FORMULATION DEVELOPMENT OF IBUPROFEN USING DIFFERENT GEL BASES

BY

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DECLARATION

This dissertation is my original work and has not been presented for a degree award in any university or published anywhere else.

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DEDICATION

This work is dedicated to my family; my wife Susan and my three children John, Isabel and Solomon for making my life have a meaning.

ABBREVIATIONS AND ACRONYMS

BP	British Pharmacopoeia
cfu	Colony forming Units
NSAID	Non Steroidal Antiflammatory Drugs
API	Active Pharmaceutical Ingredient
g	gram
mg	milligram
uv	Ultra violet
rpm	rotations per minute
^{0}C	degrees Celsius
USP NF	United States Pharmacopoeia National Formulary
µg	Micrograms
TSA	Tryptone Soya Agar
SDA	Sabouraud Dextrose agar
MDT	Mean Dissolution time
MP	Melting Point
HEC	Hydroxyl ethyl cellulose
C940	Carbopol 940, Cross linked polyacrylic acid
MP	Melting Point
min	minutes

DEFINITION OF TERMS

Compatibility: ability of more than one ingredients to be mixed in a formulation without chemical or physical interaction.

Cosolvency: increasing solubility of a substance by use of one or more solvents jointly.

Dissolution: transfer of molecules or ions from a solid state to solution.

Dosage formulation: process of combining different chemical excipients and the active pharmaceutical ingredient(s) to produce a finished pharmaceutical product.

Drug release: ability of a vehicle to deliver the active drug substance (API) for the intended site in the body.

Penetration enhancers: substances that temporarily alter the barrier function of the skin allowing better drug penetration.

Pharmacokinetics: study of dry Absorption, distribution, excretion and metabolism in the body.

Pre-formulation: characterization of the physical, chemical and mechanical properties of a drug in order to choose the appropriate ingredients (excipients) to do a stable formulation with good drug release properties.

Rheological properties: flow and deformation properties of matter.

Solid dosage form: drug formulations with rheological properties.

Transdermal delivery: drug transport through the skin i.e. percutaneous absorption.

Viscosity: resistance of a fluid to flow.

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ABSTRACT

Introduction: This study was done to formulate and evaluate the topical drug release variation of Ibuprofen 5% w/w gel using different gel bases. Dermatological biopharmaceutics aims at designing active drugs and incorporating them in vehicles to allow transdermal delivery.

This study was undertaken to formulate Ibuprofen gel using different polymer bases and investigate the effect of the different polymers on release profile of the Ibuprofen. Hydroxy ethyl cellulose and carbopol 940 were used in the different formulations and in each in three different concentrations.

Materials and methods: Ibuprofen was obtained from Lab & Allied, Kenya. Carbopol 940 was obtained from Oxford Labchem, India. Hydroxy ethyl cellulose was given as a kind donation from Stedam Pharma Manufacturing Ltd. Kenya. The equipments used electric stirrer (Jencos Scientific Ltd Bedfordshire) Water bath (Clifton unstirred serial no. 50689 Nick Electro Ltd England) Viscometer (NDJ-55 Rotating Viscometer) Dissolution tester (Erweka DT6 Serial No. 68062 Germany), Spectrophoyometer (Genesys 105 Serial No. ZL9R 130 209) pH Meter (Jenway 3510 Bibby Scientific Ltd, Uk).

A total of 18 formulations were prepared all with the same concentrations of Ibuprofen and other ingredients but with varying amounts of the polymer bases HEC and C940.
The formulations were subjected to tests for pH, viscocity microbial load, drug content assay and drug release profile.

Results and discussion: The pH, viscosity and drug content assay were all found to be within the expected range. Drug release was evaluated over one hour. The release rate was found to be directed by polymer concentration for both HEC and C940. Higher polymer concentration in the polymer matrix decreased the rate of drug release. A burst drug release was obtained in the first 15 minutes giving an immediate release profile especially with HEC based formulations. C940 was found to give slow prolonged drug release rate. Formulations with proper adjustment of these polymers in the gel formulations of Ibuprofen can offer desirable release characteristics.

CHAPTER ONE: INTRODUCTION

1. BACKGROUND OF THE STUDY

The skin is the largest organ of the human body with a surface area of about $80m^2$. This offers a large opportunity for transdermal drug delivery. The skin offers an important role in selective entrance of molecules and preventing entry of harmful ones. Therefore, topical drug administration has proved to be a suitable drug delivery system. Transdermal drug delivery system has several advantages over conventional methods. Oral drug delivery poses a myriad of shortcomings like first pass metabolism, variable absorption rates, gastric irritation, drug instability in gastric pH among others. Drug delivery through the skin i.e. Transdermal drug delivery may also be compared to continuous intravenous infusion for some systematic medication though in some others, this drug delivery method minimizes systematic toxicity (Shahida A. et al., 2012)

Many antiflammatory drugs are used topically due to improved local effects and also to avoid their gastro-irritating effects. Ibuprofen has been rated as the safest conventional NSAID in the UK (Rabia B. et al., 2010) and is widely used for the symptomatic treatment of rheumatoid arthritis, ankylosing spondylitis and osteoarthritis. Ibuprofen suitability in the treatment of the various types of arthritis is dependent on maintenance of effective drug concentration in the inflamed part of the body and a constant and uniform drug supply as desired (Sudhamani T.et al., 2010) Topical application of Ibuprofen allows higher local concentration of the drug at the site of pain and inflammation and lower or negligible systematic drug levels thus producing fewer or no adverse drug toxic effects (Reddy et al., 2011) When administered orally, Ibuprofen is extensively metabolized in the liver leading to a short biological half life. This leads to the need to administer the drug frequently. Further more, Ibuprofen causes extensive gastric irritation and has been known to cause gastric ulceration when used over a long duration of time especially considering that arthritis is a long term disease condition requiring long term administration of NSAIDs.

Other NSAIDs utilizing transdermal drug delivery include ketoprofen, naproxen sodium, aceclofenac (Sugit K. et al., 2008) and diclofenac sodim (Shirhane U. et al.,) among others. Transdermal gel and patches of Ibuprofen have also been formulated (Bazigha K. et al., 2010).

Due to the importance and significant popularity of Ibuprofen in terms of its safety and being a drug of choice for the treatment of arthritis and other forms of local inflammation, more work need to be done in the optimal formulation of Ibuprofen as a topical gel. The present study involves formulation of Ibuprofen topical gel and evaluation of drug release rate when different polymer bases are used.

1.1 Problem Statement

Ibuprofen continues to be one of the most preferred NSAID for the management of various forms of arthritis and management of local inflammatory conditions. However, due to its vulnerability to extensive metabolism and hence a short biological half life and its side effect of gastric irritation coupled with the need to provide and maintain high local levels of the drug for an extended period of time, a well formulated topical gel remains a major requirement. This study is therefore aimed at coming up with a well formulated Ibuprofen topical gel.

1.2 Purpose of the Study

To formulate and evaluate Ibuprofen topical gel using different polymer bases.

1.3 Objectives

General Objective

To formulate and evaluate Ibuprofen topical gel.

Specific objectives

1. To develop a topical gel of Ibuprofen.
2. To evaluate the drug release properties of Ibuprofen gel when different polymer bases are used.

1.4 Significance of Study

This study resulted in well formulated Ibuprofen topical gel. It also came up with a validated drug release profile for the topical dosage form.

1.5 Anticipated output

The anticipated output of this study is an Ibuprofen gel well formulated and evaluated in terms of release characteristics.

1.6 Delimitations

Preformulation study of the Ibuprofen API and the excipients was limited to physical characteristics only. Extrudability and spreadability tests were not done due to unavailability of tubes and equipment.

1.7 Limitations

Stability testing of the finished product was not done due to unavailability of a stability chamber with stress conditions.

The cellulose nitrate filter papers were used to simulate the human skin. Use of animal models to study drug release properties would have given close to actual results.

The antiflammatory properties were also not carried out on animal models.

CHAPTER TWO

LITERATURE REVIEW

Transdermal drug delivery or percutaneous absorption of drugs depends on the topical bioavailability of medical products applied on the skin. The medicament is released from the topical formulation (cream, ointment, gel, powder, patch e.t.c) and penetrates through the stratum corneum into the viable epidermis. Dermatological biopharmaceutics aims at designing active drugs or prodrugs and to incorporate them into vehicles or devices which deliver the medication to the active site through the skin (Bronaugh R. et al., 1992). The skin has the functions of protecting the internal body structure from hostile external environment by limiting passage of chemicals, stabilizing body temperature, mediating sensations of heat, cold, touch and pain among others. It may be damaged mechanically, chemically, biologically and by radiation.

The human skin comprises of tissue layers which include epidermis that contains the stratum corneum, the dermis, the subcutaneous tissue and the skin appendages. The barrier function of the skin can be manipulated to allow drug molecules to pass through topical application e.g. antibiotics or by directing drugs through viable skin tissue e.g. topical antiflammatories like Ibuprofen as an alternative to oral route or by skin delivery for systematic treatment e.g. drugs for angina, pain or motion sickness (Mark R. et al., 2009).

The rate limiting step in transdermal drug delivery is the stratum corneum. The entire horny layer provides diffusional resistance of drugs. Once they pass this layer, drug molecules permeate rapidly through the living tissue and sweep into the systemic circulation. Topical drugs pass through the skin via sweat ducts, across the stratum corneum and through hair follicles with their associated sebaceous glands (Michael E.A., 2008).

The fraction of a drug that penetrates the skin via any of the above routes will largely depend on physicochemical nature of the drug particularly its size, solubility and partition coefficient, timescale of observation, site and condition of the skin, formulation and how vehicle components temporarily change the properties of the stratum corneum. Clinical results of a topical preparation applied on the skin depend on a sequence of events which

are, the release of medicament from the vehicle, followed by penetration through the skin barriers and then the activation of the intended pharmacological response. Effective therapy optimizes the above sequence, affected by three components, the drug, the vehicle and the skin. Biological factors that affect transdermal drug delivery may include the skin condition, where healthy skin is a tough barrier and injured skin has compromised barrier qualities. Skin types of young and old people are more permeable than adult tissue. Blood flow and regional skin sites affect drug delivery. Plantar and palmar callus are 400 – 600 µm thick compared to 10 – 20 µm for other sites. Skin hydration, temperature and pH also affect the rate of drug penetration. Other factors affecting the penetration through skin are drug concentration, partition co-efficient and molecular size and shape where absorption is inversely related to molecular weight. Small molecules penetrate faster than large molecules. (Michael E.A., 2005).

Drug transport through the skin is by passive diffusion or active transport. In passive diffusion, the drug moves from the high concentration topical formulation to low concentration in the skin as expressed by Fick's first law of diffusion.

$$J = D\frac{dc}{dx} \qquad \text{Equation 1}$$

Where J is the rate of transfer per unit area of surface (the flux), C is the concentration of the diffusing drug substance, x is the space coordinate measured normal to the section and D is the diffusion co-efficient.

When a membrane is applied in experimental designs, with a concentration gradient operating designs, during a run and sink conditions, the cumulative mass of diffusant m, which passes per unit area is as follows: -

$$\frac{dy}{dx} = \frac{DCoK}{h} \qquad \text{Equation 2}$$

Where C_o is constant concentration of the drug in the donor, *k* is the partition co-efficient of the solute between the membrane and the bathing solution, and h is the thickness of the membrane. If a steady state, plot is extraporated to the time axis, the intercept so obtained at m = 0 is the lag time L.

$$L = \frac{h^2}{6D} \qquad \text{Equation 3}$$

In perfect sink conditions and where only one drug is the penetrant, the diffusion co-efficient does not alter with time and when the penetrant is absorbed instantaneously on reaching the skin, the relationship becomes;

$$M \approx 2 C_o \sqrt{\frac{D_v t}{\prod}} \qquad \text{Equation 4}$$

where M is the quantity of drug released to the sink per unit area of application, Co as the initial concentration of drug in the vehicle, Dv the diffusion co-efficient of the drug in the vehicle and t the time after application.

Differentiating this application provides the release rate:

$$\frac{dm}{dt} \approx Co\sqrt{\frac{Dv}{\prod t}} \qquad \text{Equation 5}$$

(Michael E.A., 2007)

Ibuprofen [2-(4-isobutylphenyl) propionic acid] is a potent non steroidal anti-inflammatory (NSAID) drug commonly indicated for the treatment of acute and chronic arthritic conditions trauma, swelling of soft tissues and other forms of pain due to its good analgesic properties. It also has antipyretic properties.

Structure of Ibuprofen.

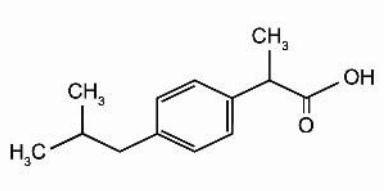

Figure 1: structure of Ibuprofen

Molecular formular $C_{13}H_{18}O_2$

Molecular weight 206.28082 g/mol (Mizumoto .T. et al., 2005)

Ibuprofen is poorly water soluble (log p value 3.6) and this limits its entry into systematic circulation before gastric emptying (30 minutes to 2 hours) despite its good gastric permeability (Patel R. et al., 2010).

During gastric emptying, Ibuprofen enters the small intestines where it cannot permeate through the membrane despite being solubilised (Greenhalgh D.J. et al., 1999). This

results in low bioavailability due to erratic or incomplete absorption from the gastrointestinal tract (Vasconolos T. et al., 2007, Mehlisch D.R. et al., 2002).

Ibuprofen also causes gastric mucosal damage that may result in gastric ulceration and bleeding (Rainsford K.D. et al., 2003). It also causes nausea, dyspepsia, nose bleeding and dizziness (Rossi S. 2013).

It also causes heart failure, renal impairment and can exacerbate asthma (Ayres J.G. et al., 1987).

Mode of action of Ibuprofen:

Arachidonic Acid

↓ Cyclo-oxygenase enzyme

Prostaglandin (mediates inflammatory process)

Like other NSAIDs it works by inhibiting the synthesis of prostaglandins derived from arachidonic acid which mediate inflammation, pain and fever. This happens by inhibiting enzyme cyclo-oxygenase present in various body tissues.

Potential oral formulations like inclusion of complexes, prodrug, solid dispersion method and microcapsulation have been explored, all trying to increase bioavailability and safety.

Transdermal delivery provides an increased bioavailability by avoiding first pass metabolism by the liver and a consistent delivery for an extended period. (Prausnit M. et al., 2008), (Prausnitz M. et al., 2004).

Topical delivery vehicles like gels and transdermal delivery agents e.g. dermal patches improve patient compliance due to decrease dosage frequency and avoiding gastric irritation. The permeability problems at the skin surface may be solved by use of drug carriers and penetration enhancers. This enhances ibuprofen permeability and transdermal absorption of Ibuprofen (British Journal of Dermatology, 1993).

Topical preparations can be applied directly to an external body surface by spreading, rubbing and spraying. The topical route has been utilized either to produce local effect for treating skin disorder or to produce systemic effects within the body. Major groups of

semisolid preparations have expanded both in cosmetic and pharmaceutical formulations. Gels often provide a faster release of drug substance, independent of the water solubility of the drug as compared to creams and ointments. Gels are highly biocompatible with a lower risk of inflammation or adverse reactions and are easy to apply. They have several favorable properties such as being thixotropic, greaseless, easily spreadable, easily removed emollient, non staining and compatible with several excipients (Shaik A. B. et al., 2013).

Gels are semisolid systems in which a liquid phase is constrained within a three dimensional polymeric matrix in which a high degree of physical cross linking has been introduced. The polymers are used between 0.5 – 15% and in most of the cases they are usually at the concentration between 0.5 – 2%. They are usually clear, transparent semi solids containing solubilised active substances (Lachman L. et al., 1987).

Ideally, gelling agents in pharmaceutical formulations should have certain characteristics. They should be inert, safe and non reactive with other formulation components. They should exhibit little viscosity change under temperature variations. Gels should not be tacky especially when intended for dermatological use. They should also have good rheological properties.

Gel forming substances are classified as; natural polymers e.g. agar, guar, semisynthetic polymers e.g. hydroxyl ethyl cellulose, hydroxyl propyl methyl cellulose e.t.c synthetic polymers e.g. carbomer, carbopol e.t.c inorganic substances e.g. microcrystalline silica, clays e.t.c and other gallants e.g. beeswax, cetyl ester wax e.t.c (Rowe C. et al., 2003).

Gels contain the active pharmaceutical agent and other excipients that have various functions in the formulation. These include solvents and co-solvents for the API, humectants, penetration enhancers among others. Penetration enhancers temporarily diminish the impermiability of the skin. They are accelerants and sorption promoters. An ideal penetration enhancer should be pharmacologically inert, non toxic, non irritant, non allergic. Its action should be immediate and predictable, should not cause loss of fluid, should be compatible with other formulation ingredients, is cosmetically acceptable, should be odourless and colourless. Penetration enhancers include water, sulfoxides, pyrrolidones, fatty acids, urea, alcohols and glycols, essential oils and surfactants.

Some penetration enhancers work by binding to surface proteins of the skin, denaturing skin surface proteins, solubilising intercellular lipids of the skin, penetrating through epidermal lipid barrier and interacting with living cells (Barel A. et al., 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

This was an experimental study design.

3.2 Location of the study

The study was carried out at the Department of Pharmaceutics and Pharmacy Practice and Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi, Kenya.

3.3 Equipment/ apparatus

The following equipment were used:

Electric stirrer (Jencos Scientific Ltd, Bedfordshire) Water bath (Clifton Unstirred Serial No. 50689 Nick Eletro Ltd, England) Viscometer (NDJ-5S Rotating Viscometer) Dissolution Tester (Erweka DT 6 Serial No. 68062 Germany) Spectrophotometer (Genesys 105 Serial No. 2L9R130209) pH meter (Jenway 3510 Bibby Scientific Ltd UK) Electric light microscope (Olympus, 264667 Tokyo Japan) Melting apparatus (Barloworld scientific ltd, Staffordshire UK), Top loading electronic balance model Tx 3202 L, (Shimadzu).

3.4 Materials

Ibuprofen (Borrowed from Lab & Allied, Kenya), carbopol 940 (Oxford Labchem, India), Propylene Glycol (Oxford Labchem, India), Hydroxy Ethyl Cellulose (Fisher Chemicals), Ethyl Alcohol (BDH Laboratory Supplies, UK), Propylene glycol (Oxford Labchem, India), Glycerine (Oxford Labchem, India) Menthol (Fisher Chemicals) Methyl Paraben (Oxford Labchem, India) Propyl Paraben (Oxford Labchem, India), Triethanolamine (Oxford Labchem, India) Sodium Chloride (Ram Chem, India), Sodium Hydroxide (Ram Chem, India), Potassium Dihydrogen Phosphate (Lote Chemie, India). Hydroxy ethyl cellulose was given as a kind donation from Stedam Pharma Manufacturing Ltd Kenya.

3.5 Methods

3.5.1 Pre-formulation studies of Ibuprofen

Colour observation – visual examination was done and results recorded.

Odour – due to lack of an offactometer, odour concentration and strength of Ibuprofen powder was determined by smelling using natural senses.

Melting point determination

The melting point procedure was done as described in section <741> Pg 2033-2034 of the USP NF.

USP compatible capillaries are specified for melting determination: 10 cm length 0.8-1.2 mm interval diameter and 0.2-0.3mm thickness. The capillary tubes were charged with sufficient amount of dry powder to form a column in the bottom of the tube 2.5 – 3.5 mm high when packed down as tightly as possible by tapping on the solid surface. The capillaries were inserted in the melting apparatus (Barloworld Scientific Ltd Staffordshire UK) and heated at a rate of 1-2^{0}C until the melt is complete. The clear point i.e. the temperature at which sample became completely liquid was recorded as the melting point.

3.5.2 Preparation of Ibuprofen gel in various concentrations of polymer base

Table 1 illustrates the formulae used to prepare Ibuprofen in various concentrations of polymer. Sodium Chloride, the preservatives methyl paraben and propyl paraben were first dissolved in the water used for wetting the polymer. Carbopol 940 based gel formulations were prepared by first soaking the carbopol 940 for 24 hours to hydrate it. Hydroxy ethyl cellulose was allowed to gel by adding water and sodium chloride. Menthol was dissolved in ethanol to form a co-solvent for Ibuprofen. Ibuprofen was then added with continuous stirring until full solubility. The Ibuprofen mixture was then added to the various gel concentrations, polyethylene glycol and glycerine were then added with continuous mixing using the electric stirrer. Triethanolamine was added. Hydroxy ethyl based gels were allowed to bubble out. The prepared formulations were filled into jars. For hydroxyl ethyl based gels, air bubbles were allowed to diffuse out. The formulations were stored in a cool place.

Guar gum gel was made by dispersing the guar gum powder into propylene glycol and then mixed with water heated at 60^{o}C, Ibuprofen was dissolved in a little ethanol and propylene glycol then mixed into the gel in small amounts with stirring.

During the preparation of the gels, the following were the critical process parameters: Mixing time (10 minutes after every addition), mixing speed (100 rpm), position of the stirrer (75^0) and the order of addition of various ingredients.

In process tests done were physical appearance and pH.

3.5.3 Preparing gel formulations

Table 1: Composition of the gel formulation (% w/w)

	F1			F2			F3			F4			F5			F6		
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
Ibuprofen	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
HEC	0.75	0.75	0.75	1.5	1.5	1.5	3	3	3	-	-	-	-	-	-	-	-	-
Carbopol	-	-	-	-	-	-	-	-	-	0.75	0.75	0.75	1.5	1.5	1.5	3	3	3
Ethyl Alcohol	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
Propylene glycol	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Glycerine	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Menthol	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Propyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Triethanolamine	0.25	0.25	0.25	0.25	0.25	0.25	0.4	0.4	0.4	0.25	0.25	0.25	0.25	0.25	0.25	0.4	0.4	0.4
Nacl	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14

Each formulation was made upto 100 g. It was found that the order of mixing various components was very critical in the formulation.

Ethyl alcohol and menthol acted as co-solvents and penetration enhancers.

Ionic strength of water was found to be critical in the gelling of the polymers. Distilled water alone could not form the gels. Sodium Chloride and purified water provided the right conditions for gel formulation.

Plate 1: Electric Mixer

Plate 2: Formulated Gels

3.5.4 Guar Gum was found to display instability with high volumes of ethyl alcohol and high API concentrations. The result was a gel that could not follow the factorial design. Table 3 shows the guar gum gel that was formulated.

Table 2: composition of guar gum based Ibuprofen formulation

Ingredient	% w/w
Ibuprofen	2.5
Guar Gum	3
Propylene glycol	15
Propyl Paraben	0.2
Methyl Paraben	0.2
Nacl	0.2
Menthol	1.0
Water	77.9

Plate 3: Guar gum plain

Plate 4: Guar gum Ibuprofen formulation

This formulation was found to be very turbid and had some degree of grittiness. No other studies were done on the guar gum.

3.5.5 Batch Weight determination

Each of the formulations in the pre-weighed jars were weighed using the weighing balance (Top loading electronic balance model Tx 3202 L, Shimadzu). The yield weight was determined and recorded.

3.5.6 Determination of pH

1g of gel formulation was accurately weighed and dispersed in 100 ml distilled water. This was left to settle for 2 hours and the pH measured using the digital pH meter (Jen Way 3510, Bibby Scientific Ltd, UK).

3.5.7 Determination of Viscosity

The viscosity of the formulations was determined using a digital viscometer NDF-5S at 25^{o}C using spindle / Rotor No. 4 and a rotation of 60 rpm, after a 3 minute rest time (Dai et. at., 2009) (Rasool et al., 2010).

3.5.8 Determination of Microbial Load

3.5.8.1 Bacterial load determination

12g of Tryptone Soya Agar was mixed in 400 ml distilled water and boiled on a hot plate to dissolve. 20 ml of the hot medium was measured into 20 universal bottles. The 20 universal bottles were sterilized in the autoclave set at 121^{0}c for 15 minutes after which it was switched off and allowed to cool.

10 ml of gel sample was diluted to 100 ml with alcohol/ water solvent. 10 ml of this was added into 90 ml peptone broth.

Inoculation was done in the laminar flow chamber, 1 ml sample in peptone broth was poured into each Petridish, then 20 ml Tryptone Soya Agar media was added and allowed to set.

Bacillus subtillis was used as the positive control. The Petridishes were put in an incubator set at 37^{0}C for 3 days. Bacterial count was done by counting the number of colonies. Excess peptone broth and the Petridishes were all sterilized in the autoclave at 121^{0}c for 15 minutes to kill all the microbes.

3.5.8.2 Fungal load determination

26 g of Sabouraud Dextrose Agar was weighed and mixed with 400 ml distilled water, then boiled on a hot plate. 20 ml was dispensed into 20 universal bottles and sterilized in an autoclave set at 121^0C for 15 minutes. 10ml of gel sample was diluted to 100 ml with alcohol/ water solvent. 10 ml of this was added into 90 ml peptone broth.

Inoculation was done in the laminar flow chamber, 1 ml sample in peptone broth was poured into each petridish, then 20 ml Sabouraud Dextrose Agar medium was added and allowed to set.

Candida albicans was used as the positive control.

The petridishes were put in an incubator set at room temperature for 7 days after which fungal colonies count was determined. Excess peptone broth and the petridishes were all sterilized in the autoclave at 121^0c for 15 minutes to kill all the microbes.

3.5.9 Determination of Gel Clarity; grittiness, odour and colour change.

The clarity of various gel formulations was determined by inspection under an electric microscope (Olympus, 264667 Tokyo Japan) and was graded as turbid +, clear ++, very clear +++

Grittiness, odour and colour change were observed and recorded.

3.5.10 Determination of Drug Content/ Assay of gel formulation.

The label claim for all the formulation as per Table 1 is:

Active Ingredient: Ibuprofen 5% w/w.

500mg of gel (equivalent to 5mg of drug) was dissolved in 100ml of Phosphate buffer pH 7.4. The volumetric flask were shaken for 15 minutes. Subsequently, the solution was diluted by taking 3 ml and mixing with 25ml buffer. This diluted solution was filtered using whatman filter paper no. 42. Drug content was measured with the UV spectrophotometer at 222 nm (Genesys 10S serial no. 2L9R130209)

A standard was prepared by dissolving 5 mg of pure Ibuprofen in 100ml pH 7.4 phosphate buffer. 3 ml of this solution was diluted into 25 ml the same buffer and absorbance measured at 222 nm.

3.5.11 Analysis of drug release and dissolution.

Preparation of phosphate Buffer pH 7.4 was done by dissolving potassium dihydrogen phosphate in 1500 ml distilled water and separately dissolving 9.6 g odium hydroxide in 1200 ml distilled water. The two were mixed and made up to 6000 ml with distilled water. pH was then adjusted to 7.4 using 2M sodium hydroxide.

In vitro drug release studies of the gels was conducted for a period of 1 hour using six station USP XX11 type 1 apparatus (Ereweka DT6 serial No. 68062, Germany) equilibrated at 37 ± 0.5^0C and 100 rpm. A modified holding cell was used and cellulose nitrate filter paper was soaked for 24 hours to hydrate. This was used to mimic human skin .Validations studies were done on this modified cell to ensure reproducibility of outcomes during the study.

The drug release studies were carried out in 900 ml phosphate buffer at Ph 7.4 under sink conditions. 1.8g of gel was loaded into the modified cell. At every 15 minutes interval, an aliquot of 10 ml was withdrawn from the dissolution medium and replaced with a fresh medium to maintain the volume (sink conditions). After dilution of 3ml to 10ml using the buffer solution and filtration using Whatman filter paper no.42, the solution was analysed at 222nm by UV spectrophotometer (Genesys 10S serial no. 2L9R13029). The amount of drug released at different intervals was calculated by comparing it with previously prepared standard of ibuprofen in pH 7.4 phosphate buffer medium.

Pharmacokinetic modeling of drug release (Zero-order and first order) were applied to interpret the drug release kinetics from the gel matrix with the help of equation 6-8)

$M = M_o - K_o t$ Equation 6 Zero order

$InM = InM_o - K_1 t$ Equation 7

M = C = Amount of Drug

$LnC_p = lnC_o - k_o t$ Equation 8 First order

$Q = Kn\sqrt{t}$

$^{Mt}/_{Mx} = K_k t^n$

To characterize drug release rate in different experimental conditions MDT (Mean dissolution time) T25%, T50% and T80% values is obtained by the following calculations:

$T25\% = (0.25/K)^{1/n}$

$T50\% = (0.5/K)^{1/n}$

$T80\% = (0.8/K)^{1/n}$

MDT can also be calculated by the following equation (Mockel et at., 1993)

$$MDT = \frac{n}{n+1} \cdot K^{-1/n}$$

MDT values are used to characterize drug release from dosage form and the retarding efficiency of the polymer. A higher value of MDT indicates a higher drug retaining ability of the polymer and vice versa. It is a function of polymer loading, polymer nature and physico-chemical properties of the drug molecule (Shahida J. et al., 2012).

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 Preformulation Studies

The Ibuprofen powder (API) was subjected to preformulation studies.

4.1.1 Colour – Ibuprofen is a colourless, crystalline solid. (O'Niel M.J. (ed). 2001).

4.1.2 Odour – Ibuprofen has characteristic odour (McEvoy G. K(ed). 1990).

4.1.3 Melting Point

The melting point of Ibuprofen was found to be 75 – 77^{0}c. This indicates that the API used was of high purity due to small range of MP (O'Niel M.J. (Ed) 2001)

Plate 5 – Melting Point Apparatus.

4.1.4 Solubility

Ibuprofen is readily soluble in most organic solvents. It is very soluble in ethyl alcohol (Osol, A. (ed) 1980).

Poor solubility in water was observed. The solubility in water is 21mg/ litre at 25^{0}C.

Table 3: Solubility of Ibuprofen in various solvents.

Solvent	Solubility
Ethanol	Very Soluble
Water	Poorly Soluble
Water/ Ethanol Mixture	Soluble
Methanol	Very Soluble
Propylene glycol	Soluble

All the results of preformulation studies were consistent with findings of previous researchers.

4.2 Batch Weight determination

The weights of each jar was taken and then the final weight after filling. The anticipated weight was 100g.

Table 4: Batch Mean Weight of the formulations

Formulation	Mean Weight (g)	Deviation from anticipated weight
F1	102.0	+2
F2	101.7	+1.7
F3	103	+3
F4	104	+4
F5	101.3	+1.3
F6	102.3	+2.3

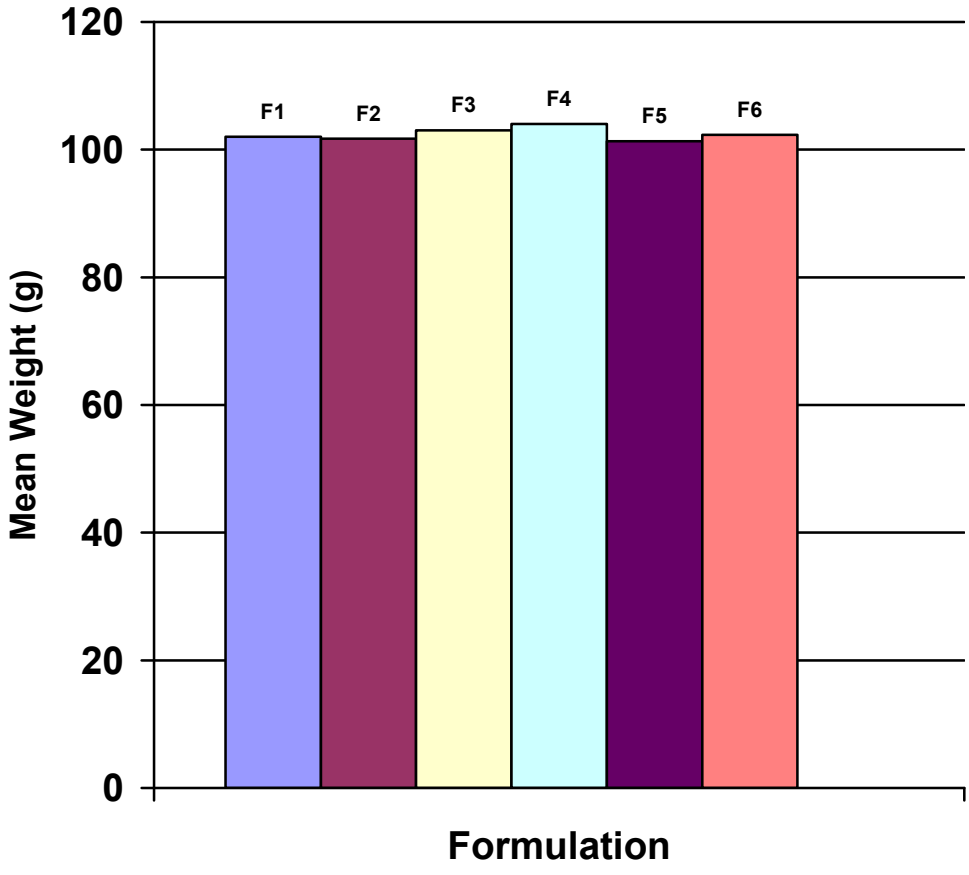

Figure 2: Mean Batch weight of the formulations

Variation in weight was attributed to spirage while mixing due to small size of batch (100g) and the fact that the alcohol content was high and its evaporation during stirring could make the final weight vary. Adjustment of pH was done by dissolving Triethanolamine in small volume of water which also made the final weights vary.

4.3 Results of pH determination

Table 5: Mean pH of formulations

Formulation	Mean pH
F1	6.4
F2	6.5
F3	6.4
F4	6.5
F5	6.5
F6	6.5

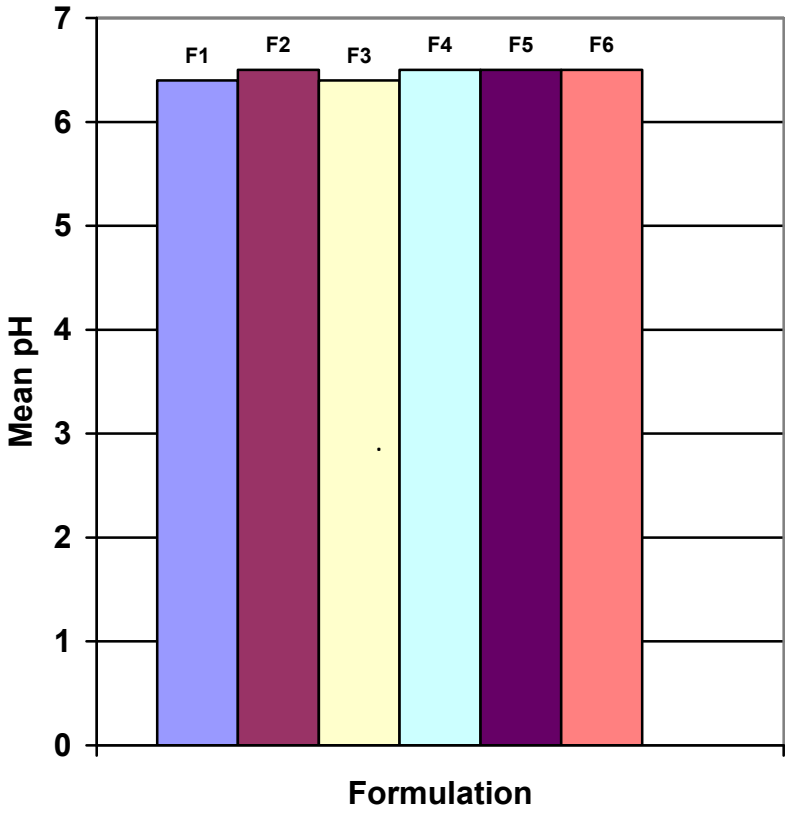

Figure 3: Mean pH of different formulations

Mean pH = 6.5

4.4 Results of Determination of Viscosity

Table 6: Mean viscosity of gel formulations

Formulation	Viscosity (mPa)
F1	3780
F2	9350
F3	9360
F4	Undetected
F5	470
F6	9330

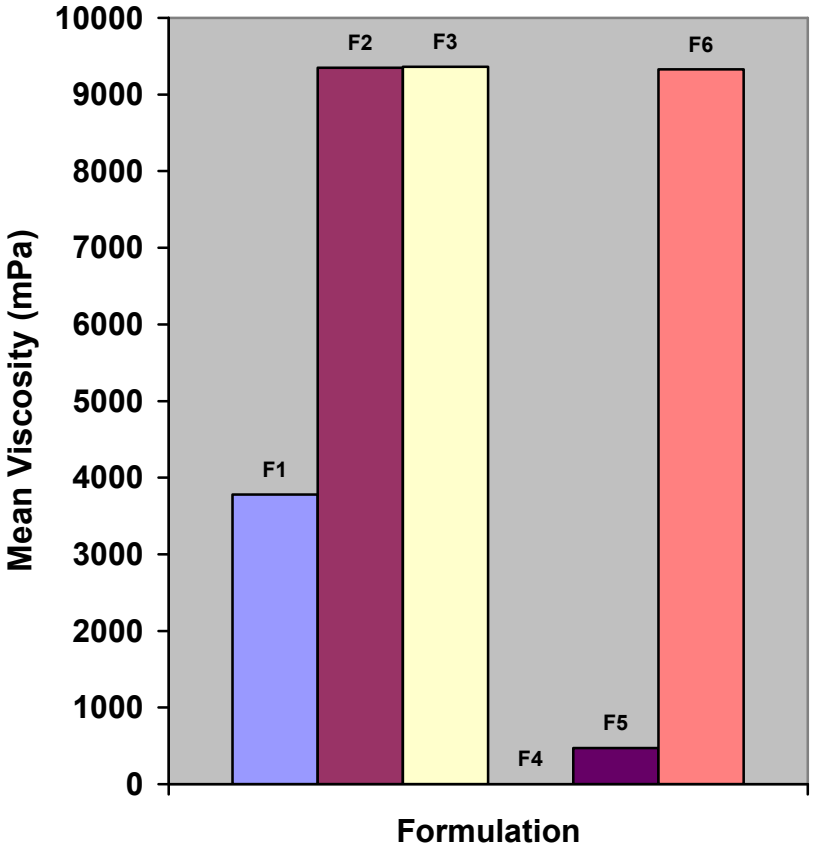

Figure 4: Mean Viscosity of different formulations using spindle No.4

Plate 6: Viscometer

Plate 7: Spindles

Limits for viscosity is 10 – 90 % when using spindle no.4

Ideal viscosity of gel is dependent on the use (skin, ophthalmic, oral, vaginal e.t.c)

The viscosity of formulation no. 4 was undetected using spindle no.4.

4.5 Results of determination of microbial load.

4.5.1 Bacterial load

Table 7 Bacterial load of different formulations

Formulation		No. of Colonies	Calculated Bacterial load	Pass/ Fail
F1	Batch 1	1	100	Pass
	Batch 2	1	100	Pass
	Batch 3	2	200	Pass
F2	Batch 1	3	300	Pass
	Batch 2	1	100	Pass
	Batch 3	3	300	Pass
F3	Batch 1	2	200	Pass
	Batch 2	2	200	Pass
	Batch 3	1	100	Pass
F4	Batch 1	5	500	Pass
	Batch 2	2	200	Pass
	Batch 3	1	100	Pass
F5	Batch 1	4	400	Pass
	Batch 2	uncountable	unquantifiable	Fail
	Batch 3	4	400	Pass
F6	Batch 1	1	100	Pass
	Batch 2	2	200	Pass
	Batch 3	2	200	Pass
	Positive control	4	400	Pass
	Negative Control	0	0	Pass

Limits: Not more than 1000 cfu/ml for bacteria load(BP 2008).

4.5.2 Fungal load

Table 8: Fungal load of different formulations

Formulation		No. of Colonies	Calculated Fungal load	Pass/ Fail
F1	Batch 1	1	100	Pass
	Batch 2	1	100	Pass
	Batch 3	1	100	Pass
F2	Batch 1	1	100	Pass
	Batch 2	1	100	Pass
	Batch 3	1	100	Pass
F3	Batch 1	1	100	Pass
	Batch 2	1	100	Pass
	Batch 3	1	100	Pass
F4	Batch 1	1	100	Pass
	Batch 2	1	100	Pass
	Batch 3	1	100	Pass
F5	Batch 1	1	100	Pass
	Batch 2	5	500	Fail
	Batch 3	1	100	Pass
F6	Batch 1	1	100	Pass
	Batch 2	1	100	Pass
	Batch 3	1	100	Pass
	Positive control	1	100	Pass
	Negative Control	0	0	Pass

Limits: Not more than 100 cfu/ml for fungal load (BP 2008)

4.6 Results of determination of gel clarity, grittiness, presence of odour and colour change

Gel clarity (Turbid+, clear++, very clear+++)

Table 9: Organoleptic properties of gel formulations

Formulation		Clarity	Gritness	Odour	Colour Change
F1	Batch 1	+++	None observed	None observed	None observed
	Batch 2	+++	None observed	None observed	None observed
	Batch 3	+++	None observed	None observed	None observed
F2	Batch 1	+++	None observed	None observed	None observed
	Batch 2	+++	None observed	None observed	None observed
	Batch 3	+++	None observed	None observed	None observed
F3	Batch 1	+++	None observed	None observed	None observed
	Batch 2	+++	None observed	None observed	None observed
	Batch 3	+++	None observed	None observed	None observed
F4	Batch 1	+++	None observed	None observed	None observed
	Batch 2	+++	None observed	None observed	None observed
	Batch 3	+++	None observed	None observed	None observed
F5	Batch 1	+++	None observed	None observed	None observed
	Batch 2	++	None observed	None observed	None observed
	Batch 3	++	None observed	None observed	None observed
F6	Batch 1	++	None observed	None observed	None observed
	Batch 2	+	None observed	None observed	None observed
	Batch 3	+	None observed	None observed	None observed

Clarity of the gel may be used to indicate that all the ingredients were able to dissolve. Lack of odour and colour change indicates stability of the gel. Carbopol based gels become abit turbid due to evaporation of ethanol. This explains why they make a good film on application to the skin.

4.7 Results of formulation Assay (Drug content)

Table 10: Percentage drug content for various formulations

Formulation	Mean absorbance	Percentage Drug Content
F1	0.781	99.24
F2	0.782	99.40
F3	0.788	100.13
F4	0.786	99.87
F5	0.784	99.62
F6	0.784	99.62

Standard 0.787

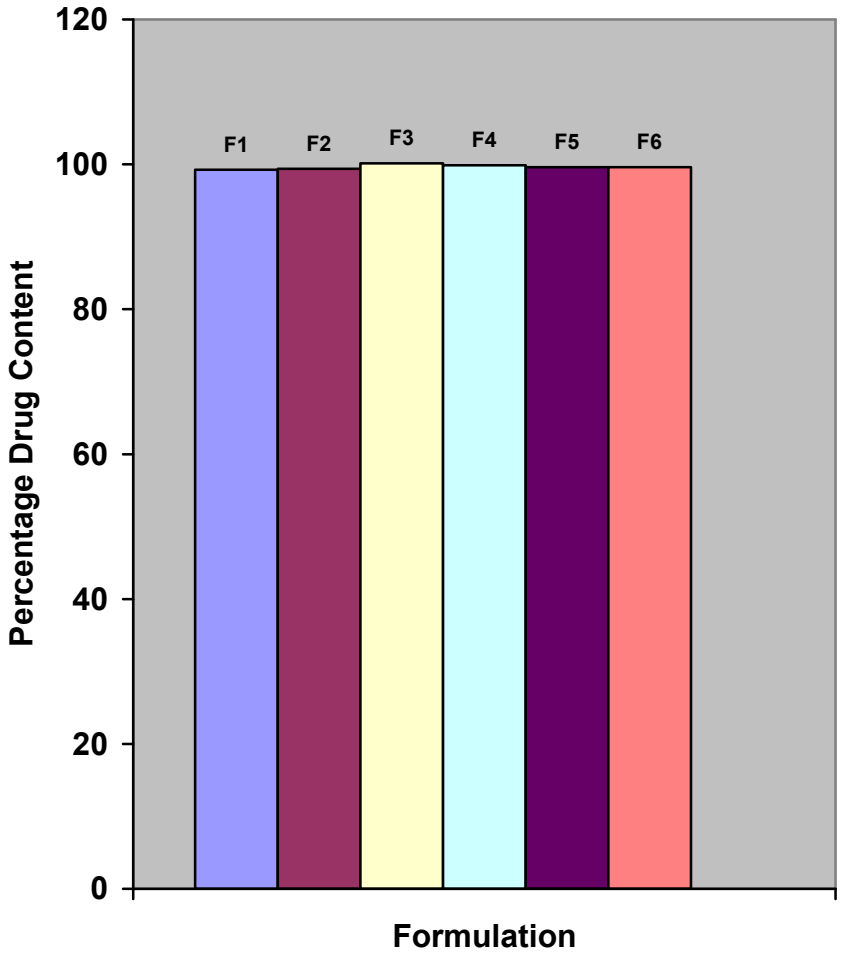

Figure 5: Percentage drug content for each of the formulations.

All formulations were found to have drug content within the limits of 90 – 110%. (BP 2008).

4.8 Results of analysis of drug release and dissolution studies.

4.8.1 Validation of modified drug release cells

3 samples were randomly selected and run 3 times each for 1 hour. Reading of absorbance was taken at 15min, 30min, 45min and 60min and recorded after dilutions and filtering.

Table 11: Absorbance results for validation testing at 222 nm

Formulation	Time **(min)**											
	15	30	45	60	15	30	45	60	15	30	45	60
F1 Batch 1	0.429	0.548	0.650	0.736	0.428	0.549	0.652	0.735	0.428	0.550	0.650	0.738
F3 Batch 1	0.218	0.299	0.357	0.421	0.218	0.299	0.358	0.419	0.219	0.297	0.357	0.420
F1 Batch 1	0.202	0.220	0.253	0.308	0.205	0.224	0.254	0.309	0.203	0.221	0.252	0.310

Table 12: Mean Absorbance for validation data

Formulation	**Time (min)**			
	15	**30**	**45**	**60**
F1 Batch 1	0.428	0.549	0.650	0.736
F3 Batch 1	0.218	0.298	0.357	0.420
F1 Batch 1	0.203	0.221	0.253	0.309

Table 13: Standard deviation for validation data

Formulation	Time **(min)**											
	15	30	45	60	15	30	45	60	15	30	45	60
F1 Batch 1	0.01	0.01	0	0	15	0	0.02	-0.01	0	0.01	0	0.02
F3 Batch 1	0	0	0	0.1	0	0.1	0.01	-0.01	0.1	-0.01	0	0
F1 Batch 1	-0.01	-0.01	0	-0.01	0	-0.03	0.001	0	0	0	-0.01	0.01

The standard deviation from the mean was found to be within a small range. The results were found to be reproducible and the modified cell was employed in the drug release and dissolution testing.

4.8.2 The results for absorbance of the samples drawn from drug release study

Table 14: Drug release for different gel formulations

Time (min)	Formulations																	
	F1			F2			F3			F4			F5			F6		
	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3	Batch 1	Batch 2	Batch 3
15	0.428	0.412	0.429	0.401	0.404	0.406	0.217	0.209	0.216	0.216	0.216	0.218	0.210	0.208	0.212	0.200	0.204	0.202
30	0.550	0.555	0.551	0.531	0.530	0.533	0.298	0.298	0.290	0.300	0.299	0.312	0.252	0.250	0.251	0.220	0.222	0.223
45	0.652	0.650	0.649	0.640	0.642	0.648	0.358	0.355	0.350	0.359	0.358	0.354	0.299	0.297	0.300	0.255	0.256	0.254
60	0.741	0.742	0.740	0.68	0.680	0.690	0.419	0.420	0.418	0.419	0.418	0.422	0.341	0.340	0.340	0.310	0.312	0.310

Standard absorbance = 0.796

Plate 8: Dissolution tester

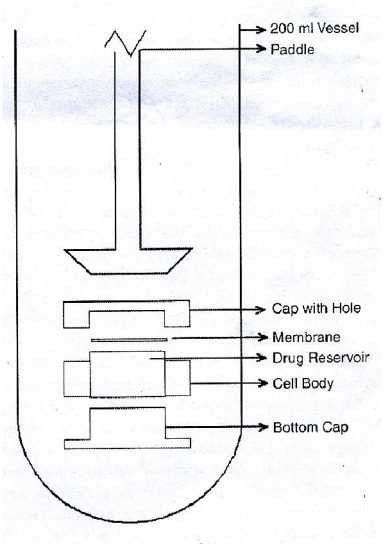

Figure 6: Dissolution cell for semisolids

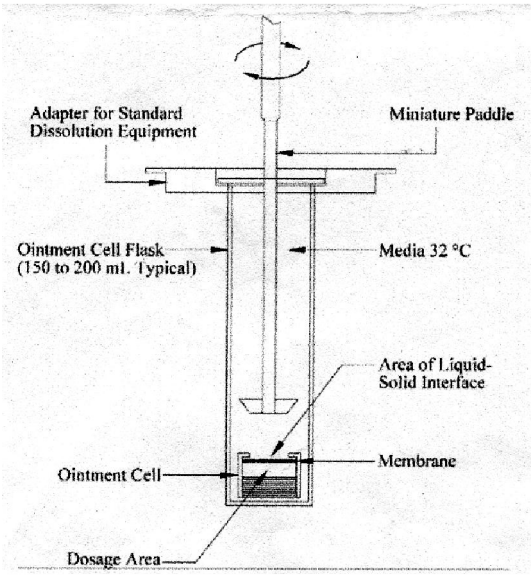

Figure 7: Modified holding cell for semisolids

Adopted from Journal of Food and Drug Analysis, Vol. 12. 1, 2004

Plate 9: Modified cell for *in vitro* drug release and dissolution testing.

4.8.3 Pharmacokinetic Modelling

Batches were selected randomly for pharmacokinetic modeling.

Table 15: Cumulative percent drug release of selected formulations

Time (Min)	Cumulative percent of release					
	F1 Batch 1	F2 Batch 1	F3 Batch 2	F4 Batch 2	F5 Batch 3	F6 Batch 1
15	53.7	50.4	26.2	27.4	26.6	25.1
30	69.1	66.7	37.4	39.2	31.4	27.6
45	81.9	80.4	44.6	44.5	37.7	32.0
60	93.1	85.4	52.8	53.0	42.7	38.9

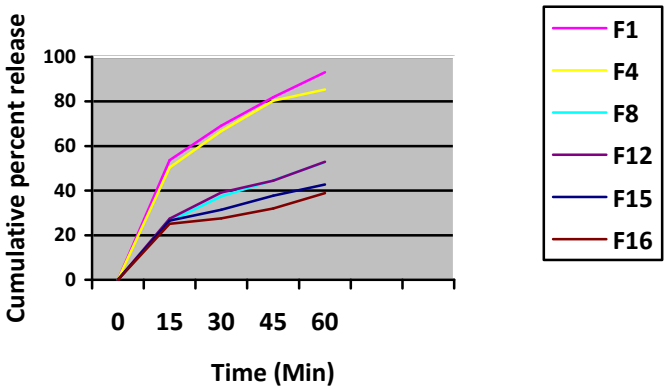

Fig 8: In vitro percentage release Profile of ibuprofen from selected gels of HEC and C940 (First order plot)

Formulation 1 Batch 1 absorbance results were used to draw the first order plot.

Table 16: Cumulative concentration of drug released with time for Formulation 1 Batch 1.

Time (min)	Percent Drug Released	Cumulative Concentration Released (mg)	ln Cumulative concentration
15	53.7	48.33	3.88
30	69.1	62.19	4.13
45	81.9	73.71	4.30
60	93.1	83.79	4.43

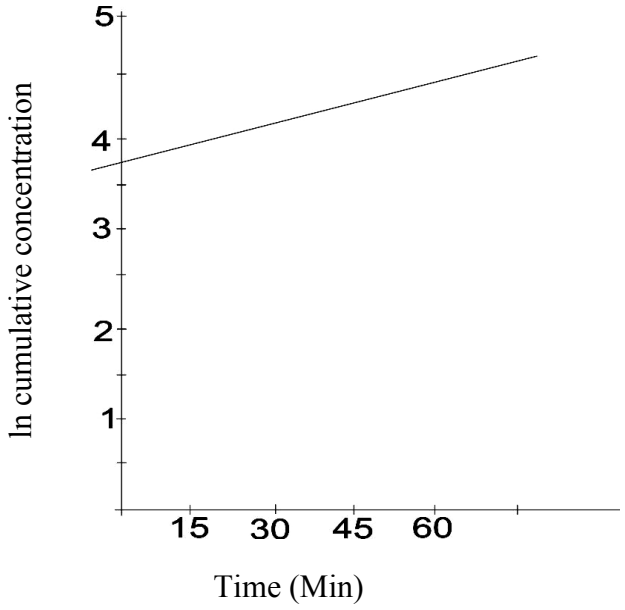

Figure 9: Plot of ln concentration against time (first-order plot) for formulation 1 batch 1.

$K_o = \frac{dy}{dx} = \frac{0.82}{1} = 0.82\ h^{-1}$

$C = C_o - K_o t$

$InC_p = InC_o - K_o t$

$lnC_p = In3.61 - 0.82t$ (Equation 9)

These graphs (Figure 7 and 8) show first order drug release pharmacokinetics.

4.9 Discussion

As fully described in this study ibuprofen was formulated as topical gels with mean pH of 6.5. The label claim of the gel is 5% w/w of Ibuprofen, 15% non volatile solvents mainly propylene glycol, 45% ethanol and 0.75 % to 3% gelling agent, in this case hydroxyl ethyl cellulose and carbopol 940. Ethanol and menthol were used both also co-solvents to help in the solubility of ibuprofen and together with glycerine they were used as penetration enhancers. Ionic concentration of water was found to be critical in the gelling process.

All the formulations were found to be able to produce a transparent to colourless gel. The mean weight of the formulations were found to be within 1 to 4 deviation from the expected 100g final weight. This could be attributed to loss due to volatiles (ethanol) and spillages during stirring and excess weight due to pH adjustment. Drug content was well within 90 – 110% label claim. The viscosity of the formulations show variation depending on the concentration of the polymer. All gel formulations had mean viscosity between 470 - 9360 mPa except formulation 4 with 0.75% carbopol940 which were undetected when using spindle no. 4 (Table 6). Microbial load for one gel batch formulation (F5 Batch 2) was found to be too high above the limits of 1000 cfu/ml for bacteria and 100 cfu/ml for fungi. Possible sources of microbial contamination were most likely the raw materials especially water, the vessels used, the equipment like the stirrer and the environment in the laboratory where the air is not filtered.

Modeling produced first order drug release pharmacokinetics. In vitro drug release profile obtained for formulations containing hydroxyl ethyl cellulose were found to be rapid. Carbopol 940 produced slower drug release profile. The concentration of each polymer was also found to determine the release characteristic. The higher the concentration of the polymer, the lower the drug release rate. This can be attributed to the drug release retarding effect of the polymer where the drug takes time to be released from the polymer matrix. Hydroxy ethyl cellulose has high solubility at pH 7.4 and this allowed the drug to be released from the matrix at a higher rate. Carbopol 940 on the other hand has lower solubility at pH 7.4 (Vijaya R. et al., 2011) and hence the release rate was low. Due to the presence of more ions in the buffer solution and the pH, Hydroxy ethyl cellulose dissolves faster and the drug is released,

similar findings were observed by Rita and Mohamed (2011) who observed the gradual rate retarding effect of Ketorolac tromethanine from transdermal film prepared with kollidon SR at increasing order. The burst effect (high initial release) might be due to the initial migration of the drug towards the surface of the matrix (Pratibha S.P. et al., 2010).

4.10 Conclusion

Ibuprofen can be formulated as a topical dosage form using different polymers, in this case hydroxyl ethyl cellulose and carbopol 940. From the in vitro release data, it can be concluded that various polymers release drugs at various rates and the concentration of the polymer affects the release profiles of the drug from the formulation. Formulations F2 (hydroxyl ethyl cellulose base) and F6 (carbopol 940 base) were found to have good properties in their finished form.

4.11 Recommendations

Further research work can proceed to combine various polymers to get the best formulation to give fast onset (Burst effect) and prolonged release of Ibuprofen from the gel formulation. In vivo performance of these formulations should also be investigated.

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