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FORMULATION AND EVALUATION OF *CHANNA STRIATUS* EXTRACT AND FUSIDIC ACID AEROSOL FOR TREATMENT OF WOUNDS AND BURN

by

FEBRIYENTI

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FEBRIYENTI

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

Page

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Description
AA	arachidonic acid
AABA	alfa amino butyric acid
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
AQC	aminoquinolyl-N-hydroxysuccinimidyl carbamate
Asc	ascending curve of rheology
BHA	butylated hydroxyanisole
BITC	butylisothiocyanate
C.	Channa
^{0}C	Degree Celsius
CDER	Centre for Drug Evaluation and Research
CFCs	chlorofluorocarbons
cm	centimeter
CMC	carboxymethylcellulose
cps	centipoise
CV	correlation variance
DCM	dichloromethane
Desc	descending curve of rheology
DHA	docosahexaenoic acid
DME	dimethyl ether
DNA	deoxyribonucleic acid
DOT	Department of Transportation
DPA	docosapentaenoic acid
ED	edema
EPA	Enviromental Protection Agency
ER	erythema
ETA	eicosatrienoic acid
FAME	fatty acid methyl ester
FDA	Food and Drug regulation Authority
FID	flame ionization detector

Abbreviation	**Description**
FMOC-Cl	9-fluorenylmethyl-chloroformate
GC	gas chromatography
HC	hydrocarbon
HFA	hydrofluoroalkanes
HFC	hydrofluorocarbon
HPLC	high performance liquid chromatography
HPMC	hydroxypropyl methylcellulose
ICH	International Committee on Harmonisation
ID	inner diameter
kg	kilogram
LA	linoleic acid
LOD	limit of detection
LOQ	limit of quantification
LPG	liquefied petroleum gas
LVI	limited volume insert
M	molar
MDIs	metered dose inhalers
mg	milligram
min.	minute
ml	millilitre
mm	millimetre
mN	milli Newton
MUFA	monounsaturated fatty acid
µm	micrometer
N	normal; number of replicate/samples; Newton
NBD-F	4-fluoro-7-nitrobenzo-2-oxa-1,3-diazole
ng	nanogram
nm	nanometre
NO	nitric oxide
OPA	o-phthaldialdehyde
Pa	pascal

Abbreviation	Description
PDGF	platelet-derived growth factor
PEG	polyethylene glycol
PGE_2	prostaglandin E_2
pH	power of hydrogen ion concentration
PII	primary irritation index
PITC	phenylisothiocyanate
pmol	picomole
ppm	part per million
psig	pounds-force per square inch gauge
PUFA	polyunsaturated fatty acid
R^2	correlation coefficient
RH	relative humidity
rpm	rotation per minute
RSD	relative standard deviation
SD	standard deviation; Sprague-dawley
sec.	second
TGF-β	transforming growth factor beta
w/v	weight/volume
WVP	water vapour permeability
WVT	water vapour transmission
w/w	weight/weight

FORMULASI DAN PENILAIAN AEROSOL EKSTRAK *CHANNA STRIATUS* DAN ASID FUSIDIK UNTUK RAWATAN LUKA DAN LUKA KEBAKARAN

ABSTRAK

Aerosol ialah sistem penyampaian drug yang baru untuk pembalutan dan perawatan luka. Aerosol mempunyai beberapa manfaat apabila dibandingkan dengan sistem penyampaian yang lain seperti plaster dan balutan untuk pembalutan luka dan krim atau gel untuk perawatan luka. Aerosol pada badan dapat mengurangkan kesakitan yang disebabkan kesan gosokan mekanikal salap dan krim pada kulit. Aerosol mampu meliputi pelbagai saiz luka yang tidak mampu diliputi oleh filem kerana filem mempunyai saiz yang tetap. *Channa striatus* telah digunakan secara tradisional sejak dahulu untuk mempercepatkan penyembuhan luka. Ekstrak *Channa striatus* mengandungi asid amino dan asid lemak yang penting untuk proses penyembuhan. Asid fusidik ialah agen antimikrob untuk applikasi permukaan. Ekstrak *Channa striatus* dan campurannya dengan asid fusidik telah digunakan sebagai bahan aktif dalam kajian ini. Ekstrak *Channa striatus* mengandungi kira-kira 3.61% protein. Sistem HPLC untuk kaedah analisis asid amino dan sistem GC untuk kaedah analisis asid lemak yang digunakan dalam kajian ini telah mencapai keperluan validasi. Sistem HPLC ini boleh dipercayai dan boleh dihasilkan semula. Ekstrak *Channa striatus* mengandungi asid amino yang boleh mempercepatkan proses penyembuhan luka. Ekstrak *Channa striatus* juga mengandungi omega-3 dan omega-6 yang amat penting dalam proses penyembuhan luka. Nisbah diantara omega-3 dan omega-6 asid lemak adalah 0.85. Omega-6 asid lemak diperlukan sebagai pencetus radang dalam fasa awal proses penyembuhan luka. HPMC adalah yang terbaik antara kesemua polimer yang telah diuji untuk menghasilkan kepekatan

untuk aerosol dan filem dengan kualiti yang dijangka. Glyserin digunakan sebagai agen pemplastik untuk formula E dan PEG 400 untuk formula G. Tokoferol digunakan sebagai antioksidan. Tin aerosol aluminium digunakan sebagai bekas. Berdasarkan kepada aspek keselamatan dan ekonomi, butana telah dipilih untuk digunakan sebagai agen pendorong untuk aerosol *Channa striatus* dan asid fusidik. Formula E2 (mengandungi ekstrak *Channa striatus*) dan G1(mengandungi ekstrak *Channa striatus* dan asid fusidik) tidak mempunyai sebarang potensi untuk menyebabkan iritasi pada kulit arnab, menunjukkan kedua-dua formula aerosol *Channa striatus* sesuai digunakan sebagai balutan luka. Perbandingan antara kumpulan-kumpulan yang dirawat dengan formula E2 dan G1 untuk kajian hirisan luka, menunjukkan formula E2 memberikan kekuatan regangan yang lebih baik berbanding formula G1 yang mengandungi asid fusidik sebagai bahan aktif. Ini mungkin disebabkan oleh kehadiran asid fusidik di dalam formula G1 yang merencat sintesis protein dan melambatkan proses penyembuhan. Peratus penutupan luka dalam kumpulan yang dirawat dengan formula E2 lebih tinggi berbanding kumpulan-kumpulan lain (formula G1 dan blank). Dari hari ke-4 sehingga hari ke-12, rawatan menggunakan formula E2 menunjukkan peratus penutupan luka yang tinggi dan signifikan berbanding kumpulan-kumpulan lain. Kumpulan yang dirawat menggunakan formula E2 mencapai 82% penutupan luka pada hari ke-10 dan 98% penutupan luka pada hari ke-15, manakala dua kumpulan yang lain mencapai 82% dan 98% penutupan masing-masing hanya pada hari ke-12 dan hari ke-18. Ini menunjukkan bahawa formula aerosol yang mengandungi ekstrak *Channa striatus* boleh mempercepatkan proses penyembuhan luka berbanding formula aerosol G1 dan blank.

FORMULATION AND EVALUATION OF *CHANNA STRIATUS* EXTRACT AND FUSIDIC ACID AEROSOL FOR TREATMENT OF WOUNDS AND BURN

ABSTRACT

Aerosol is a new drug delivery system for wound dressing and wound treatment. Aerosol has several benefits when compared with other delivery systems such as plasters or bandages for wound dressing and cream or gel for wound treatment. When applied to the body aerosol reduces pain that may result from the mechanical rubbing of gel and creams onto the skin. Aerosol could cover any size of wound which could not be covered by films due to films have a fixed size. *Channa striatus* has been used since a long time ago traditionally to accelerate the wound healing. *Channa striatus* extract contained amino acids and fatty acids which important for healing process. Fusidic acid is an antimicrobial for topical application. *Channa striatus* extract and in combination with fusidic acid have been used as active ingredients in this study. *Channa striatus* extract contained about 3.61% protein. HPLC systems for amino acids analysis and GC systems for fatty acids analysis method used in this study met the requirements of validation. These HPLC systems are reliable and reproducible. *Channa striatus* extract contained amino acids that could promote the wound healing process. *Channa striatus* extract also contained omega-3 and omega-6 that are very important in wound healing process. Ratio between omega-3 and omega-6 fatty acids was 0.85. Omega-6 fatty acid is needed as inflammatory inducer in the early phase of wound healing process. HPMC is the best among polymers tested to produce concentrate for aerosols and films with the expected qualities. Glycerine has been used as plasticizer for formula E and PEG 400

for formula G. Tocopherol was used as antioxidant. Aerosol aluminium can was used as container. Based on safety and economic aspect, butane was chosen to be used as the propellant for *Channa striatus* and fusidic acid aerosols. Formulas E2 (containing *Channa striatus* extract) and G1 (containing *Channa striatus* extract and fusidic acid) did not have any potential irritant ability to the rabbits skin, indicating that both of *Channa striatus* aerosol formulas can be applied as a wound dressing without any undesirable effect. Comparison between the groups treated with formula E2 and G1 for incision wound study, showed that formula E2 gave a better tensile strength than formula G1 which contained fusidic acid as additional active ingredient. This might be due to the presence of fusidic acid in formula G1 which inhibited the protein synthesis and delayed the healing process. Percentage of wound closure of the group which treated with formula E2 is higher than other groups (formula G1 and blank). From day 4 until day 12, formula E2 treatment significantly showed a higher percentage of wound closure compared to the other groups. Group which treated with formula E2 reached 82% wound closure on day-10 and 98% wound closure on day-15, whereas other two groups reached 82% and 98% closure only on day-12 and on day-18 respectively. It means that the aerosol formula containing *Channa striatus* extract could accelerate the wound healing process as compared to aerosol formula G1 and blank.

CHAPTER 1
INTRODUCTION

1.1 AEROSOL

Aerosol dosage form for oral and topical application was developed for use in the mid-1950s. Since that time it has found widespread acceptance because of its ease of use and therapeutic efficacy. The first textbook devoted exclusively to the subject of aerosol science and technology appeared in 1958 and was authored by Herzka and Pickthall (Sciarra, 1974).

Pharmaceutical aerosols are dosage forms containing therapeutically active ingredients intended for topical administration, introduction into body cavities or by inhalation via the respiratory tract (Sciarra and Cutie, 1990b). The dosage form is packaged in a metal or glass container and sealed with either a metered or continuous-spray valve. The aerosol product itself consists of four components: concentrate (containing the active ingredient(s), propellant(s), container and valve-actuator (Sciarra, 1976). The propellant provides the internal pressure that forces the product out of the container when the valve is opened and delivers the product in its desired form. For metered dose inhalers (MDIs) the product is delivered as a finely dispersed mist (particles less than 8 µm in diameter). Topical aerosol delivers its content as a spray, foam, or semisolid (Sciarra, 1996; Sciarra and Stoller, 1974).

Topical pharmaceutical aerosols have been accepted by both patients and physicians because of their aesthetic properties, ease of application, maintainability of sterility (if the package is sterile), tamperproof system, prevention of contamination of the unused contents, and increased stability. Topical aerosols have been dispensed as sprays, foams, and semisolids. When applied to the body they avoid or reduce pain that normally may result from the mechanical rubbing of ointments and creams onto the skin. Through use of a metered dose valve, an accurate amount of medication can be dispensed each time the valve is actuated. Topical products that have been formulated as aerosol include first-aid products containing local anaesthetics and antiseptics, adhesive tape removers and bandage adherents, products used in athletic and sports, burn remedies, foot preparations, germicidal and disinfectant products, spray-on bandages, protective, topical dermatologic, including antibiotics and steroids, veterinary products, body liniments and rubs, products for vaginal and rectal applications which include contraceptive foams and rectal foams, edible foams and saline solutions to cleanse contact lenses (Sciarra, 1996; Sciarra and Stoller, 1974).

1.1.1 Concentrate

The concentrate can be of the solution, dispersion, emulsion, or semisolid type. The concentrate is made up of active ingredient(s) and may include solvent(s) and dispersing agent. Depending on the type of product, various inert ingredient(s) are used as additive(s) to prepare solutions, suspensions, and emulsions (Sciarra, 1996; Sciarra and Stoller, 1974).

(a) Solution Systems

This type of aerosol system consists of two distinct phases: liquid and vapour. The solvent is used to dissolve the active ingredient and/or to retard the evaporation of the propellant. Solution aerosols are relatively easy to formulate, provided that the ingredients are soluble in the propellant. However, the liquefied gas propellants are nonpolar in nature and in most cases are poor solvents for some of the commonly used medicinal ingredients. Through use of a solvent that is miscible with the propellant, one can achieve varying degrees of solubility. Ethyl alcohol has found widespread use for this purpose. Other solvents use in pharmaceuticals may also be used with topical aerosols (Sciarra, 1996).

When the valve of a solution aerosol is depressed, a mixture of active ingredients, solvents, and propellants which has a very high vapour pressure is forced and emitted into the atmosphere. As the liquid propellant encounters the surrounding atmospheric (very much lower vapour pressure) air, it tends to vaporize and, in so doing, breaks up the active ingredients and solvents into fine particles. Depending on their size, the particles remain suspended in air for relatively long period of time. The particles sizes of aerosol can vary from as small as 5 to 10 µm or less to as large as 50 to 100 µm (Sciarra and Stoller, 1974). The size of aerosol droplets produced will depend on the nature of the propellant, the amount of propellant, the nature of the product concentrate, and the valve design. Metered-dose inhalers require particles of less than 8 µm whereas nasal aqueous aerosols generally have particles in the range of 50 to 75 µm. Topical aerosols have a particle size of about 100 µm (Sciarra, 1996).

Table 1.1: Prototype formulation for topical aerosol solutions (Sciarra and Cutie, 1990b)

Active ingredient(s)	Dissolved in system
Solvents	Ethyl and isopropyl alcohol Glycols Isopropyl esters Surfactants
Antioxidants	Ascorbic acid
Preservative	Methyl and propyl parabens
Propellant(s)	Isobutane Propane/butane Propane/isobutene Propellant 22 Propellant 152/142 Propellant 22/142 Dimethyl ether

(b) Suspension systems

For substances that are insoluble in the propellant or the mixture of propellant and solvent, or in cases where a cosolvent is not desirable, the active ingredients can be suspended in the propellant vehicle. When the valve is depressed, the suspension is emitted, followed by rapid vaporization of the propellant, leaving behind the finely dispersed active ingredients. This system has been used successfully to dispense anti-asthmatic aerosol as well as topical aerosol containing antibiotics. However, the formulation of this type of aerosol is not without difficulty. Problems involving caking, agglomeration, particle size growth and clogging of the valve arise (Sciarra and Stoller, 1974). Salt of the active ingredient having very low solubility or not soluble in the propellant and solvents should be selected. It is the slight solubility of the active ingredients in the propellants and solvents that contributes to particle size growth. This phenomenon, known as Ostwald Ripening occurs because the small particles have higher equilibrium solubility than larger particle of the same substance. These small particles will gradually dissolve and deposited onto the surface of the larger particles, resulting in an increase in the particle size of the originally micronized active drug substances. By adjusting the density of both the propellant and/or the insoluble material so that they are approximately equal, the rate of sedimentation can be reduced substantially. This can be accomplished by using a mixture of different propellants of varying densities as well as by addition of an inert powder to the active ingredients. Final consideration should be given to the use of a surfactant or dispersing agent. Sorbitan oleate, lecithin, oleic acid, and oleyl alcohol have been used in oral and metered-dose inhalers; isopropyl myristate has been used primarily in topical aerosols. Although it may be easier to formulate a solution system compared to a suspension system, the latter is generally preferred because one can obtain closer control over the particle size distribution of droplets dispersed in the suspension aerosol. Suspensions generally show greater stability of the active ingredient as compared with solution (Sciarra, 1996; Sciarra and Stoller, 1974).

Table 1.2: Prototype formulation for topical aerosol suspensions (Sciarra and Cutie, 1990b)

Active ingredient(s)	Pass through a 325 mesh screen
Dispersing agents	Isopropyl myristate
	Mineral oil
	Sorbitan esters
	Polysorbates
	Glycerol ethers and derivatives
Propellant(s)	12/11; 12/114 (only if exempted)
	Hydrocarbons
	142, 152, 22
	Dimethyl ether

(c) Emulsion systems

Water and hydrocarbon or fluorinated hydrocarbon propellants are not miscible. In order to formulate a suitable aerosol using these materials, various techniques can be used. An emulsion aerosol consists of active ingredient(s), aqueous or non aqueous vehicle, surfactant and propellant. Depending on the choice of ingredients, the product can be emitted as stable or quick-breaking foam or as a spray (Sciarra, 1996; Sciarra and Stoller, 1974).

Table 1.3: Prototype formulation for topical aerosol emulsions/foam (Sciarra and Cutie, 1990b)

Active ingredient(s)	Solubilised in fatty acid, vegetable oil, glycol
Emulsifying agents	Fatty acid soaps (triethanolamine stearate) Polyoxyethylene Sorbitan esters Emulsifiable waxes Surfactants
Other modifiers	Emollients Lubricants Presevatives Perfumes
Propellant(s)	12/114 (only if exempted) Hydrocarbons 22/152 22/142 152/142 Dimethyl ether

(i) Foam system

The propellant used in an emulsion is an important part of this system and determines the type of foam produced. The propellant is generally considered part of the immiscible phase and as such can be in the internal or external phase. When the propellant is included in the internal phase, typical stable or quick-breaking foam is emitted. When the propellant is in the external phase, the product is dispensed as a spray (Sciarra, 1996; Sciarra and Stoller, 1974).

(ii) Stabilized foams

In an emulsion system where the propellant is in the internal phase (generally part of the oil phase of the emulsion), water makes up the external or dispersing phase. The propellant is generally used to the extent of about 7% to 10% of the total weight. When a hydrocarbon propellant is used (such as isobutane/propane blend) as little as 3% to 4% is sufficient to produce suitable foam. These propellants are emulsified in the aqueous or nonaqueous emulsion. Some of the propellant will vaporize in the container and be present in the head space to produce the necessary vapour pressure. The pressure should be approximately 40 psig, depending on the propellant used. When the valve is depressed, the pressure provided by the vaporised propellant forces the emulsion up the dip tube and out the valve. The low atmospheric pressure cause the propellant trapped in the emulsion droplets to expand and vaporises to form stable foam, e.g. shaving foams (Sciarra, 1996; Sciarra and Stoller, 1974).

(iii) Quick-breaking foams

These foams consist of ethyl alcohol, water, and a surfactant that is soluble in either alcohol or water but not in both. Other miscible solvents can be used in place of alcohol and water. The surfactant can be non-ionic, anionic, or cationic. The product is dispensed as foam but quickly collapse, so that there is no further injury by mechanical dispersion of the product.

Steroid, burn, and other topical preparations can be applied in this manner. One advantage of a foam system over an aerosol system is the fact that the area with which the product comes into contact is limited or can be controlled. Preparations containing irritating ingredients may also be dispensed in this manner. The incidence of airborne particles can be substantially reduced, thereby lowering the incidence of toxicity of the sprayed products which may cause irritation on release and may be inhaled (Sciarra, 1996; Sciarra and Stoller, 1974).

(iv) Spray emulsion

The base for this product is a water-in-oil emulsion. A fairly large amount of propellant (about 25% to 30%) is miscible with the outer oil phase so that the propellant remains in the external phase of the final emulsion. When this system is dispensed, the propellant vaporizes, leaving behind droplets of water-in-oil emulsion with no foaming. Because the propellant and concentrate phase tend to separate on standing, products formulated using this system must be shaken before use. A hydrocarbon propellant or a mixed hydrocarbon/fluorocarbon propellant is preferred for this system, because the specific gravity of the propellant is less than 1 and the propellant will float on the aqueous layer. In addition, such systems use a vapour tap valve, which tends to produce finely dispersed particles (Sciarra, 1996; Sciarra and Stoller, 1974).

1.1.2 Propellants

The propellant can be either a liquefied or a compressed gas. The propellant is responsible for developing the proper pressure within the container and for expulsion of the product when the valve is opened. It also is responsible (together with the valve) for dispensing the product as a spray, foam, or semisolid. Various types of propellants are utilized. The fluorinated hydrocarbons, such as trichloromonofluoromethane (propellant 11), dichlorodifluoromethane (propellant 12), and dichlorotetrafluoro-ethane (propellant 114) found widespread use in most aerosol for oral, nasal, and inhalation use. Topical pharmaceutical aerosols utilize hydrocarbons (propane, butane, and isobutene) a limited number of hydrofluorocarbons and hydrochlorofluorocarbons (142b, 152a, 22), and compressed gases such as nitrogen and carbon dioxide (Sciarra, 1996; Sanders, 1979).

(a) Chlorofluorocarbons (CFCs)

The use of chlorinated fluorocarbons for aerosols and other commercial uses has been seriously curtailed and, in certain cases, banned. These compounds have been implicated in causing a depletion of the ozone layer and partially responsible for the "greenhouse" effect (increase in earth's temperature, rising sea levels, and altered rainfall patterns) (Sciarra, 1996).

Prior to 1978, fluorinated hydrocarbons were used almost exclusively as the propellants for all types of pharmaceutical aerosols. Their chemical inertness, lack of toxicity, lack of flammability and explosiveness and their safe record of use made them ideal candidates for use. The publication of the "ozone depletion theory" in the mid-1970s, however, and the alleged implication of the fluorocarbons in depleting the ozone levels in the atmosphere, led to the phasing out and ban of the use of fluorocarbon propellants in aerosols (with few exceptions) in 1978. This ban, promulgated by the Environmental Protection Agency (EPA), Food and Drug Administration (FDA) and the Consumer Products Safety Commission, became fully effective in April 1979, when manufacturers could no longer ship aerosol products containing fluorocarbons unless the product carried a specific federal exemption. While some propellant manufacturers indicated that there were other suitable replacements for propellants 11, 12, and 114, the only ones that have survived the necessary toxicity tests (long- and short-range) are fluorocarbons 152a, 142b and 22 which may be of limited value. The other alternatives include hydrocarbons, compressed gases, and mechanical devices and pumps. Of these alternatives, hydrocarbons were restricted to use with foams and water-based aerosols while compressed gases were of limited value in aqueous products where the propellant and water were not miscible. When compressed gases overcame the immiscibility of the components, other problems such as loss of propellant and to a lesser degree the change in the dispersion of the spray became apparent. Since compressed gas systems do not have chilling effect, they are applicable to topical preparations. With the development of newer valve technology (the vapour tap and the Aquasol valve), it was found that hydrocarbon propellants, such as butane, propane, isobutene and their mixtures could be safely used not only with aqueous products but with solvent-based aerosol as well. At present, hydrocarbons can be used for all types of topical aerosols (Sciarra and Cutie, 1986).

All MDIs marketed prior to 1995 contained CFCs as a propellant. These are also implicated in the depletion of stratospheric ozone. Except for some specific exemptions; their production has been banned since 1996 under the terms of the Montreal Protocol (UNEP, 2000). Hydrofluoroalkanes have been identified as suitable alternatives for MDI propellants but their physico-chemical properties differ significantly from CFCs and an extensive redevelopment and testing programme has been required to demonstrate the safety, quality and efficacy of hydrofluoroalkanes (HFAs) containing MDIs. HFAs contribute to global warming but the benefit to human health through continued MDI availability currently outweighs the environmental concern. Several HFA-MDIs have reached the market and the transition to replace existing CFC-MDIs is now underway (Smyth et al., 2005; McDonald and Martin, 2000).

(b) Hydrochlorofluorocarbons and hydrofluorocarbons

Topical pharmaceutical products must be formulated using a propellant other than the chlorofluorocarbons. For this purpose, a series of hydrochlorofluorocarbons and hydrofluorocarbons are available. These propellants do not present a hazard to the environment and can be used successfully to formulate topical pharmaceuticals. Table 1.4 illustrates these propellants along with some of their more useful physicochemical properties. As can be seen from Table 1.4, these propellants have suitable vapour pressures making them useful for a variety of different products. Propellant 152a and 142b may be blended to yield a vapour pressure within the useful range of from 35 to 50 psig. They may also be blended with propellant 22 to give the desired nonflammability to the final mixture as well as to obtain a higher vapour pressure (Sciarra, 1996).

Table 1.4: Propellants useful for topical pharmaceutical aerosol

Designation	Propellant 22	Propellant 142b	Propellant 152a
Formula	$CHClF_2$	CH_3CClF_2	CH_3CHF_2
Molecular weight	86.5	100.5	66.1
Boiling Point, ^{0}F (^{0}C)	-41.4(-40.8)	14.4(-9.44)	-11.2(-23.0)
Vapour pressure (psig), 70 ^{0}F	121	29	62
Vapour pressure (psig), 130 ^{0}F	297	97	176
Density (g/mL), 70 ^{0}F	1.21	1.12	0.91
Solubility in water (wt.%), 70 ^{0}F	3.0	0.5	1.7
Kauri-butanol value	25	20	11
Flammability limits in air (vol. %)	Nonflammable	6.3-14.8	3.9-16.9
Flash point, ^{0}F	-	-	-

(c) Hydrocarbons

The hydrocarbon propellants; butane, propane, and isobutene have replaced the chlorofluorohydrocarbons as the propellant for most consumer aerosol products (other than MDIs). They have also been used for topical pharmaceuticals. They are ideal for use with foams because they are nontoxic, nonreactive, and relatively inexpensive. They yield satisfactory foam and are environmentally acceptable. The chief drawback to their use is their flammability. However, this is of greater concern to the manufacturer than to the consumer. The advantage of hydrocarbons is their greater range of solubility and lower cost compared to fluorinated hydrocarbons. Their density of less than 1 and their immiscibility with water, make them useful in formulation of three-phase (two-layer) aerosols. Being lighter than water, the hydrocarbon remains on top of the aqueous layer and serves to push the contents out of the container. They are not subject to hydrolysis, making them useful with water-based aerosols (Sciarra and Cutie, 1990a).

Table 1.5: Selected properties of hydrocarbons and dimethyl ether

Designation	Propane	Isobutane	n-Butane	DME
Formula	C_3H_8	$i\text{-}C_4H_{10}$	$n\text{-}C_4H_{10}$	CH_3OCH_3
Molecular weight	44.1	58.1	58.1	46.07
Boiling Point, ^{0}F (^{0}C)	-43.7(-42.0)	10.9(-11.7)	31.1(-0.56)	-12.7
Vapour pressure (psig), 70 ^{0}F	109	31	17	63
Vapour pressure (psig), 130 ^{0}F	257	97	67	174
Density (g/mL), 70 ^{0}F	0.50	0.56	0.58	0.66
Solubility in water (wt.%), 70 ^{0}F	0.01	0.01	0.01	34
Kauri-butanol value	15	17	20	60
Flammability limits in air (vol.%)	2.2-9.5	1.8-8.4	1.8-8.5	3.4-18
Flash point, ^{0}F	-156	-117	-101	-42

As can be seen in Table 1.5, they all have a density of about 0.5 to 0.6 g/mL and therefore less is required as compared to a fluorocarbon (generally 1.2 to 1.4 g/mL). They can also be blended with each other and with hydrochlorofluorocarbons and hydrochlorocarbons so as to obtain different vapour pressures. Recently, dimethyl ether has been used to formulate aerosols. As can be seen in Table 1.5, its main advantage over all other materials used in aerosol formulations is its rather high miscibility with water (about 34%). This allows the formulator greater flexibility in developing different types of aerosol systems (Sciarra, 1996).

(d) Compressed gases

The compressed gases nitrogen, nitrous oxide, and carbon dioxide are limited in use. They are used in those cases where large quantity of water is present and the product must be dispensed as a spray. Such is the case with contact lens cleaners. Nitrogen is used because its inertness and its lack of solubility and miscibility with water. It is used to push the contents out of the container and to dispense a fine stream of solution that can be directed to the contact lens. Nitrous oxide and carbon dioxide are used with products where solubility of the gas in the product is desirable. Their main use at the present time is with foams. Table 1.6 indicates some of the other properties of these gases. Unlike liquefied gases, there is a drop in pressure as the contents of a product using compressed gas as propellant are dispensed (Sciarra, 1996). Since compressed gases are utilized in the gaseous state and not in the liquid state which may act as depot for the propelling gas, a higher initial pressure is required, as well as a relatively larger head space as in liquefied-gas aerosols. While the pressure of a liquefied-gas aerosol remains constant during use because the pressure is not influenced by the head space but by the mole ratio of the gas which remain constant during use and the gas is constantly replaced by the evaporating propellant liquid. A drop in pressure is noted during use of a compressed-gas aerosol because the gas pressure drop as the volume of head space increase (Sciarra and Cutie, 1990a).

Table 1.6: Properties of the compressed gases

Designation	Carbon dioxide	Nitrous oxide	Nitrogen
Formula	CO_2	N_2O	N_2
Molecular weight	44.0	44.0	28.0
Solubility in water (%w/w, 70 ^{0}F, 100 psig)	1.5	0.7	-
Solubility in isobutene	5.3	7.3	-
Solubility in ethyl alcohol	5.6	5.7	-
Solubility in Propellant 11	3.5	4.9	-

For safety reasons, the package should not be filled full to the top with liquid or concentrate. There must always be sufficient space for the propellant gas to occupy. The actual amount of liquid or concentrate that can be filled into an aerosol container is controlled by legislation, and there has to be a greater head space when using compressed gas propellants.

1.1.3 Containers

The concept of an aerosol originated as early as 1790, when self-pressurized carbonated beverages were introduced in France. In 1837, Perpigna invented a soda siphon incorporating a valve. Metal aerosol cans on the other hand were being introduced and tested as early as 1862. They were constructed from heavy steel and were too bulky to be commercially successful. In 1899, inventors Helbling and Pertsch patented aerosols pressurized by methyl and ethyl chloride as propellants. Department of Agriculture researchers, Lyle Goodhue and William Sullivan, developed a small aerosol can pressurized by a liquefied gas (a fluorocarbon) in 1943 (Bellis, 1997; Stoller, 1974).

In 1949, Robert H. Abplanalp's invention of a crimp on valve enabled liquids to be sprayed from a can under the pressure of an inert gas. In 1953, Robert H. Abplanalp patented his crimp-on valve "for dispensing gases under pressure." In the mid-1970s, concern over the use of fluorocarbons adversely affecting the ozone layer drove Abplanalp back into the lab for a solution. Robert H. Abplanalp invented both the first clog-free valve for aerosol cans and the "Aquasol" or pump aerosol, which used water-soluble hydrocarbons as the propellant source (Bellis, 1997).

(a) Glass

Glass bottle are not recommended for suspension aerosol because of the visibility of the suspended particles may present an aesthetic problem for the patient. Glass, being inert, has always been preferred for use with all types of pharmaceuticals, but with the advent and introduction of many newer materials, glass has been replaced by aluminium containers for most aerosols.

Glass does have several advantages (Chapman, 2004):

(i) It is inert to most medicinal products

(ii) It is impervious to air and moisture

(iii) It allows easy inspection of the container contents

(iv) It can be coloured to protect contents from harmful wavelengths of light

(v) It is easy to clean and sterilized by heat

(vi) It is available in variously shaped containers

The disadvantages of glass (Chapman, 2004) are:

(i) It is fragile: glass fragments can be released into the product during transport or contaminants can penetrate the product by way of cracks in the container.

(ii) Certain types of glass release alkali into the container contents.

(iii) It is expensive when compared to the price of plastic.

(iv) It is heavy resulting in increased transport costs.

The chemical stability of glass for pharmaceutical use is governed by the resistance of the release of soluble minerals into water contacting the glass. This is known as the hydrolytic resistance. Details of four types of glass are given in the BP (2007) and Ambrosio (2002).

(b) Aluminium

Aluminium is extremely lightweight and also is essentially inert. Aluminium can be used without an internal organic coating for certain aerosol formulations (especially those that contain only active ingredient and propellant) but many are available with an internal coating made from an epon- or expoxy-type resin (Sciarra, 1996; Sciarra and Cutie, 1986).

(c) Tin-Plated steel

These containers are used for most nonpharmaceutical aerosol and are the least expensive and most versatile of all containers (Sciarra, 1996; Sciarra and Cutie, 1986).

(d) Other container

There are many additional container systems available that allow for special dispensing of some product. The viscosity of the product, incompatibility of the product concentrate and propellant and desired dispensing characteristics of the finished product represent a few of the reasons why the typical aerosol systems may be unsuitable (Sciarra, 1996; Sciarra and Cutie, 1986).

1.1.4 Valves

A Valve is an important component of all aerosols and is responsible together with the propellant for the delivery of the product in the desired form whether as a spray, foam, semisolid, or as a fine mist having particles below 8 µm that suitable for inhalation (Sciarra, 1996; Sciarra and Cutie, 1986).

(a) Metered Valves

Metered valves, fitted with a 20 mm ferrule, are used with glass bottles and aluminium canisters for all MDIs (Sciarra, 1996).

(b) Continuous-Spray Valves

These valves are used primarily with topical pharmaceuticals. Table 1.7 indicates some of the commonly used materials for each of the subcomponents. These materials must be tested with the specific formulation in order to determine its compatibility with the formulation. Leakage and/or absorption (or adsorption) of the active ingredient are sometimes noted with these valves and generally can be overcome through proper selection of the material of construction (Sciarra, 1996; Sciarra and Cutie, 1986).

Table 1.7: Materials used in valve construction

Subcomponent	Material
Actuator (button, spout)	Polypropylene, polyethylene
Mounting cup	Tinplate, aluminium
Mounting cup gasket	Flowed-in or polyethylene sleeve
Stem	Nylon, delrin, acetal
Stem gasket	Buna N, neoprene, butyl
Spring	Stainless steel-passivated, stainless steel
Body	Nylon, delrin, acetal
Dip tube	Polyethylene, polypropylene

Depending on the design of the actuator or spout, the product can be dispensed as a spray, foam, or semisolid (Sciarra, 1996; Sciarra and Cutie, 1986).

1.2 AEROSOL FORMULATION, MANUFACTURING PROCEDURE AND QUALITY CONTROL

An aerosol formulation consists of two essential components: product concentrate and propellant. The product concentrate consists of active ingredients, or a mixture of active ingredients and other necessary agents such as solvents, antioxidants and surfactants. The propellant may be a single propellant or a blend of various propellants and is selected to give the desired vapour pressure, compatibility and dispensing characteristics (Sciarra, 1996; Sciarra and Cutie, 1986).

Both manufacturing procedures and packaging must be considered simultaneously, as part of the manufacturing operation takes place during the packaging of the product. The concentrate, which contains the active ingredients, solvents and cosolvents, other inert ingredients and may even contain a small portion of the propellant are compounded separately and then mixed with the remainder of the propellant. Two methods are available for use that includes a cold-fill and a pressure-fill method. Present-day technology and the availability of good equipment give preference to the pressure method over the cold-fill method. Less propellant may escapes into the atmosphere from the pressure fill method thus is more environmentally friendly (Sciarra, 1996).

A quality control system for aerosol is no different from the system used for nonaerosol pharmaceuticals except that several in-process tests are necessary in order to ensure that the concentrate has been properly prepared. In most cases this will include an assay to determine the level of active ingredient present. Other tests are dependent on the nature of the product. Weights of both the concentrate and the propellant must be checked routinely throughout the manufacturing process. Any errors and variation will affect the strength of active ingredient present in the final product and will result in product's rejection. Other essential tests that must be carried out on topical aerosol include: pressure, weight loss, delivery-amount/second, specific gravity, density, viscosity, interaction of product with valve, interaction of product with container, aerosol valve discharge rate, spray pattern, net contents, particle size, leakage and others depending on nature of product (Sciarra, 1996; Sciarra and Cutie, 1986).

Aerosol to be formulated in this study is a drug delivery system which delivers spray-bandage. Aerosol sprayed on the wound surface would release the active ingredient and additives onto the wound and form a thin layer of bandage that would cover and protect the wound. The aerosol to be formulated would contain the suitable type and amount of film-forming polymer and plasticizer which could produce the proper film for covering the wound and function as dressing.

Films prepared from pure polymers frequently are brittle and crack on drying. To correct this deficiency, the polymer can be chemically modified or other ingredients can be added to make the film more pliable. As a general rule, the film will become more flexible and more resistant to mechanical stress when a plasticizer is added to a film composition. There is an optimal concentration of plasticizer to be used for any film composition (Seitz, 1988).

1.3 *CHANNA STRIATUS*

Figure 1.1: *Channa striatus* fish

Taxonomy of *Channa striatus* fish are (Zipcodezoo, 2010):

Domain : *Eukaryota*

Kingdom : *Animalia*

Subkingdom : *Bilateria*

Branch : *Deuterostomia*

Infrakingdom : *Chordonia*

Phylum : *Chordata*

Subphylum : *Vetebrata*

Infraphylum : *Gnathostomata*

Superclass : *Osteichthyes*

Class : *Actinoterygii*

Subclass : *Actinopterygii*

Infraclass : *Actinopteri*

Cohort : *Clupeocephala*

Superorder : *Acanthopterygii*

Order : *Perciformes*

Suborder : *Channoidei*

Family : *Channidae*

Genus : *Channa*

Specific name : *striata*

Scientific name : *Channa striata*

There are 52 species in Channa genus i.e. *C. africanus, C. amphibeus, C. arga*, *C. argus* (Northern Snakehead), *C. argus argus* (Snakehead), *C. argus warpachowskii* (Northern Snakehead), *C. asiatica* (Small Snakehead), *C. aurantimaculata*, *C. bankanensis*, *C. baramensis*, *C. barca* (Barca Snakehead), *C. bistriata*, *C. bleheri*, *C. burmanica*, *C. cyanospilos*, *C. diplogramma*, *C. elliptica*, *C. fasciata*, *C. formosa*, *C. gachua*, *C. grandinosa*, *C. harcourtbutleri* (Burmese Snakehead), *C. leucopunctatus*, *C. lucius*, *C. maculata* (Blotched Snakehead), *C. maculatus*, *C. marulioides*, *C. marulius* (Bullseye Snakehead), *C. marulius ara*, *C. marulius marulius* (Bullseye Snakehead), *C. melanoptera*, *C. melanosoma*, *C. melasoma* (Black Snake Mackerel), *C. microlepis*, *C. micropeltes* (Indonesian Snakehead), *C. micropeltis* (Indonesian Snakehead), *C. nox*, *C. obscura*, *C. obscurus*, *C. ocellata*, *C. orientalis* (Smooth-Breasted Snakefish), *C. panaw*, *C. pleurophthalma*, *C. punctata* (Spotted Snakehead), *C. punctatus*, *C. sinensis*, *C. spp*, *C. stewartii* (Assamese Snakehead), *C. striata* (Striped Snake Head Murrel), *C. striatus*, *C. theophrasti*, and *C. argus* subsp. *warpachowskii* (Zipcodezoo, 2010)

Unambiguous Synonyms for *Channa striata* are:

1. *Channa striatus* Bloch, 1793
2. *Ophicephalus planiceps* Cuvier, 1831
3. *Ophicephalus striatus* Bloch, 1793
4. *Ophiocephalus chena* Hamilton, 1822
5. *Ophiocephalus philippinus* Peters, 1869
6. *Ophiocephalus planiceps* Cuvier, 1831
7. *Ophiocephalus striatus* Bloch, 1793

8. *Ophiocephalus vagus* Peters, 1869
9. *Ophiocephalus wrahl* Lacepède, 1801

Original description of *Channa striatus* is *Ophicephalus striatus* Bloch, 1793 in Naturgeschichte der Auslandischen Fische, 7:I-xiv + 1-144, pls. 325-360. *Channa striatus* fish has size up to 91.4 cm and can attain a length of 30-36 cm in 1 year. *Channa striatus* has habitat in freshwater ponds and streams, usually in stagnant muddy waters; primarily found on plains in India. Nevertheless, in Malaysia this species is reported to exist in rivers, lakes, swamps, paddy fields, mining pools, and roadside ditches. *Channa striatus* is an obligate air breather; spend up to 15 percent of the time in surfacing and related activities. This species is carnivorous, feeding on worms, prawns, frogs and fishes especially of other species (Courtenay et al., 2007).

In Malaysia, it has always be a strong belief that *Channa striatus* enhance wound healing and a very powerful tool for recovery of health and injury of mothers after giving birth. Since 1931 there has been in Malaysian literature about wound treatment using *Channa striatus*. Several studies have been carried out to examine the efficacy and contents of *Channa striatus* meat. Indeed, the *Channa striatus* did contain all the essential amino acids and fatty acids uniquely capable of accelerating the wound healing. Early in January 2003, Eddy Suprayitno from Indonesia studied about *Channa striatus* extract for wound healing. The study was conducted by using *Channa striatus* extract as a substitute for serum albumin which is normally used for surgical wound healing. *Channa striatus* extract was prepared by steaming the cleaned *Channa striatus* fish and water extract. The water extract was drunk immediately to new patients after surgery. The results of Suprayitno study showed that the wounds of patients treated with *Channa striatus* extract healed within three days faster than the wounds of patients which treated with serum albumin (Yellowfin, 2004).

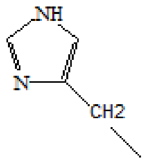
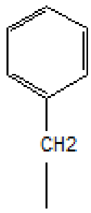
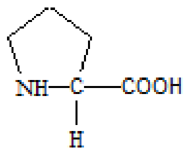
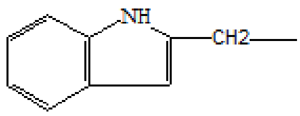
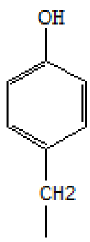
Channa striatus, a fresh water fish indigenous to many tropical countries have long been regarded as valuable food fish in the Far East. Their flesh is claimed to be rejuvenating, particularly in recuperation from serious illness and in a post-natal diet. It is consumed for its putative effects on wound healing (Mat Jais, 2007; Mat Jais et al., 1994). It is also used by the patients in the post-operative period in the belief that it promotes wound healing and reduces post operative pain and discomfort. This fish is known to contain polyunsaturated fatty acids that can regulate prostaglandin synthesis and hence induce wound healing (Turek, 2007). Certain amino acids like glycine, aspartic and glutamic acid are also known to play important roles in the process of wound healing (Ahuja et al., 2007; Dylewski and Yu, 2007; Schoemann et al., 2007). Despite the wide-spread uses of this fish for medicinal purposes, there have been hardly any studies to establish the scientific basis for its claimed wound healing effects. Previously (Mat Jais et al., 1994) reported that the fatty acid composition of *Channa striatus* may account for the promotion of wound healing process. Gam et al. (2005) reported that there are no significant differences in the content of amino acid and fatty acid compositions in this snakehead fish of various sizes and obtained at different times of the year (Courtenay et al., 2007). Cream extracts of *Channa striatus* tissues contain high levels of arachidonic acid, a precursor of prostaglandin, essential amino acids (particularly glycine) and polyunsaturated fatty acids necessary to promote prostaglandin synthesis. Treating wounds with these extracts has been demonstrated to promote synthesis of collagen fibers better than standard use of Cetrimide, an antimicrobial quaternary ammonium compound. In that study, *Channa striatus* extract was shown to increase the tensile strength of the surgically stitched wounds when compared to those treated with Cetrimide cream (Baie and Sheikh, 2000a).

1.3.1 Amino acids in *Channa striatus*

Amino acids are molecules containing an amine group, a carboxylic acid group and a side chain that vary between different amino acids. These molecules contain the key elements of carbon, hydrogen, oxygen, and nitrogen. These molecules are particularly important in biochemistry and referred to as alpha-amino acids with the general formula $H_2NCHRCOOH$, where R is an organic substituent. In an alpha amino acid, the amino and carboxylate groups are attached to the same carbon atom, which is called the α-carbon. The various alpha amino acids differ in the side chain (R group) which is attached to their alpha carbon. These R groups can vary in size from just a hydrogen atom in glycine, to a methyl group in alanine, through to a large heterocyclic group in tryptophan (Banga, 2006; Womack and Rose, 1947).

Amino acids are critical to life and have many functions in metabolism. One particularly important function is as the building blocks of proteins, which are linear chains of amino acids. Every protein is chemically defined by this primary structure, its unique sequence of amino acid residues, which in turn define the three-dimensional structure of the protein ("The Structures of Life," 2008). Amino acids are also important in many other biological molecules, such as forming parts of coenzymes, as in S-adenosylmethionine, or as precursors for the biosynthesis of molecules such as heme (Womack and Rose, 1947).

Table 1.8: Structure of 20 L-α-amino acids found in proteins (Woodbury, 2006; Thornton and Barlow, 1991)

No.	Amino acid	Three- and one-letter codes	Side chain/structure
1	Alanine	ALA (A)	$-CH_3$
2	Arginine	ARG (R)	$-(CH_2)_3-NH-C-(NH)NH_2$
3	Asparagine[f]	ASN (N)	$-CH_2-CONH_2$
4	Aspartic acid[f]	ASP (D)	$-CH_2-COOH$
5	Cysteine	CYS (C)	$-CH_2-SH$
6	Glutamic acid[f]	GLU (E)	$-(CH_2)_2-COOH$
7	Glutamine[f]	GLN (Q)	$-(CH_2)_2-CONH_2$
8	Glycine	GLY (G)	-H
9	Histidine	HIS (H)	
10	Isoleucine	ILE (I)	$-CH(CH_3)CH_2CH_3$
11	Leucine	LEU (L)	$-CH_2-CH(CH_3)_2$
12	Lysine	LYS (K)	$-(CH_2)_4-NH_2$
13	Methionine	MET (M)	$-(CH_2)_2-S-CH_3$
14	Phenylalanine	PHE (F)	
15	Proline[g]	PRO (P)	
16	Serine	SER (S)	$-CH_2-OH$
17	Threonine	THR (T)	$-CH(CH_3)OH$
18	Tryptophan	TRP (W)	
19	Tyrosine	TYR (Y)	
20	Valine	VAL (V)	$-CH-(CH_3)_2$