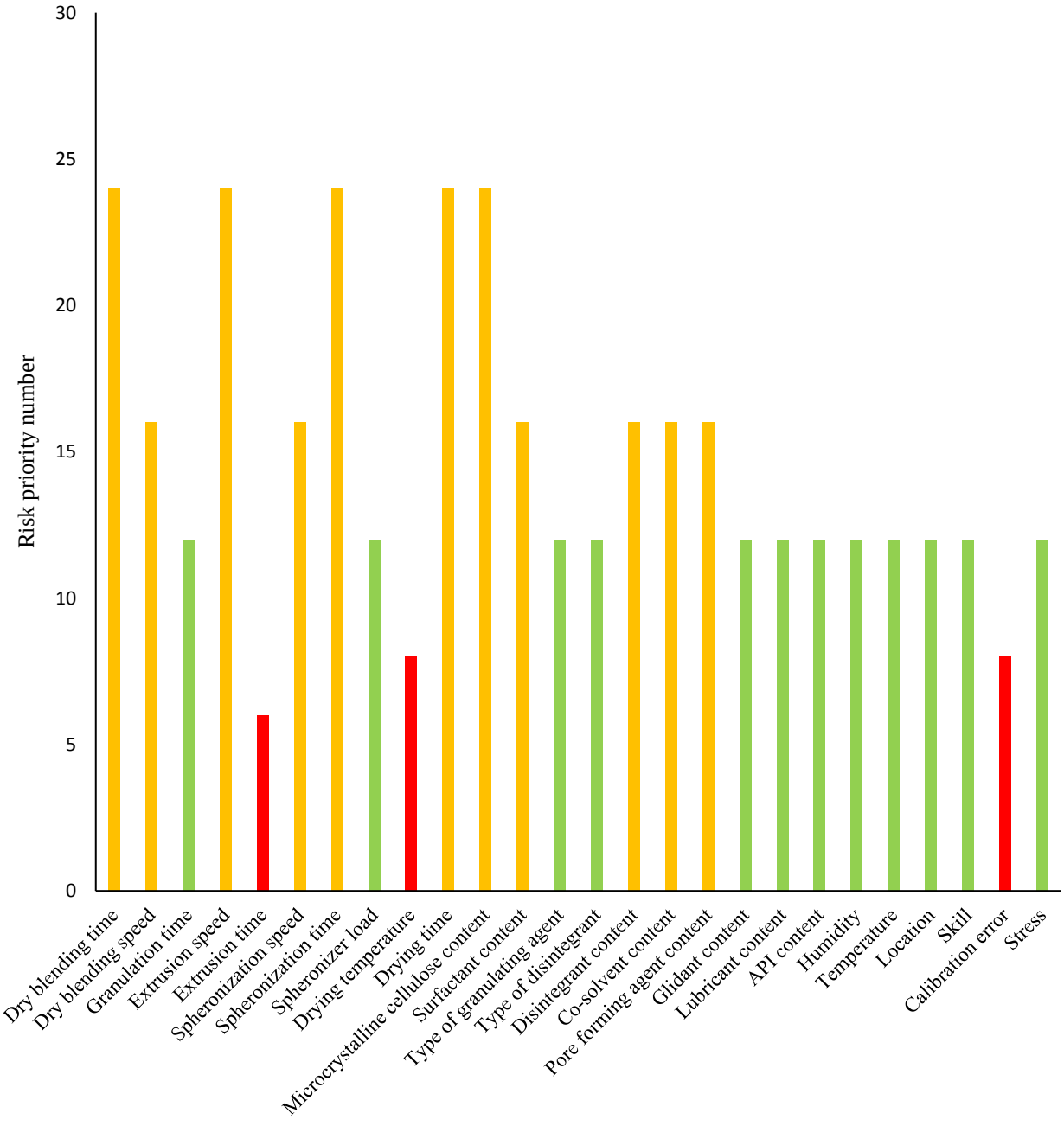


Figure 5.4 Bar graph of RPN scores following FMEA

Following FMEA, potential risk factors were classified qualitatively in order of impact from high to low and the results are summarized in Table 5.8.

Table 5.8 Qualitative rankings of potential risk factors

Impact	Potential risk factor
High	Microcrystalline cellulose content
	Surfactant content (Tween® 80)
	Pore forming agent content (Polyethylene glycol 400)
	Disintegrant content (Sodium starch glycolate)
	Co-solvent content (Eudragit® RL 15 D)
	Dry blending speed
	Dry blending time
	Extrusion speed
	Spheronization speed
	Spheronization time
	Drying time
Medium	Granulation time
	Spheronizer load
	Type of granulating agent
	Type of disintegrant
	Glidant content
	Lubricant content
	API content
	Humidity
	Temperature
	Location
	Skill
	Stress
Low	Extrusion time
	Drying temperature
	Calibration error

The results suggest that microcrystalline cellulose, Tween® 80, polyethylene glycol 400, sodium starch glycolate and Eudragit® RL 30D content, dry blending speed, dry blending time, extrusion speed, spheronization speed, spheronization time and drying time exhibited RPN > 15 implying that these factors may have a potentially high impact on product quality, safety and efficacy. A total of eleven potentially significant factors were identified. The factors were used in a Plackett-Burman screening study for further investigation.

In addition to the potential risk of failure posed by varied excipient content, a change of supplier also poses significant risk. Depending on their origin some excipients may contain traces of natural contaminants. Moreover, different manufacturing processes for the same excipients by different

suppliers may result in different process derived impurities. The current controls which are used to prevent this failure are to maintain the same supplier and bulk purchasing to avoid running out of excipients. The extruder and spheronizer also pose significant risk. A potential failure mode is a steep change in speed during extrusion and/or spheronization. The potential cause of failure is probably human error. As a result a potential effect of failure is poor quality of extrudates and pellets. The current controls which are used to prevent this failure are frequent monitoring of speed settings during extrusion and/or spheronization. Another potential failure mode is the sudden stop of operation of the extruder or spheronizer and the potential effect is incomplete extrusion or spheronization. A potential cause of failure is a short circuit in the electrical supply or old age of the machine. The current controls which are used to prevent this failure mode are routine maintenance and observation by qualified personnel. In addition to the failure mode posed by varied residence time in the oven (drying time), another potential failure mode is a steep change in oven temperature. As a result the potential effect of failure is poor pore formation due to uneven contraction and poor porosity has a negative impact on dissolution. The potential cause of failure is probably human error and the current controls which are used to prevent this failure mode are frequent monitoring of oven temperature.

Granulation time, spheronizer load, type of granulating agent, type of disintegrant, glidant content, lubricant content, API content, humidity, temperature, location, skill and stress exhibited RPN scores between 10 and 15 implying that these factors had potentially moderate impact on product quality, safety and efficacy. If not monitored closely these factors may affect product quality. Therefore, good manufacturing practices must be observed at all times to minimize risk due to these factors.

Extrusion time, drying temperature and calibration error exhibited RPN < 10 implying that these factors did not possess a significant impact on product quality. Risks posed by these factors can be easily avoided or corrected.

This section has only dealt with defining the QTPP, identifying CQAs and the risk assessment conducted to identify potential CPPs and CMAs. The DoE conducted for screening and optimization studies together with establishing the design space are covered in the next chapter.

5.6 CONCLUSIONS

QbD is a knowledge based approach to manufacturing that is largely dependent on good understanding of sources of variability and the manufacturing process. QbD tools ensure that product quality is built into the design and this guarantees quality at the end of the production line. This chapter provided a brief description of QbD principles and their implementation thereof. In addition, risk assessment studies conducted prior to formulation development were presented. An Ishikawa fishbone diagram was used to classify potential risk factors under broad categories as follows: manufacturing process variables, formulation variables, environmental and operator based variables. A total of twenty six potential risk factors were identified. All were assessed under FMEA and assigned a RPN score. Only eleven factors were established to have a potentially high impact on product quality, safety and efficacy *viz.*, microcrystalline cellulose content, Tween® 80 content, polyethylene glycol 400 content, sodium starch glycolate content, Eudragit® RL 15 D content, dry blending speed, dry blending time, extrusion speed, spheronization speed, spheronization time and drying time. These factors were further investigated.

CHAPTER 6
FORMULATION DEVELOPMENT AND OPTIMIZATION OF A MULTIPLE UNIT PELLET SYSTEM CONTAINING PREDNISONE

6.1 INTRODUCTION

Design of Experiments (DoE) was invented by the statistician and geneticist Sir Ronald A Fisher during the 1920s [446, 447]. Fisher argued that complex multi-factorial designs were more efficient at generating knowledge when compared to the traditional one factor at time approach. Fisher detailed the effectiveness of DoE in generating information with less investment of time, effort and money [447, 448]. Fisher argued that valid conclusions could be drawn from experimental data sets in the presence of what he called “nuisance” variables. According to Fisher, known “nuisance” variables generally result in systematic bias leading to batch to batch variation, whereas unknown “nuisance” variables generally cause random variability of results and are referred to as inherent variability or noise [446]. Fisher suggested that the outcomes of multi-factorial experiments could be analyzed by performing ANOVA. This approach enabled researchers to simultaneously assess and answer questions regarding multiple variables concurrently [449, 450].

Multiple variables are assessed during pharmaceutical development and hence it is critical to use a DoE approach in order to save time and minimize cost. In addition, the International Council for Harmonization (ICH) Q8 guidelines recommend use of a DoE approach when manufacturing pharmaceutical dosage forms since multivariate experiments have the ability to generate comprehensive knowledge in a relatively short time and with minimum number of experiments [451]. Several researchers have reported success in using DoE and statistical modeling techniques to address pharmaceutical manufacturing and formulation development challenges [452-454].

The purpose of this study was to establish a design space for manufacturing a multiple-unit pellet system for prednisone as an alternative to currently marketed prednisone tablets. Prior to establishing a design space, studies were conducted to identify an optimum formulation composition and process settings for manufacturing prednisone pellets. A Plackett-Burman screening study was used to identify the most significant factors and a Box-Behnken study was used to optimize the few most significant factors. After identifying optimum conditions, a design space was established.

6.2 MATERIALS AND METHODS

6.2.1 Materials

Prednisone powder was purchased from Skyrun (Taizhou, China). Microcrystalline Cellulose (Comprecel® M102 D+) was purchased from Mingtai chemicals (Taoyuan Hsien, Taiwan). Eudragit® RL 30 D was donated by Rohm Pharma (Darmstadt, Germany). Sodium starch glycolate, talc, magnesium stearate, Tween® 80 were donated by Aspen Pharmacare (Port Elizabeth, South Africa). Polyethylene glycol (PEG) 400 was purchased from Merck chemicals (Johannesburg, South Africa). Hydrochlorothiazide was purchased from Skyrun (Taizhou, China). HPLC far UV grade acetonitrile was purchased from ROMIL-SpS™ (Port Elizabeth, South Africa). The HPLC grade water used for analyses and granulation was prepared using a Milli-Q® Academic A10 water purification system (Millipore®, Bedford, MA, USA), consisting of an Ionex® ion-exchange cartridge and a quantum EX-ultrapore organex cartridge, which was fitted with a 0.22 μm Millipak® 40 sterile filter (Millipore®, Bedford, MA, USA).

6.2.2 Methods

6.2.2.1 Preparation of pellets

Prednisone pellets were manufactured by the extrusion-spheronization method (Figure 6.1). Prednisone powder, microcrystalline cellulose (Comprecel® M102 D+), sodium starch glycolate, talc and magnesium stearate were separately weighed on a Mettler AG 135 top loading balance (Mettler Instruments, Zurich, Switzerland) according to working formulas for each batch. The powders were transferred to a Kenwood Multi-Pro FP580 planetary mixer (Kenwood Ltd. Maraisburg, South Africa) and blended for 4 minutes. Tween® 80, PEG 400 and a 50 % v/v aqueous dilution of Eudragit® RL 30 D were added to the planetary mixer and the contents blended until a uniform paste had formed. Water was then added slowly with blending until a powder mass of optimal wetness had formed. To ensure uniform mixing materials were repeatedly scraped from the walls of the mixing bowl during the granulation process.

The resultant mass was passed through a Model 20 Caleva® extruder (Schlueter, Neustadt am Ruebenberge, Germany) fitted with co-rotating impellers and a screen of aperture pore size 1 mm (diameter). Extrusion was conducted at speeds 25, 30 or 35 rpm. Sufficient time was allowed to harvest the maximum yield of extrudate that were then transferred to a Caleva® MBS 250 spheronizer (Schlueter, Neustadt am Ruebenberge, Germany) immediately following extrusion and spheronized for 1, 2 or 3 minutes (min).

The spheronizer was fitted with a 250 mm diameter crosshatched friction plate of 3 x 3 mm pitch and 1.2 mm depth. Preliminary experiments revealed that high speeds > 1000 rpm resulted in a low pellet yield due to formation of fine material and therefore a low speed of 642 rpm was used throughout this study. Following manufacture, the pellets were collected and dried at 40 ˚C in a size one hotbox oven (Gallenkamp®, Weiss Technik, Loughborough, UK). After drying, the pellets were collected and stored in tightly sealed 100 mL glass containers (Lasec® Solutions, Cape Town, South Africa) and some were filled into opaque yellow size 1 gelatin capsules for further investigation. Each capsule was loaded with pellets equivalent to 5 mg prednisone and stored in a cool, dry place away from light.

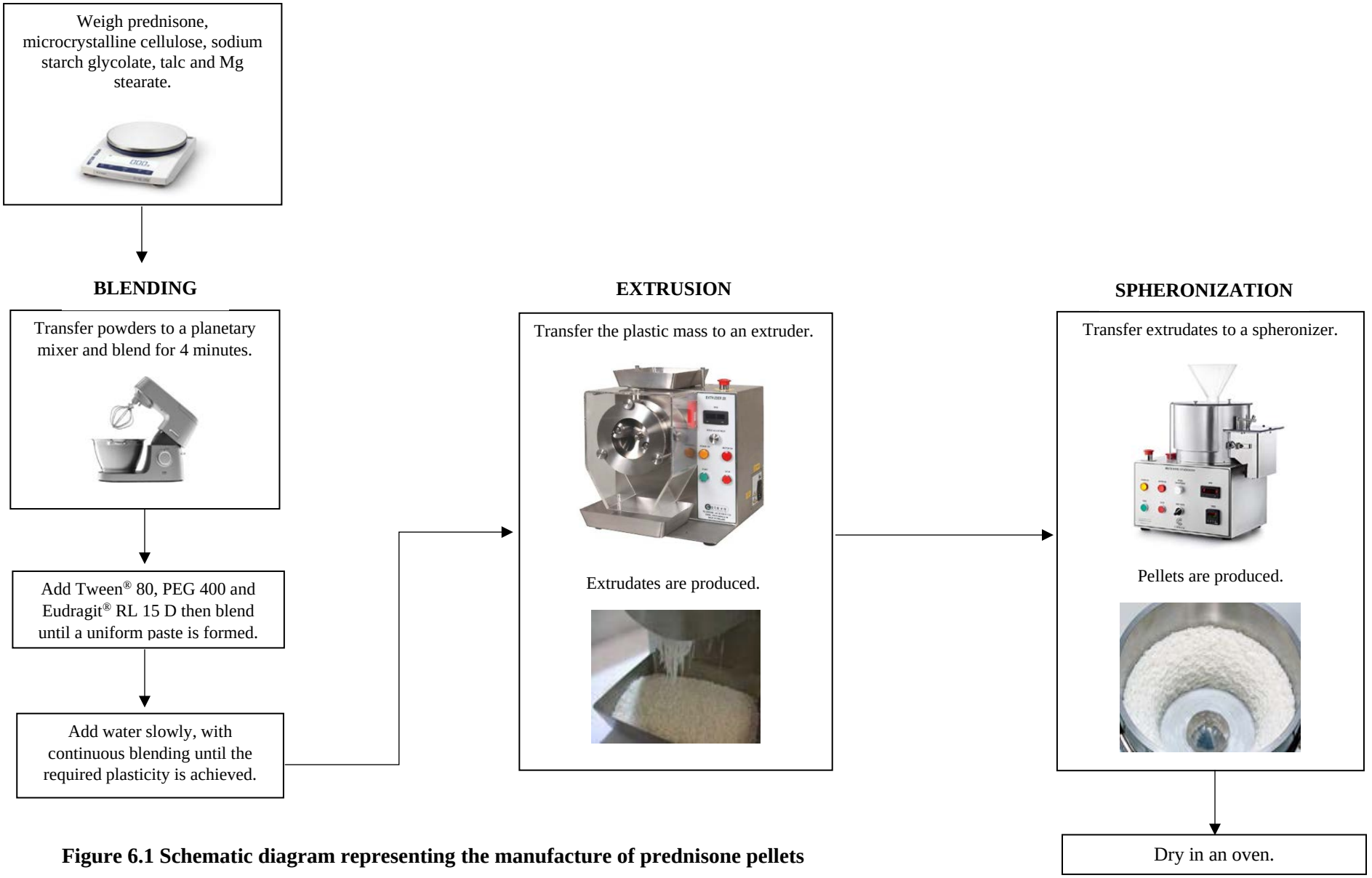


Figure 6.1 Schematic diagram representing the manufacture of prednisone pellets

6.3 EVALUATION OF PREDNISONE PELLETS

6.3.1 Size and shape

6.3.1.1 Scanning Electron Microscopy

A scanning electron microscope (VEGA LMU©, Tescan, Czechoslovakia Republic) was used to observe the shape and surface morphology of the pellets. Samples were mounted onto aluminum stubs using double sided adhesive tape and sputter coated with gold for 20 minutes in a Hitachi vacuum coating unit. Coated samples were viewed at an accelerated voltage of 20 kV and a probe current of 20 nA at 960 x 1280 pixels.

6.3.1.2 Aspect ratio

The aspect ratio was used to assess pellet sphericity. Aspect ratio is defined as the ratio of maximum Feret diameter to the diameter perpendicular to the maximum Feret diameter [455]. Ideally a value of 1 indicates a perfect sphere, but any ratio ≤ 1.2 is considered acceptable [455]. The images generated using SEM were transferred to analySIS docu® software (Olympus, Hamburg, Germany) for size analysis and the aspect ratio was calculated using Equation 6.1.

$$Aspect\ ratio\ (AR) = \frac{d_{max}}{d_{90}} \qquad \textit{Equation 6.1}$$

Where,

d_{max} = maximum Feret diameter
d_{90} = Feret diameter perpendicular to *dmax*

An illustration of how d_{max} and d_{90} values of individual pellets were obtained from SEM images is depicted in Figure 6.2.

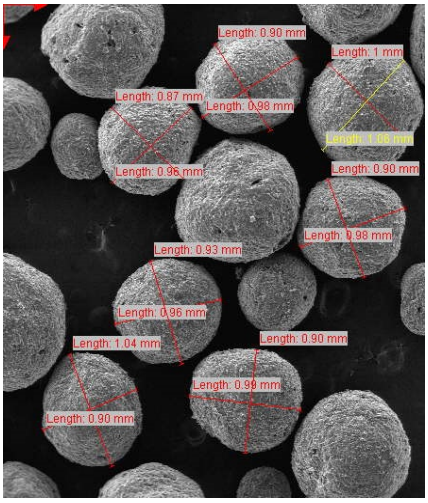


Figure 6.2 Depiction of d_{max} and d_{90} values generated from prednisone pellets

6.3.2 Yield

Yield was calculated using Equation 6.2 and presented as a percentage of the mass of pellets manufactured to the theoretical yield [456].

$$Yield\ (\%) = \frac{Amount\ of\ pellets\ obtained\ (g)}{Theoretical\ amount\ of\ pellets\ (g)}\ x\ 100 \qquad \textit{Equation 6.2}$$

Where,

Amount of pellets obtained = Weight of dry pellets after manufacture
Theoretical amount of pellets = Total weight of excipients

6.3.3 *In vitro* release

Ensuring that a product performs according to USP specifications is important in a quality control system. *In vitro* release testing reveals the cumulative amount of drug released over a specific time and it generates useful information that can be used to postulate *in vivo* bioavailability [457]. Drug release is assessed through a process known as dissolution. Key aspects of the process can be explained by Equation 6.3 which was described by Noyes-Whitney in 1897 [458, 459].

$$\frac{dC}{dt} = \frac{DA(Cs-C)}{h} \qquad \textit{Equation 6.3}$$

Where,

dC/dt = Rate of dissolution
D = Diffusion coefficient
A = Surface area of the solid
h = Thickness of the diffusion layer
Cs = Concentration of the solid in the diffusion layer surrounding the solid
C = Concentration of the solid in dissolution medium

The equation reveals that physicochemical properties of an API have an impact on dissolution rate [458, 459]. Generally water or aqueous based buffers are used as dissolution media. Buffers can be tailored to mimic gastro-intestinal conditions by modifying pH [460]. In order to achieve specific release patterns, excipients must be carefully selected and prior knowledge regarding performance of excipients can be acquired from a literature review and preliminary experimentation.

In vitro release studies were performed using the method described in USP XXIV [461] using a Model SR 8 PLUS USP Apparatus 2 (Hanson Research, California, United States of America) that was fitted with an Autoplus™ Multifill™, maximizer syringe fraction collector and a digitally controlled water circulation heater. All tests were conducted with pellets containing an equivalent of 5 mg prednisone (125 mg of pellets) that were loaded into size 1 gelatin capsules. Preliminary experimentation conducted with buffers of pH 1.2 and 6.8 as dissolution media revealed pH to not have an impact on the rate of prednisone release. Therefore we maintained the method specified in USP XXIV in which water was used as the dissolution medium [461]. The target optimum formulation was intended to be bioequivalent to currently marketed prednisone tablets and therefore it made sense to use a method that is currently used to assess marketed solid oral prednisone dosage forms.

To prevent capsules from floating, spiral capsule sinkers (Hanson Research, California, United States of America) were used. The capsules were dropped into 500 mL of HPLC grade water maintained at 37 ± 0.5 $^{\circ}$C. The paddles were set to rotate at 50 rpm and at 5, 10, 15, 20, 25, 30, 45, and 60 minutes 5 mL aliquots of dissolution fluid were automatically collected and placed into test tubes for further analysis. After each collection equal volumes of dissolution media were replaced into the vessels to maintain sink conditions. Aliquots (1 mL) of each sample were analyzed using the validated RP-HPLC method developed in our laboratory (§ 2.5). Prior to analysis, all aliquots were mixed with hydrochlorothiazide (0.5 mL) as described in Chapter 2.

6.4 PLACKETT-BURMAN DESIGN

Screening studies are conducted when many factors potentially affect final product quality and when researchers are unsure of which factors produce the most optimum results [462-464]. Traditionally, the one factor at a time approach is used to assess factors. During the process, only one factor is investigated whilst keeping all other factors constant. The process is tedious, time consuming and not cost effective as a result more researchers now resort to using statistical techniques to aid the process. The use of statistical models has been proven to reduce the number of experiments and cost whilst still generating vital information [465, 466]. One such statistical model is the Plackett-Burman design [462, 464, 467].

In 1946 Plackett and Burman designed a type of a two level screening design which allows the study of 'n' number of factors using only 'n + 1' experiments [467, 468]. The total number of experiments in a Plackett-Burman design is always a multiple of 4 [467, 468]. Possible factors are identified from literature review and preliminary experimentation. Once determined, all process factors are investigated at high and low level whilst all formulation factors are investigated at high and low concentration. To allow for computational equivalence all low and high level factors are coded as -1 and +1 respectively [467, 469]. Each factor is tested equal number of times at both low and high levels and because of this equal allocation, a balance exists between each and every pair of factors throughout the design [469, 470]. Table 6.1 is a depiction of the different coding levels in a typical eleven-factor Plackett-Burman design.

Table 6.1 Equal allocation of low and high level tests in a typical 11-factor Plackett-Burman design

Run	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}
1	+	+	+	-	-	-	+	-	+	+	-
2	+	-	+	+	-	+	+	+	-	-	-
3	-	-	+	-	+	+	-	+	+	+	-
4	-	-	-	+	-	+	+	-	+	+	+
5	+	-	+	+	+	-	-	-	+	-	+
6	-	+	+	+	-	-	-	+	-	+	+
7	-	-	-	-	-	-	-	-	-	-	-
8	+	+	-	-	-	+	-	+	+	-	+
9	+	+	-	+	+	+	-	-	-	+	-
10	-	+	+	-	+	+	+	-	-	-	+
11	-	+	-	+	+	-	+	+	+	-	-
12	+	-	-	-	+	-	+	+	-	+	+

Where,

S_1, S_2, S_3 … S_n = different factors
Run = an experiment with different factor interactions [467]

Extrusion-spheronization is a multi-step process involving many factors. Following risk assessment (§ 4.3.5), eleven factors were found to have a significant impact on final pellet quality. Nevertheless eleven factors are still too many and would result in a large number of experiments, hence a screening study was used to assess the eleven factors with the hope of finding a few most impactful factors. The objective of the screening study was not to find absolute numerical values for each factor but rather to establish if a factor was significant or not.

The Plackett-Burman design was generated and analyzed using Design Expert Software (Version 8.0.7.1, Minneapolis, MN, USA). The formulation variables assessed were microcrystalline cellulose (S_1), Tween® 80 (S_2), polyethylene glycol 400 (S_3), sodium starch glycolate (S_4) and Eudragit® RL 15 D (S_5) whereas the process variables assessed were dry blending speed (S_6), dry blending time (S_7), extrusion speed (S_8), spheronization speed (S_9), spheronization time (S_{10}) and drying time (S_{11}). Actual and coded levels of the eleven factors investigated are presented in Table 6.2.

Table 6.2 Actual and coded factors investigated in Plackett-Burman screening studies

Variables	Symbol	Low level (-1)	High level (+1)
Microcrystalline cellulose (g)	S_1	25	35
Tween® 80 (mL)	S_2	2.5	5
Polyethylene glycol 400 (mL)	S_3	2.5	5
Sodium starch glycolate (g)	S_4	1	2
Eudragit® RL 15 D (mL)	S_5	5	10
Dry blending speed	S_6	1	2
Dry blending time (min)	S_7	2	4
Extrusion speed (rpm)	S_8	25	35
Spheronisation speed (rpm)	S_9	642	742
Spheronisation time (min)	S_{10}	1	3
Drying time (hrs)	S_{11}	6	12

Many factors are analyzed simultaneously in a Plackett-Burman screening design, as a result the total number of experiments and cost is reduced. Hence using a Plackett-Burman screening design is more effective than a traditional one factor at a time approach [465, 466]. Furthermore, equal allocation of factors in a Plackett-Burman model makes the screening study more efficient and comprehensive [462, 463]. Following the study, mathematical computations of the form presented in Equation 6.4 were used to represent the effects of different factors on output responses [462, 471].

$$T = A_0 + A_1S_1 + A_2S_2 + A_3S_3 + \ldots + A_nS_n \qquad \textit{Equation 6.4}$$

Where,

T = Predicted response
A_0 = Constant
$A_{1, 2 \ldots n}$ = Coefficients of responses
$S_{1, 2 \ldots n}$ = Factors under investigation

Valuable information can be gathered from the equations. Positive coefficients indicate that a factor has a positive effect on the response and *vice versa* [472, 473].

The output responses were aspect ratio (T_1), yield (T_2), drug release at 15 min (T_3), 30 min (T_4), 45 min (T_5) and 60 min (T_6). Batch production records for screening studies (Batch number Pred-PB-001 to Pred-PB-012) detailing actual parameters used, output responses, dissolution profiles and SEM images for each batch are attached in Appendix I. The input factors investigated during Plackett-Burman screening studies are presented in Table 6.3.

Table 6.3 Input factors investigated during Plackett-Burman screening studies

Run	Microcrystalline cellulose (S_1) % w/w	Tween® 80 (S_2) mL	PEG 400 (S_3) mL	Sodium starch glycolate (S_4) % w/w	Eudragit RL 15D (S_5) mL	Dry blending speed (S_6)	Dry blending time (S_7) min	Extrusion speed (S_8) rpm	Spheronization speed (S_9) rpm	Spehronization time (S_{10}) min	Drying time (S_{11}) hrs
1	25.00	5.00	5.00	1.00	10.00	2	4.00	25.00	642	1.00	12.00
2	25.00	5.00	2.50	2.00	10.00	1	4.00	35.00	742	1.00	6.00
3	35.00	2.50	5.00	2.00	10.00	1	2.00	25.00	742	1.00	12.00
4	35.00	5.00	5.00	1.00	5.00	1	4.00	25.00	742	3.00	6.00
5	25.00	2.50	2.50	1.00	5.00	1	2.00	25.00	642	1.00	6.00
6	35.00	2.50	2.50	1.00	10.00	1	4.00	35.00	642	3.00	12.00
7	25.00	2.50	2.50	2.00	5.00	2	4.00	25.00	742	3.00	12.00
8	35.00	2.50	5.00	2.00	5.00	2	4.00	35.00	642	1.00	6.00
9	25.00	5.00	5.00	2.00	5.00	1	2.00	35.00	642	3.00	12.00
10	35.00	5.00	2.50	1.00	5.00	2	2.00	35.00	742	1.00	12.00
11	35.00	5.00	2.50	2.00	10.00	2	2.00	25.00	642	3.00	6.00
12	25.00	2.50	5.00	1.00	10.00	2	2.00	35.00	742	3.00	6.00

6.5 RESULTS

The responses observed from Plackett-Burman screening studies are listed in Table 6.4.

Table 6.4 Experimental responses from Plackett-Burman screening studies

Run	Aspect ratio (T_1)	Yield (T_2) %	Drug release 15 min (T_3) %	Drug release 30 min (T_4) %	Drug release 45 min (T_5) %	Drug release 60 min (T_6) %
1	1.11	37.3	80.9	89.2	87.4	85.9
2	1.05	42.9	103.2	104.2	103.8	103.8
3	1.15	24.7	94.6	94.9	92.0	92.0
4	1.07	41.1	65.7	78.2	80.6	82.3
5	1.10	23.8	81.6	88.2	87.7	90.7
6	1.07	48.4	13.2	32.4	41.5	53.1
7	1.07	38.9	76.2	88.0	91.9	89.4
8	1.15	56.1	47.8	73.5	69.9	85.1
9	1.11	37.9	49.5	90.4	98.7	99.6
10	1.21	54.2	61.8	79.4	82.4	90.2
11	1.12	57.0	74.7	90.2	87.9	87.7
12	1.09	37.8	62.2	79.0	90.7	85.9

The aspect ratio ranged between 1.05 and 1.21, yield between 23.8 % and 57.0 %, drug release at 15 min between 13.2 % and 103.2 %, 30 min between 32.4 % and 104.2 %, 45 min between 41.5 % and 103.8 % and 60 min between 53.1 % and 103.8 %.

6.5.1 Model fitting and statistical analysis

Following experimentation, a number of statistical tools were used to assess the significance of each factor. Data generated from ANOVA were used to evaluate model adequacy and fitness. The R^2 value was used to evaluate goodness of model fit [474]. Results from this study revealed that most R^2 values were close to 1 indicating good model fitness. The adjusted R^2 (Adj R^2) value corrects the ordinary R^2 value for sample size and the number of terms in the model [475] and was used to evaluate model adequacy and fitness [476]. Adjusted R^2 values for the significant responses, aspect ratio (T_1) and drug release at 15 min (T_3) were found to be in close agreement with predicted R^2 (Pred R^2) values indicating good correlation between experimental and predicted

responses. Adequate precision (Adeq precision) is a comparison between the range of predicted values at design points and the mean prediction error and measures the signal to noise ratio, with a ratio > 4 desirable [477]. All ratios for Adeq precision were > 20 which indicates an adequate signal. The coefficient of variation (CV) measures the unexplained variability between experimental data and predictions from the second order polynomial models [478]. The CV is presented as a percentage and low values are desirable since they indicate good precision and reliability of the experiments. The standard deviation (SD) was also investigated, where low values indicated better accuracy and precision of a model. The probability value (p-value) was used as a means to qualify significance and to determine whether to accept or reject the null hypothesis, where a p-value < 0.05 implies the model is significant, and that the evidence favours the alternative hypothesis [479]. The models for aspect ratio and drug release (15 min) exhibited $p < 0.05$ and were significant. All other models ($p > 0.05$) were deemed insignificant. The model F-value determines the utility of a model and establishes if the data set is best fitted to that model. In essence, the F-value is a ratio of explained to unexplained variability. Explained variability is based on the R^2 value and unexplained variability is based on $1 - R^2$ and each are divided by the corresponding degrees of freedom. The larger the F-value, the more useful the model [480, 481]. F-values for the model varied with each response under investigation. A summary of ANOVA data generated from the results is presented in Table 6.5.

Table 6.5 ANOVA data from Plackett-Burman screening studies

Response	R^2	Adj R^2	Pred R^2	Adeq Precision	CV	F-value	P-value	SD
T_1	0.9999	0.9992	0.9895	132.363	0.11	1365.34	0.0211	$1.270e^{-003}$
T_2	0.9990	0.9888	0.8535	30.646	2.77	98.19	0.0784	1.15
T_3	1.0000	0.9998	0.9980	314.054	0.43	7224.73	0.0092	0.29
T_4	0.9964	0.9602	0.4791	20.382	4.23	27.54	0.1473	3.46
T_5	0.9971	0.9681	0.5822	21.821	3.32	34.37	0.1320	2.80
T_6	0.9977	0.9752	0.6750	26.106	2.16	44.20	0.1166	1.88

*Data written in purple indicates significant factors.

Mathematical computations of the outcomes are presented as first-order linear Equations 6.5 - 6.10.

$$T_1 = 1.14 + 0.048S_1 + 3.567e^{-003}S_2 + 5.300e^{-003}S_3 - 0.011S_5 + 0.016S_6 - 0.022S_7 + 5.583e^{-003}S_8 - 1.650e^{-003}S_{10} - 0.019S_{11} + 0.012S_{12}$$ *Equation 6.5*

$$T_2 = 49.54 + 13.10S_1 + 3.39S_2 - 2.52S_3 + 1.24S_4 + 5.22S_6 + 2.44S_7 + 4.53S_8 - 1.74S_{10} + 1.84S_{11} - 1.42S_{12}$$ *Equation 6.6*

$$T_3 = 55.78 - 19.29S_1 + 4.75S_2 - 0.57S_3 + 6.45S_4 + 3.58S_5 - 3.38S_7 - 11.60S_8 + 9.40S_{10} - 10.43S_{11} - 4.65S_{12}$$ *Equation 6.7*

$$T_4 = 71.18 - 17.96S_1 + 5.95S_2 + 2.25S_3 + 7.55S_4 + 1.27S_6 - 5.07S_7 - 6.17S_8 + 4.63S_{10} - 5.58S_{11} - 2.90S_{12}$$ *Equation 6.8*

$$T_5 = 71.46 - 21.27S_1 + 5.28S_2 + 2.32S_3 + 5.84S_4 - 0.97S_5 - 5.68S_7 - 3.69S_8 + 5.38S_{10} - 2.34S_{11} - 1.91S_{12}$$ *Equation 6.9*

$$T_6 = 79.19 - 12.73S_1 + 4.13S_2 + 1.64S_3 + 5.48S_4 - 2.72S_5 - 4.19S_7 - 1.18S_8 + 3.14S_{10} - 3.82S_{11} - 1.79S_{12}$$ *Equation 6.10*

Pareto charts were used to rank factors from most significant to least significant and the magnitude of significance was represented by a difference in column height [470]. Pareto charts are graphical tools used to manage model selection for two level factorial designs. Positive and negative factors are designated different colours on the Pareto chart, with orange bars representing positive factors and blue bars representing negative factors [482]. Two horizontal lines in red and black are observed across the Pareto chart. The red line represents the Bonferroni limit and the black line represents the *t*-value limit. The Bonferroni and *t*-value limit are statistically based acceptance limits similar to the 95 % confidence interval for each bar in the Pareto chart. They provide an indication of statistical significance of a factor [482, 483].

The Bonferroni limit is located higher than the *t*-value limit and factors above the Bonferroni limit are more significant than those above the *t*-value limit. Nonetheless, factors above the *t*-value limit possess 95 % significance whereas factors below the *t*-value limit are insignificant and are considered to be of least importance towards the response under investigation. The Bonferroni correction is normally used in statistical modelling to control false discoveries, counteract the problem of multiple comparisons and to eliminate the possibility of rejecting the correct null hypothesis known as a type 1 error [483, 484]. Following assessment of Pareto charts, positive

significant factors are generally carried over for further analysis and negative factors are dropped. However the decision is left to the researcher's discretion depending on the responses [482].

The significance of factors was further elicited using half-normal probability plots. In half-normal probability plots, large effects appear on the upper right section of the plot, further away from the near-zero line (red line) [485]. Factors located on the near-zero line are unimportant and have no significant impact on the response [483]. Positive and negative factors are designated different colours on the half-normal probability plot, with orange squares representing positive factors and blue squares representing negative factors.

6.5.1.1 Aspect ratio

ANOVA data generated for the model of aspect ratio are presented in Table 6.6.

Table 6.6 ANOVA data for aspect ratio - Plackett-Burman screening studies

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob > F
Model	0.022	10	$2.203e^{-003}$	1365.34	0.0211
S_1	$4.439e^{-003}$	1	$4.439e^{-003}$	2751.48	0.0121
S_2	$1.527e^{-004}$	1	$1.527e^{-004}$	94.62	0.0652
S_3	$3.371e^{-004}$	1	$3.371e^{-004}$	208.93	0.0440
S_5	$1.487e^{-003}$	1	$1.487e^{-003}$	921.95	0.0210
S_6	$3.015e^{-003}$	1	$3.015e^{-003}$	1868.60	0.0147
S_7	$5.941e^{-003}$	1	$5.941e^{-003}$	3682.28	0.0105
S_8	$3.741e^{-004}$	1	$3.741e^{-004}$	231.87	0.0417
S_{10}	$3.267e^{-005}$	1	$3.267e^{-005}$	20.25	0.1392
S_{11}	$4.516e^{-003}$	1	$4.516e^{-003}$	2799.37	0.0120
S_{12}	$1.733e^{-003}$	1	$1.733e^{-003}$	1074.05	0.0194
Residual	$1.613e^{-006}$	1	$1.613e^{-006}$		
Cor Total	0.022	11			
R^2					0.9999
Adj-R^2					0.9992
Pred-R^2					0.9895
Adeq-Precision					132.363
CV %					0.11
SD					$1.27e^{-003}$

*Data written in purple indicates significant factors.

The model for aspect ratio exhibited an R^2 = 0.9999 which is close to 1 implying good model fitness. In addition, an Adj-R^2 = 0.9992 was observed which was in close agreement with the Pred-R^2 = 0.9895 implying good correlation between experimental and predicted responses. A low CV of 0.11 % indicates that the model was highly reliable. The model for aspect ratio resulted in an F-value of 1365.34 and p-value of 0.0211 implying that the model was significant. Dry blending time (S_7), spheronization time (S_{11}) and microcrystalline cellulose (S_1) exhibited large F-values of 3682.28, 2799.37 and 2751.48 respectively. In addition, they exhibited p-values of 0.0105, 0.0120 and 0.0121 respectively which are all < 0.05 indicating that these factors had the most significant impact on aspect ratio. Dry blending speed (S_6), drying time (S_{12}) and Eudragit® RL 15 D (S_5) exhibited relatively large F-values of 1868.60, 1074.05 and 921.95 with p-values of 0.0147, 0.0194 and 0.0210 respectively indicating that they had moderate significance on aspect ratio. Extrusion speed (S_8) and PEG 400 (S_3) had the least significance as indicated by low F-values of 231.87 and 208.93, and p-values of 0.0417 and 0.0440 respectively. The lowest F-values of 94.62 and 20.25 were observed for Tween® 80 (S_2) and spheronization speed (S_{10}) respectively. They were coupled with p-values of 0.0652 and 0.1392 which are > 0.05 hence Tween® 80 and spheronization speed were insignificant.

Based on the Pareto chart (Figure 6.3) generated from the study, Eudragit® RL 15 D (E), dry blending time (G), spheronization speed (J) and spheronization time (K) had antagonistic effects on aspect ratio whereas microcrystalline cellulose (A), Tween® 80 (B), PEG 400 (C), dry blending speed (F), extrusion speed (H) and drying time (L) had agonistic effects on aspect ratio. Microcrystalline cellulose (A), PEG 400 (C), Eudragit® RL 15 D (E), dry blending speed (F), dry blending time (G), extrusion speed (H), spheronization time (K) and drying time (L) were above the *t*-value limit implying that they had 95 % confidence level. Tween® 80 (B), sodium starch glycolate (D) and spheronization speed (J) were below the *t*-value limit implying that they were insignificant. No factors were above the Bonferroni limit.

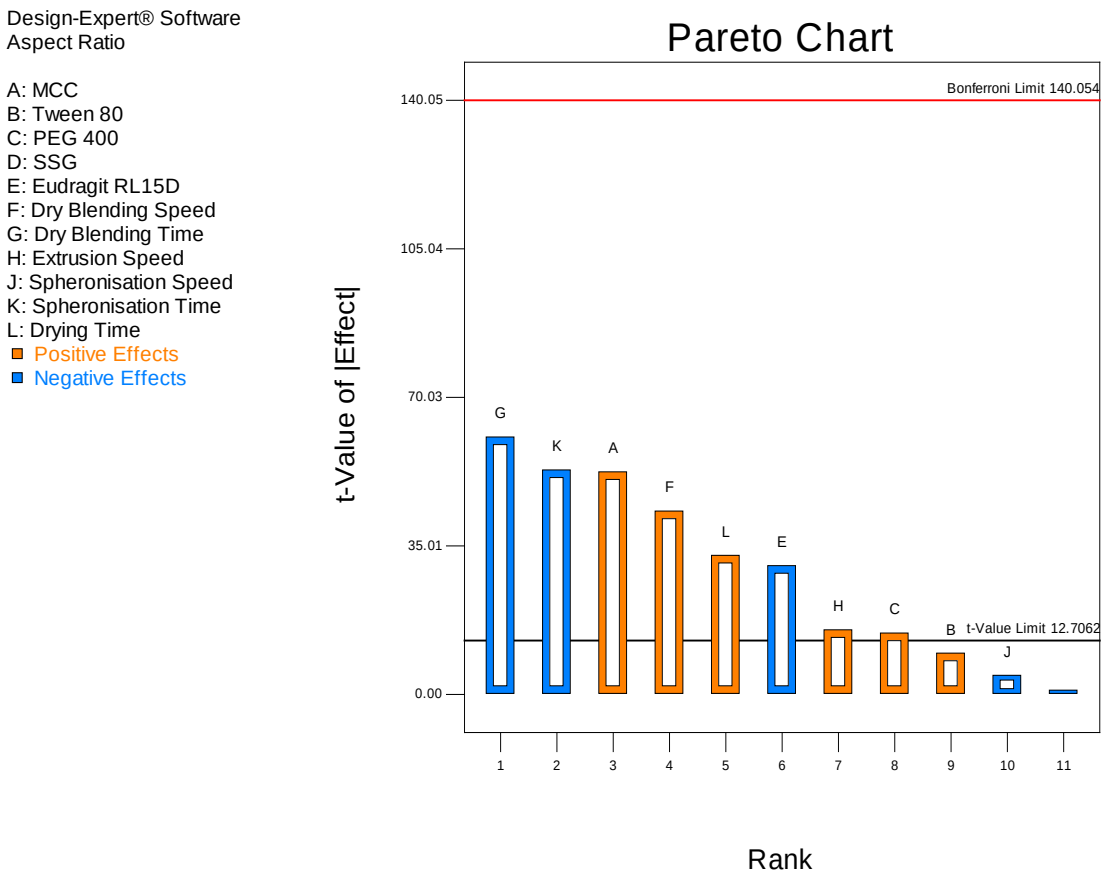


Figure 6.3 Pareto chart for aspect ratio

The half-normal probability plot (Figure 6.4) for standardized effects of factors on aspect ratio revealed dry blending time (G) to be the most significant factor since it was located furthest from the near-zero line. Other important factors were spheronization time (K), microcrystalline cellulose (A), dry blending speed (F), drying time (L), Eudragit® RL 15 D (E), extrusion speed (H) and PEG 400 (C) in descending order of significance. Tween® 80 (B) and spheronization speed (J) were too close to the near-zero line implying that they were insignificant. Sodium starch glycolate (D) was located on the near-zero line indicating that it was completely unimportant towards aspect ratio.

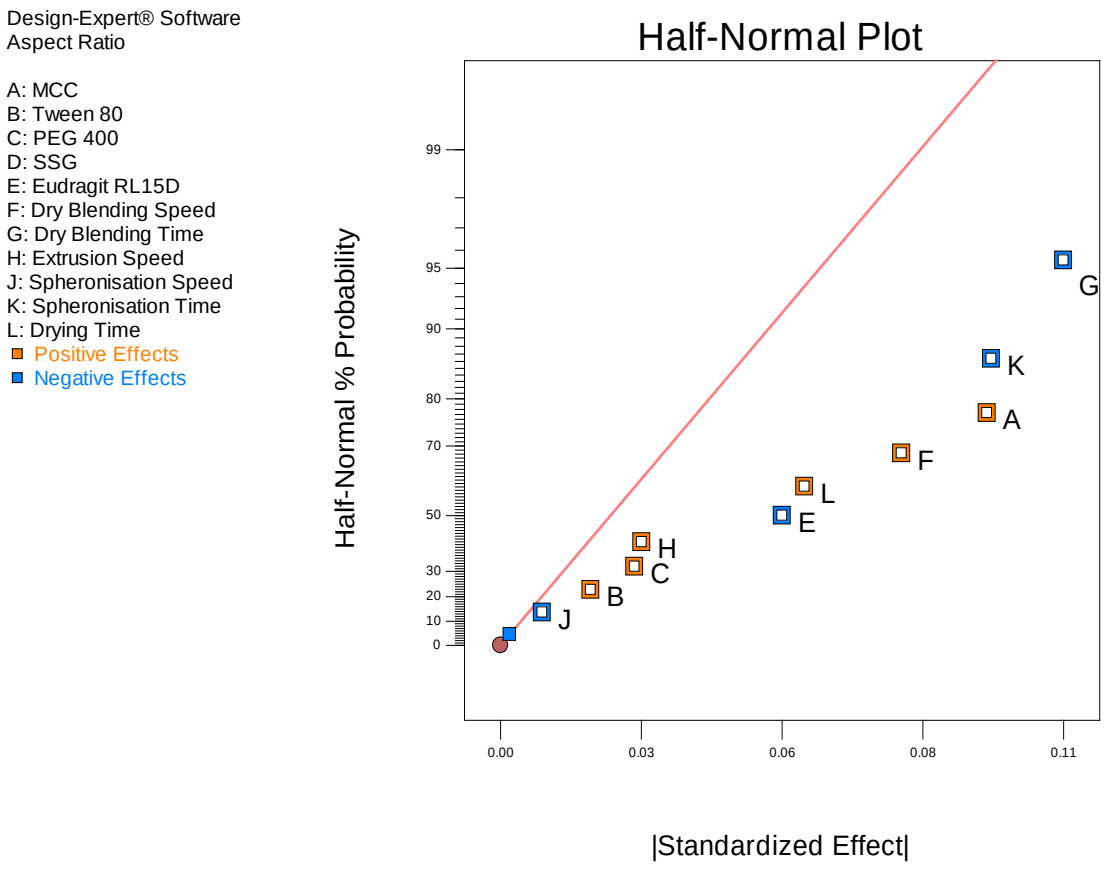


Figure 6.4 Half-normal probability plot of the standardized effect of factors on aspect ratio

Pareto rankings and half-normal probability plots indicate that dry blending time (G) and spheronization time (K) were the most significant negative factors on aspect ratio. Generally, dry blending must be conducted long enough to generate a uniform powder blend. A short dry blending time increases the risk of producing a non-uniform powder blend which potentially results in inconsistent and non-uniform pellets. Findings from analyzing the impact of spheronization time on aspect ratio were in line with previously reported data [392]. Generally, long spheronization time is associated with smoother, more spherical pellets whereas asymmetrical, non-spherical and often ‘dumb-bell’ shaped pellets are typically produced when spheronization is conducted for a short time. Poor pellet rounding associated with short spheronization time is due to inadequate interactions between particles, spheronizer walls and the friction plate [392, 486].

Pareto rankings and half-normal probability plots indicate that microcrystalline cellulose content and dry blending speed were the most significant positive factors. The commercial grade Comprecel® M102 D+ was used throughout the study. In addition to bulking up the formulation, microcrystalline cellulose was incorporated into the formulation to work as a spheronization aid because of its excellent binding properties [487]. As a result changes in microcrystalline cellulose content had a direct impact on pellet consistency and roundness. Generally, increasing the concentration of microcrystalline cellulose was found to increase mean particle size of pellets and *vice versa*. The impact of dry blending speed was correlated to uniformity of pellets. Both extremely low and high dry blending speed often result in non-uniform mixing which leads to varied pellet consistency and roundness.

Even though it was less significant, drying time exhibited a positive impact on aspect ratio. During drying, granulation fluid evaporates and in the process liquid bridges are replaced by inter-particular solid bridges. Drying temperature, rate and time control the shrinking process hence they contribute to the final roundness of pellets. Following the study, microcrystalline cellulose and spheronization time were selected for further investigation.

6.5.1.2 Drug release (15 min)

ANOVA data generated for drug release at 15 min are presented in Table 6.7.

Table 6.7 ANOVA data for drug release at 15 min

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob > F
Model	6020.61	10	602.06	7224.73	0.0092
S_1	714.56	1	714.56	8574.76	0.0069
S_2	270.75	1	270.75	3249.00	0.0112
S_3	3.85	1	3.85	46.24	0.0930
S_4	499.23	1	499.23	5990.76	0.0082
S_5	154.08	1	154.08	1849.00	0.0148
S_7	137.36	1	137.36	1648.36	0.0157
S_8	1614.72	1	1614.72	19376.64	0.0046
S_{10}	1060.32	1	1060.32	12723.84	0.0056
S_{11}	1306.25	1	1306.25	15675.04	0.0051
S_{12}	259.47	1	259.47	3113.64	0.0114
Residual	0.083	1	0.083		
Cor Total	6020.69	11			
R^2					1.0000
Adj-R^2					0.9998
Pred-R^2					0.9998
Adeq-Precision					314.054
CV %					0.43
SD					0.29

*Data written in purple indicates significant factors.

The model for drug release at 15 min exhibited an $R^2 = 1$ implying perfect model fitness. In addition, an Adj-$R^2 = 0.9998$ was observed which was similar to the Pred-$R^2 = 0.9998$ implying good correlation between experimental and predicted responses. A low CV of 0.43 % indicates that the model was highly reliable. The model for drug release at 15 min resulted in an F-value of 7224.73 and a p-value of 0.0092 implying that the model was significant. Extrusion speed (S_8), spheronization time (S_{11}), spheronization speed (S_{10}), microcrystalline cellulose (S_1) and sodium starch glycolate (S_4) exhibited large F-values of 19376.64, 15675.04, 12723.84, 8574.76 and 5990.76 respectively. In addition they exhibited p-values of 0.0046, 0.0051, 0.0056, 0.0069 and 0.0082 respectively which are all < 0.05 implying that these factors had the most significant effect on the rate of prednisone release. Tween® 80 (S_2), drying time (S_{12}), Eudragit® RL 15 D (S_5) and dry blending time (S_7) exhibited relatively large F-values of 3249.00, 3113.64, 1849.00 and 1648.36 with p-values of 0.0112, 0.0114, 0.0148 and 0.0157 respectively implying that these

factors had moderate significance on prednisone release. PEG 400 was the only insignificant factor indicated by the lowest F-value of 46.24 and a p-value of 0.0930 which is > 0.05.

Based on the Pareto chart (Figure 6.5) generated from the study, microcrystalline cellulose (A), PEG 400 (C), dry blending time (G), extrusion speed (H), spheronization time (K) and drying time (L) had antagonistic effects on prednisone release whereas Tween® 80 (B), sodium starch glycolate (D), Eudragit® RL 15 D (E) and spheronization speed (J) had agonistic effects on prednisone release. Microcrystalline cellulose (A), Tween® 80 (B), sodium starch glycolate (D) Eudragit® RL 15 D (E), dry blending time (G), extrusion speed (H), spheronization speed (J), spheronization time (K) and drying time (L) were above the *t*-value limit implying that they had 95 % confidence level. PEG 400 (C) and dry blending speed (F) were below the *t*-value limit implying that they were insignificant. No factors were above the Bonferroni limit.

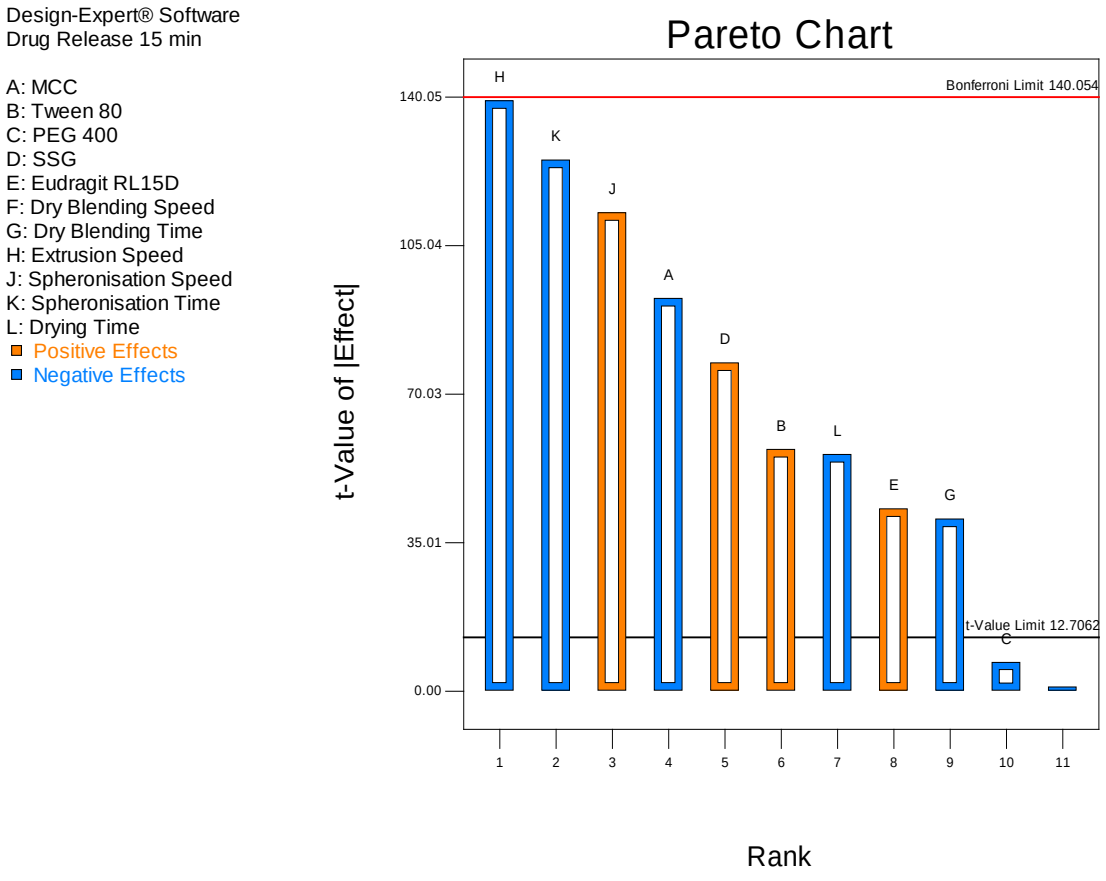


Figure 6.5 Pareto chart for drug release after 15 minutes

The half-normal probability plot (Figure 6.6) for standardized effects of factors on drug release at 15 min revealed extrusion speed to be the most significant factor due to its location relative to the near-zero line. Other important factors were spheronization time (K), spheronization speed (J), microcrystalline cellulose (A), sodium starch glycolate (D), Tween® 80 (B), drying time (L), Eudragit® RL 15 D (E) and dry blending time (G) in descending order of significance. PEG 400 (C) and dry blending speed (F) were located on the near-zero line implying that they were insignificant.

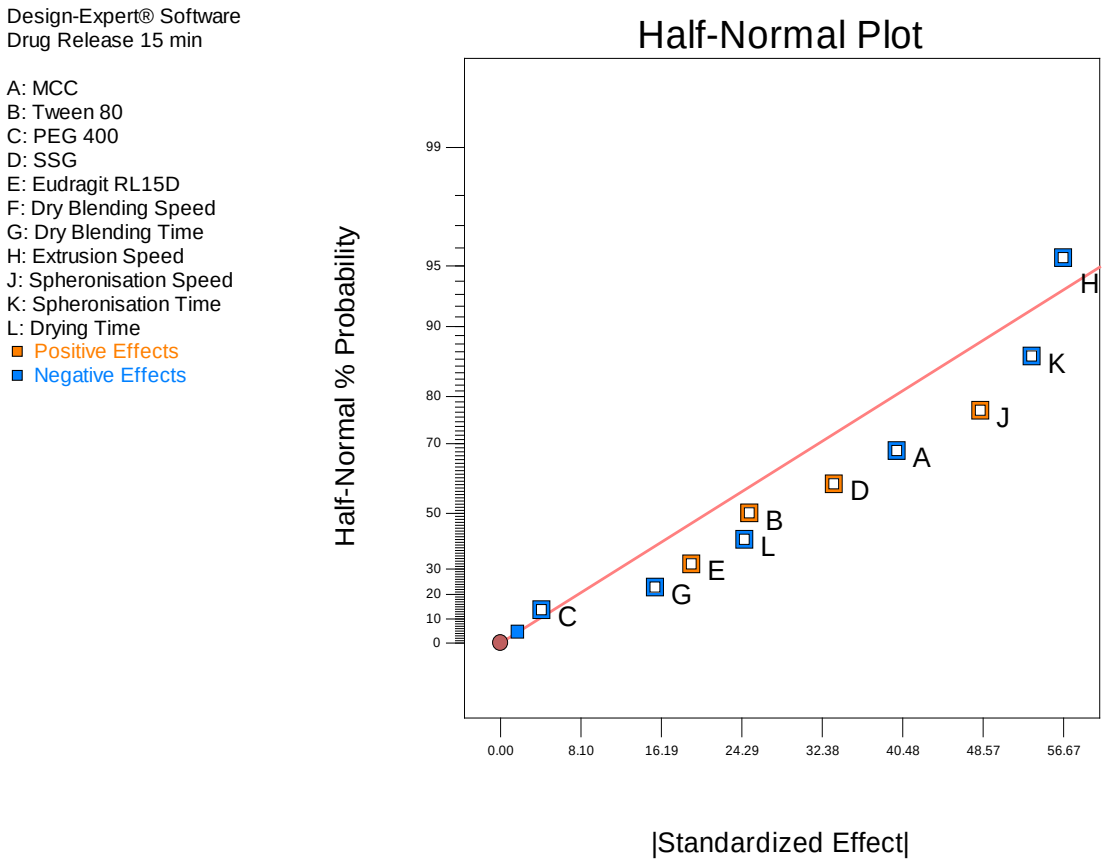


Figure 6.6 Half-normal probability plot of the standardized effect of factors on drug release (15 min)

Pareto rankings and half-normal probability plots indicate that extrusion speed, spheronization time and microcrystalline cellulose content were the most significant negative factors on drug release. Extrusion speed has an impact on surface texture of extrudates. Generally, a high extrusion speed is associated with surface defects such as roughness and shark-skinning which often result in formation of poor quality pellets [400]. Pellets resulting from these extrudates normally break up unevenly causing uneven drug release patterns. In order to identify settings that produce good quality extrudates, extrusion speed was selected for further investigation.

The impact of spheronization time was largely dependent on formulation composition. Formulations containing high Tween® 80 and PEG 400 content produced extrudates that were visually appealing however, the high viscosity resulting from Tween® 80 and PEG 400 inclusion resulted in sticking, prompting a degree of coalescence and globulation during spheronization especially when longer residence times were used. Formulations containing low Tween® 80 and PEG 400 content fractured easily during spheronization causing large formation of fines. Both phenomena resulted in uneven pellet consistency and drug release. Hence spheronization time was selected for further investigation. The goal was to determine an optimum residence time, one that maximizes yield whilst producing spherical pellets that have a narrow particle size distribution.

Adhesive forces that held pellet units together were mainly due to the binding property of microcrystalline cellulose [488]. Generally microcrystalline cellulose does not disintegrate but forms an inert matrix around the formulation and dissolution occurs through the matrix *via* diffusion [489]. This phenomenon significantly slows down dissolution of microcrystalline cellulose based pellets hence the negative effect on drug release. In order to establish an optimum relationship between microcrystalline cellulose and other excipients, microcrystalline cellulose was selected for further investigation.

Pareto rankings and half-normal probability plots indicate that spheronization speed and sodium starch glycolate were the most significant positive factors on drug release. When extrudates are loaded into the spheronizer, contact with the friction plate in combination with inter-particulate collisions and collisions with the spheronizer wall cause the extrudates to break [374]. During the process they deform gradually into a spherical shape. The rotating friction plate is responsible for inducing kinetic energy which facilitates continuous movement of particles until processing is terminated [383]. The kinetic energy generated can be controlled by altering speed. Hence

spheronization speed has a direct effect on pellet quality. Generally high spheronization speed produces small and spherical pellets that have a high surface area to volume ratio, conditions which favour dissolution, whereas an extremely low spheronization speed may fail to generate enough shear to cause a significant change in shape [405, 408]. In these studies, high spheronization speed was observed to reduce yield due to large formation of fines. However, a relatively low speed of 642 rpm was found to be effective. Hence a speed of 642 rpm was used throughout these studies. Emphasis was rather put on establishing an ideal residence time to go along with a speed of 642 rpm in order to produce pellets of acceptable sphericity, yield and quality.

Sodium starch glycolate is a super-disintegrant [276] and was incorporated into the formulation to facilitate pellet dissociation. Sodium starch glycolate exerts its effect *via* a swelling mechanism. When exposed to aqueous media it swells overcoming inter-particulate adhesive forces that hold the pellet together resulting in dissociation into smaller fragments thereby releasing prednisone trapped in each pellet [276, 490]. Changes in sodium starch glycolate content drastically impacted pellet disintegration dynamics. Increasing the amount of sodium starch glycolate used improved the rate and extent of prednisone released and *vice versa*. Visual observation of pellets during dissolution testing revealed that the magnitude of swelling was greatest and disintegration time slowest for pellets containing large amounts of sodium starch glycolate. The smaller fragments have large surface area to volume ratio which favors dissolution. Therefore changes in sodium starch glycolate content directly impacted dissolution rate. In order to identify the ideal concentration of sodium starch glycolate required, it was selected for further investigation.

6.6 DISCUSSION

The extrusion-spheronization method is a multi-step process that involves many input factors. In order to save time and reduce cost, a screening study was conducted to narrow down significant factors. Preliminary risk assessment generated eleven potentially significant factors. A Plackett-Burman screening design was used to assess these factors. Each factor was assessed at high (+1) and low (-1) level. The eleven factors were microcrystalline cellulose (S_1), Tween 80 (S_2), polyethylene glycol 400 (S_3), sodium starch glycolate (S_4), Eudragit® RL 15 D (S_5), dry blending speed (S_6), dry blending time (S_7), extrusion speed (S_8), spheronization speed (S_9), spheronization time (S_{10}) and drying time (S_{11}). The output responses were aspect ratio (T_1), yield (T_2), drug release 15 min (T_3), drug release 30 min (T_4), drug release 45 min (T_5) and drug release 60 min (T_6). Design-Expert® software (Version 8.0.7.1, State-Ease Inc., Minneapolis, MN, USA) was used

to analyze experimental data, perform ANOVA and to generate Pareto and half-normal plots. R^2, Adj-R^2 and Pred-R^2 values were used to evaluate model fitness and correlation between experimental and predicted responses. The CV was used to establish whether a model was reliable or not. Only aspect ratio (T_1) and drug release 15 min (T_3) exhibited p-values < 0.05 hence they were the only significant responses. From these models F-values and p-values were used to establish the significance of the impact of each factor on output responses. Pareto charts were used to graphically represent significance by ranking factors from most significant to least significant. Half-normal probability plots were used to confirm the importance of factors based on their location relative to the near-zero line. Based on the results microcrystalline cellulose (S_1), sodium starch glycolate (S_4), extrusion speed (S_8) and spheronization time (S_{10}) were the most significant factors. Hence these factors were selected for further investigation.

6.7 RESPONSE SURFACE METHODOLOGY (RSM)

Box and Behnken proposed a three level design for fitting response surfaces [491] and the design is a combination of a two factorial and incomplete block design. The result was a cube shaped, revolving design that consists of a central point and points at the middle of each edge [491, 492]. These designs are either rotatable or nearly rotatable and are very efficient in terms of the number of experimental runs required to elucidate a solution [492]. In these studies, a Box-Behnken design was used to generate multivariate experiments for pellet manufacture as it is rotatable. The purpose of the study was to find an optimum experimental region and therefore a design that provided equal precision of estimation in all directions was preferred [493].

A four-factor Box-Behnken design was used for optimization. This design is suitable for exploring quadratic response surfaces and constructing second order polynomial models. Each independent variable was coded at three levels *viz*., -1, 0 and +1. A total of twenty nine experimental runs were conducted. The total number of experiments was calculated according to Equation 6.11.

$$N = 2k\,(k-1) + C_0 \qquad \textit{Equation 6.11}$$

Where,

N = the number of experiments
k = the number of factors
C_0 = the number of central points

The non-linear quadratic model generated by the design is of the form presented as Equation 6.12.

$$Y = \beta_0 + \beta_i X_1 + \beta_i X_2 + \beta_i X_3 + \beta_i X_4 + \beta_{ij} X_1 X_2 + \beta_{ij} X_1 X_3 + \beta_{ij} X_1 X_4 + \beta_{ij} X_2 X_3 + \beta_{ij} X_2 X_4 + \beta_{ij} X_3 X_4 + \beta_{ii} X_1^2 + \beta_{ii} X_2^2 + \beta_{ii} X_3^2 + \beta_{ii} X_4^2 \quad \textit{Equation 6.12}$$

Where,

Y = Measured response
β_0 = Intercept
β_i = Coefficient of a first order term
β_{ij} = Coefficient of a second order interaction
β_{ii} = Coefficient of a quadratic interaction [494]

The experimental design and levels for formulation and process variables selected for optimization are listed in Table 6.8.

Table 6.8 Experimental design levels for formulation and process variables

Variables	**-1**	**0**	**+1**
MCC, (X_1) % w/w	50	60	70
SSG, (X_2) % w/w	1	1.5	2
Spheronization time, (X_3) min	1	2	3
Extrusion speed, (X_4) rpm	25	30	35

Design Expert® software (Version 8.0.7.1, State-Ease Inc., Minneapolis, MN, USA) was used to generate multivariate experiments for the Box-Behnken design and the formulation and process parameters investigated are listed in Table 6.9. The target batch size was 50 g. For all batches the prednisone content was 4 % w/w, talc content 1.5 % w/w and magnesium stearate content 0.5 % w/w. Preliminary investigations revealed formulation variables such as microcrystalline cellulose (X_1) and sodium starch glycolate (X_2) content and process variables such as spheronization time (X_3) and extrusion speed (X_4) to be the main factors affecting pellet quality and drug release. These factors were varied to investigate their impact on pellet production and performance. The remaining excipients *viz*., Tween® 80 (T), PEG 400 (P) and Eudragit® RL 30 D (E) were used in the same ratio of 2 : 1 : 2 respectively in all formulations, but actual quantities varied depending on the microcrystalline cellulose and sodium starch glycolate content used. The output responses were aspect ratio (Y_1), yield (Y_2), drug release at 15 min (Y_3), 30 min (Y_4), 45 min (Y_5) and 60 min (Y_6). Batch production records for optimization studies (Batch number Pred-BB-001 to Pred-BB-

029) detailing actual parameters used, output responses, dissolution profiles and SEM images for each batch are attached in Appendix II.

Table 6.9 Formulation and process variables used to manufacture prednisone pellets

Run	Microcrystalline cellulose (X_1) % w/w	Sodium starch glycolate (X_2) % w/w	Spheronization time (X_3) min	Extrusion speed (X_4) rpm	Tween® 80 (T) % w/w	PEG 400 (P) % w/w	Eudragit® RL 30 D (E) % w/w
1	60	2.0	1	30	12.8	6.4	12.8
2	60	1.0	2	25	13.2	6.6	13.2
3	60	1.0	1	30	13.2	6.6	13.2
4	70	1.0	2	30	9.20	4.6	9.20
5	70	1.5	2	25	9.00	4.5	9.00
6	50	1.5	1	30	17.0	8.5	17.0
7	60	2.0	3	30	12.8	6.4	12.8
8	60	2.0	2	25	12.8	6.4	12.8
9	60	1.5	2	30	13.0	6.5	13.0
10	60	1.5	2	30	13.0	6.5	13.0
11	70	1.5	3	30	9.00	4.5	9.00
12	50	2.0	2	30	16.8	8.4	16.8
13	60	1.5	2	30	13.0	6.5	13.0
14	60	1.5	1	35	13.0	6.5	13.0
15	60	1.5	2	30	13.0	6.5	13.0
16	60	1.5	3	35	13.0	6.5	13.0
17	60	2.0	2	35	12.8	6.4	12.8
18	60	1.0	3	30	13.2	6.6	13.2
19	50	1.0	2	30	17.2	8.6	17.2
20	50	1.5	2	25	17.0	8.5	17.0
21	50	1.5	2	35	17.0	8.5	17.0
22	50	1.5	3	30	17.0	8.5	17.0
23	70	1.5	2	35	9.00	4.5	9.00
24	70	1.5	1	30	9.00	4.5	9.00
25	60	1.5	3	25	13.0	6.5	13.0
26	70	2.0	2	30	8.80	4.4	8.80
27	60	1.5	1	25	13.0	6.5	13.0
28	60	1.0	2	35	13.2	6.6	13.2
29	60	1.5	2	30	13.0	6.5	13.0

6.8 RESULTS

Box-Behnken experiments were conducted to establish optimum formulation composition and process settings required to produce good quality pellets. Quality was measured against aspect ratio and *in vitro* release performance. In addition, yield was assessed to determine the efficiency of the manufacturing method. The results of these studies are presented in Table 6.10.

Table 6.10 Experimental responses from Box-Behnken studies

Run	Aspect ratio (Y_1)	Yield (Y_2) %	Drug release 15 min (Y_3) %	Drug release 30 min (Y_4) %	Drug release 45 min (Y_5) %	Drug release 60 min (Y_6) %
1	1.09	55.7	75.3	88.2	100.1	100.3
2	1.07	54.8	31.1	53.3	66.8	84.6
3	1.21	58.3	37.1	56.0	65.0	75.2
4	1.20	47.5	24.6	39.8	51.3	59.4
5	1.19	58.4	32.8	51.8	66.5	74.6
6	1.40	41.2	80.4	89.7	90.7	90.2
7	1.08	56.4	71.5	82.8	86.2	90.5
8	1.09	52.5	77.5	88.2	95.1	99.1
9	1.16	58.9	63.4	78.8	85.2	87.9
10	1.17	63.2	47.2	62.3	71.9	77.6
11	1.19	73.9	18.5	32.8	43.3	51.1
12	1.53	34.3	80.2	82.0	91.3	80.9
13	1.45	60.8	64.0	76.7	82.8	84.8
14	1.27	60.4	68.7	83.5	86.2	86.7
15	1.27	62.8	72.6	82.6	86.6	86.5
16	1.24	79.3	50.8	67.1	73.9	80.8
17	1.11	56.0	64.1	80.0	85.3	90.5
18	1.08	55.7	25.1	46.4	61.4	71.7
19	1.25	39.2	63.7	79.9	85.3	84.0
20	1.53	70.9	58.9	77.1	80.9	81.5
21	1.17	36.4	67.3	72.3	77.1	77.2
22	1.15	72.4	55.6	72.1	76.6	75.4
23	1.28	74.1	17.8	33.0	44.4	53.9
24	1.45	78.3	24.7	43.2	55.8	65.2
25	1.09	62.1	70.7	82.3	85.4	87.0
26	1.10	56.7	46.4	75.7	91.5	99.0
27	1.20	76.9	57.9	74.8	81.0	83.2
28	1.12	60.9	43.2	68.7	91.2	99.3
29	1.18	63.5	61.6	74.6	77.7	85.9

The aspect ratio ranged between 1.07 and 1.53, yield between 34.3 % and 79.3 %, drug release at 15 min between 17.8 % and 80.4 %, 30 min between 33.0 % and 89.7 %, 45 min between 43.3 % and 100.1 % and 60 min between 51.1 % and 100.3 %.

6.8.1 Model fitting and statistical analysis

Design-Expert® software (Version 8.0.7.1, State-Ease Inc., Minneapolis, MN, USA) was used to compute mathematical models that best described the relationship between input factors and responses. The final second-order polynomial equations in terms of coded factors are presented in Equations 6.13 - 6.18.

$$Y_1 = 1.25 - 0.052X_1 + 5.833e^{-003}X_2 - 0.066X_3 + 1.667e^{-003}X_4 - 0.095X_1X_2 - 2.5e^{-003}X_1X_3 + 0.11X_1X_4 + 0.03X_2X_3 - 7.5e^{-003}X_2X_4 + 0.020X_3X_4 + 0.095X_1^2 - 0.094X_2^2 - 0.029X_3^2 - 0.040X_4^2 \quad \textit{Equation 6.13}$$

$$Y_2 = 61.84 + 7.87X_1 - 0.4X_2 + 2.42X_3 - 0.71X_4 + 3.53X_1X_2 - 8.90X_1X_3 + 12.55X_1X_4 + 0.82X_2X_3 - 0.65X_2X_4 + 8.43X_3X_4 - 4.35X_1^2 - 11.27X_2^2 + 6.56X_3^2 + 3.07X_4^2 \quad \textit{Equation 6.14}$$

$$Y_3 = 61.76 - 20.11X_1 + 15.85X_2 - 4.33X_3 - 1.42X_4 + 1.32X_1X_2 + 4.65X_1X_3 - 5.85X_1X_4 + 2.05X_2X_3 - 6.38X_2X_4 - 7.68X_3X_4 - 11.35X_1^2 - 2.73X_2^2 - 3.17X_3^2 - 2.61X_4^2 \quad \textit{Equation 6.15}$$

$$Y_4 = 75.00 - 16.40X_1 + 12.73X_2 - 4.33X_3 - 1.91X_4 + 8.45X_1X_2 + 1.80X_1X_3 - 3.50X_1X_4 + 1.05X_2X_3 - 5.90X_2X_4 - 5.98X_3X_4 - 11.35X_1^2 + 0.096X_2^2 - 2.67X_3^2 - 1.02X_4^2 \quad \textit{Equation 6.16}$$

$$Y_5 = 80.84 - 12.43X_1 + 10.70X_2 - 4.33X_3 - 1.47X_4 + 8.55X_1X_2 + 0.4X_1X_3 - 4.57X_1X_4 - 2.55X_2X_3 - 8.55X_2X_4 - 4.17X_3X_4 - 9.92X_1^2 + 4.54X_2^2 - 3.57X_3^2 - 0.037X_4^2 \quad \textit{Equation 6.17}$$

$$Y_6 = 84.54 - 7.17X_1 + 7.15X_2 - 3.67X_3 - 1.80X_4 + 10.68X_1X_2 + 0.17X_1X_3 - 4.10X_1X_4 - 1.50X_2X_3 - 5.82X_2X_4 - 2.42X_3X_4 - 11.60X_1^2 + 6.13X_2^2 - 3.52X_3^2 + 1.65X_4^2 \quad \textit{Equation 6.18}$$

The coefficients of each factor reveal its effect on the response. A positive coefficient indicates an agonistic effect whereas a negative coefficient indicates an antagonistic effect [495-497]. Based on the polynomial equations microcrystalline cellulose exhibited an agonistic effect on yield but antagonistic effects on both aspect ratio and drug release. Sodium starch glycolate exhibited agonistic effects on drug release and aspect ratio but exhibited an antagonistic effect on yield. Spheronization time exhibited an agonistic effect on yield but exhibited antagonistic effects on aspect ratio and drug release. Extrusion speed exhibited an agonistic effect on aspect ratio but exhibited antagonistic effects on yield and drug release.

Even though the polynomial equations provided an indication of the nature of effects caused by each factor, information generated from them was limited and inconclusive hence a more thorough analysis was conducted by performing ANOVA. F-values, p-values, R^2 values, adjusted R^2 values, predicted R^2 values, standard deviation and coefficient of variance were used to evaluate model

fitness and adequacy. In addition, 2D contour plots and 3D response surface plots were used to present input-output relationships in a visual manner. Design-Expert® software was used to conduct the tests *via* a stepwise regression method and a summary of ANOVA data generated from the study is presented in Table 6.11.

Table 6.11 ANOVA data generated for Box-Behnken studies

Response	R^2	Adj R^2	Pred R^2	Adeq precision	CV %	F-value	P-value	SD
Y_1	0.6594	0.3188	-0.4673	5.530	9.12	1.94	0.1144	0.11
Y_2	0.9128	0.8256	0.5137	13.129	8.42	10.47	0.0001	5.00
Y_3	0.8885	0.7769	0.4885	10.775	17.33	7.97	0.0002	9.28
Y_4	0.8606	0.7213	0.3201	10.612	13.03	6.18	0.0008	8.97
Y_5	0.8405	0.6811	0.1800	10.297	10.90	5.27	0.0018	8.41
Y_6	0.8143	0.6286	-0.0071	9.671	9.35	4.39	0.0046	7.62

*Data written in purple indicates significant factors.

Results from the study revealed that most R^2 values were close to 1 indicating good model fitness. The adjusted R^2 value for most responses was found to be in close agreement with the predicted R^2 value indicating good correlation between experimental and predicted responses. All ratios for Adeq precision were > 5.5 which indicates an adequate signal. The relatively low values for CV and SD indicate good precision and reliability of the model, where low values indicated better accuracy. The model for aspect ratio ($p > 0.05$) was deemed insignificant. All other models exhibited $p < 0.05$. The model for drug release at 60 min exhibited a negative Pred R^2 value hence the model was not suitable for predicting responses. A negative Pred R^2 implies that the overall mean is a better predictor of a response than the current model.

Drug release after 30 min of dissolution was the most important output response. Pharmacopoeial guidelines were used to determine the suitability of prednisone release profiles. According to pharmacopoeial guidelines, immediate release oral dosage forms must release at least 80 % of the active ingredient within 30 minutes [498]. The effect of formulation and process variables on drug release (30 min) is discussed here in detail. For all other time points investigated the same trend and conformation was observed. ANOVA data generated for the model of drug release at 30 min is presented in Table 6.12.

Table 6.12 ANOVA data for prednisone release at 30 min

Source	Sum of Squares	df	Mean Square	F-value	p-value Prob > F
Model	6956.38	14	496.88	6.18	0.0008
X_1	3227.52	1	3227.52	40.11	<0.0001
X_2	1945.65	1	1945.65	24.18	0.0002
X_3	224.47	1	224.47	2.79	0.1171
X_4	43.70	1	43.70	0.54	0.4733
X_1X_2	285.61	1	285.61	3.55	0.0805
X_1X_3	12.96	1	12.96	0.16	0.6942
X_1X_4	49.00	1	49.00	0.61	0.4482
X_2X_3	4.41	1	4.41	0.055	0.8183
X_2X_4	139.24	1	139.24	1.73	0.2095
X_3X_4	142.80	1	142.80	1.77	0.2041
X_1^2	836.22	1	836.22	10.39	0.0061
X_2^2	0.060	1	0.060	$7.404e^{-004}$	0.9787
X_3^2	46.13	1	46.13	0.57	0.4615
X_4^2	6.70	1	6.70	0.083	0.7771
Residual	1126.50	14	80.46		
Lack of Fit	889.96	10	89.00	1.50	0.3691
Pure Error	236.54	4	59.14		
Cor Total	8082.88	28			
R^2					0.8606
Adj-R^2					0.7213
Pred-R^2					0.3201
Adeq-Precision					10.6120
CV %					13.03
SD					8.97

*Data written in purple indicates significant factors.

The model for drug release at 30 min exhibited an F-value of 6.18 and a p-value of 0.0008 which is < 0.05 implying that it was significant. Microcrystalline cellulose and sodium starch glycolate exhibited the largest F-values of 40.11 and 24.18 with p-values of < 0.0001 and 0.0002 respectively which are < 0.05 hence they had the most significant impact on drug release.

Response surfaces were plotted as 2D and 3D response surface curves to provide better visualization and understanding of the interaction of variables. Response surface plots are plotted as a function of two factors, where all other factors are constant in order to provide better understanding of the most significant factors and the interactive effects thereof.

The results from these studies revealed that microcrystalline cellulose and sodium starch glycolate were the most significant factors impacting prednisone release. An inverse relationship was observed between microcrystalline cellulose and sodium starch glycolate. It was observed that dissolution was more rapid when the concentration of sodium starch glycolate was 2 % w/w and the concentration of microcrystalline cellulose was at 50 % w/w. The 2D and 3D response plots depicted in Figures 6.7 and 6.8 respectively provide a visual representation of the effects of microcrystalline cellulose and sodium starch glycolate on prednisone release at 30 min and for all other time points investigated the same trend and conformation was observed.

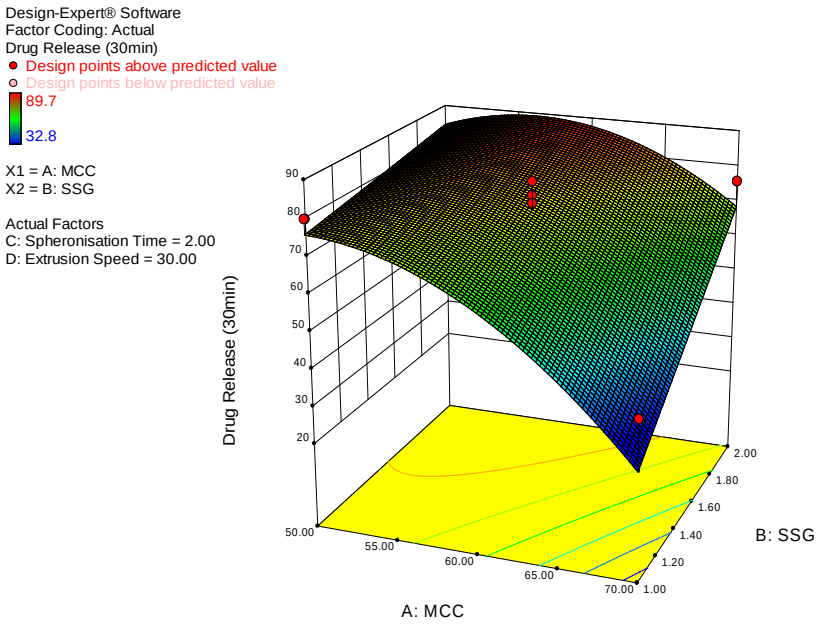


Figure 6.7 3D response surface plot depicting the impact of microcrystalline cellulose and sodium starch glycolate content on prednisone release at 30 minutes

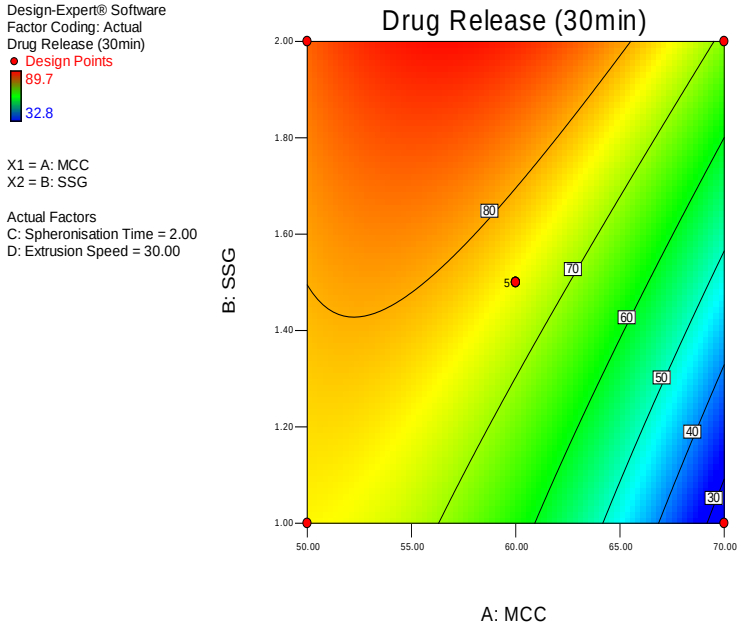


Figure 6.8 Contour plot depicting the impact of microcrystalline cellulose and sodium starch glycolate content on prednisone release at 30 minutes

Microcrystalline cellulose was incorporated into the formulation as a diluent and pelletization aid [499]. Microcrystalline cellulose is highly hygroscopic and has a tendency to adsorb and retain large volumes of water during granulation that imparts adequate plasticity to the wet mass thereby facilitating pelletization [499]. Increasing the microcrystalline cellulose content retarded prednisone release. A possible explanation for this phenomenon may be postulated using the crystallite-gel model proposed by Kleinebudde [295] which suggests that microcrystalline cellulose particles disaggregate eventually forming single crystallites due to shear stress forces encountered during granulation and extrusion. In the presence of liquid, these crystallites form a crystallite-gel held together in a framework by cross linked hydrogen bonds at the amorphous ends of the molecules. Hydrogen bonding is even more apparent when water is used as a granulating fluid and an even stronger matrix is formed. Since microcrystalline cellulose forms the gel, the strength and extent of adhesive forces that maintain pellet integrity are highly dependent on the fraction of microcrystalline cellulose used in a formulation. In addition, the water required to form the gel increases with increasing microcrystalline cellulose content further influencing the extent of hydrogen bonding. When water eventually evaporates during drying, new hydrogen bonds are formed, and the interweaving of microcrystalline cellulose fibers continues to hold the pellet

structure together. Due to this phenomenon the time for pellets to dissociate increases with increasing microcrystalline cellulose content due to extensive bonding causing slower prednisone release.

Sodium starch glycolate is a super-disintegrant [276] and was incorporated into the formulation to facilitate pellet dissociation. Sodium starch glycolate exerts its effect *via* a swelling mechanism [276]. When exposed to aqueous media the swelling overcomes inter-particulate adhesive forces that hold the pellet together resulting in dissociation into smaller fragments thereby releasing prednisone trapped in each pellet [500]. Changes in sodium starch glycolate content drastically impacted pellet disintegration dynamics. Increasing the amount of sodium starch glycolate used improved the rate and extent of prednisone released and *vice versa*. Visual observation of pellets during dissolution testing revealed that the magnitude of swelling was greatest and disintegration time slowest for pellets containing large amounts of sodium starch glycolate. The smaller fragments have large surface area to volume ratio which favours dissolution, therefore changes in sodium starch glycolate content affect the rate and extent of fragmentation which ultimately affects the rate of dissolution of prednisone. These results are consistent with the findings of Kilor et al. [501] who observed an improvement in dissolution rate of immediate release aceclofenac pellets when sodium starch glycolate was incorporated into formulations.

Previous studies have shown that incorporating surfactants alone or in combination with glycerides can improve the aqueous solubility of hydrophobic drugs [368-370]. Tween® 80 was added to the formulation to facilitate solubilization of prednisone. PEG 400 was used as a pore-forming agent to ensure the ingress of aqueous fluid into pellets *via* capillary action [370]. In addition, PEG 400 was incorporated to improve the solubility of prednisone as it is hydrophilic nature [370]. A 50 % v/v aqueous dilution of Eudragit® RL 30 D was prepared to reduce the viscosity and permit easy spraying during the granulation process and was incorporated into the formulation to improve solubility and to impart tensile strength. Talc and magnesium stearate were added as anti-adherents to minimize friction during manufacturing. The combination of excipients resulted in rapid wetting and hydration of the pellets, exposing the disintegrant to aqueous media within seconds. As a result, disintegration was rapid and dissolution of prednisone from most formulations tested complied to the pharmacopoeial limits.

Based on the model of drug release at 30 min, extrusion speed and spheronization time exhibited low F-values of 0.54 and 2.79 with p-values of 0.4733 and 0.1171 respectively which are > 0.05 implying that they were insignificant. Their impact on prednisone release was minimal, however they had a significant impact on extrudate and pellet quality. Generally, an ideal extrudate must be non-adhesive to itself and must be rigid enough to retain the shape imposed by a die, yet be sufficiently brittle to be broken into short lengths by the spheronizer without disintegrating completely [295]. An extrusion speed of 25 rpm was found to produce extrudate conforming to these criteria. The quality of the resultant extrudate was not only a function of extrusion speed, but rather a combination of the formulation composition and ideal speed to confer sufficient shear to produce extrudates that met the aforementioned criteria. The impact of spheronization time on pellet quality varied according to formulation composition however acceptable sphericity was observed after the first minute. Additionally, formulation composition had an impact on pellet size. Formulations containing high Tween® 80, PEG 400 and Eudragit® RL 15 D content had a degree of sticking, prompting coalescence and globulation due to the high viscosity resulting from Tween® 80, PEG 400 and Eudragit® RL 15 D inclusion. Formulations containing low Tween® 80, PEG 400 and Eudragit® RL 15 D content fractured easily during spheronization causing large formation of fines.

Whilst the yield and aspect ratio were not the main attributes investigated in the study they were monitored to determine the efficiency of production and if acceptable sphericity of pellets was achieved. The yield increased with increasing microcrystalline content since it is a pelletization aid. In addition, the yield also improved with increasing spheronization time possibly due to more extrudate converting to a pellet state. ANOVA data and response surface plots for yield, aspect ratio and other dissolution time points are presented in Appendix IV.

6.9 ESTABLISHMENT OF A DESIGN SPACE

Risk assessment and process development experiments lead to improved understanding of the relationships between input variables and final product quality. In addition, they facilitate determination of key variables and ranges within which a product of consistent quality can be achieved [502]. In line with QbD principles, ICH guidelines recommend establishing a design space [503]. In a design space, specific ranges for formulation and process variables are prescribed. The goal is to ensure that any navigation within the design space yields a good quality product which meets the desired quality target profile. The design space is specified during regulatory approval, hence any movement within the design space is not considered a change and does not require regulatory approval [504]. However, any movement out of the predefined design space requires regulatory approval [504]. The design space is therefore considered a final achievement of process understanding in the development of a new product. It may however be updated as more knowledge is acquired throughout the product's life cycle [502, 505]. In the pharmaceutical industry the design space is conceptualized into knowledge space, design space (DS) and control space [506, 507]. In the knowledge space, the impact of formulation and process variables on critical quality attributes is measured, whereas the design space represents a region where products satisfy all the requirements for safety and efficacy for all levels in that space [506]. The control space is the operating range and lies within the design space. Generally, it is desirable for the control space to be smaller than the design space, possibly centered as far as possible from the edges of the design space to minimize the probability of any undesirable factor combinations [506].

Design-Expert® software (Version 8.0.7.1, State-Ease Inc., Minneapolis, MN, USA) was used to generate optimized (OPT) values and to establish the design space lower limit (DS-LL) and upper limit (DS-UL) for each factor. The limits established for each factor are presented in Table 6.13 and Figure 6.9. Lower and upper limits from Box-Behnken design experiments are represented in the knowledge space (Figure 6.9) as BB-LL and BB-UL respectively.

Table 6.13 Design space limits for manufacturing prednisone pellets

Formulation or Process Variable	Coded form	Design space lower limit	Optimized	Design space upper limit
Microcrystalline cellulose (% w/w)	A	58	60	62
Sodium starch glycolate (% w/w)	B	1.9	2.0	2.0
Spheronization time (min)	C	1.4	2.0	2.2
Extrusion speed (rpm)	D	25	25	26

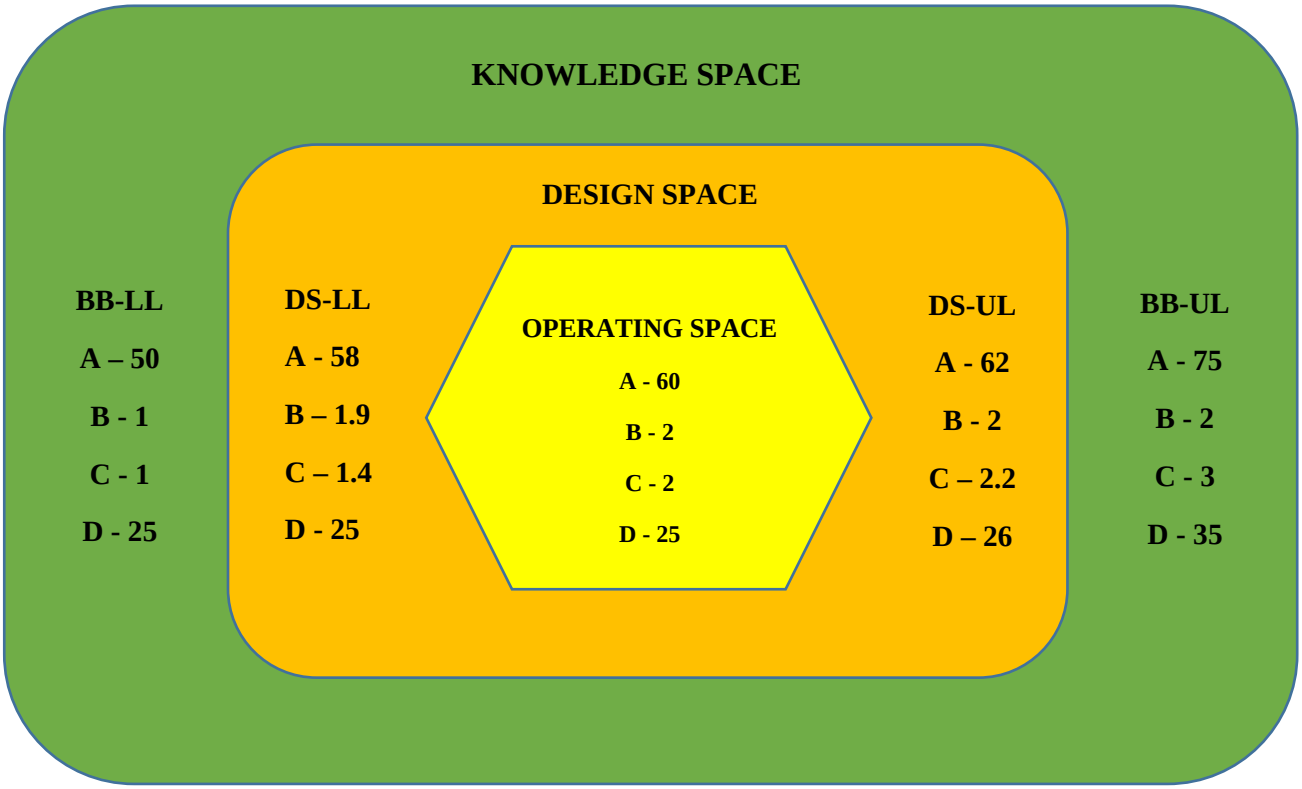


Figure 6.9 Schematic representation of design space limits

Key:

Knowledge space = Experimental or characterization range.

Design space = Acceptable range.

Control space = Operating range.

All formulations were analyzed in replicate (n = 3) and the responses observed are listed in Table 6.14. The output responses were aspect ratio, yield, prednisone release at 15, 30, 45 and 60 min. Batch production records for DS-LL, DS-UL and OPT formulations are reported in Appendix III.

Table 6.14 Responses for optimized and design space formulations

Batch	Aspect ratio	Yield %	Drug release 15 min %	Drug release 30 min %	Drug release 45 min %	Drug release 60 min %
OPT 1	1.12	70.20	77.48	89.98	95.14	100.45
OPT 2	1.11	69.34	75.92	86.46	94.35	100.26
OPT 3	1.09	71.25	78.68	88.10	94.50	99.10
DS-LL 1	1.08	56.72	65.24	82.20	86.56	95.50
DS-LL 2	1.07	55.83	67.58	84.92	87.00	94.90
DS-LL 3	1.09	59.33	68.00	85.50	86.90	96.22
DS-UL 1	1.13	70.12	69.44	83.26	90.45	95.53
DS-UL 2	1.11	69.50	68.80	86.59	89.65	95.44
DS-UL 3	1.09	68.98	69.76	85.67	88.78	95.80

The aspect ratio for OPT 1-3, DS-LL 1-3 and DS-UL 1-3 ranged between 1.09 - 1.12, 1.07 - 1.09 and 1.09 - 1.13 respectively. All values were < 1.20 implying that all pellets were of acceptable sphericity. In addition, the aspect ratios obtained were in close proximity indicating the tightness of the design space. The percentage yield of OPT 1-3, DS-LL 1-3 and DS-UL 1-3 ranged between 69.34 - 71.25 %, 55.83 - 59.33 % and 68.98 - 70.12 % respectively. Pellets from OPT and DS-UL limit formulations were harvested in higher yield compared to DS-LL formulations. OPT and DS-UL formulations contained a higher microcrystalline cellulose content hence more pellets were obtained since microcrystalline cellulose is a pelletization aid. Drug release at 30 min for OPT 1-3, DS-LL 1-3 and DS-UL 1-3 ranged between 86.46 - 89.26 %, 82.20 - 85.50 % and 83.26 - 86.59 %. All formulations released > 80 % of prednisone in 30 min which conforms to USP specifications.

The optimized formulations (OPT 1-3) exhibited the highest drug release following 60 minutes of dissolution, with a mean (n = 3) drug release of 99.94 %. In addition, the yield and aspect ratio were within acceptable limits. Therefore the optimized formula resulted in products that met the desired quality target product profile (QTPP) set at the beginning of the development process. Furthermore, all formulations within the design space from the lower to the upper limit exhibited acceptable drug release, aspect ratio and yield, implying that QbD principles were successfully used to produce an acceptable design space and that any navigation or movement within the design

space will result in a good quality product. *In vitro* release profiles for OPT 1-3, DS-LL 1-3 and DS-UL 1-3 are presented in Figures 6.10 - 6.12.

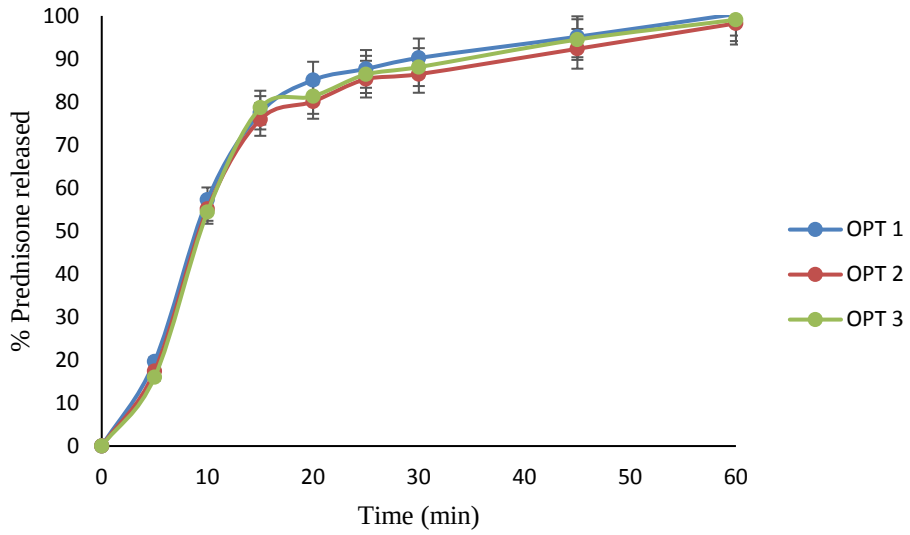


Figure 6.10 Dissolution profiles for optimized batches (n = 3)

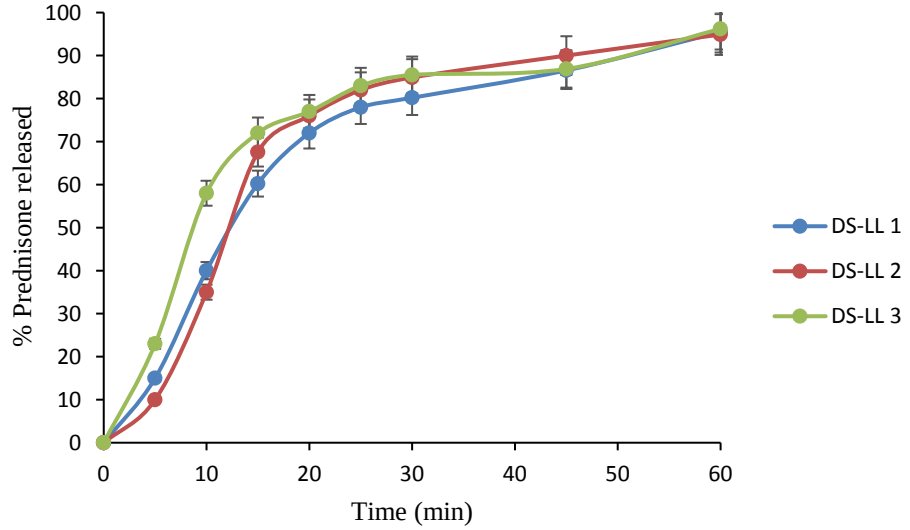


Figure 6.11 Dissolution profiles for DS-LL batches (n = 3)

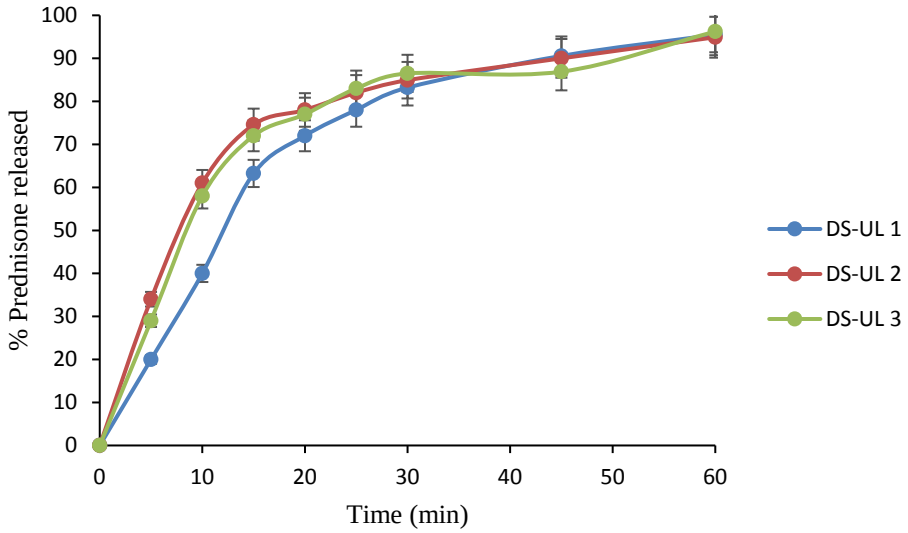


Figure 6.12 Dissolution profiles for DS-UL batches (n = 3)

A list of excipient content for the optimized formulation that resulted in optimum prednisone release with an acceptable aspect ratio and yield is presented in Table 6.15. Optimum process settings are listed in Table 6.16.

Table 6.15 Optimum formulation composition for prednisone pellets

Material	Content %	Function
Prednisone	4.0	Active ingredient
Tween 80	12.8	Surfactant, Solubilizer.
Polyethylene glycol 400	6.4	Pore-former, solubilizer, Imparts hydrophilicity.
Eudragit® RL 30 D (50 % aqueous dilution)	12.8	Improves pellet tensile strength, Imparts hydrophilicity.
Comprecel® M102 D+	60.0	Bulking agent, spheronization aid.
Sodium starch glycolate	2.0	Disintegrant.
Talc	1.5	Glidant, anti-adherent.
Magnesium stearate	0.5	Lubricant, anti-adherent.

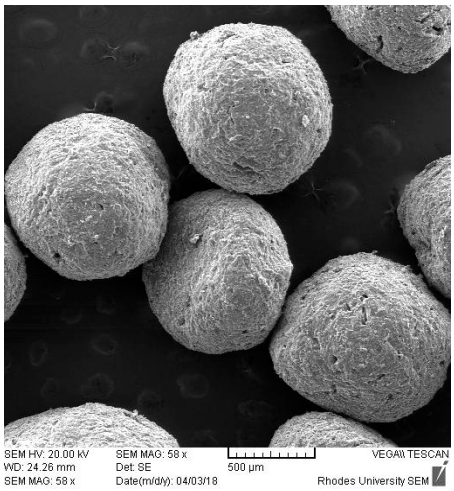
*Granulation fluid - water.

Table 6.16 Optimum extrusion-spheronization settings

Process	Settings
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 minutes
Drying temperature	40 °C

The use of the optimized composition and process variables resulted in the formation of (I) spherical and (II) porous pellets as depicted in Figure 6.12.

(I)



(II)

Figure 6.13 SEM images for the (I) optimized formulation and (II) cross section of the optimized formulation revealing a porous internal structure

6.10 CONCLUSIONS

An attempt has been made to prepare an immediate release multipartite dosage form for oral administration of prednisone. The availability of an alternative oral drug delivery system for prednisone will address some of the concerns associated with oral delivery of prednisone observed using conventional technologies. A Plackett-Burman screening study was conducted to narrow down potentially significant factors from eleven to four. The four factors, considered to be the most significant were further analyzed using a Box-Behnken design. Based on experimental results and statistical analysis, the following important conclusions were derived from this investigation. Only two factors, microcrystalline cellulose content and sodium starch glycolate content were found to be the most impactful towards prednisone release. All other excipients and process settings contributed to the production of an immediate release formulation that performed within pharmacopoeial specifications for such dosage forms. Following the Box-Behnken study a design space was established and optimum formulation content and process settings were determined. In conclusion, this work illustrates that QbD principles can be successfully used to manufacture an immediate release multipartite dosage form intended for oral administration. QbD principles allow for a more knowledge based approach to manufacturing and therefore result in good quality products.

CHAPTER 7
CONCLUSIONS

Even though prednisone has multiple therapeutic benefits, its oral delivery is associated with many challenges. Its poor solubility and low dissolution rate in gastrointestinal fluids often causes insufficient bioavailability. Furthermore it has harsh effects on the GIT and has an extremely bitter taste which needs to be masked in order to achieve better palatability. Many attempts have been made to improve the efficiency of delivering prednisone and its derivatives. Incorporation of prednisone into micro-emulsions, polymeric micelles, polymeric implants and microspheres has been reported in literature. This study explored the effectiveness of incorporating prednisone into a multiple-unit pellet system to address the aforementioned challenges regarding its oral delivery. To our knowledge incorporation of prednisone into a multiple-unit pellet system has not been reported in currently published literature. Principles of QbD were used to produce a good quality product. The aim was to achieve optimum product quality with consistent dosage form performance and minimal risk of failure in patients. In accordance with ICH guidelines, multivariate experiments under the RSM framework were used to study the relationships between formulation and process variables and final product quality.

A multipartite dosage form was investigated because compared to traditional single unit dosage forms, multi-particulate dosage forms have lower incidences of gastrointestinal irritation due to a decrease in the local concentration of API in the GIT following oral administration. Furthermore lower individual variability in plasma concentrations is observed compared to tablets since there is a reduced risk of dose dumping. In addition, the presence of many individual units increases surface area leading to improved solubility and bioavailability. The use of discrete units also offers a simple solution to minimizing potential API-API or API-excipient interactions in some cases, and the free flowing nature of pellets facilitates reproducible capsule filling and content uniformity of doses.

A RP-HPLC method capable of quantifying prednisone from solid dosage forms was developed using principles of AQbD and validated according to ICH guidelines. A DoE approach which included the use of CCD was used to develop and optimize the method. Quadratic equations, ANOVA, 3D response surface and contour plots were used to determine the relationships between input variables and output responses. The input variables were column temperature, column type

and mobile phase ratio and the output responses were retention time, peak tailing and resolution. The results indicate that ACN content and column type exhibited the highest impact on retention time. Increasing ACN content led to a decrease in retention time and *vice versa*. A Phenomenex Synergi™ Polar-RP 80Å 250 mm x 4.6 mm x 4 µm column produced sharp, well resolved peaks and hence it was selected for use in all further studies. The effect of column temperature on retention time was negligible. Both ACN content and column temperature exhibited very negligible effects on peak tailing, however an increase in both resulted in tailing factors closer to 1 which are more ideal. Following selection of optimum chromatographic conditions, the optimized RP-HPLC method was validated according to ICH guidelines and was found to be selective, sensitive, precise and accurate for use in the assessment of solid dosage forms containing prednisone. When forced degradation studies were conducted, prednisone was found to degrade in 0.1 M HCl by 59.3 % and in water by 25.4 % after 8 hours of refluxing at 90 $^{\circ}$C. Degradation in 0.1 M NaOH was extremely rapid, 99.71 % after refluxing for 1.5 hours at 90 $^{\circ}$C and no degradation peaks were observed following exposure to 90 $^{\circ}$C of dry heat for 8 hours.

The pre-formulation studies conducted were assessment of particle size, particle shape, powder flow properties and compatibility studies. CI, HR and AOR were used to evaluate powder flow properties and results generated from all of them suggested the need for adding a glidant and lubricant to improve flow. DSC and FT-IR were used to evaluate drug-excipient compatibility. All excipients were found to be compatible and suitable for formulation. Prednisone was found to degrade at temperatures $\geq$ 248 $^{\circ}$C however formulation was undertaken at room temperature so the risk of degradation occurring under normal manufacturing conditions was highly unlikely. When a binary mixture of prednisone and Eudragit® RL 30 D was assessed under DSC, peaks from both compounds disappeared from the thermogram suggesting an unwanted interaction at elevated temperatures. However characteristic peaks from both compounds were present when the same binary mixture was assessed under FT-IR at room temperature suggesting that the risk of an unwanted interaction occurring under normal manufacturing conditions was highly unlikely. To confirm compatibility, long term and accelerated stability studies would have to be conducted to determine if all the excipients were indeed compatible in the final dosage form.

The extrusion-spheronization method was used to manufacture prednisone pellets. Manufacturing pellets *via* extrusion-spheronization is a multi-step process that involves many variables. QRM

principles were used to identify and narrow down potentially significant factors. An Ishikawa fishbone diagram was used to classify factors under broad categories and FMEA was used to quantify risk according to RPN scores. A set RPN threshold was used to determine whether a factor was of high risk or not and factors above the threshold value were regarded as highly risky. A total of eleven high risk factors were identified, but eleven factors are still too many hence the factors were further analysed using a Plackett-Burman screening study with the intention of identifying a few most impactful factors. The results from Plackett-Burman screening studies indicate that microcrystalline cellulose content, sodium starch glycolate content, extrusion speed and spheronization speed were the four most significant factors. These factors were used as input variables for the final optimization study using a Box-Behnken design. Aspect ratio, yield and drug release were monitored as output responses. Drug release was analyzed using a USP Apparatus 2 and aliquots collected at each sampling time were analysed using the validadated RP-HPLC method.

Data generated from Box-Behnken studies were fitted to different statistical models to establish the relationship between input factors and output responses. Based on experimental results and statistical analysis, the following important conclusions were derived from this investigation. Only two factors, microcrystalline cellulose content and sodium starch glycolate content were found to be the most impactful towards prednisone release. All other excipients and process settings contributed to the production of an immediate release formulation that performed within pharmacopoeial specifications for such dosage forms. Following the Box-Behnken study a design space was established and optimum formulation content and process settings were determined. In conclusion, this work illustrates that QbD principles can be successfully used to manufacture an immediate release multipartite dosage form intended for oral administration. QbD principles facilitate a knowledge based approach to manufacturing and therefore lead to the developmemt of good quality products.

Future work will involve masking the bitter taste of prednisone. This limitation of prednisone can adversely impact patient acceptability and may result in non-adherence to dosage regiments. The future work will utilize a novel drug delivery technology known as ion exchange resins for taste masking.

APPENDIX - I
BATCH PRODUCTION RECORD
PLACKETT-BURMAN SCREENING STUDIES

Formulation development, manufacture and assessment of all batches (Pred-PB-001 to Pred-PB-012) was undertaken at the Faculty of Pharmacy, Department ofPpharmaceutics, Rhodes University using current Good Manufacturing Practice (cGMP) guidelines.

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-001	Start time	09.00 hrs
Date of manufacture	07/09/17	End time	10.00 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	25
Sodium starch glycolate (g)	1
Tween® 80 (mL)	5
Polyethylene glycol 400 (mL)	5
Eudragit® RL 15 D (mL)	10
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

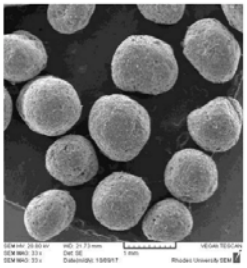
Process settings

Process	**Setting**
Dry blending speed	2
Dry blending time (min)	4
Extrusion speed (rpm)	25
Spheronization speed (rpm)	642
Spheronization time (min)	1
Drying time (hrs)	12

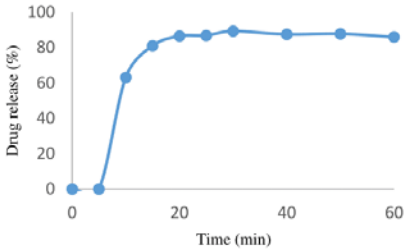
Characteristics

1. Aspect ratio – 1.11

2. Yield – 37.3 %



SEM image



***In vitro* dissolution profile**

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-002	Start time	09.00 hrs
Date of manufacture	08/09/17	End time	10.05 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	25
Sodium starch glycolate (g)	2
Tween® 80 (mL)	5
Polyethylene glycol 400 (mL)	2.5
Eudragit® RL 15 D (mL)	10
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

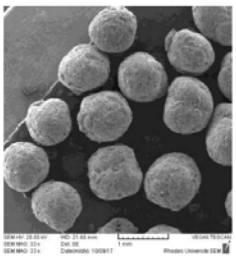
Process settings

Process	**Setting**
Dry blending speed	1
Dry blending time (min)	4
Extrusion speed (rpm)	35
Spheronization speed (rpm)	742
Spheronization time (min)	1
Drying time (hrs)	6

Characteristics

1. Aspect ratio – 1.05

2. Yield – 42.9 %



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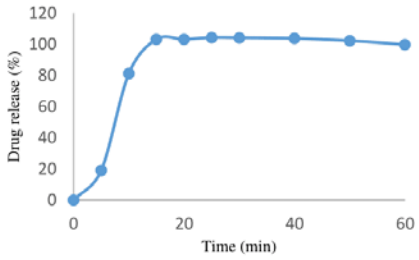

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-003	Start time	10.15 hrs
Date of manufacture	08/09/17	End time	11.15 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	35
Sodium starch glycolate (g)	2
Tween® 80 (mL)	2.5
Polyethylene glycol 400 (mL)	5
Eudragit® RL 15 D (mL)	10
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

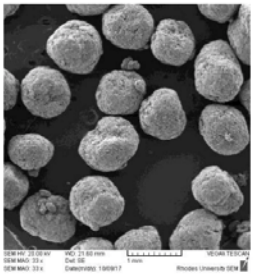
Process settings

Process	**Setting**
Dry blending speed	1
Dry blending time (min)	2
Extrusion speed (rpm)	25
Spheronization speed (rpm)	742
Spheronization time (min)	1
Drying time (hrs)	12

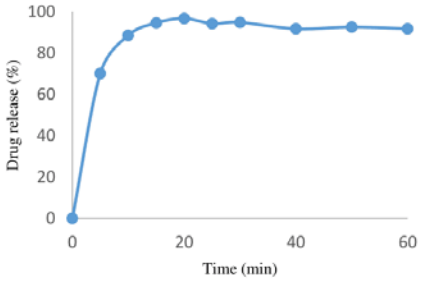
Characteristics

1. Aspect ratio – 1.15

2. Yield – 24.7 %



SEM image



***In vitro* dissolution profile**

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-004	Start time	11.30 hrs
Date of manufacture	08/09/17	End time	12.40 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	35
Sodium starch glycolate (g)	1
Tween® 80 (mL)	5
Polyethylene glycol 400 (mL)	5
Eudragit® RL 15 D (mL)	5
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

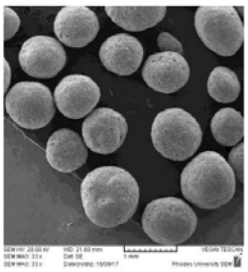
Process settings

Process	**Setting**
Dry blending speed	1
Dry blending time (min)	4
Extrusion speed (rpm)	25
Spheronization speed (rpm)	742
Spheronization time (min)	3
Drying time (hrs)	6

Characteristics

1. Aspect ratio – 1.07

2. Yield – 41.1 %



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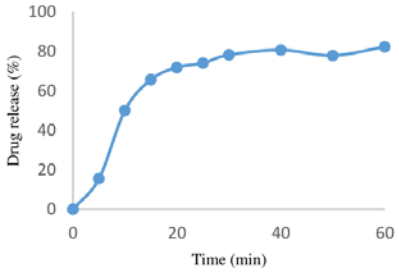

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-005	Start time	09.00 hrs
Date of manufacture	11/09/17	End time	10.10 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	25
Sodium starch glycolate (g)	1
Tween® 80 (mL)	2.5
Polyethylene glycol 400 (mL)	2.5
Eudragit® RL 15 D (mL)	5
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

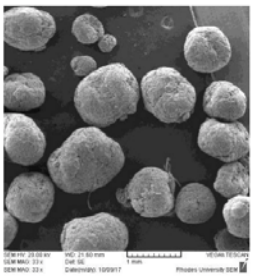
Process settings

Process	**Setting**
Dry blending speed	1
Dry blending time (min)	2
Extrusion speed (rpm)	25
Spheronization speed (rpm)	642
Spheronization time (min)	1
Drying time (hrs)	6

Characteristics

1. Aspect ratio – 1.10

2. Yield – 23.8 %



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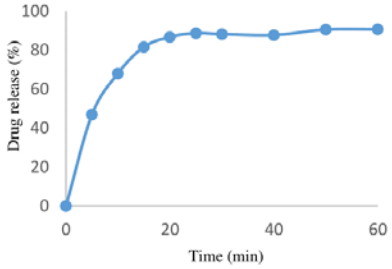

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-006	Start time	09.00 hrs
Date of manufacture	13/09/17	End time	10.15 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	35
Sodium starch glycolate (g)	1
Tween® 80 (mL)	2.5
Polyethylene glycol 400 (mL)	2.5
Eudragit® RL 15 D (mL)	10
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

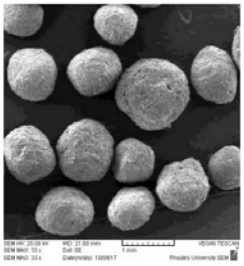
Process settings

Process	**Setting**
Dry blending speed	1
Dry blending time (min)	4
Extrusion speed (rpm)	35
Spheronization speed (rpm)	642
Spheronization time (min)	3
Drying time (hrs)	12

Characteristics

1. Aspect ratio – 1.07

2. Yield – 48.4 %



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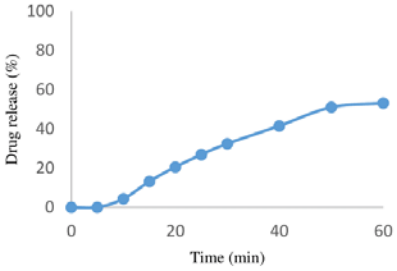

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-007	Start time	10.30 hrs
Date of manufacture	11/09/17	End time	11.30 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	25
Sodium starch glycolate (g)	2
Tween® 80 (mL)	2.5
Polyethylene glycol 400 (mL)	2.5
Eudragit® RL 15 D (mL)	5
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

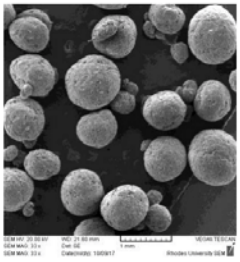
Process settings

Process	**Setting**
Dry blending speed	2
Dry blending time (min)	4
Extrusion speed (rpm)	25
Spheronization speed (rpm)	742
Spheronization time (min)	3
Drying time (hrs)	12

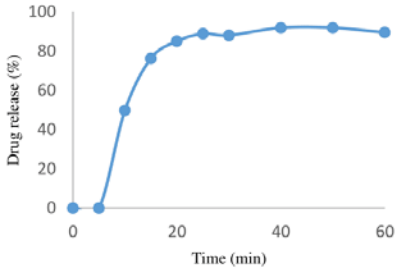
Characteristics

1. Aspect ratio – 1.07

2. Yield – 38.9 %



SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-008	Start time	09.05 hrs
Date of manufacture	12/09/17	End time	10.15 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	35
Sodium starch glycolate (g)	2
Tween® 80 (mL)	2.5
Polyethylene glycol 400 (mL)	5
Eudragit® RL 15 D (mL)	5
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

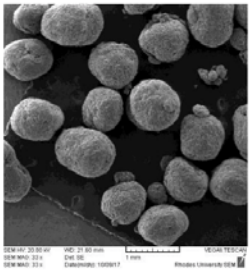
Process settings

Process	**Setting**
Dry blending speed	2
Dry blending time (min)	4
Extrusion speed (rpm)	35
Spheronization speed (rpm)	642
Spheronization time (min)	1
Drying time (hrs)	6

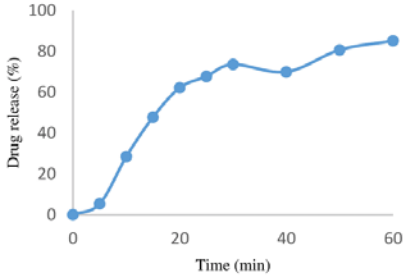
Characteristics

1. Aspect ratio – 1.15

2. Yield – 56.1 %



SEM profile



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-009	Start time	12.00 hrs
Date of manufacture	11/09/17	End time	13.05 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	25
Sodium starch glycolate (g)	2
Tween® 80 (mL)	5
Polyethylene glycol 400 (mL)	5
Eudragit® RL 15 D (mL)	5
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

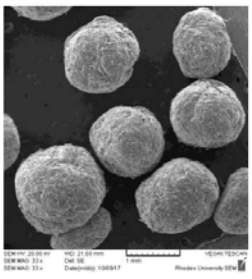
Process settings

Process	**Setting**
Dry blending speed	1
Dry blending time (min)	2
Extrusion speed (rpm)	35
Spheronization speed (rpm)	642
Spheronization time (min)	3
Drying time (hrs)	12

Characteristics

1. Aspect ratio – 1.11

2. Yield – 37.9 %



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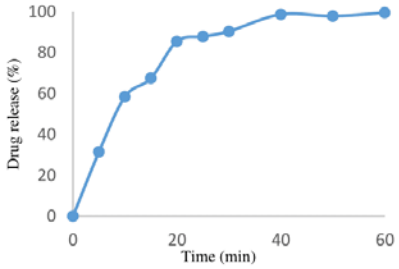

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-010	Start time	10.30 hrs
Date of manufacture	13/09/17	End time	11.35 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	35
Sodium starch glycolate (g)	1
Tween® 80 (mL)	5
Polyethylene glycol 400 (mL)	2.5
Eudragit® RL 15 D (mL)	5
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

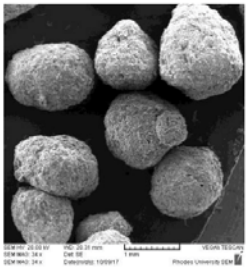
Process settings

Process	**Setting**
Dry blending speed	2
Dry blending time (min)	2
Extrusion speed (rpm)	35
Spheronization speed (rpm)	742
Spheronization time (min)	1
Drying time (hrs)	12

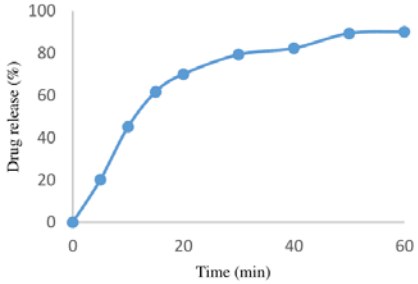
Characteristics

1. Aspect ratio – 1.21

2. Yield – 54.2 %



SEM images



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-011	Start time	10.30 hrs
Date of manufacture	12/09/17	End time	11.45 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	35
Sodium starch glycolate (g)	2
Tween® 80 (mL)	5
Polyethylene glycol 400 (mL)	2.5
Eudragit® RL 15 D (mL)	10
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

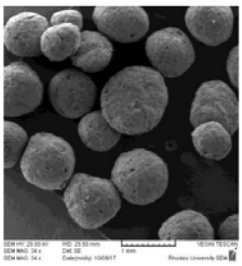
Process settings

Process	**Setting**
Dry blending speed	2
Dry blending time (min)	2
Extrusion speed (rpm)	25
Spheronization speed (rpm)	642
Spheronization time (min)	3
Drying time (hrs)	6

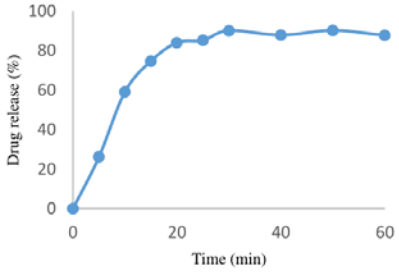
Characteristics

1. Aspect ratio – 1.12

2. Yield – 57.0 %



SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-PB-012	Start time	12.00 hrs
Date of manufacture	12/09/18	End time	13.15 hrs
Target strength/capsule	5 mg		

Formula

Material	**Amount added**
Prednisone (g)	2
Microcrystalline cellulose (g)	25
Sodium starch glycolate (g)	1
Tween® 80 (mL)	2.5
Polyethylene glycol 400 (mL)	5
Eudragit® RL 15 D (mL)	10
Talc (g)	0.5
Magnesium stearate (g)	0.5

*Granulation fluid - water

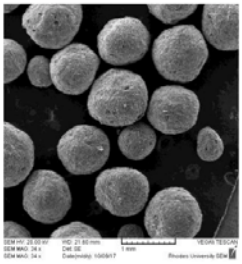
Process settings

Process	**Setting**
Dry blending speed	2
Dry blending time (min)	2
Extrusion speed (rpm)	35
Spheronization speed (rpm)	742
Spheronization time (min)	3
Drying time (hrs)	6

Characteristics

1. Aspect ratio – 1.09

2. Yield – 37.8 %



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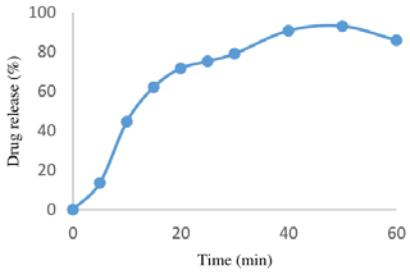

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APPENDIX - II

BATCH PRODUCTION RECORD

BOX-BEHNKEN OPTIMIZATION STUDIES

Formulation development, manufacture and assessment of all batches (Pred-BB-001 to Pred-BB-029) was undertaken at the Faculty of Pharmacy, Department of Pharmaceutics, Rhodes University using current Good Manufacturing Practice (cGMP) guidelines.

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-001	Target strength/capsule	5 mg
Date of manufacture	22/11/17	Start time	09.00 hrs
Batch size	50 g	End time	10.10 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	2	1
Tween® 80	12.8	6.40 mL
Polyethylene glycol 400	6.4	3.20 mL
Eudragit® RL 15 D	12.8	6.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

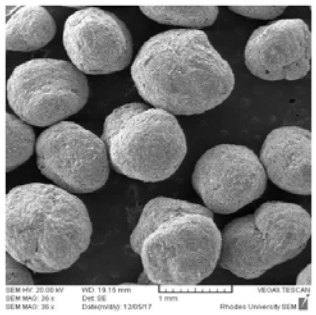
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	1 min

Characteristics

1. Aspect ratio – 1.09

2. Yield – 55.7 %



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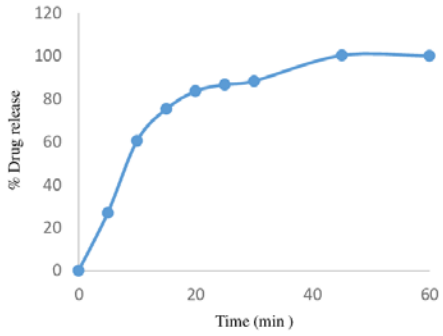

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-002	Target strength/capsule	5 mg
Date of manufacture	24/11/17	Start time	09.00 hrs
Batch number	Pred-BB-001	End time	10.00 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1	0.5
Tween® 80	13.2	6.60 mL
Polyethylene glycol 400	6.6	3.30 mL
Eudragit® RL 15 D	13.2	6.60 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

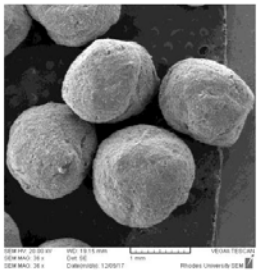
Process settings

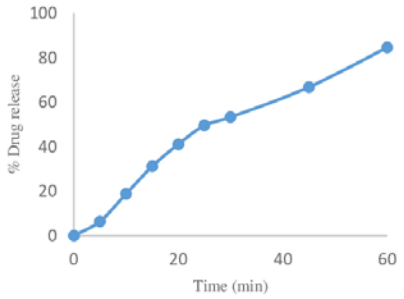
Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.07

2. Yield – 54.8 %





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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-003	Target strength/capsule	5 mg
Date of manufacture	24/11/17	Start time	10.30 hrs
Batch number	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1	0.5
Tween® 80	13.2	6.60 mL
Polyethylene glycol 400	6.6	3.30 mL
Eudragit® RL 15 D	13.2	6.60 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

Process settings

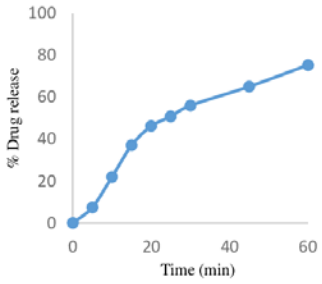
Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	1 min

Characteristics

1. Aspect ratio – 1.21

2. Yield – 58.3 %

SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-004	Target strength/capsule	5 mg
Date of manufacture	04/12/17	Start time	09.00 hrs
Batch number	50 g	End time	10.15 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	70	35
Sodium starch glycolate	1	0.5
Tween® 80	9.2	4.60 mL
Polyethylene glycol 400	4.6	2.30 mL
Eudragit® RL 15 D	9.2	4.60 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

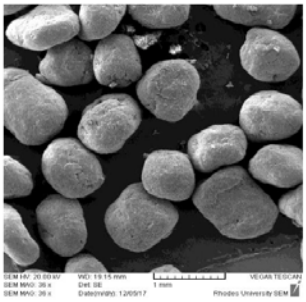
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 min
Spheronization speed	642 rpm
Spheronization time	2 min

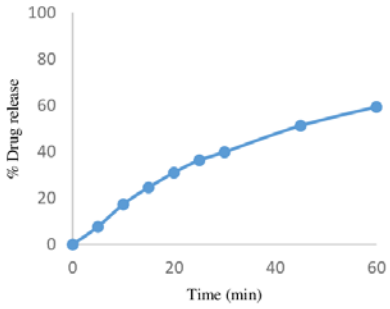
Characteristics

1. Aspect ratio – 2.10

2. Yield – 47.5 %



SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-005	Target strength/capsule	5 mg
Date of manufacture	29/11/17	Start time	09.00 hrs
Batch size	50 g	End time	10.15 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	70	35
Sodium starch glycolate	1.5	0.75
Tween® 80	9	4.50 mL
Polyethylene glycol 400	4.5	2.25 mL
Eudragit® RL 15 D	9	4.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

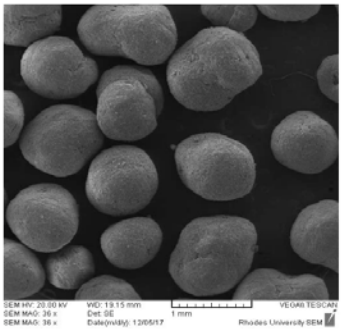
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.19

2. Yield – 58.4 %



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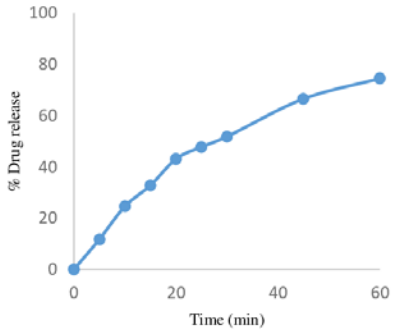

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-006	Target strength/capsule	5 mg
Date of manufacture	28/11/17	Start time	09.00 hrs
Batch size	50 g	End time	10.05 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	50	25
Sodium starch glycolate	1.5	0.75
Tween® 80	17	8.50 mL
Polyethylene glycol 400	8.5	4.25 mL
Eudragit® RL 15 D	17	8.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

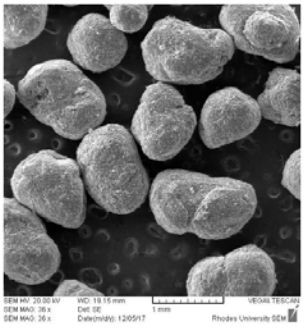
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	1 min

Characteristics

1. Aspect ratio – 1.40

2. Yield – 41.2 %



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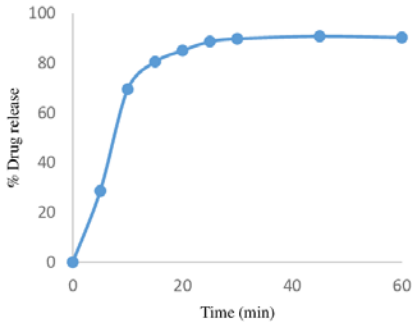

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-007	Target strength/capsule	5 mg
Date of manufacture	22/11/17	Start time	10.30 hrs
Batch size	50 g	End time	11.30 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	2	1
Tween® 80	12.8	6.40 mL
Polyethylene glycol 400	6.4	3.20 mL
Eudragit® RL 15 D	12.8	6.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

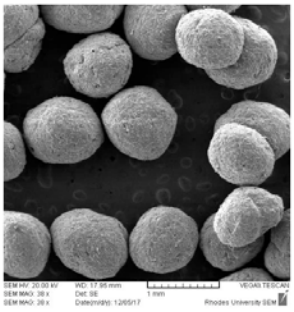
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	3 min

Characteristics

1. Aspect ratio – 1.08

2. Yield – 56.4 %



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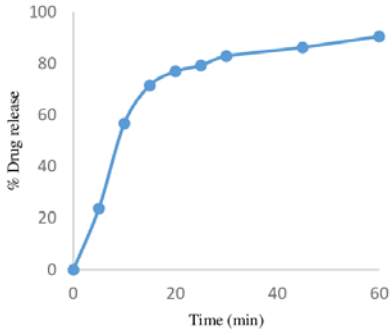

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-008	Target strength/capsule	5 mg
Date of manufacture	22/11/17	Start time	12.00 hrs
Batch size	50 g	End time	13.05 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	2	1
Tween® 80	12.8	6.40 mL
Polyethylene glycol 400	6.4	3.20 mL
Eudragit® RL 15 D	12.8	6.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

Process settings

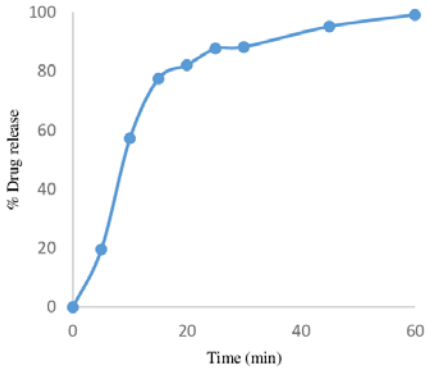
Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.09

2. Yield – 52.5 %

SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-009	Target strength/capsule	5 mg
Date of manufacture	26/11/17	Start time	09.00 hrs
Batch size	50 g	End time	10.00 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

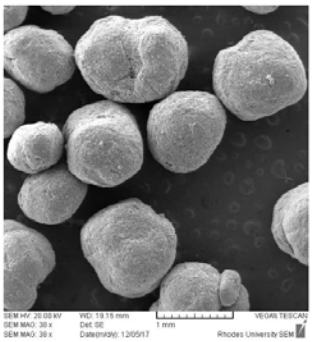
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.16

2. Yield – 58.9 %



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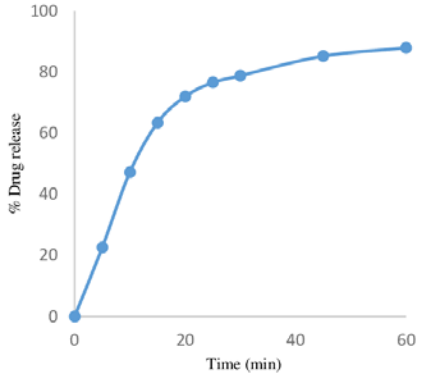

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-010	Target strength/capsule	5 mg
Date of manufacture	26/11/17	Start time	10.30 hrs
Batch size	50 g	End time	11.30 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

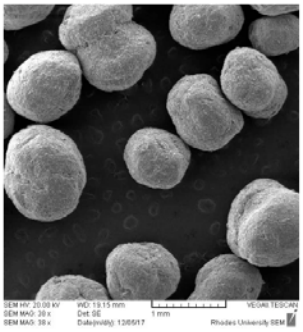
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.17

2. Yield – 63.2 %



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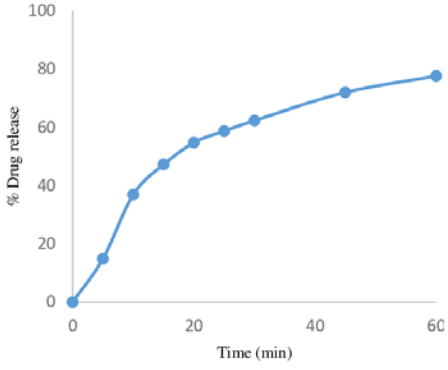

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-011	Target strength/capsule	5 mg
Date of manufacture	29/11/17	Start time	10.30 hrs
Batch size	50 g	End time	11.35 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	70	35
Sodium starch glycolate	1.5	0.75
Tween® 80	9	4.50 mL
Polyethylene glycol 400	4.5	2.25 mL
Eudragit® RL 15 D	9	4.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

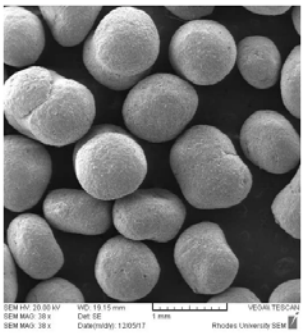
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	3 min

Characteristics

1. Aspect ratio – 1.19

2. Yield – 73.9 %



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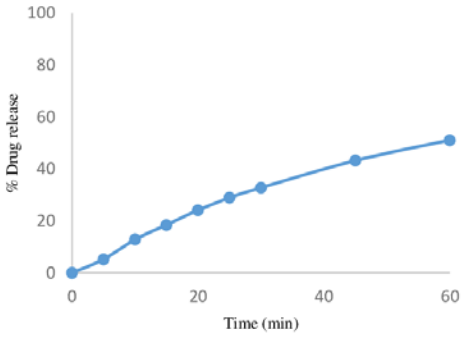

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-012	Target strength/capsule	5 mg
Date of manufacture	03/12/17	Start time	09.00 hrs
Batch size	50 g	End time	10.15 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	50	25
Sodium starch glycolate	2	1
Tween® 80	16.8	8.40 mL
Polyethylene glycol 400	8.4	4.20 mL
Eudragit® RL 15 D	16.8	8.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

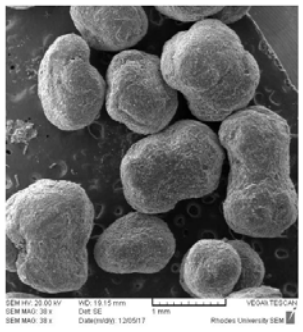
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.53

2. Yield – 34.3 %



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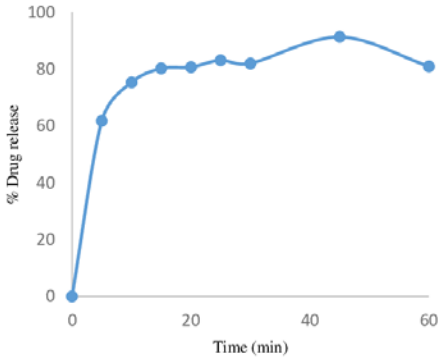

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-013	Target strength/capsule	5 mg
Date of manufacture	26/11/17	Start time	12.00 hrs
Batch size	50 g	End time	13.10 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

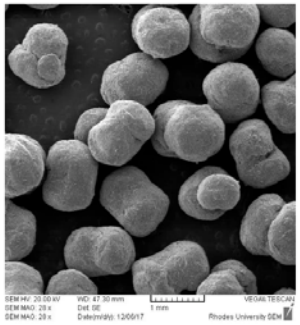
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

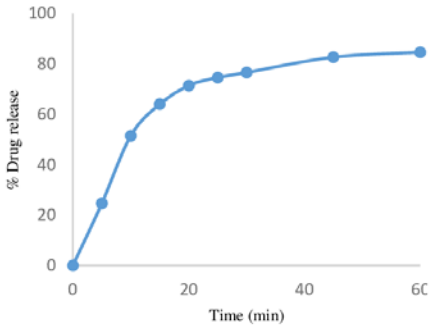
Characteristics

1. Aspect ratio – 1.45

2. Yield – 60.8 %



SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-014	Target strength/capsule	5 mg
Date of manufacture	26/11/17	Start time	14.00 hrs
Batch size	50 g	End time	15.00 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

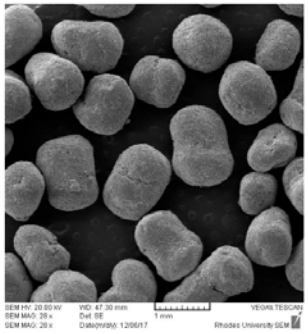
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	35 rpm
Spheronization speed	642 rpm
Spheronization time	1 min

Characteristics

1. Aspect ratio – 1.27

2. Yield – 60.4 %



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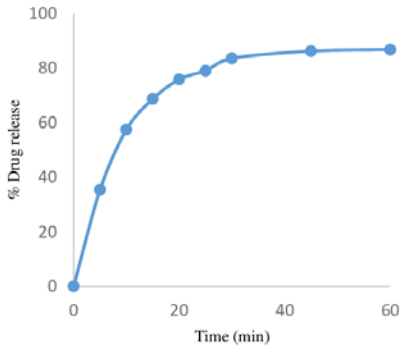

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-015	Target strength/capsule	5 mg
Date of manufacture	26/11/17	Start time	15.15 hrs
Batch size	50 g	End time	16.30 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

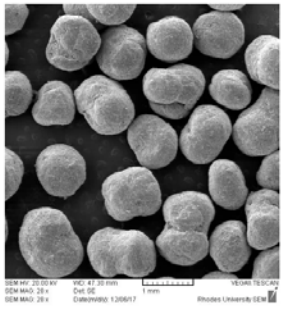
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.27

2. Yield – 62.8 %



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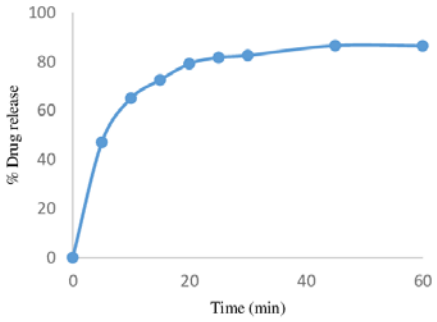

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-016	Target strength/capsule	5 mg
Date of manufacture	26/11/17	Start time	17.00 hrs
Batch size	50 g	End time	18.05 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

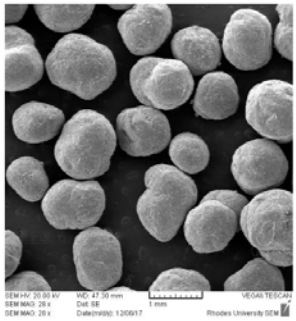
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	35 rpm
Spheronization speed	642 rpm
Spheronization time	3 min

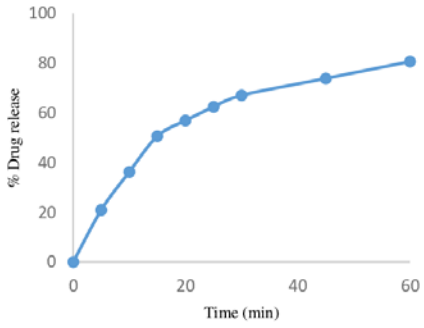
Characteristics

1. Aspect ratio – 1.24

2. Yield – 79.3 %



SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-017	Target strength/capsule	5 mg
Date of manufacture	22/11/17	Start time	13.30 hrs
Batch size	50 g	End time	14.35 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	2	1
Tween® 80	12.8	6.40 mL
Polyethylene glycol 400	6.4	3.20 mL
Eudragit® RL 15 D	12.8	6.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

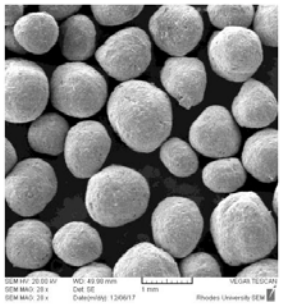
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	35 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.11

2. Yield – 56.0 %



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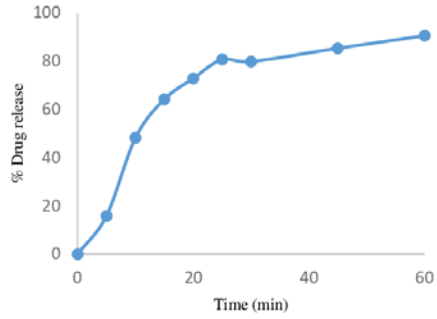

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-018	Target strength/capsule	5 mg
Date of manufacture	25/11/17	Start time	09.00 hrs
Batch size	50 g	End time	10.00 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1	0.5
Tween® 80	13.2	6.60 mL
Polyethylene glycol 400	6.6	3.30 mL
Eudragit® RL 15 D	13.2	6.60 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

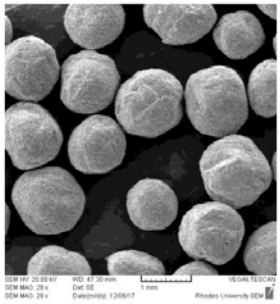
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	3 min

Characteristics

1. Aspect ratio – 1.08

2. Yield – 55.7 %



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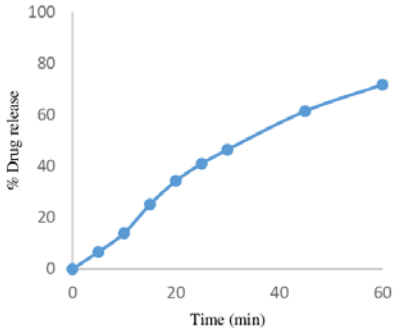

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-019	Target strength/capsule	5 mg
Date of manufacture	04/12/17	Start time	10.30 hrs
Batch size	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	50	25
Sodium starch glycolate	1	0.5
Tween® 80	17.2	8.60 mL
Polyethylene glycol 400	8.6	4.30 mL
Eudragit® RL 15 D	17.2	8.60 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

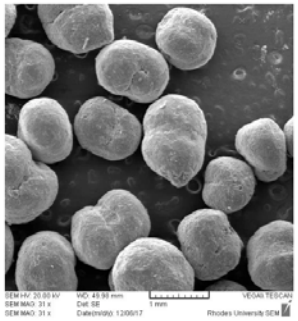
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.25

2. Yield – 39.2 %



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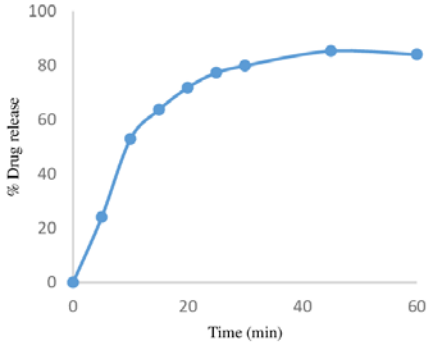

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-020	Target strength/capsule	5 mg
Date of manufacture	28/11/17	Start time	10.30 hrs
Batch size	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	50	25
Sodium starch glycolate	1.5	0.75
Tween® 80	17	8.50 mL
Polyethylene glycol 400	8.5	4.25 mL
Eudragit® RL 15 D	17	8.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

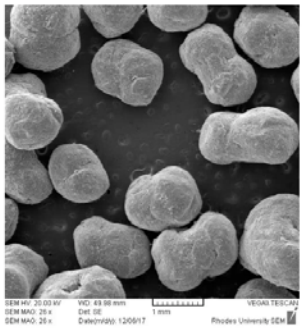
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.53

2. Yield – 70.9 %



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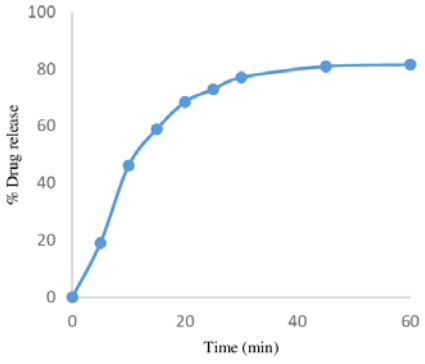

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-021	Target strength/capsule	5 mg
Date of manufacture	29/11/17	Start time	12.00 hrs
Batch size	50 g	End time	13.10 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	50	25
Sodium starch glycolate	1.5	0.75
Tween® 80	17	8.50 mL
Polyethylene glycol 400	8.5	4.25 mL
Eudragit® RL 15 D	17	8.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

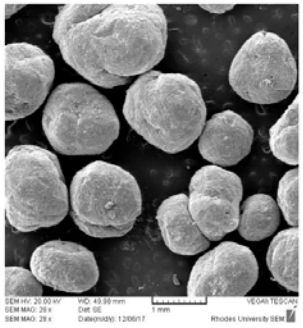
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	35 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.17

2. Yield – 36.4 %



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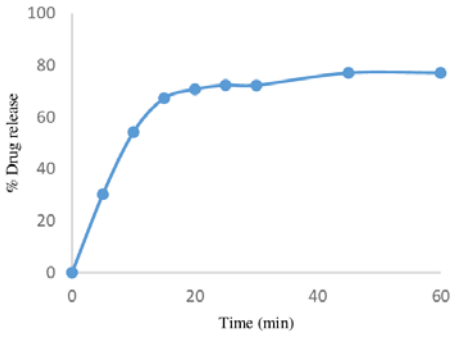

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-022	Target strength/capsule	5 mg
Date of manufacture	29/11/17	Start time	14.00 hrs
Batch size	50 g	End time	15.00 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	50	25
Sodium starch glycolate	1.5	0.75
Tween® 80	17	8.50 mL
Polyethylene glycol 400	8.5	4.25 mL
Eudragit® RL 15 D	17	8.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

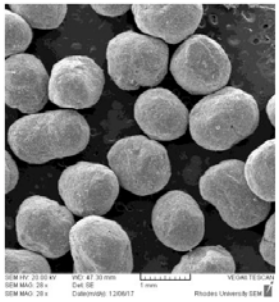
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	3 min

Characteristics

1. Aspect ratio – 1.15

2. Yield – 72.4 %



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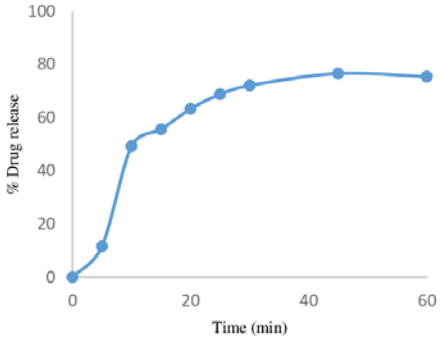

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-023	Target strength/capsule	5 mg
Date of manufacture	29/11/17	Start time	15.15 hrs
Batch size	50 g	End time	16.20 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	70	35
Sodium starch glycolate	1.5	0.75
Tween® 80	9	4.50 mL
Polyethylene glycol 400	4.5	2.25 mL
Eudragit® RL 15 D	9	4.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

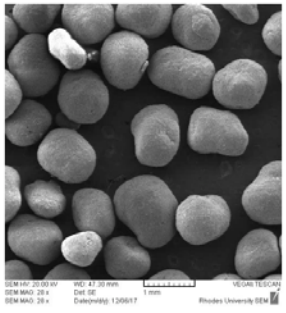
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	35 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.28

2. Yield – 74.1 %



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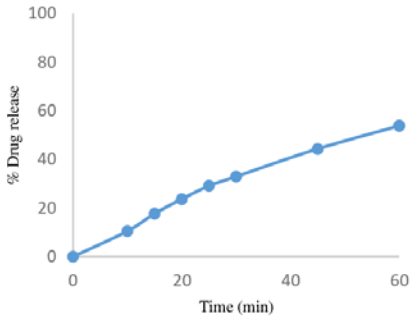

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-024	Target strength/capsule	5 mg
Date of manufacture	29/11/17	Start time	16.45 hrs
Batch size	50 g	End time	18.00 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	70	35
Sodium starch glycolate	1.5	0.75
Tween® 80	9	4.50 mL
Polyethylene glycol 400	4.5	2.25 mL
Eudragit® RL 15 D	9	4.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

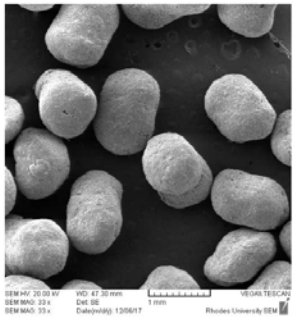
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	1 min

Characteristics

1. Aspect ratio – 1.45 %

2. Yield – 78.3 %



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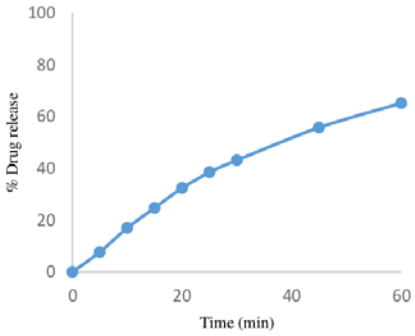

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-025	Target strength/capsule	5 mg
Date of manufacture	27/11/17	Start time	09.00 hrs
Batch size	50 g	End time	10.05 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

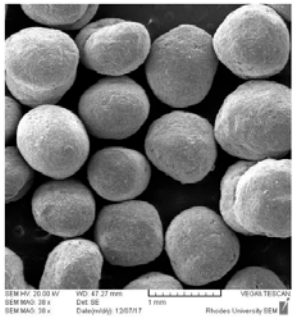
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	3 min

Characteristics

1. Aspect ratio – 1.09

2. Yield – 62.1 %



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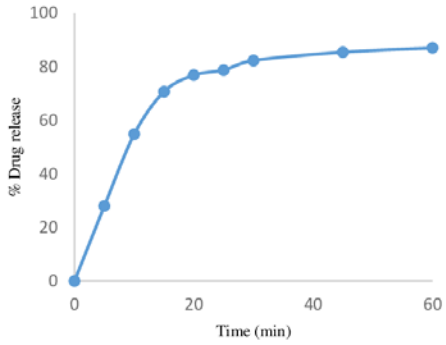

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-026	Target strength/capsule	5 mg
Date of manufacture	04/12/17	Start time	12.00 hrs
Batch size	50 g	End time	13.05 hrs

Formula

Material	**% w/w**	**Amount added**
Prednisone	4	2
Microcrystalline cellulose	70	35
Sodium starch glycolate	2	1
Tween® 80	8.8	4.40 mL
Polyethylene glycol 400	4.4	2.20 mL
Eudragit® RL 15 D	8.8	4.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

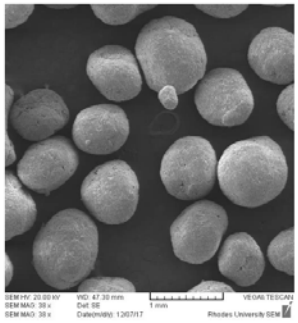
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.10

2. Yield – 56.7 %



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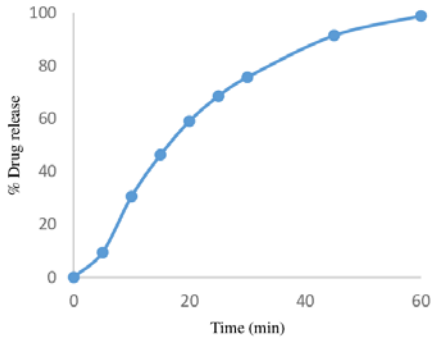

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-027	Target strength/capsule	5 mg
Date of manufacture	27/11/17	Start time	10.30 hrs
Batch size	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

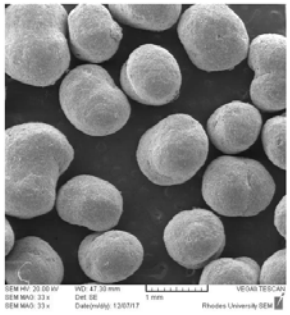
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	1 min

Characteristics

1. Aspect ratio – 1.20

2. Yield – 76.9 %



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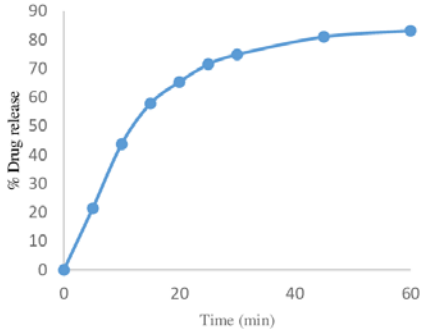

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-028	Target strength/capsule	5 mg
Date of manufacture	25/11/17	Start time	10.30 hrs
Batch size	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1	0.5
Tween® 80	13.2	6.60 mL
Polyethylene glycol 400	6.6	3.30 mL
Eudragit® RL 15 D	13.2	6.60 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

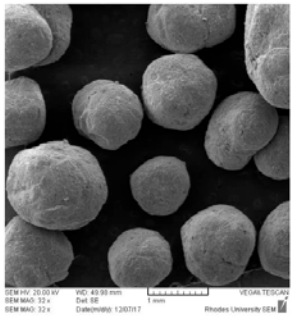
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	35 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.12

2. Yield – 60.9 %



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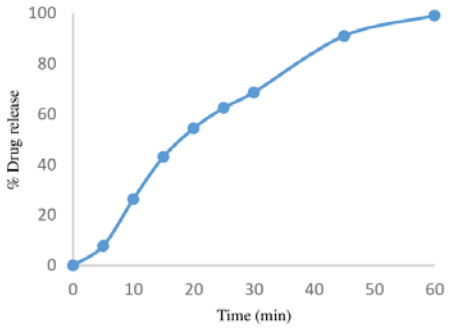

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	Pred-BB-029	Target strength/capsule	5 mg
Date of manufacture	03/12/17	Start time	10.30 hrs
Batch size	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4	2
Microcrystalline cellulose	60	30
Sodium starch glycolate	1.5	0.75
Tween® 80	13	6.50 mL
Polyethylene glycol 400	6.5	3.25 mL
Eudragit® RL 15 D	13	6.50 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

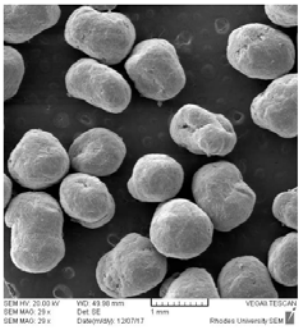
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	30 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.18

2. Yield – 63.5 %



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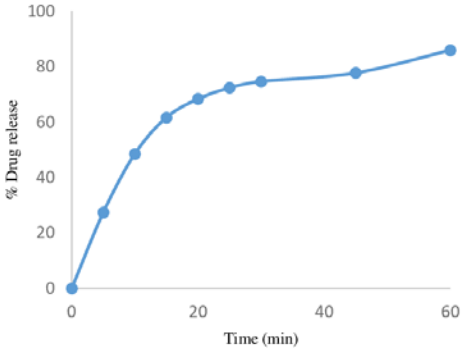

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APPENDIX - III
BATCH PRODUCTION RECORD
DESIGN SPACE

Formulation development, manufacture and assessment of all batches (OPT 1-3, DS-LL 1-3 and DS-UL 1-3) was undertaken at the Faculty of Pharmacy, Department of Pharmaceutics, Rhodes University using current Good Manufacturing Practice (cGMP) guidelines.

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	OPT-01	Target strength/capsule	5 mg
Date of manufacture	07/12/17	Start time	09.00 hrs
Batch size	50 g	End time	10.05 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	60.0	30.0
Sodium starch glycolate	2.0	1.0
Tween® 80	12.8	6.40 mL
Polyethylene glycol 400	6.4	3.20 mL
Eudragit® RL 15 D	12.8	6.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

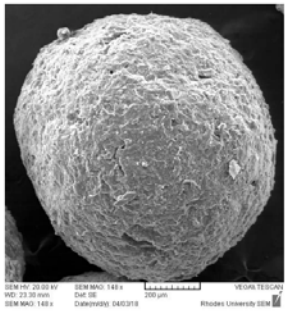
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.12

2. Yield – 70.20 %



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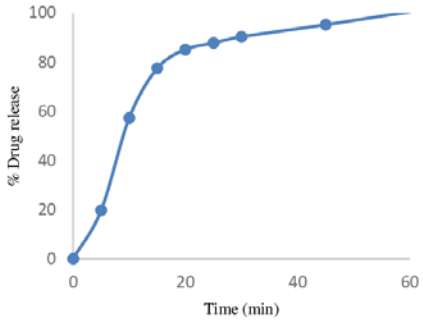

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	OPT-02	Target strength/capsule	5 mg
Date of manufacture	07/12/17	Start time	10.30 hrs
Batch size	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	60.0	30.0
Sodium starch glycolate	2.0	1.0
Tween® 80	12.8	6.40 mL
Polyethylene glycol 400	6.4	3.20 mL
Eudragit® RL 15 D	12.8	6.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

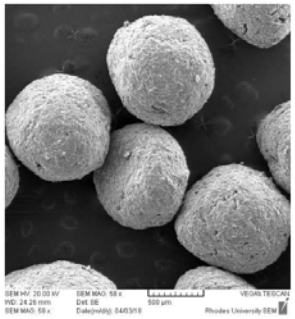
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.11

2. Yield – 69.34 %



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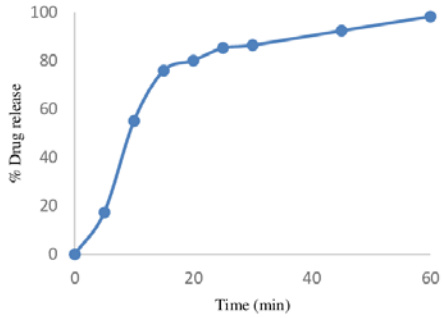

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	OPT-03	Target strength/capsule	5 mg
Date of manufacture	07/12/17	Start time	12.00 hrs
Batch size	50 g	End time	13.10 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	60.0	30.0
Sodium starch glycolate	2.0	1.0
Tween® 80	12.8	6.40 mL
Polyethylene glycol 400	6.4	3.20 mL
Eudragit® RL 15 D	12.8	6.40 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

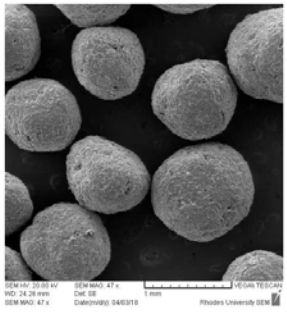
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	2 min

Characteristics

1. Aspect ratio – 1.09

2. Yield – 71.25 %



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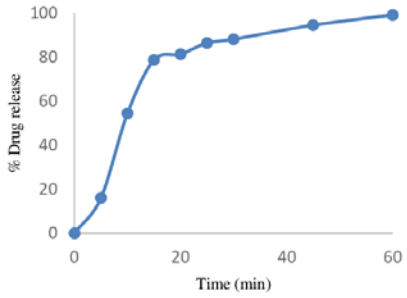

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	DS-LL-01	Target strength/capsule	5 mg
Date of manufacture	08/12/17	Start time	09.00 hrs
Batch size	50 g	End time	10.15 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	58.0	29.0
Sodium starch glycolate	1.9	0.95
Tween® 80	13.64	6.82 mL
Polyethylene glycol 400	6.82	3.41 mL
Eudragit® RL 15 D	13.64	6.82 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

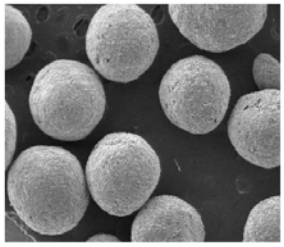
*Granulation fluid - water

Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	1.4 min

Characteristics

1. Aspect ratio – 1.08
2. Yield – 56.72 %



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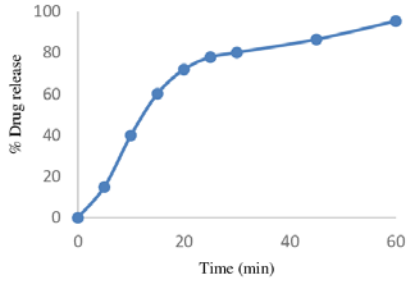

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	DS-LL-02	Target strength/capsule	5 mg
Date of manufacture	08/12/17	Start time	10.30 hrs
Batch size	50 g	End time	11.30 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	58.0	29.0
Sodium starch glycolate	1.9	0.95
Tween® 80	13.64	6.82 mL
Polyethylene glycol 400	6.82	3.41 mL
Eudragit® RL 15 D	13.64	6.82 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

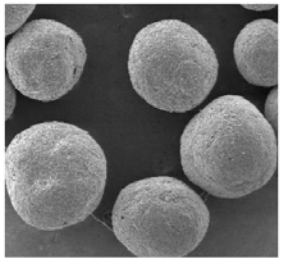
*Granulation fluid - water

Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	1.4 min

Characteristics

1. Aspect ratio – 1.07
2. Yield – 55.83 %



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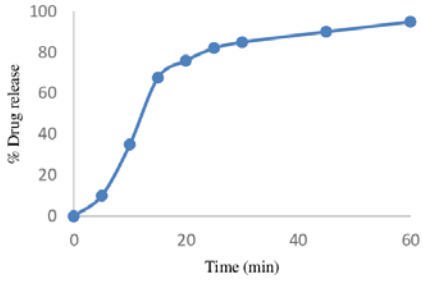

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	DS-LL-03	Target strength/capsule	5 mg
Date of manufacture	08/12/17	Start time	12.00 hrs
Batch size	50 g	End time	13.05 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	58.0	29.0
Sodium starch glycolate	1.9	0.95
Tween® 80	13.64	6.82 mL
Polyethylene glycol 400	6.82	3.41 mL
Eudragit® RL 15 D	13.64	6.82 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

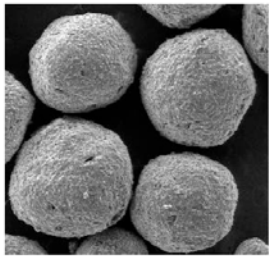
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	25 rpm
Spheronization speed	642 rpm
Spheronization time	1.4 min

Characteristics

1. Aspect ratio – 1.09

2. Yield – 59.33 %



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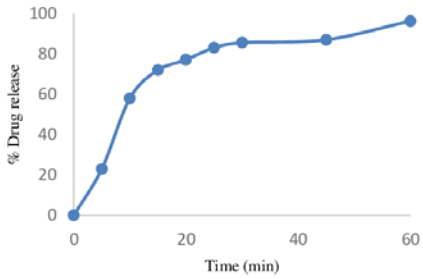

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	DS-UL-01	Target strength/capsule	5 mg
Date of manufacture	09/12/17	Start time	09.00 hrs
Batch size	50 g	End time	10.00 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	62.0	31.0
Sodium starch glycolate	2.0	1.0
Tween® 80	12.0	6.0 mL
Polyethylene glycol 400	6.0	3.0 mL
Eudragit® RL 15 D	12.0	6.0 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

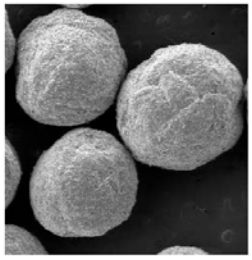
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	26 rpm
Spheronization speed	642 rpm
Spheronization time	2.2 min

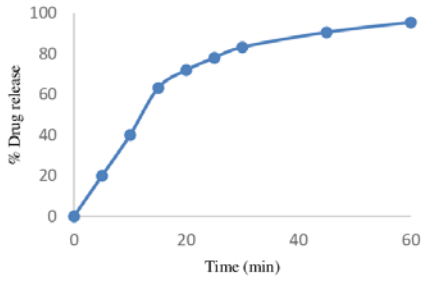
Characteristics

1. Aspect ratio – 1.13

2. Yield – 70.12 %



SEM image



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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	DS-UL-02	Target strength/capsule	5 mg
Date of manufacture	09/12/17	Start time	10.30 hrs
Batch size	50 g	End time	11.45 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	62.0	31.0
Sodium starch glycolate	2.0	1.0
Tween® 80	12.0	6.0 mL
Polyethylene glycol 400	6.0	3.0 mL
Eudragit® RL 15 D	12.0	6.0 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

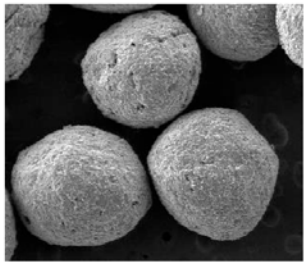
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	26 rpm
Spheronization speed	642 rpm
Spheronization time	2.2 min

Characteristics

1. Aspect ratio – 1.11

2. Yield – 69.50 %



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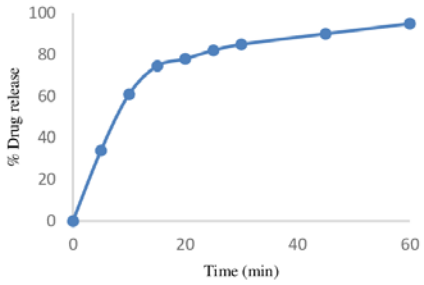

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BATCH PRODUCTION RECORD

Formulator	**Arthur Manda**	**Product**	**Prednisone pellets**
Batch number	DS-UL-03	Target strength/capsule	5 mg
Date of manufacture	09/12/17	Start time	12.00 hrs
Batch size	50 g	End time	13.15 hrs

Formula

Material	**% w/w**	**Amount added (g)**
Prednisone	4.0	2.0
Microcrystalline cellulose	62.0	31.0
Sodium starch glycolate	2.0	1.0
Tween® 80	12.0	6.0 mL
Polyethylene glycol 400	6.0	3.0 mL
Eudragit® RL 15 D	12.0	6.0 mL
Talc	1.5	0.75
Magnesium stearate	0.5	0.25

*Granulation fluid - water

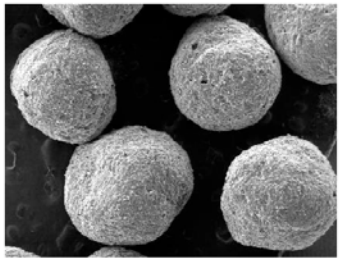
Process settings

Process	**Setting**
Dry blending speed	Level 2
Dry blending time	4 min
Extrusion speed	26 rpm
Spheronization speed	642 rpm
Spheronization time	2.2 min

Characteristics

1. Aspect ratio – 1.09

2. Yield – 68.98 %



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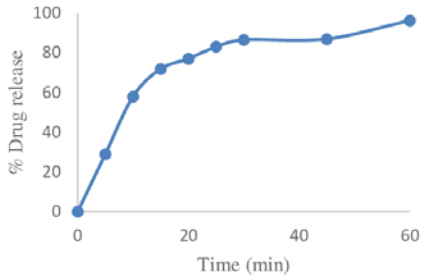

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APPENDIX IV
ANOVA

ANOVA data, 3D response surface and contour plots for aspect ratio, yield and prednisone released at 15, 45 and 60 min. These models were generated using Design expert software (Version 8.0.7.1, State-Ease Inc., Minneapolis, MN, USA) during optimization studies.

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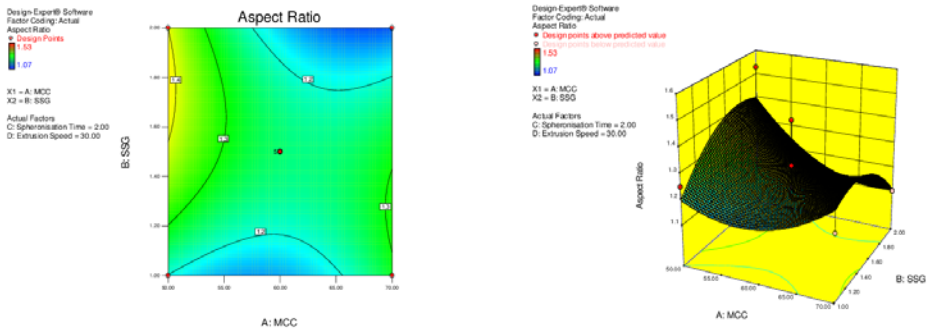
ASPECT RATIO

ANOVA data for the aspect ratio of prednisone pellets

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob > F
Model	0.33	14	0.024	1.94	0.1144
X_1	0.032	1	0.032	2.60	0.1293
X_2	$4.083e^{-004}$	1	$4.083e^{-004}$	0.033	0.8582
X_3	0.052	1	0.052	4.22	0.0592
X_4	$3.333e^{-005}$	1	$3.333e^{-005}$	$2.703e^{-003}$	0.9593
X_1X_2	0.036	1	0.036	2.93	0.1091
X_1X_3	$2.500e^{-005}$	1	$2.500e^{-005}$	$2.027e^{-003}$	0.9647
X_1X_4	0.051	1	0.051	4.11	0.0622
X_2X_3	$3.600e^{-003}$	1	$3.600e^{-003}$	0.29	0.5975
X_2X_4	$2.250e^{-004}$	1	$2.250e^{-004}$	0.018	0.8945
X_3X_4	$1.600e^{-003}$	1	$1.600e^{-003}$	0.13	0.7241
X_1^2	0.058	1	0.058	4.74	0.0471
X_2^2	0.057	1	0.057	4.63	0.0493
X_3^2	$5.393e^{-003}$	1	$5.393e^{-003}$	0.44	0.5192
X_4^2	0.010	1	0.010	0.85	0.3735
Residual	0.17	14	0.012		
Lack of Fit	0.11	10	0.011	0.76	0.6732
Pure Error	0.060	4	0.015		
Cor Total	0.51	28			



Contour and 3D plots for the effects of microcrystalline cellulose and sodium starch glycolate on aspect ratio

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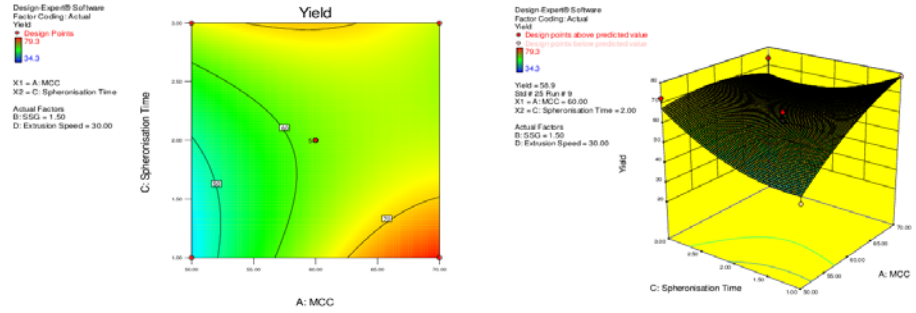
YIELD

ANOVA data for the yield of prednisone pellets

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob > F
Model	3659.10	14	261.36	10.47	<0.0001
X_1	744.19	1	744.19	29.80	<0.0001
X_2	1.92	1	1.92	0.077	0.7856
X_3	70.08	1	70.08	2.81	0.1161
X_4	6.02	1	6.02	0.24	0.6310
X_1X_2	49.70	1	49.70	1.99	0.1801
X_1X_3	316.84	1	316.84	12.69	0.0031
X_1X_4	630.01	1	630.01	25.23	0.0002
X_2X_3	2.72	1	2.72	0.11	0.7461
X_2X_4	1.69	1	1.69	0.068	0.7985
X_3X_4	283.92	1	283.92	11.37	0.0046
X_1^2	122.93	1	122.93	4.92	0.0435
X_2^2	823.26	1	823.26	32.97	<0.0001
X_3^2	279.07	1	279.07	11.18	0.0048
X_4^2	61.20	1	61.20	2.45	0.1398
Residual	349.59	14	24.97		
Lack of Fit	334.33	10	33.43	8.77	0.0254
Pure Error	15.25	4	3.81		
Cor Total	4008.69	28			



Contour and 3D plots for the effects of microcrystalline cellulose and spheronization time on pellet yield

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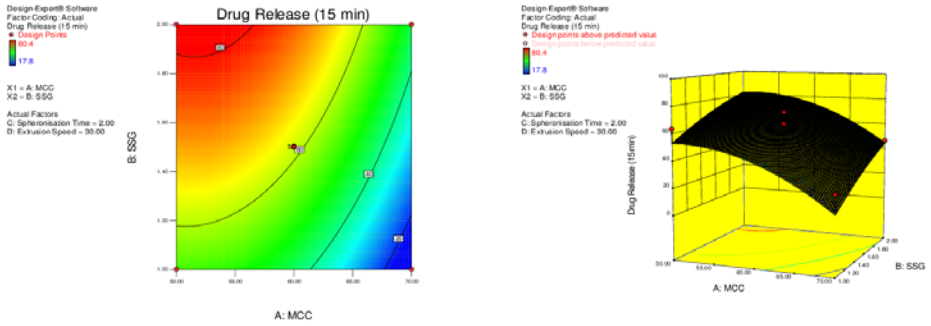
PREDNISONE RELEASE (15 MIN)

ANOVA data for prednisone release at 15 min

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob > F
Model	9604.59	14	686.04	7.97	0.0002
X_1	4852.14	1	4852.14	56.34	<0.0001
X_2	3014.67	1	3014.67	35.00	<0.0001
X_3	224.47	1	224.47	2.61	0.1288
X_4	24.08	1	24.08	0.28	0.6052
X_1X_2	7.02	1	7.02	0.082	0.7794
X_1X_3	86.49	1	86.49	1.00	0.3333
X_1X_4	136.89	1	136.89	1.59	0.2280
X_2X_3	16.81	1	16.81	0.20	0.6654
X_2X_4	162.56	1	162.56	1.89	0.1911
X_3X_4	235.62	1	235.62	2.74	0.1204
X_1^2	835.11	1	835.11	9.70	0.0076
X_2^2	48.49	1	48.49	0.56	0.4655
X_3^2	65.25	1	65.25	0.76	0.3988
X_4^2	44.16	1	44.16	0.51	0.4857
Residual	1205.82	14	86.13		
Lack of Fit	868.58	10	86.86	1.03	0.5349
Pure Error	337.23	4	84.31		
Cor Total	10810.41	28			



Contour and 3D plots for the effects of microcrystalline cellulose and sodium starch glycolate on prednisone release at 15 min

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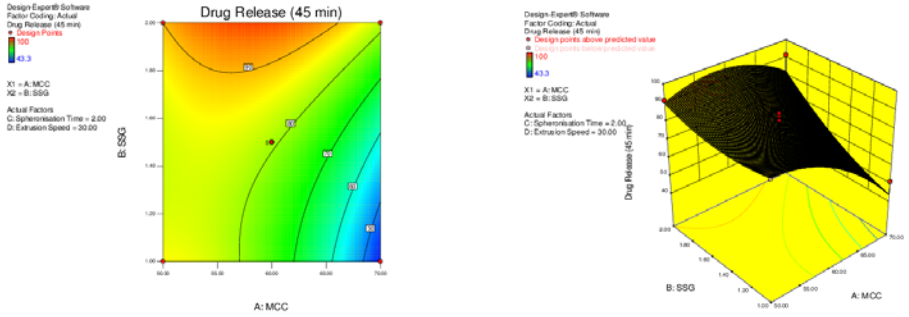
PREDNISONE RELEASE (45 MIN)

ANOVA data for prednisone release at 45 min

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob > F
Model	5218.39	14	372.74	5.27	0.0018
X_1	1852.57	1	1852.57	26.20	0.0002
X_2	1373.88	1	1373.88	19.43	0.0006
X_3	224.47	1	224.47	3.17	0.0965
X_4	25.81	1	25.81	0.37	0.5554
X_1X_2	292.41	1	292.41	4.13	0.0614
X_1X_3	0.64	1	0.64	$9.050e^{-003}$	0.9256
X_1X_4	83.72	1	83.72	1.18	0.2949
X_2X_3	26.01	1	26.01	0.37	0.5539
X_2X_4	292.41	1	292.41	4.13	0.0614
X_3X_4	69.72	1	69.72	0.99	0.3376
X_1^2	638.85	1	638.85	9.03	0.0094
X_2^2	133.60	1	133.60	1.89	0.1909
X_3^2	82.86	1	82.86	1.17	0.2973
X_4^2	$8.721e^{-003}$	1	$8.721e^{-003}$	$1.233e^{-004}$	0.9913
Residual	990.07	14	70.72		
Lack of Fit significant	844.26	10	84.43	2.32	0.2171
Pure Error	145.81	4	36.45		
Cor Total	6208.46	28			



Contour and 3D plots for the effects of microcrystalline cellulose and sodium starch glycolate on prednisone release at 45 min

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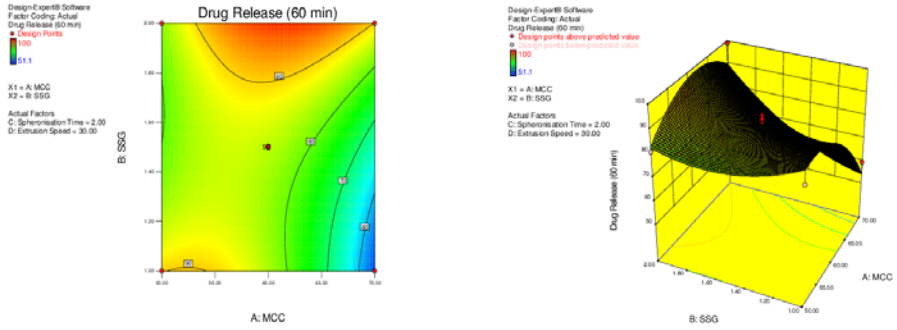
PREDNISONE RELEASE (60 MIN)

ANOVA data for prednisone release at 60 min

Source	Sum of Squares	df	Mean Square	F-value	P-value Prob > F
Model	3566.77	14	254.77	4.39	0.0046
X_1	616.33	1	616.33	10.61	0.0057
X_2	613.47	1	613.47	10.56	0.0058
X_3	161.33	1	161.33	2.78	0.1178
X_4	38.88	1	38.88	0.67	0.4270
X_1X^2	455.82	1	455.82	7.85	0.0142
X_1X_3	0.12	1	0.12	$2.108e^{-003}$	0.9640
X_1X_4	67.24	1	67.24	1.16	0.3002
X_2X_3	9.00	1	9.00	0.15	0.6998
X_2X_4	135.72	1	135.72	2.34	0.1487
X_3X_4	23.52	1	23.52	0.40	0.5349
X_1^2	872.07	1	872.07	15.01	0.0017
X_2^2	243.74	1	243.74	4.20	0.0598
X_3^2	80.37	1	80.37	1.38	0.2591
X_4^2	17.77	1	17.77	0.31	0.5890
Residual	813.39	14	58.10		
Lack of Fit	748.17	10	74.82	4.59	0.0776
Pure Error	65.21	4	16.30		
Cor Total	4380.16	28			



Contour and 3D plots for the effects of microcrystalline cellulose and sodium starch glycolate on prednisone release at 60 min

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