

**FORMULATION DEVELOPMENT OF 2% ERYHTROMYCIN AND 0.75%
METRONIDAZOLE TOPICAL GEL**

BY

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degree of Masters of Pharmacy in Industrial Pharmacy at the University of Nairobi.**

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DECLARATION

I, **NDEGEH BOB KEACY**, declare that the matter embodied in the dissertation titled **FORMULATION DEVELOPMENT OF 2% ERTHROMYCIN / 0.75% METRONIDAZOLE TOPICAL GEL** is a bonafide and genuine work carried out by me under the guidance of my supervisors at the University of Nairobi. I also declare that the same has not previously formed the basis for the award of any associate ship, fellowship and degree of any other university or institution.

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DEDICATION

Dedicated to my Family &



Friends

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This study was conducted at the Department of Pharmaceutics and Pharmacy Practice and Department of Pharmaceutical Chemistry, School of Pharmacy, University of Nairobi.

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DEFINITION OF TERMS AND SYMBOLS

λ_{max}.	:Absorption Maxima
Conc.	:Concentration
TDDS	:Topical drug delivery system
cm	:Centimeter
MIC	: Minimum inhibitory concentration.
FT-IR	:Fourier Transform Infrared Spectroscopy
g	:Grams
bis n die	:Twice a day application
μg	:Microgram
mg	:Milligram
PP	: Propyl parabene.
MP	: Methyl parabene.
ml	:Milliliter
M	:Molarity
μm	:Micrometer
%	:Percent
S.D.	:Standard Deviation
s	:Seconds
e.g.	:Example
Semel in die	: Once a day application
UV	:Ultra Violet
HPMC	:Hydroxyl Propyl Methyl Cellulose
mpa.s	:Millipascal .seconds
TEA	:Triethanolamine
Rpm	:Rotations per minute
M	: Metronidazole
w/w	:Weight/ Weight
E	: Erythromycin.

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ABSTRACT

Introduction

Topical gels that satisfy the Target product profile are approved as formulations that are stable and proved to have effective in-vitro release and therapeutic activity. The skin been the biggest defense organ creates a vast opportunity as a delivery point for topical application drugs. In acne treatment and management many drug regimens are available but not as a single combined unit formulation in both topical and oral formulations. Topical Erythromycin gel and Metronidazole gel are available as separate product in the market, at the same time this two drugs are prescribed for the treatment and management of acne, in most cases compounding takes place at most pharmacies by mixing the two product, this is an expensive affair to the patient and this might also affect the pharmacokinetics of the product. This lead to the innovation of a combined dosage form of Erythromycin /Metronidazole as a topical gel using carbomer polymers. Erythromycin as a Macrolide antibiotic is an inhibitors of protein synthesis. Metronidazole is a synthetic nitro-imidazole, the mechanism of action include anti-inflammatory properties and antibacterial as documented in most literature studies. Acne been a long term disease, patient compliant to medication is key, therapeutically effective and cosmetically elegant dug as a combination in a topical gel dosage form will enhance better treatment and management of the disease.

Methodology

The topical gel formulation of 2% Erythromycin and 0.75% Metronidazole combination was done by factorial experimental design. The product development was done first, by having the known Target product profile qualities to be achieved. Secondly the pre-formulation studies were done for the actives pharmaceutical ingredient properties on solubility in different diluents and also by combining the different diluents in different ratios to achieve maximum solubility at room temperature and pressure. Thirdly the compatibility studies, both physical compatibility and chemical compatibility for the actives and excipients were done by Physical observation and FT-IR spectroscopy respectively. The polymer used in the formulation was carbopol 940 in 0.5% and 1.5% concentration respectively. Hydroxyl Propyl methyl cellulose (HPMC) as a thickening agent was also used in 1%, 1.5% and 2% concentration respectively. The topical gel permeation enhancer employed was by using a

methanol 60%/ water 40% system. The topical gel formulation was analyzed for different parameter such as pH as an in-process parameter and end product parameter, viscosity, micro-bioassay and drug content. Solubility been a major key aspect in the formulation various vehicles were tested and the best result in a system consisting of a ratio of 60% methanol: 40% water. Post formulation studies for each formulated topical gels were organoleptic properties as per set standard in the target product profile including clarity, homogeneity, phase separation, grittiness and pH. Analysis was also done for viscosity, micro-bioassay and drug content. HPLC method was initially tried for simultaneous analysis of both API's to determine the label claim but results were inconclusive. Drug content analysis was done for Metronidazole using UV/VIS spectroscopy method.

Results

In the pre-formulation studies complete solubility for both the API, s was achieved in 60% methanol: 40% water ratio. There was no incompatibility observed from the FT-IR spectra in the API, s and excipients used from the characterized absorption encountered in the parent hydrocarbon species and the associated back-born groups. In the post formulation studies organoleptic characteristic of the formulated gels showed descriptive result in all the tests done to be within the limits set for a topical gels, the only varying descriptive observation was in the clarity test, same turbidity was observed in formulation G1, G3, G5 and G7. pH was within the limit set of between 4 - 6.5 for all the formulations .The viscosity was performed at the 2nd month and formulation G1, G3, G5 and G7 were within limits (10%-90%) as per the viscometer used NJS-5S rotating viscometer. Formulation G2, G4, G6 and G8 were not above 100% which was also considered within limit (1000mpa.s-10000mpa.s). The micro-bioassay analysis was done and the formulation activity index was calculated, the descriptive results showed formulations G1 to G8 zones of inhibition close to that of the reference drug .Formulations G1, G3, G5 and G7 indicated better descriptive zone of inhibition as that of reference drug from the microbial activity index results for both high and low concentration. Percentage label claim for Metronidazole was within limit as per BP, for formulation G1, G5, G6, G7 and G8 between 90 -110% w/w

Conclusion

The presented work envisages the feasibility of the use of the methanol 60%/water 40% system as good diluents for the above formulations.. The drugs employed Erythromycin and Metronidazole showed good solubility and also the polymer used, Carbopol 940 .The use of

HPMC as a thickening agent, effect was observed not to have descriptive variation when it came to the viscosity of the formulations. For carbopol 940 used at 0.5% the topical gel formulations physical descriptive observation were more free flowing as compared to 1.5% carbopol 940 thus need for further test on rheological properties. The stability studies after 2 month showed descriptive results in all the formulation under room temperature and pressure to be within the standard set in the target product profile.

CHAPTER ONE

INTRODUCTION

1.0 Rosacea and Acne.

Skin disorders are a common problem in childhood and adolescence, with some persisting till adulthood. Treatment of these dermatological diseases has been typified by poly-pharmacy. Rosacea is a chronic relapsing skin disorder characterized by persistent erythema, telangiectasia, facial flushing, pustules affecting the central face and inflammatory papules. The National Rosacea Society, Barrington, UK, has classified it based on 4 subtypes: erthematotelangiectatic, papulolustular, phymatous, ocular and one variant, granulomatous. Before treatment proper diagnosis should be done. Some topical pharmacotherapeutic options in the market include: erythromycin, metronidazole (metrocream or metrolotion, metrogel[®]), azaleic acid, clindamycin, 1% clindamycin 5% -benzoyl peroxide gel and 10% sodium sulfacetamide 5%-sulphure lotion. For patients with severe cases to moderate papulopustular rosacea and those with ocular infection, systemic therapy that includes erythromycin, minocycline, metronidazole, doxycycline or tetracycline is often used. The most widely used topical rosacea treatment is Metronidazole:- a nitroimidazole antibiotic, which has been available for clinical use since 1989 (Del Rosso , 2002). It's available in 0.75% gel, lotion and cream formulation bis in die application and 1% cream, gel for semel in die application (Gooderham ,M., 2009) .



Figure 1: Acne vulgarise. (Preet, L., et al, 2013)

Acne is a disorder of the pilocebaceous follicles of the skin and it is characterized by comedones, papules, cysts, pustules and scars. Mainly a disease of adolescence, It is not only a physical disorder but also has a significant psychosocial implication and contributes to lower self-esteem of the patient. It affects 85% of teenagers, 42.5% of men, and 50.9% of women between the ages of 20 and 30 years, spontaneous regression usually occurs after the age of 20, but some patients may continue suffering during adult life (Wankhade.R., et al., 2012). Major progressive factors include hyper-keratosis of the follicular epithelium, increased sebum production and proliferation of propionibacterium acne. It is classified into - : inflammatory acne and non-inflammatory or comedonal acne. Antibiotic are the most preferred treatment both oral and tropical treatment. Oral treatment takes minimum of 3 months while treatment for 6 months yields better results but may continue per physicians analysis (Simonart,T., et al., 2008).

Topical antibiotics treatment for mild to moderate acne is usually initiated as first line treatment due to low side effects. Topical antibiotics work by killing the propionibacterium acne bacteria thus indirectly keeping the pores open, by this been effective for treating mild to moderate inflammatory acne. Examples of topical antibiotics are: clindamycin, erythromycin, azithromycin, minocycline, doxycycline and tetracycline. Of the above aforementioned antibiotics, erythromycin as a topical formulation acts both as an antimicrobial and anti-inflammatory agent. It has been shown to be more effective as a combination with benzoyl peroxide that has keratolytic and desquamative properties. This erythromycin-benzoyl peroxide combination produces comedolytic and keratolytic effect. Erythromycin is safe for use even in pregnant women (Strauss, J.S., et al., 2007). The first use of erythromycin as a topical antibiotic for acne was reported in a study of the Macrolides, erythromycin group, which showed topical erythromycin had the advantage of reducing side effects, like nausea, diarrhea, vaginal yeast overgrowth and gastrointestinal upset, caused by systemic drugs (Fulton, 1974).

Topical formulations are intended for external application in the form of creams, powders, ointments, paste, gels, suspensions, lotions, foams, sprays, aerosols and solutions. Topically administered drugs have -: both local and systemic disease treatment and management. They

are advantageous in been more convenient pain-free self administration, effective and less toxic than most oral and injectable formulation in terms of side effects. It also eliminates valleys associated with frequent dosing administration and plasma level peaks associate with oral dosing and injection to maintain a constant concentration. They have also improved patient compliance due to the form of administration via the topical application route and also when long term treatment was required. Topical application has many advantages over other routes of drug administration in that the preparation avoids hepatic first –pass metabolism and the GI-irritation seen with some orally administered drugs, elimination of this first –pass effect allows the amount of drug administered to be lower, hence safer for hepato-compromised patients(Paudel, K.S, et al., 2010). Risks and inconvenience of intravenous therapy, varied conditions of absorption in the stomach like; pH changes, presence of enzymes and gastric emptying time and bioavailability for poorly aqueous soluble drug was avoided . (Preet, L., et al., 2013) .

These drugs are divided into two categories those for systemic action and local action. For systemic effect/action the product used are also known as self –adhering transdermal drug delivery systems or transdermal patches. Local action drugs include those whose action is on the skin surface(stratum corneum) and those that modulate the function of the epidermis and the dermis (Ueda, C.T., et al., 2009).

1.1 Topical gel as a pharmaceutical dosage form.

The field of pharmaceutical industry has been developing steadily over the years and through the use of topical drug delivery sytem for both systemic and local action, it has progressed more to create a better effective drug formulations at localized sites using polymers in gel formulations. A topical gel formulation as a drug delivery system has proved to have better effect in confining the manifestation of the disease and good bioavailability of the drug as compared to cream and ointments. The U.S.P defines a gel as a semisolid system consisting of dispersion made up of either small inorganic particles or large organic molecule enclosing and interpenetrated by a liquid (Hornedo, M., 1990). Gels exhibit no flow when in the steady state and consist of synthetic or natural polymers that forms a three dimensional matrix throughout a hydrophilic liquid or dispersion medium. After application the liquid evaporates leaving the drug entrapped in a thin film of the gel-forming matrix physically covering the skin (Preet,L., et al., 2013).

The Cosmetic acceptability in today's self-image conscious world, patients are looking for topical products that are not only safe and effective, but also cosmetically acceptable and easy to apply. This was especially true in acne, where the esthetic aspect was one of the primary reasons why patients seek dermatologic consultation. Moreover, acne patients are mainly comprised of teenagers or young adults, and therefore, products that offer convenience and are minimally disruptive to their daily routines increase the level of compliance, and ultimately, the efficacy of the topical therapy. For example, vehicle considerations for prescribing should take into account the application of the drug on large, hairy surfaces like the chest and the back. This may require formulations that spread easily, or in the case of facial acne, the ideal formulation should leave minimal residue or oiliness(Bhowmik, D., et al., 2012).

1.2. Problem Statement.

It has been very easy to have a dosage form comprising a single API in the market and have patients requiring combination therapy buy several of these single API dosage forms for treatment and management of the disease. This poly-pharmacy makes a bulk of the patients' treatment regimen and may lead to lack of patient compliance especially in teenagers' stage where disease is most prevalent. A dosage form comprising a combination of API's would in the treatment and management, patient acceptance and compliance, cut cost in the manufacturing process thus creating a much cost-effective package for the industry and also the patient.

1.3. Objectives.

1.3.1 General objective.

To formulate 2% Erythromycin / 0.75% Metronidazole into a topical gel combination medication.

1.3.2 Specific objectives.

- ❖ To derive a target product profile for a topical gel of 2% Erythromycin / 0.75% Metronidazole.

- ❖ To carry out pre-formulation studies to aid the development of 2% Erythromycin / 0.75%Metronidazole topical gel.
- ❖ To formulate the topical gel using different polymers concentrations and permeation enhancers.
- ❖ To evaluate the quality of the formulated 2% Erythromycin / 0.75% Metronidazole topical gel.

1.4. Significance and anticipated outcomes.

The topical gel of 2%Erythromycin/0.75%Metronidazole formulations were expected to be within the limits set for a topical gel organoleptic properties, have good viscosity, label claim and to exhibit maximum antibacterial properties. It should also have improved use in its combined form in therapy to the patient. It should cut cost in term of manufacturing by having a single unit product.

1.5 Delimitations.

- ❖ A Target Product Profile for 2%Erythromycin / 0.75%Metronidazole was set up.
- ❖ A Compatibility studies with the different API's and excipients determined.
- ❖ The different formulae for compounding were set up for a topical gel of 2%Erythromycin/ 0.75%Metronidazole and were evaluated for its quality.

1.6 Limitations.

- ❖ Some post formulation evaluation test such as spreadability and extrudability were not done due to lack of equipment.
- ❖ In-vitro drug release profile was a challenge due to lack of a validated simultaneous analytical method and also apparatus required for the study.
- ❖ Stability studies for accelerated and long term conditions were not performed due to time constraint and equipment (stability chamber) was not available, within the project time frame.

CHAPTER TWO

LITERATURE REVIEW

2.0. Introduction.

A topical dermatological formulation is said to be successful if it satisfies the target product profile properties such as: 1) Chemically and physically stable, 2) Contains excipients that are only necessary and approved by law, 3) Releases and delivers the API from the formulation and into the skin as required for the target indication, 4) Easy to apply, 5) compatible with desired packaging and commercially it can be manufactured with a process that is scalable.

Before formulation of a product pre-formulation studies are conducted and form the basis for the rational formulation design. The API's stability at different pH ranges was assessed and also the solubility in different solvents determined and then compatibility studies with other, API's and excipients done. The information gathered helped to formulate a prototype formulation, this is then evaluated for the required standards set for the particular formulation in the pharmacopeia (Lee, R.W., et al., 2010, Chang, R.K., et al., 2013).

2.1. Anatomy of the skin.

The skin is the biggest external defense system and organ in the body that prevents microorganism from entering the body; it also acts as a mechanical barrier between the inner part of the body and external environment. Skin temperature is between 30-40°C depending on the environment. Skin pH is of 4.2 - 5.6. Its large surface area presents a vast opportunity as a drug delivery point. The skin is structured in 3 distinct layers namely the epidermis outer layer, dermis middle layer and the hypodermis inner most layer. The epidermis consists of epithelial cells that are connected by desmosomes. Desmosomes are in contact with intracellular keratin filates which produce keratin. Keratin cells accumulate and crosslink with the keratin cells in the cytosol during their maturation. When the older cell dies ,this network of keratin fibres remain and provide a tough protective layer in epidermis known as protective keratinized layer .In diseased skin ,particularly burns, epidermis is destroyed causing potential loss of body fluid and increased susceptibility to microbial infection (Premjeet, S., et al., 2012) .

The epidermis consists of five layers namely (from outside to inside): stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, stratum germinativum (basal layer). In the epidermis there exist same cell types namely langerhans cells found in the basal layer which are important immunological cells and are also found in the middle dermis (El Maghraby, G.M., et al., 2008). Keratinocytes make up 95% of cell types in epidermis, melanocytes are pigment producer cell in basal layer of epidermis. The dermis has a thickness of 3-5mm. It is characterized by many elastin fibers that provide stretching ability as well as lots of collagen that provides strength to the skin. It also plays a major role in temperature regulation. Blood vessels found in the dermis provide nutrients, and nerves present play the role for pain and pressure sensation. The dermis also has interfibrillar gel of glycosaminoglycan, salt, water, sweat glands and lymphatic cells. Others cell types are fibroblast which produces collagen, macrophages also known as scavenger cells and mast cells responsible for interaction with eosinophils and responsible for immunological reactions (Habif, D.A., et.al, 2004).

The hypodermis is the inner most layer of the skin and the contact layer between the underlying tissue and the skin.

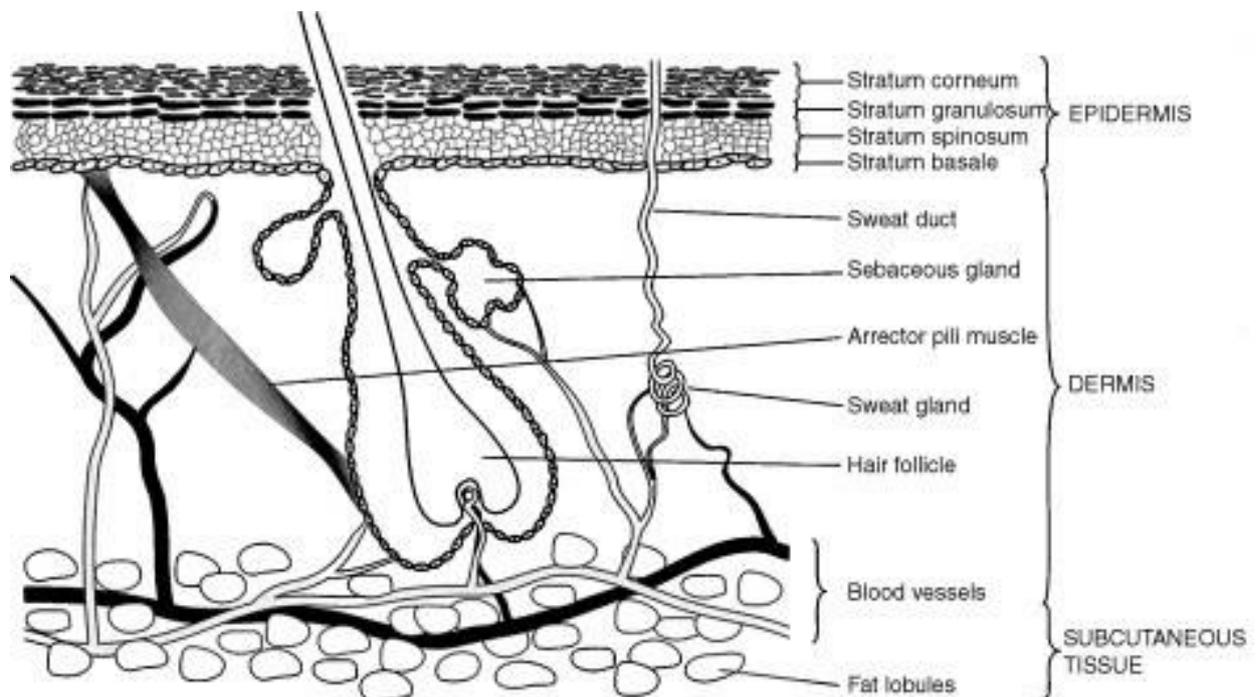


Figure 2: Diagrammatic presentation of the skin layers. (Preet, L., et al, 2013)

2.1.1 Penetration pathways through the stratum corneum.

Permeation of molecules through the skin can be through two pathways: 1) Trans-appendageal route where the molecules permeate through skin sweat gland and across hair follicles. 2).Trans-epidermal pathway which has other two micro pathways namely: intra-cellular/ trans-cellular micro pathway and inter-cellular/ para-cellular micro pathway.(Bhowmick, D.B., et al., 2013).

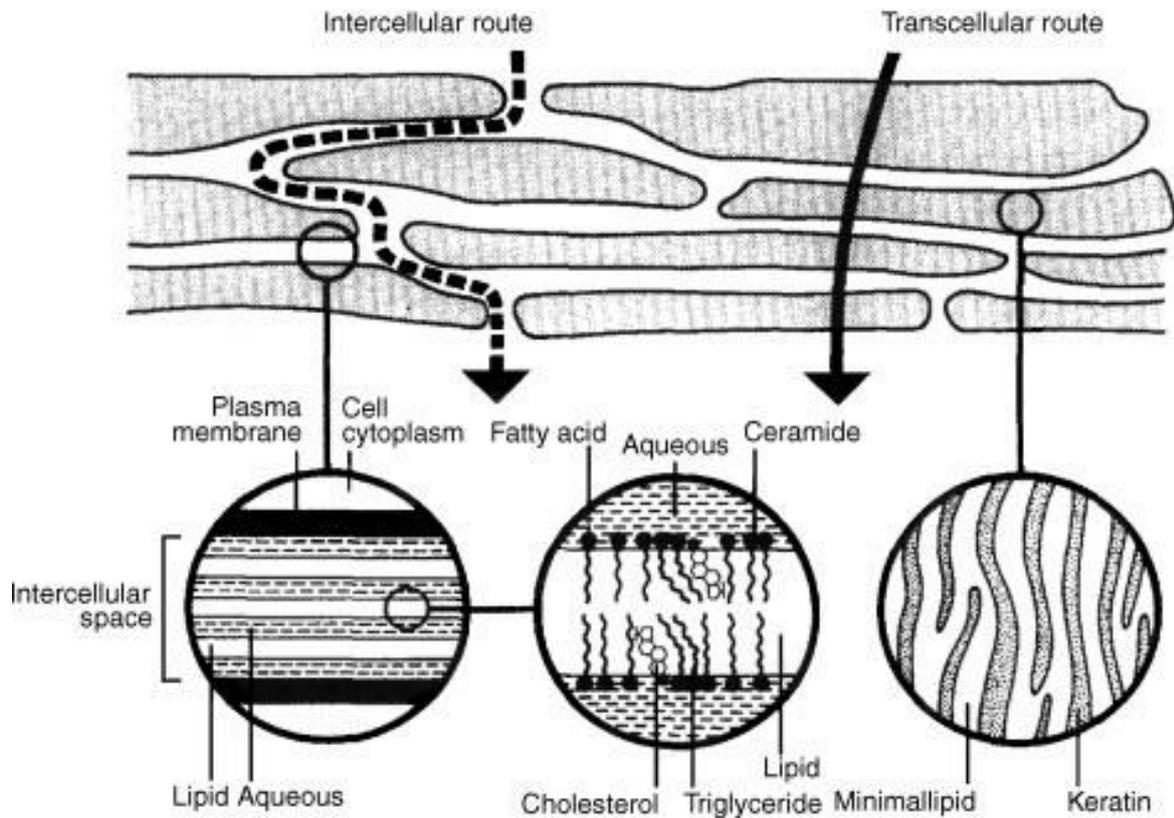


Figure 3: Possible micro route for drug Penetration across human skin (El Maghraby, G.M., et al., 2008) .

Rate and extent of absorption in the skin is also influenced by the following factors:

- ❖ **Skin pH**:- Normal human skin pH is 4.2 – 5.6. Additives to a topical formulation affect the extent of dissociation of ionizable drug molecules and thus their thermodynamic activity, partitioning and skin penetration.

- ❖ **Temperature-**: Changes in temperature on the skin are followed by normal physiological reaction like increased moisture content, increased blood flow, this may also increase solubility and diffusivity of the drug.
- ❖ **Skin microflora** - Skin surface supports a vast microbial population that can carry out bio-transformation of topically applied formulation.
- ❖ **Skin surface lipids** –Sebacous gland secrete a lipid mixture that forms a film of 0.4 μm to 4μm on the surface of the skin.
- ❖ **Effect of age-**: Reduced dissolution of topical formulations due to reduced surface lipid content of old skin. While in infants permeability of topical formulation is greater (Mithal, B.M., 1976).

2.1.2 Kinetics of drug absorption and percutaneous absorption.

TDDS goes through the following process from time of application on the skin to its absorption into the systemic circulation. 1) Dissolution within and release from the formulation 2) Partitioning into the outermost layer of the stratum corneum. 3) Diffusion through the stratum corneum 4) Partitioning from the stratum corneum into the aqueous viable epidermis 5) Diffusion through the viable epidermis and into the upper dermis and then uptake into the capillary network and eventually the systemic circulation.(Robinson, J., et al., 1987).

The drug has to have certain physico-chemical properties for permeation across the skin .The rate of permeation across the skin (dQ / dt) is given by:

$$dQ / dt = P_s (C_d - C_r) \dots \dots \dots \text{Equation 1}$$

Where:

C_d -is the concentration of the drug on the surface of (stratum corneum).

C_r - is the concentration of the drug in the body (blood).

P_s - overall permeability constant of the skin tissue to the drug.

$$P_s = (K_s D_{ss}) / h_s \dots \dots \dots \text{Equation .2}$$

Where:

K_s - It is the partition coefficient for the interfacial partitioning of the drug from a solution medium onto the stratum corneum.

D_{ss} - It is the apparent diffusivity for the steady state diffusion of the penetrant drug through a skin thickness (h_s).

h_s -Skin thickness

K_s , D_{ss} and h_s are constants under given conditions thus P_s can be considered to be a constant.

From Eq.1 it is clear that a constant rate of drug permeation can be only obtained when $C_d \gg C_r$. Normally the C_d is consistently and substantially greater than the C_r then Equation 1 becomes:

$$dQ / dt = P_s C_s \dots\dots\dots \text{equation 3}$$

Permeability coefficient = $(K_s D_{ss}) / h_s = 1 / \text{resistance}$

component of resistance occurs as follows: Vehicle > Stratum corneum > Epidermis > Dermis (Robinson, J., et al., 1987).

2.1.3 Skin pH.

The pH of the skin follows a sharp gradient across the stratum corneum which is suspected to be important in controlling enzymatic activities and skin renewal. pH being defined as the negative logarithm(base ten) of the concentration of the free hydrogen ions in aqueous solution having a range of 0-14 (acidic to alkaline) and 7 been neutral. Various investigation have revealed low pH values in the extracellular space play an important role in the regulation of enzymatic activity ,especially in keratinization and barrier regeneration thus in this respect topical formulation having acid buffer substances contribute to maintenance of skin.(Schmid-Wendtner, M.H., et al., 2006)

2.2 Rheological properties of a gel.

Rheology describes the flow of liquid and the deformation of solids. This includes the measurement of viscosity and are monitored for quality control, consistency and pharmacopeia regulations ensuring formulation of gels .Viscosity is not a single value rather it's a property of fluid that depends on the conditions of measurement e.g. the rate of deformation (shear rate).Viscosities influence the drug delivery and also drug diffusion rate

.Gels perceive a non Newtonian behavior. The yield stress is important in the product in determining the shelf life, ease of application for the end use performance and stability of the product. Yield stress been the minimum force required to initiate flow can be measured using a viscometer. Semisolid are most difficult to characterize rheologically because they combine both liquid and solid properties within the same material

Carbomer gels (carbopol) are synthetic, high-molecular weight polymer composed of acrylic acid cross linked with ether allyl sucrose or allyl ether of pentaerythrotol and they occur as white –colored fluffy, acidic, hygroscopic powder with slightly characteristic odor. Carbopol 940 0.5%-1% has a pH range of 2.5 to 3.5. Carbomer are soluble in water, after neutralization they are soluble in 95% ethanol and glycerin. When carbomers are dispersed in water, an acidic colloidal solution of low viscosity forms that will thicken when the alkaline material triethanolamine (TEA) is added .To ease the initial dispersion process, the carbomer should be sprinkled on rapidly agitated water(Loyd ,V.A., 2010). Carbopol have extensively been investigated for as a function of concentration, cross-link density and pH. the choice of solvent is also of utmost importance, most studies have demonstrated use of water as a solvent and very few have been reported on hydro-alcoholic solvents system, this systems can modify the hydrogen bonding characteristics between the polymer, water solvent, alcohol solvent, thereby affecting the swelling and viscoelastic properties of the formulation. The physical properties of the carbopol gel, the time they remain on the application surface and the drug release are very sensitive to the rheological behavior of the gel formulation. Therefore the flow behavior of these systems as a function of neutralization and polymer concentration is essential to evaluate the carbopol polymer ability to gel at a desired pH value and their potential use as a topical gel. (Islam, M.T., et al, 2004.)

2.3 Topical gel and Gellation methods.

2.3.1 Gel forming substances.

❖ Semi-synthetic polymers namely:

a) Cellulose derivatives such as hydroxypropyl methyl cellulose, methyl cellulose, hydroxyethyl cellulose.

❖ Synthetic polymers namely:

a) Carbomer such as carbopol-940, carbopol-914 and carbopol-934.

b) Polyvinyl alcohol

- c) Poloxamer
- d) Polyacrylamide
- e) Polyethylene and its co-polymers.
- ❖ Natural polymers namely:
 - a) Polysaccharides such as agar, tragacanth, potassium or sodium carrageenan, pectin, alginic acid, gellum gum, xanthin, guar gum
 - b) Protein such as collagen and gelatin.
- ❖ Inorganic substances namely bentonite and aluminium hydroxide

2.3.2 Classification of gels: based on-

- ❖ Rheological properties-they usually exhibit non-Newtonian flow properties. This can be classified into: plastic gels, pseudo-plastic gels, and thixotropic gels.
- ❖ Based on colloidal phases namely: two phase system and single phase system.
- ❖ Based on nature of solvent namely: water based (hydro gel), non-aqueous solvent (organic gel), xerogels (solid gels with low solvent concentration).
- ❖ Based on the physical nature namely; rigid gels-formed from macromolecules is linked by primary valance bonds example: silica gel, elastic gels-formed from fibrous molecules linked at the point of junction by relatively weak bonds such as hydrogen bonds and dipole attraction example: agar, guar gum (Preet. L., et al, 2013).

2.3.3 Gel formulation methods.

Gels are prepared using the following methods but some polymers may require special pre-formulation processing.

- ❖ Flocculation-in this process gellation is achieved by adding just sufficient quantity of salt while mixing to precipitate, to produce age state but not excess to bring about complete precipitation. Gels formed by this method are thixotropic in nature (Shelke, S., et al., 2013).
- ❖ Thermal changes- lipophilic colloids (solvated polymers) when subjected to thermal changes causes gellation. Many hydrogen former are more soluble in hot water than cold water. If the temperature is reduced, the degree of hydration also reduces and gellation occurs (Helal, D.A., et al., 2012).
- ❖ Chemical reactions-in the preparation of the gel by this method there is chemical interaction between solvent and solute at given concentrations. For example aluminium hydroxide gel preparation involves:- interaction of aqueous solution of aluminium salt

and sodium bicarbonate and increased concentration of the reactants produce a gel. (Helal, D.A., et al., 2012).

The Penetration enhancers are incorporated that temporarily disrupt the skin barrier, fluidize the lipid channel between corneocytes, alter the partitioning of the drug into the skin structure or otherwise enhance delivery into the skin. Propylene glycol is a multifunctional excipients in a topical formulation, having humectants, solvent and antimicrobial properties. Others are detergents and emulsifiers. They function by disrupting the barrier to encourage migration of active drug through the stratum corneum. Studies show Isopropyl myristate to provide benefit by fluidizing stratum corneum lipids and partially dissolving them. Oleic acid as a penetration enhancer works through fluidizing the intercellular lipids of the stratum corneum (Leon, H., et al., 2010).

2.4 Formulation Development.

2.4.1 Pharmaceutical product development.

The aim is to design a quality product and its manufacturing process to consistently deliver the intended performance of the product. The information and knowledge gained from pharmaceutical development studies and manufacturing experience provide scientific understanding to support the establishment of the design space, specification and manufacturing controls at a minimum in the aspects of the drug substance, excipients, container closure system, and manufacturing process that are critical to the quality of the drug product. Although chemical testing for preservative content is the attribute normally included in the drug product specification, antimicrobial preservative effectiveness should be demonstrated during development. The lowest specified concentration of antimicrobial preservative should be demonstrated to be effective in controlling microorganisms by using an antimicrobial preservative effectiveness test. The concentration used should be justified in terms of efficacy and safety ,such that the minimum concentration of preservatives that gives the required level of efficacy throughout the intended shelf life of the product is used (Lawrence, X.Y., 2008).

2.4.2 Target product profile (TPP).

Target Product Profile (TPP) ensures product meets expected specifications and attributes. This helps to identify project goals and potential risks in manufacturing, regulatory, research and quality before and during development. TPP should have the following criteria: a).The overview of the product, the intended use, target population. b). Deliverability which entails the route of administration, infrastructure requirements, user-action required, product presentation, dosage schedule. c) Usage which entails typical use efficacy, side-effects profile, reversibility, pregnancy/lactation consideration, health benefits. D) Storage which entails shelf life, and packaging.(Guideline, 2009; Lawrence, 2008, Lawrence, X.Y., et al., 2007).

2.4.3 Identification of critical quality parameters and design specification.

It is important to note that any excipients used should be of analytical grade and will comply with the pharmacopeia requirements. It should also be safe and accepted by the regulatory authorities. These performance limits and function/user test limits should be specified in the product design report that the product should meet at the time of manufacture till it is a finished product and at the end of the shelf life, even though the product has not yet been developed. This include pH, appearance (color, particle free), antimicrobial activity from knowledge of already developed similar type of product as per pharmacopeia (Gibson,M., 2009). In design space a relationship between the material characteristic and manufacturing input should also be put into consideration like temperature, speed of mixer and humidity for hygroscopic drugs. Setting of the control strategy, risk management and management of the product lifecycle with continual improvement is done through continual batch to batch production challenges and improvements can be made in the product (Lawrence, X.Y., et al., 2007).

2.4.4 .Pre-formulation studies.

The candidate drug physicochemical properties such as molecular weight , molecular volume melting point, aqueous solubility and log p will determine if the compound will penetrate the skin (stratum corneum) and as such are important in topical drug delivery .pH also plays a major role in compound that are weak acid or bases. Zwitterionic drugs permeability through the skin has been enhanced through the forming of a salt (Mazzenga, M., et al. 1992) . Solubility of the compound in the vehicle to be used is a key factor, to avoid problems such

as crystal growth, if the system was super saturated as was demonstrated in phenylbutazone cream and also observed in needle like crystals of the hydrate of betamethasone -17 valerate when creams were placed in storage. Chemical and even physical stability (Gibson, M., 2009).

2.4.5 Compatibility studies.

This type of studies is usually oriented toward the final target product. In the early stage of development the interaction of the API with different possible excipients is tested to show how it is influenced and its stability at different conditions(Bohanec, S., et al., 2010).

2.4.6 Process optimization.

This is where in manufacturing the processes that are most critical are given specific set of parameters so that those product characteristics can be achieved and also have an impact on the product quality.

2.4.7 Quality testing

This test include: content uniformity, pH, microbial limits, antimicrobial preservative content, identification, impurities, assay, viscosity and tube content uniformity. (Ueda, C.T., et al., 2009).

2.5 Active Pharmaceutical ingredients.

2.5.1 Erythromycin ethyl succinate BP.

Chemical structure:

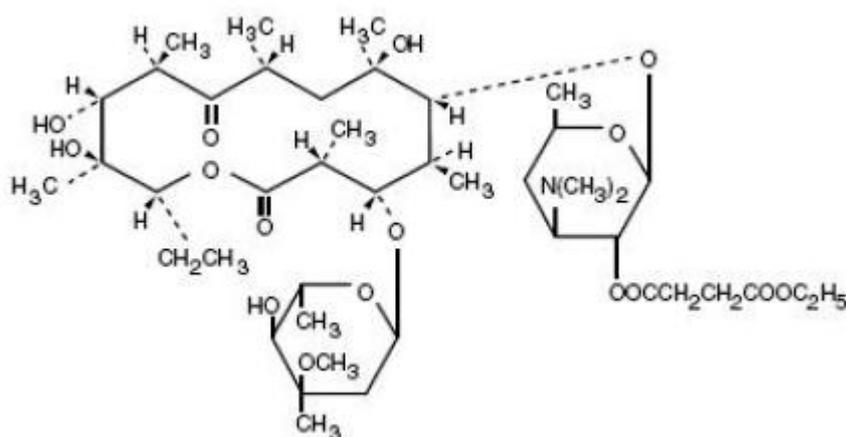


Figure 4: Erythromycin ethyl succinate BP. structure (Jigar, V., et al., 2011).

Chemical name (IUPAC name): 4-O-[(2S,3R,4S,6R)-4-(dimethylamino)-2-[[[(3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-14-ethyl-7,12,13-trihydroxy-4-[(2R,4R,5S,6S)-5-hydroxy-4-methoxy-4,6-dimethyloxan-2-yl]oxy-3,5,7,9,11,13-hexamethyl-2,10-dioxo-oxacyclotetradec-6-yl]oxy]-6-methyloxan-3-yl] 1-O-ethyl butanedioate.

Molecular formula: C₄₃H₇₅NO₁₆

Molecular weight: 862.0527G/MOL

2.5.2 Description

Color: white or slightly yellow, crystalline powder, solubility, freely sol in acetone & chloroform; soluble in 95% ethanol & benzene; sparingly soluble in ether; very slightly soluble in water. Antibiotic Class: Macrolide antibiotic, it is the ethyl succinate salt form of erythromycin.

2.5.3 Antimicrobial activity

Gram-positive bacteria, mycoplasma pneumoniae, chlamydia trachomatis, chlamydia pneumoniae, chlamydia psittaci, ureaplasma urealyticum, legionella pneumophila, campylobacter jejuni, bordatella pertussis

2.5.4 Mechanism of Action

Macrolide are inhibitors of protein synthesis. They impair the elongation cycle of the peptidyl Chain by specifically binding to the 50 S subunit of the ribosome. Specificity towards prokaryotes relies upon the absence of 50S ribosomes in eukaryotes.

2.5.5 Pharmacokinetics

Macrolides are considered time-dependent antibiotics, which mean that their efficacy will be related to the time interval during which their concentration at the infected site remains above the MIC of the offending organism.

2.5.6 Adverse Effects

Oral: Gastrointestinal: abdominal pain, nausea, vomiting, and diarrhea.

Cardiovascular System: prolongation of QT interval, ventricular fibrillation.

Hepatic: hepatotoxicity.

Otic: auditory and vestibular dysfunction.

Hematologic: eosinophilia.

Dermatologic: skin rashes, pain at injection site, thrombophlebitis.

2.5.7 Dosage:

Capsule: 250mg, Topical gel: 2%, Granules for oral suspension: 200mg/5ml Injection, powder for reconstitution: 500mg, 1g ,Ophthalmic ointment: 2%, Topical ointment: 2%, Powder for oral suspension: 200mg/5ml, 400mg/5ml, 100mg/2.5ml, Topical solution: 1.5%, 2%, Oral suspension: 125mg/5ml, 250mg/5ml, 200mg/5ml, 400mg/5ml, Swab: 2%, Chewable tablet: 200mg, Delayed release tablet: 250mg, 333mg, 500mg, Film coated tablet: 250mg, 333mg, 400mg, 500mg.

Pregnancy: Category B: No evidence of risk in humans but studies inadequate.

2.6: METRONIDZOLE BENZOATE .BP

Chemical structure:

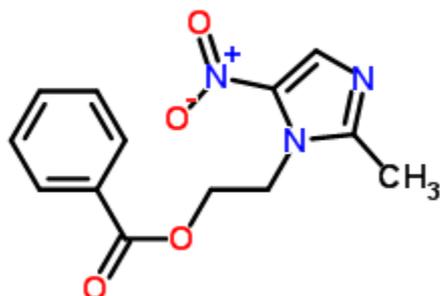


Figure 5: Metronidazole benzoate Bp. Structure.(Rosenkranz, H.S., et al., 1976)

Molecular formula: C₁₃H₁₃N₃O₄

Chemical name: 2-Methyl-5-nitro-1*H*-imidazole-1-ethyl benzoate.

Molecular weight: 275.26

2.6.1 Characteristics

Metronidazole is a white or yellowish, crystalline powder. It is slightly soluble in water, in alcohol, in acetone and in dichloromethane. It is very slightly soluble in ether. It darkens on exposure to light and should be protected from light.

2.6.2 Mode of action

Metronidazole is a synthetic nitro-imidazole derivative. It is an anaerobic antibacterial agent and an antiprotozoal (trichomoniasis, amoebiasis, and giardiasis). However, the mechanisms by which Metronidazole Gel acts in reducing inflammatory lesions of rosacea are unknown, but may include an antibacterial and/or anti-inflammatory mechanism.

2.6.3 Studies done on topical use

Metronidazole Gel is indicated for the treatment of inflammatory papules and pustules of Rosacea, it is most widely used and available as a 0.75% gel, lotion and cream format. For bis n die daily use, and a 1% cream or gel for once daily use. a recent review and a condensed

version of work (Van Zuuren .E.J. et al., 2007), summarizes 9 high and intermediate quality trials, which show clear evidence that topical Metronidazole is significantly effective. Most studies showed 0.75% Metronidazole trial that ranged from 8-9 weeks in duration, with 1 lasting up to 6 months. Reductions in erythema scores were noted: as was an improvement in physician global evaluation, and patient-assessed measures. Although data are limited, 2 studies have shown that topical Metronidazole may be as effective as oral tetracycline in reducing the inflammatory component of Rosacea the drug also plays an important role in maintenance therapy either with or without prior concomitant systemic antibiotic therapy (Gooderham, M., 2009).

2.6.4 Dosage and Administration

2.6.4.1 General Instructions

Areas to be treated should be cleansed before application of Metronidazole Gel. To minimize the risk of local irritation, application of the gel can be delayed for 15-20 minutes after cleansing of the skin. Patients may use cosmetics after application of the gel. A moisturizer can also be used if the skin is dry.

2.6.4.2 Adults & Elderly

For the treatment of inflammatory lesions (papules and pustules) of rosacea, a thin film of Metronidazole Gel should be applied and rubbed in to the cleansed, affected areas twice daily. Clinical improvement usually occurs within three weeks. Patients should be monitored to ensure clinical benefit continues and that no local or systemic events occur. In the absence of a clear clinical improvement therapy should be stopped.

Children: Not recommended.

2.7 Polymer

2.7.1: Carbopol 940 laboratory grade

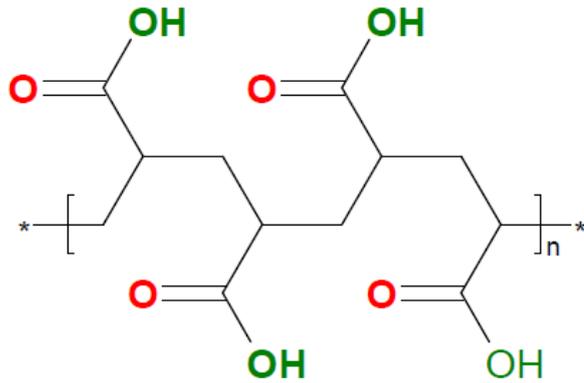


Figure 6: Structure of carbopol polymer (polyacrylic acid). (Koca K., etal. 2003)

2.7.2 Hydroxyl Propyl Methyl Cellulose (HPMC) laboratory grade.

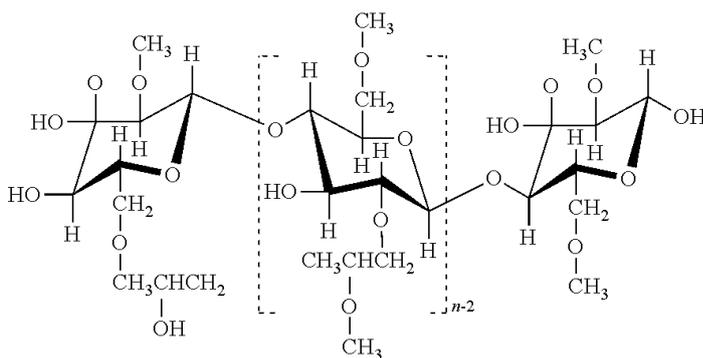


Figure 7: Structure of HPMC.(Koca K. etal., 2003)

CHAPTER THREE

METHODOLOGY

3.0. Study design.

This was a factorial experimental study

3.1. Study location.

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

3.2. Label claim: 2% Erythromycin and 0.75% Metronidazole.

3.3. Active Pharmaceutical Ingredient (API) and Excipients

Table 1: List of active pharmaceutical ingredients (API) and excipients.

S.no	Ingredients	Company	
1	Erythromycin (Pharmaceutical grade)	Donation from Regal pharmaceuticals	API
2	Metronidazole (pharmaceutical grade)	Donation from Regal pharmaceuticals	API
3	Carbopol 940 (laboratory grade)	Purchased Oxford lab chemical	Gelling polymer
4	Hydroxypropyl methyl cellulose (HPMC) (laboratory grade)	Purchased Oxford lab chemicals	thickening polymer
5	Glycerin (laboratory grade)	Purchased Oxford lab chemicals	Humectants
6	Ethanol (laboratory grade)	Purchased Oxford lab chem.	Diluents
7	Triethanolamine (TEA) (laboratory grade)	Donation University of Nairobi.	pH adjuster

8	Methyl parabene (pharmaceutical grade)	Donation from Regal pharmaceuticals	Preservative
9	Propyl parabene (pharmaceutical grade)	Donation from Regal pharmaceuticals	Preservative
10	Distilled water (analytical grade)	Pharmaceutical chemistry laboratory	Diluents

3.4. Instruments.

Table 2: List of instruments.

S.no	Instruments	Company
1	Electric stirrer	Jencos scientific ltd, Bedfordshire
2	UV/ VIS spectrophotometer	Shimandzu co.
3	Analytical weighing balance	Sartorius
4	Digital pH meter 720 SERIES	Mettler Toledo co.
5	FT-IR	Shimandzu co.
6	Laminar air flow	Arrows scientific
7	Sonicator	Wiseclean co.
8	Digital weighing balance	Jenways bibby scientific ltd, United Kingdom.
9	Light microscope	Wiseclean co.
10	Autoclave	Erweka DT 6
11	NDJ-5S digital rotating Viscometer	Sincery international
12	Incubator	

3.5 Experimental Section

3.5.1 Target Product Profile (TPP) of 2% Erythromycin /0.75% Metronidazole topical gel.

Table 3: Target product profile E/M topical gel.

Target product profile for: 2% Erythromycin/ 0.75% Metronidazole Topical gel			
S.no	Quality attributes	Optimal indication /decision criteria	Minimum acceptable indication and criteria
1.	Identity/API	Erythromycin/Metronidazole	Erythromycin/Metronidazole
2	Pharmacotherapeutic group	Macrolide antibiotic	Macrolide antibiotic
3	Indication	Treatment of acne and Rosacea	Treatment of acne and Rosacea
4	Dose/application frequency	2 time a day	2 times a day
5	Drug concentration	0.75% Metronidazole/2% Erythromycin	0.75% Metronidazole/2% Erythromycin
6	Excipients compatibility	Compatible with API affordable and available	Compatible with API affordable and available
7	Storage condition	Room temperature and pressure	Room temperature and pressure
8	pH	4.2- 6.5	4.2- 6.5
9	Shelf life	Minimum 24 months at room temperature (15-30°C)	Minimum 24 months at room temperature (15-30°C)
10	Legal status	POM	POM
11	Dosage route/route of delivery	Topical application on the skin	Topical application on the skin
12	Dosage form	Viscous Gel	Viscous Gel

3.6. General Evaluation Tests.

3.6.1. Pre-formulation studies of API.

The API characteristic was carried out for Erythromycin and Metronidazole and the excipients used.

Solubility: - Solubility of both 2 gram Erythromycin and 0.75 grams Metronidazole was done by dissolving the given amount in 50ml of these diluents: distilled water, ethanol, methanol and a mixture of methanol and distilled water at room temperature and pressure.

3.6.2 Compatibility Studies.

The API's chosen in the development of this formulation has previously been used in the formulation of the individual topical formulation marketed, thus the assumption that the excipients are compatible with the API from previous literature and also the notion that excipients are pharmacologically inert. Excipients from different suppliers can propagate or participate in chemical and physical interaction with drug compounds differently due to their different manufacturing conditions. The need to have a physical and chemical compatibility study for the two API's and excipients was necessary.

A) Physical compatibility:

Both API's were mixed and kept for 15 days then observed for any changes in color, precipitate, turbidity or evolution of gas.

B) Chemical compatibility:

This involved assessing if there was irreversible degradation of the drugs functional group to produce a therapeutically inactive or otherwise toxic compound.

FT-IR

Instrument used was FT-IR (Shimadzu FTIR-8700 spectrophotometer). In this study, potassium bromide disc method was employed. IR study of pure drug, physical mixtures and polymer were done. The sample was intimately mixed with analytical grade dry powdered potassium bromide in a ratio of 1:100. The disc was placed in IR spectrophotometer using sample holder and spectrum was recorded from 4000 to 600 cm⁻¹.

Table 4: IR spectra

Wave No. (cm ⁻¹)	Bond	functional group
3640–3610 (s, sh)	O–H stretch, free hydroxyl	Alcohols, phenols
3500–3200 (s, b)	O–H stretch, H-bonded	Alcohols, phenols
3400–3250 (m)	N–H stretch	Primary, secondary amines, amides
3300–2500 (m)	O–H stretch	Carboxylic acids
3330–3270 (n, s)	–C(triple bond)C–H: C–H stretch	Alkynes (terminal)
3100–3000 (s)	C–H stretch	Aromatics
3100–3000 (m)	=C–H stretch	Alkenes
3000–2850 (m)	C–H stretch	Alkanes
2830–2695 (m)	H–C=O: C–H stretch	Aldehydes
2260–2210 (v)	C(triple bond)N stretch	Nitriles
2260–2100 (w)	–C(triple bond)C– stretch	Alkynes
1760–1665 (s)	C=O stretch	Carbonyls (general)
1760–1690 (s)	C=O stretch	Carboxylic acids
1750–1735 (s)	C=O stretch	Esters, saturated aliphatic
1740–1720 (s)	C=O stretch	Aldehydes, saturated aliphatic
1730–1715 (s)	C=O stretch	Alpha, beta-unsaturated esters
1715 (s)	C=O stretch	Ketones, saturated aliphatic
1710–1665 (s)	C=O stretch	Alpha, beta-unsaturated Aldehydes, ketones
1680–1640 (m)	–C=C– stretch	Alkenes
1650–1580 (m)	N–H bend	Primary amines
1600–1585 (m)	C–C stretch (in-ring)	Aromatics
1550–1475 (s)	N–O asymmetric stretch	Nitro compounds
1500–1400 (m)	C–C stretch (in-ring)	Aromatics
1470–1450 (m)	C–H bend	Alkanes
1370–1350 (m)	C–H rock	Alkanes
1360–1290 (m)	N–O symmetric stretch	Nitro compounds
1335–1250 (s)	C–N stretch	Aromatic amines
1320–1000 (s)	C–O stretch	Alcohols, carboxylic acids,

		esters, ethers
1300–1150 (m)	C–H wag (–CH ₂ X)	Alkyl halides
1300–1150 (m)	C–H wag (–CH ₂ X)	Alkyl halides
1250–1020 (m)	C–N stretch	Aliphatic amines
1000–650 (s)	=C–H bend	Alkenes
950–910 (m)	O–H bend	Carboxylic acids
910–665 (s, b)	N–H wag	Primary, secondary amines
900–675 (s)	C–H	Aromatics
850–550 (m)	C–Cl stretch	Alkyl halides
725–720 (m)	C–H	Alkanes
700–610 (b, s)	–C(triple bond)C–H: C–H bend	Alkynes
690–515 (m)	C–Br stretch	Alkyl halides

Key: m=medium, w=weak, s=strong, n=narrow, b=broad, sh=sharp

3.7 Method of formulation.

3.7.1 Formulation of Erythromycin/Metronidazole topical gel

- ❖ Gels were prepared by **cold mechanical method**. It was compounded as indicated in [Table 5].

Step 1- The required quantity of carbopol 940 and HPMC was weighed and sprinkled slowly on the surface of 40ml, methanol 60%/ distilled water 40%, with continuously stirring by a mechanical stirrer, till the polymer soaked in the vehicle. With continuous stirring, triethanolamine was added to neutralize the gel and maintain the pH between 4.2- 6.2 for gelling.

Step 2- The appropriate quantity of Metronidazole was dissolved first in a separate beaker, methyl parabene and Propyl parabene were added and dissolved in 20ml, methanol 60%/ distilled water 40%. To this Erythromycin was added with constant stirring.

Step 3- Finally the mixture in step 2 was added to the gel with continuous stirring till it got dispersed in the gel to have a homogeneous mixture. Entrapped air bubbles were removed by keeping the gel overnight and also to hydrate. It was packed in glass jar bottles with cover and kept in a dark and cool place, with label.

Table 5: Compounding formula

S. no	Ingredients	G1	G2	G3	G4	G5	G6	G7	G8
1	Erythromycin/Me tronidazole (g)	E2g,0. 75g M							
2	Carbopol 940 (g)	0.5	1.5	0.5	1.5	0.5	1.5	0.5	1.5
3	HPMC (g)	-	-	1.5	1.5	1	1	2	2
4	Glycerin (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
5	Propylene glycol (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
6	TEA (ml)	q.s							
7	Propyl parabene	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
8	Methyl parabene (g)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
9	Methanol 60%/ water 40% up to 100 ml	Qs							

3.7.2. Post Formulation Studies**3.7.3. Visual examination (Organoleptic characteristics).**

All the developed gel formulae were evaluated / inspected for the following:

- ❖ Homogeneity -The gels was tested for homogeneity by visual inspection after the gel had settled in the container and a descriptive observation noted.
- ❖ Grittiness/ presence of lumps- By visual inspection after gel had settled in the container. It was evaluated microscopically for particulate matter and a descriptive observation noted.
- ❖ Clarity –if crystal clear or turbid and a descriptive observation noted.
- ❖ Phase separation and a descriptive observation noted.

3.7.4. pH determination.

The pH of the gel was determined using a Digital pH meter (company name). 1 gram of gel was dissolved in 100ml of distilled water and stored for 2 hours. The electrodes were inserted into the sample solution 10 minutes prior to taking the reading at room temperature. The procedure was repeated three times and average determined for all formulae at different duration during, in-process, at 14 days, 1 month and 2 months.

3.7.5 Drug content determination / Assay

3.7.5.1 Erythromycin in sample was analyzed by HPLC system.

3.7.5.2 Preparation of 0.2M Phosphate Buffer pH 7.0

A).Preparation 1: Di-potassium hydrogen phosphate anhydrous 0.2 M

17.418 g of disodium hydrogen phosphate was dissolved in sufficient water to produce 500ml.

B). Preparation 2: phosphate buffer solution 0.2M

16.3308mg of potassium hydrogen phosphate was dissolved to give 600ml. To 500ml of this potassium hydrogen phosphate was added sufficient 0.2M di-potassium hydrogen phosphate anhydrous to produce 1000ml. pH 7.0.

3.7.5.3 Preparation of reference standard.

A) Reference standard

For erythromycin at 215 nm

Standard (40mg erythromycin) mixture was dissolved in 3.0ml acetonitrile HPLC S grade, diluted with phosphate buffer (pH 7.0, 0.2M) to 10ml and injected (20 μ l) into the LC system

For Metronidazole at 310 nm

Standard mixture was dissolved in 20.0ml acetonitrile HPLC GRADE. this solution (1.0ml) was diluted with phosphate buffer (pH 7.0, 0.2M) to 100ml and injected 20 μ l into LC system.

3.7.5.4 Preparation for sample

For erythromycin at 215nm

2g of Sample (equivalent to 40mg of erythromycin) was dissolved in 3.0ml acetonitrile HPLC S grade, diluted with phosphate buffer (pH 7.0, 0.2M) to 10ml and injected (20 μ l) into the LC system

For Metronidazole at 310nm

0.267g Sample (equivalent to 20mg of Metronidazole) was dissolved in 20.0ml acetonitrile HPLC grade .this solution (1.0ml) was diluted with phosphate buffer (pH 7.0, 0.2M) to 100ml and injected 20ul into LC system.

HPLC: Instrumentation: **Column:** cyanno hypersil 5µm C18, was used as a stationary phase at 40°C.**Mobile phase:** acetonitrile -0.2M Potassium phosphate pH 7.0-water (35:5:60 v/v/v) .The mixture was purged by helium to degas. **Flow rate:** Erythromycin flow rate of 1.0ml/min and 0.5ml/min for Metronidazole.

3.7.5.5 Direct UV-Vis spectrometry method: for analysis of Metronidazole

Determination of absorbance maxima

For the standardization of the drug by using UV –VIS spectroscopy, the drug was first subjected to a wavelength scan for determination of absorbance maxima (λ_{max}). A stock solution of drug 30mg was prepared by dissolving the drug in 100ml volumetric flask and volume was made up to 100ml with diluent and scanned between 200-400nm .The wavelength with maximum absorbance observed was selected and one that dint have interference of erythromycin from the same wavelength. This method was used for analysis of Metronidazole.

0.1M HCL .BP Preparation.

It was prepared by dissolving 8.5 ml concentrated hydrochloric acid (HCL) in 1000ml distilled water.

Sample preparation:

A gel equivalent to 30mg of Metronidazole was weighed into 100ml volumetric flask and dissolved with small quantity of 0.1m HCL ,sonicated for a few minutes, then topped up to volume with 0.1M HCL and sonicated again.

From above sample, 0.3ml was withdrawn and diluted with 0.1M HCL in 10ml volumetric flask and final solution after mixing, absorbance was read at 282nm using UV-Spectroscopy. The preparation was repeated for standard and all other samples of the gel formulation

$$\% \text{ label claim} = \frac{\text{absorbance of sample}}{\text{absorbance of standard}} \times \frac{\text{weight of standard}}{100} \times \frac{0.3}{10} \times \frac{100}{4} \times \frac{10}{0.3} \times \frac{\text{potency of standard}}{100} \times 100 \times \frac{100}{0.75}$$

3.7.5.6 Viscosity.

NDJ-5S VISCOMETER, (Specification) : Rotor Speed (rpm): 6/12/30/60, Rotor No.1, 2, 3, 4, Measuring Range: $10 \sim 10^5$ mPa.s, Accuracy (Newtonian Fluid): $\pm 5\%$, Overall Dimensions: 300 x 300 x 450mm.

The viscosity of the different gel formulae was determined at room temperature and pressure using a NDJ-5S/8S digital rotating viscometer. The gel was rotated at 60 rpm with spindle no.4 at each speed the corresponding dial reading was noted. Evaluation was conducted in triplicate.

3.7.5.7 micro-bioassay of Erythromycin /Metronidazole topical gel on *Bacillus Subtilis* bacteria.

Standard preparation- 60mg of Erythromycin ethyl succinate working standard equivalent was dissolved in methanol and dilute to 100ml with the same solvent (stock solution).

S1- 10ml of stock solution was diluted to 25ml with phosphate buffer solution pH 8.0 in 25ml volumetric flask.

S2- 10 ml of stock solution was diluted to 50ml with phosphate buffer solution pH 8.0 in 50ml volumetric flask.

Standard preparation of Metronidazole: 22.5mg of Metronidazole powder working standard equivalent was dissolved in methanol and diluted to 100ml with the same solvent (stock solution).

S1- 10ml of stock solution was dissolved to 25ml with phosphate buffer solution pH 8.0 in 25ml volumetric flask.

S2- 10 ml of stock solution was dissolved to 50ml with phosphate buffer solution pH 8.0 in 50 ml volumetric flasks.

Sample preparation- 3mg of the topical gel was transferred into 100ml volumetric flask and drug extracted from polymer matrix with methanol and top up to the volume with methanol (stock solution)

T1- 10ml of the stock solution was dissolved to 25ml with buffer solution pH 8 in 25ml volumetric flask.

T2- 0.5ml of the stock solution was dissolved to 50ml with buffer solution pH 8 in 50ml volumetric flask

CHAPTER FOUR

RESULTS

4.0. Pre-formulation studies.

4.1 Solubility of the API.

Solubility of the API, s in the mixture was an important aspect of creating a conducive medium, which supported both the API's solubility at room temperature and pressure.

The solubility was done at room temperature and pressure for the API'S and was observed as follows: Complete solubility in both the API's was observed in the methanol/water system mixture at the ratio of 60:40 ml respectively.

Table 6: Solubility characteristics of the API' s.

	Water (ml)	Ethanol (ml)	Methanol (ml)	Acetone (ml)	40mlWater/60ml Methanol
Erythromycin ethyl succinate (1g)	Insoluble	soluble	soluble	Soluble	Soluble
Metronidazole (1g)	Sparingly soluble	Sparingly soluble	Slightly soluble	Soluble	Soluble
Erythromycin ethyl succinate /Metronidazole (1:1)	Sparingly soluble	Slightly soluble	Slightly soluble	Slightly soluble	Soluble

4.2. Compatibility studies:

4.2.1. Physical compatibility studies and chemical compatibility studies.

From the descriptive observation in the mixtures no color change, gas evolution was noted in the physical characteristic.

The below figures are spectra's giving an insight into the functional group and the absorption spectra of all the excipients and API's after been kept for 15 days, from the characterization of the IR absorption in the parent hydrocarbon species and associate back-born groups.

The data in the tables was of powder excipients and API's kept at room temperature, FTIR demonstrated between 4000-600 cm-1.

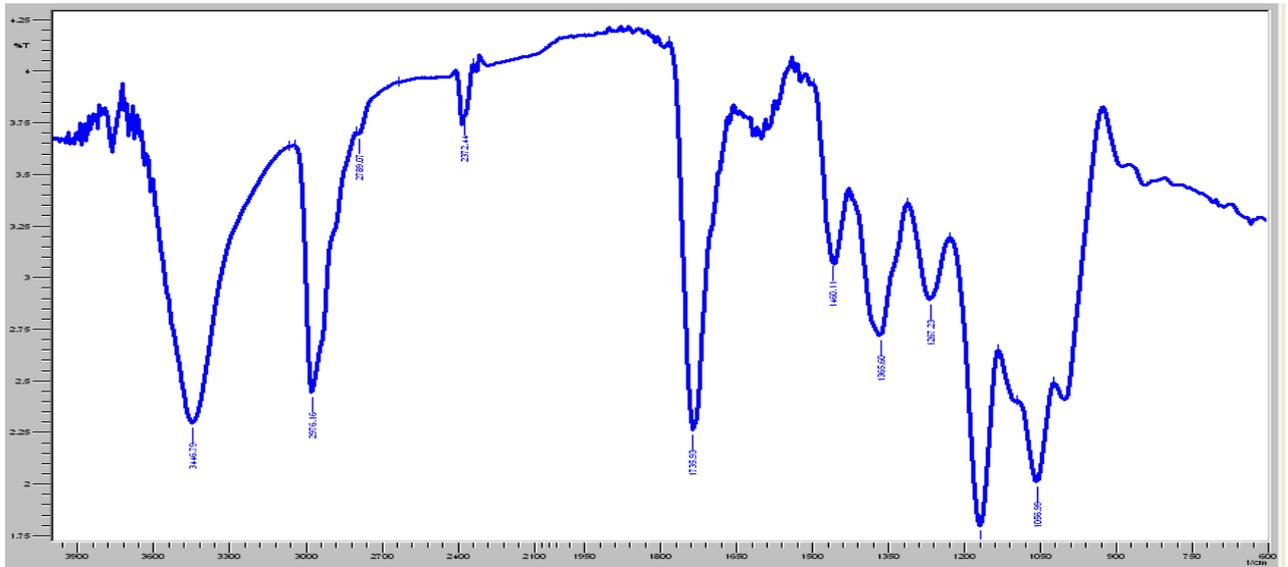


Figure 8: Erythromycin ethyl succinate API FT-IR spectra.

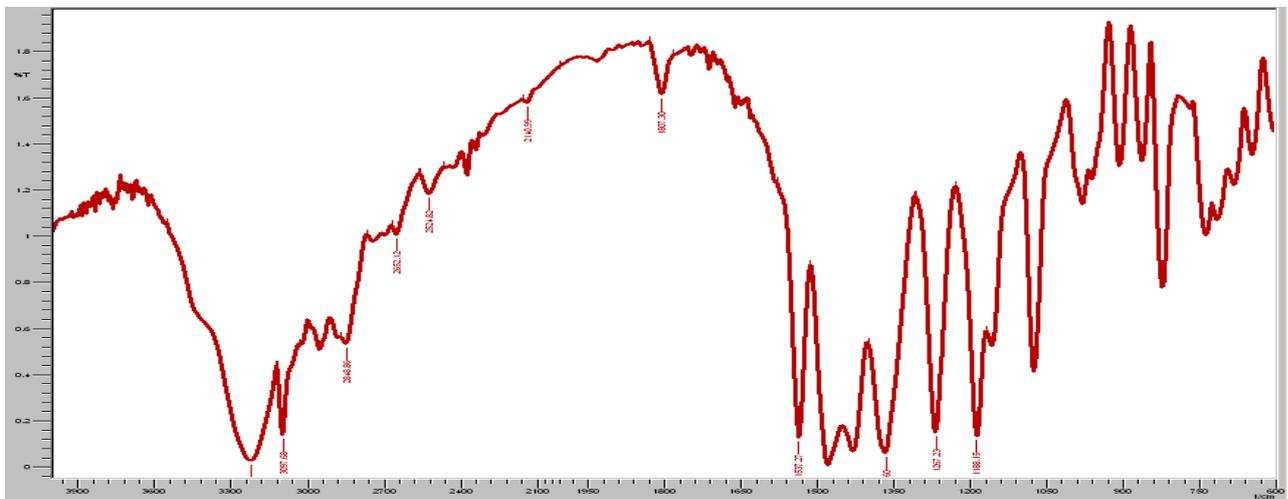


Figure 9: Metronidazole salt (API) FT-IR spectra.

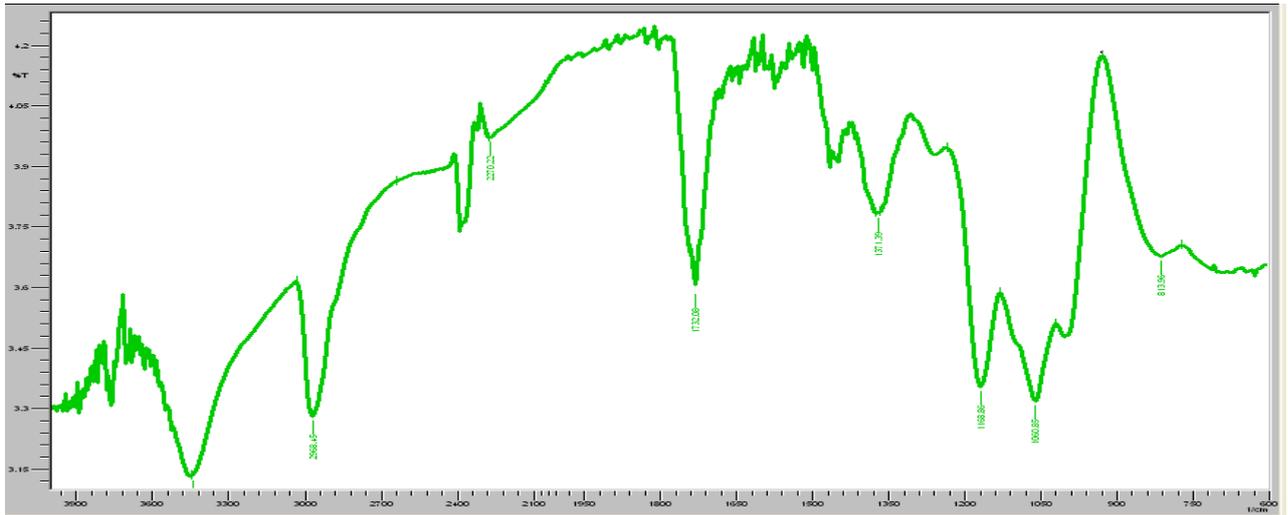


Figure 10: Erythromycin /Metronidazole combination FT-IR spectra.

Table 7: API + API's combined prominent FT-IR peaks.

Erythromycin (E)		Metronidazole (M)		E/M combination	
Peak cm ⁻¹	Groups	Peaks	Groups	Peaks	Groups
3448.79	Alcohol, phenols.	2848	Alkanes.	2968	Alkanes.
2978	Alkanes.	2521.82	Alkanes.	2270	Aldehydes.
2788	Aldehydes.	1807.30	Carbonyl.	1732.06	Aldehydes, saturated aliphatic.

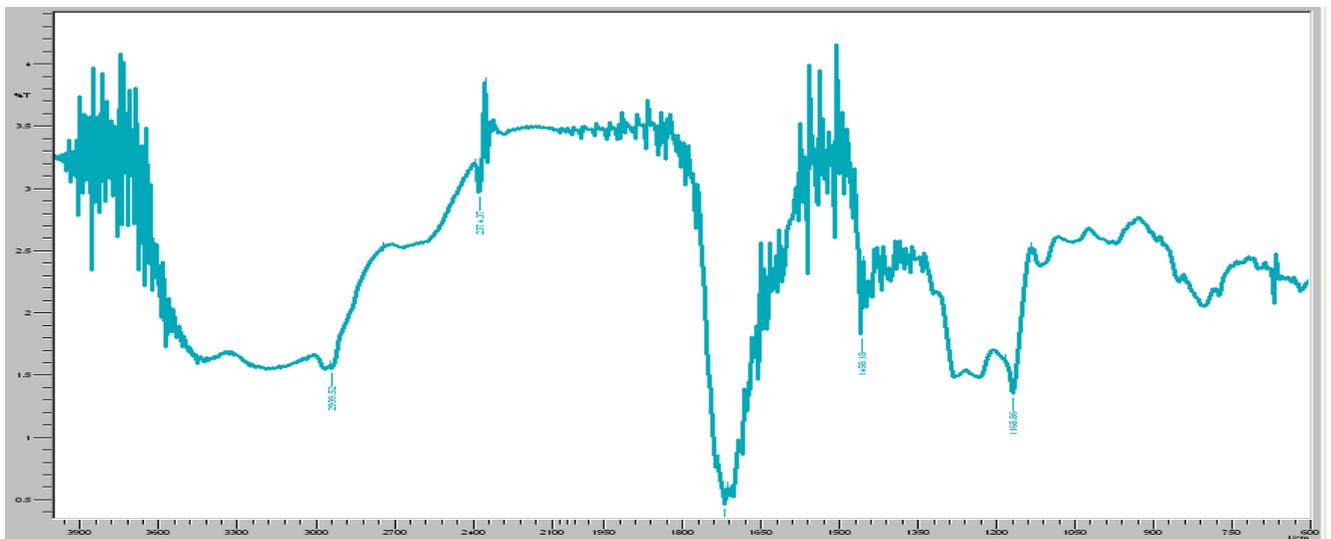


Figure 11: Carbopol (polymer) FT-IR spectra.

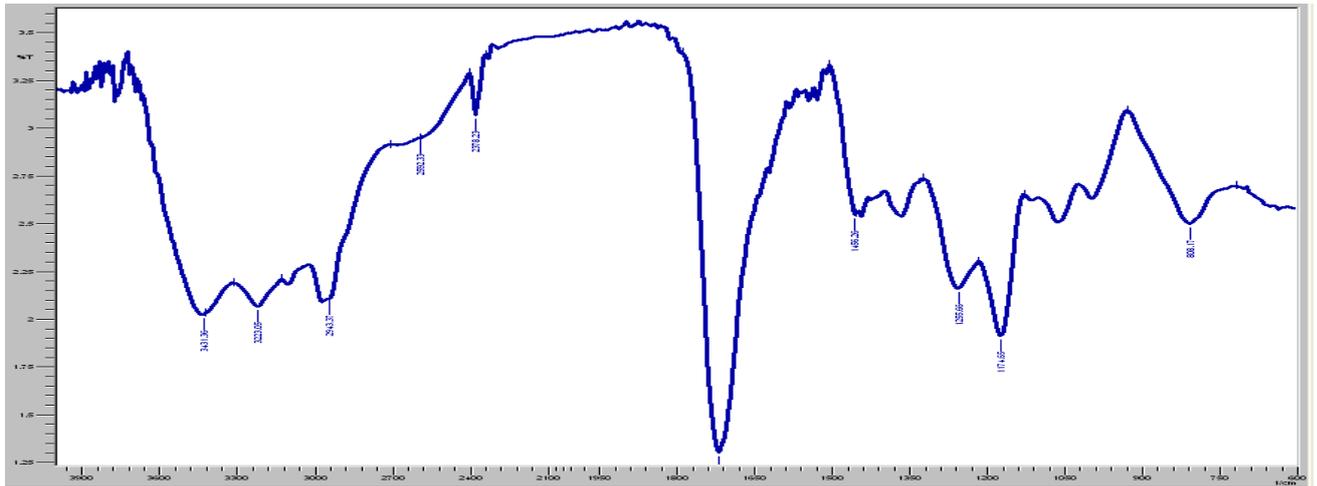


Figure 12: Erythromycin/Metronidazole combination + Carbopol 940 FT-IR spectra.

Table 8: API's + Carbopol 940 prominent FT-IR peaks.

Carbopol 940		E/M combination + carbopol	
Peaks cm^{-1}	Groups	Peaks	Groups
2960.73	Alkanes	3431.36	Alcohol, phenol
1712.79	Alpha, beta-unsaturated aldehydes, ketones	2943.37	Alkanes
1452.40	Alkanes	2592.33	Aldehydes
1246.02	Aliphatic amines	1706.25	Alpha, beta-unsaturated aldehydes, ketones
1172.72	Aliphatic amines	1174.65	Aliphatic amines

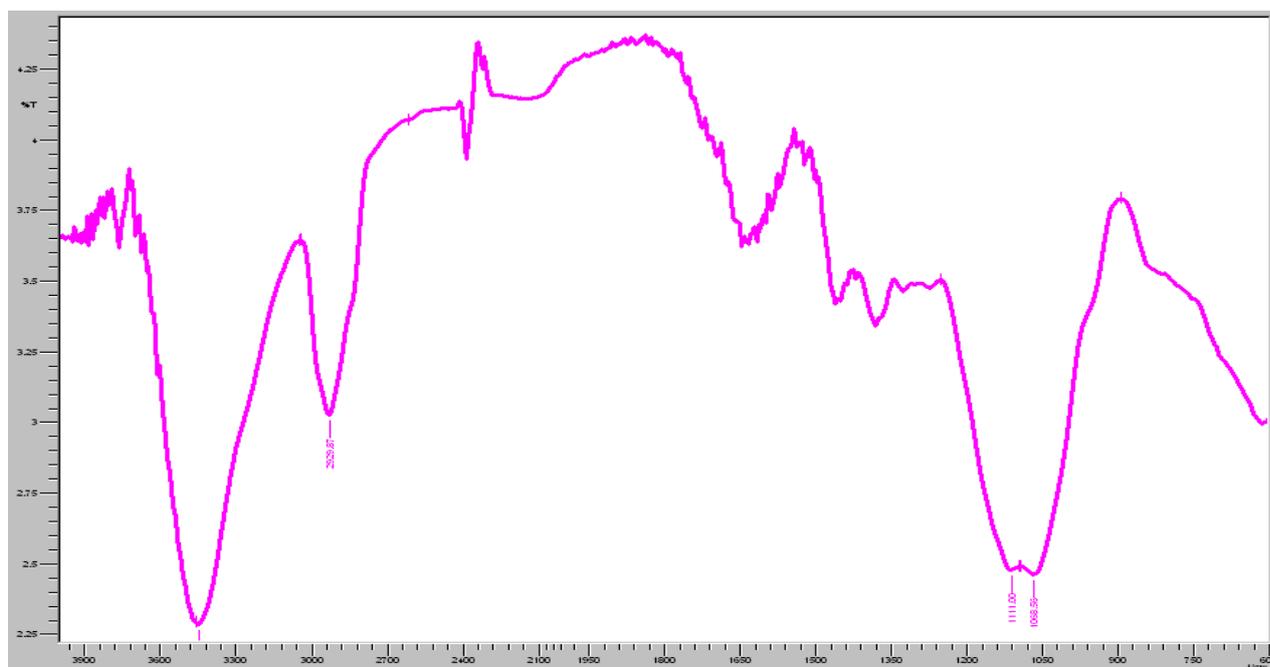


Figure 13: Hydroxyl propyl methyl cellulose (HPMC) FT-IR spectra

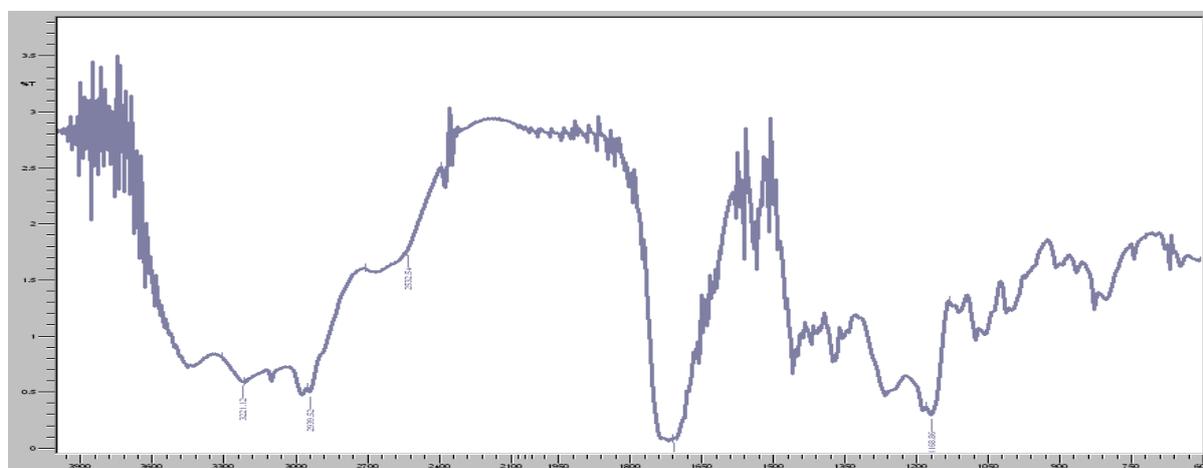


Figure 14: Erythromycin /Metronidazole combination + HPMC FT-IR spectra.

Table 9: Combined API's + HPMC prominent FT-IR peaks.

Hydroxyl propyl methyl cellulose (HPMC)		E/M combination + HPMC	
Peaks cm ⁻¹	Groups	Peaks	Groups
3560.25	Alcohol, phenol	2930.50	Alkanes
2929.57	Alkanes	2525.40	Aldehydes
1111.00	Aliphatic amines	1706.25	Carboxylic acid
1058.20	Aliphatic amines	1168.86	Aliphatic amines

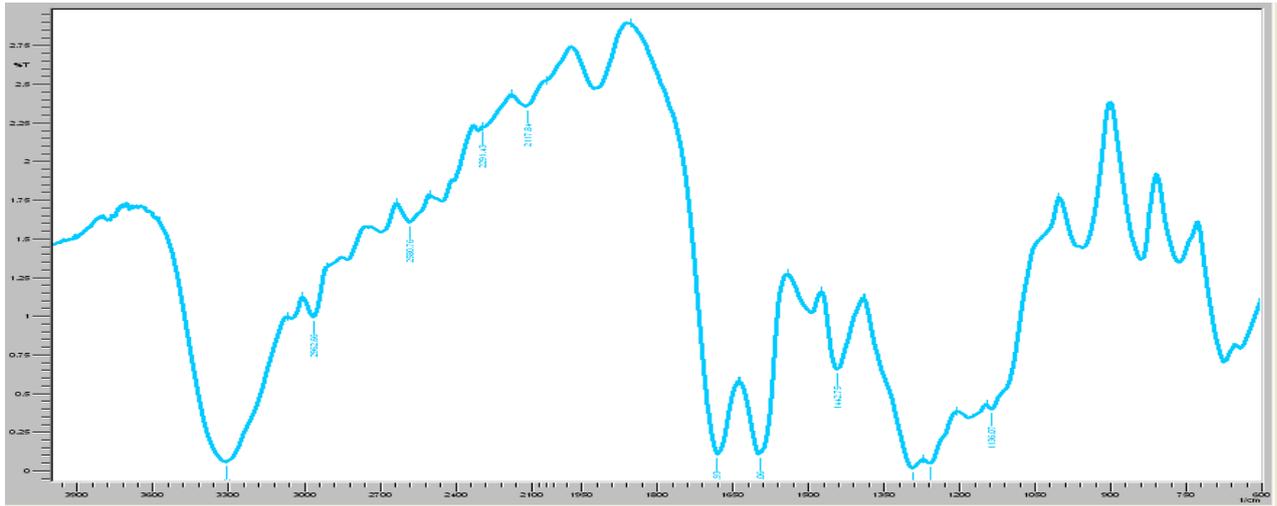


Figure 15: Methyl parabene FT-IR spectra.

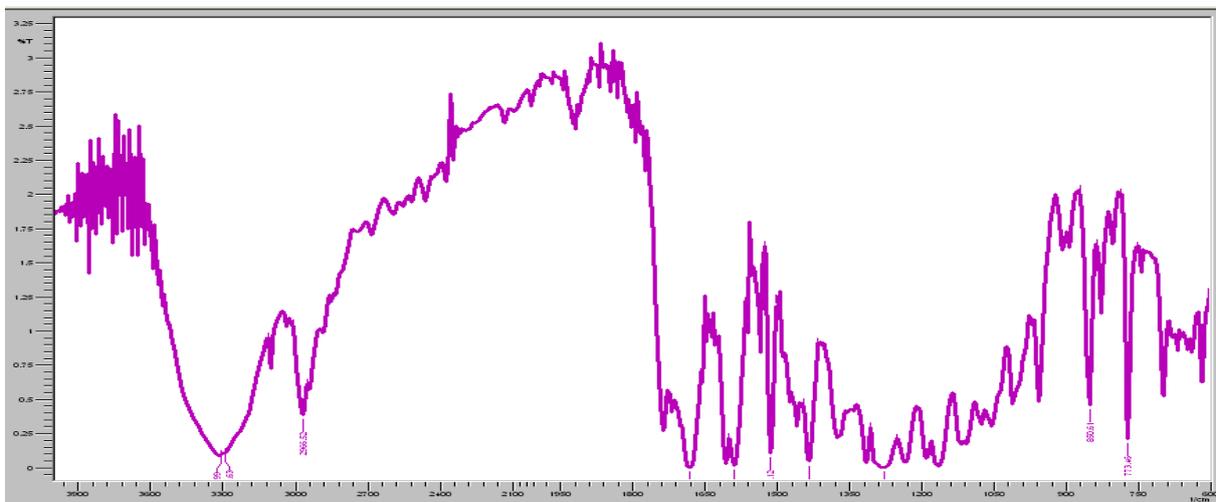


Figure 16: Erythromycin/ Metronidazole combination + Methyl parabene FT-IR spectra.

Table 10: Combined API's + MP prominent FT-IR peaks.

Methyl Parabene (MP)		E/M combination + MP	
Peaks cm^{-1}	Groups	Peaks	Groups
2942.60	Alkanes	2942.52	Alkanes
2580.70	Aldehydes	1760.25	Carboxylic acid
2291.43	Alkanes	1136.07	Aliphatic amines

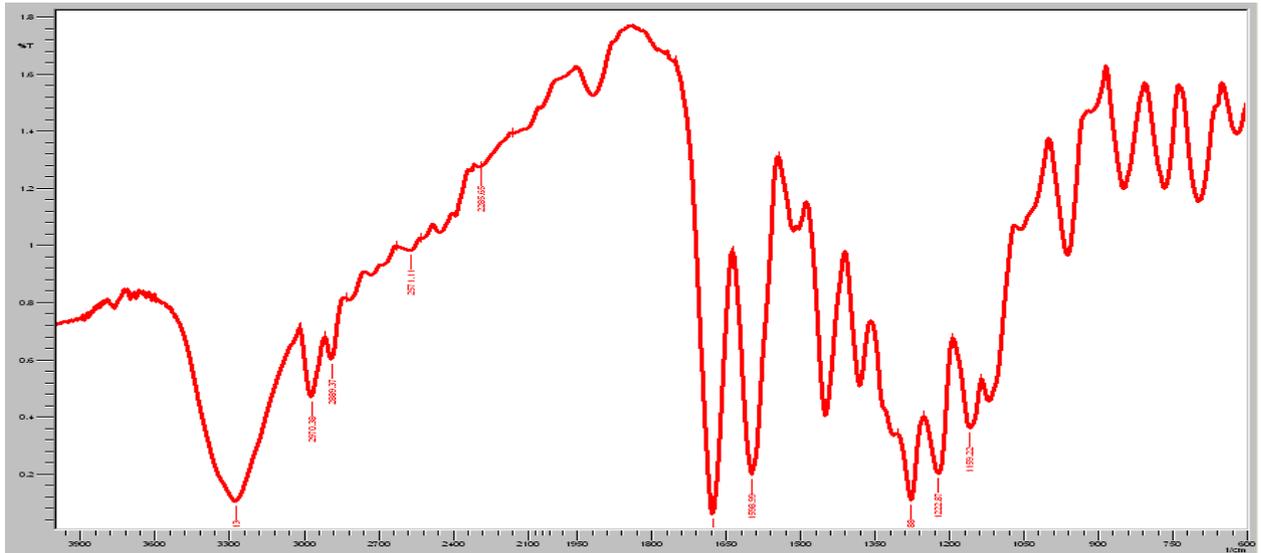


Figure 17: Propyl parabene FT-IR spectra

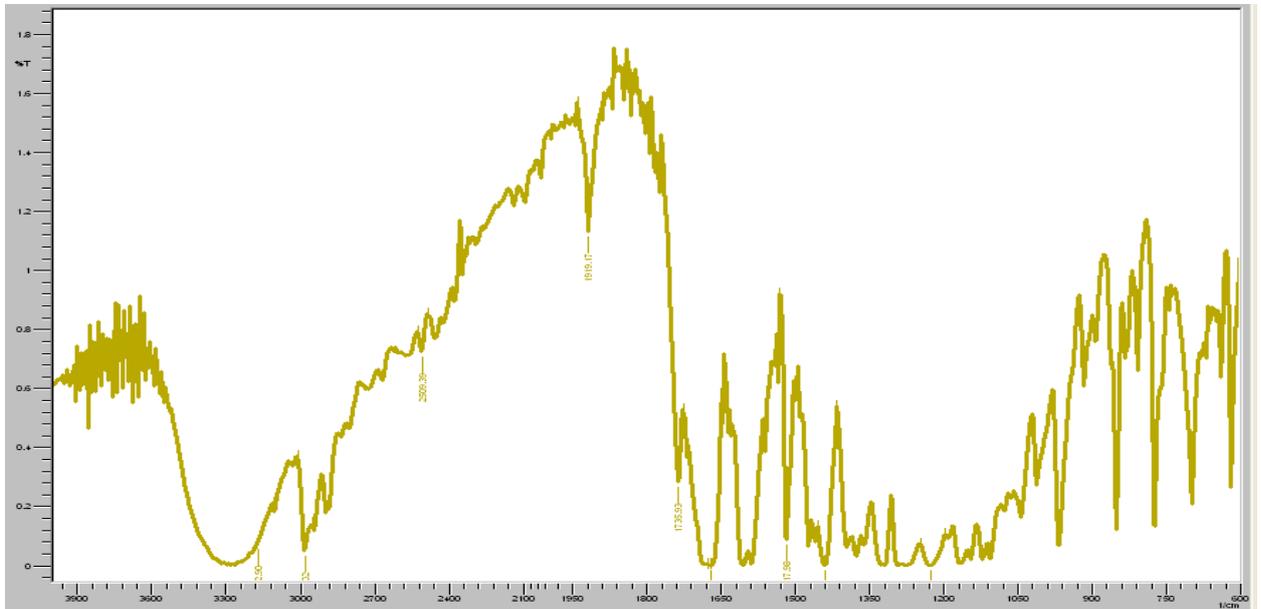


Figure 18: Erythromycin/Metronidazole combination + Propyl Parabene FT-IR spectra

Table 11: Combined API's + PP prominent FT-IR peaks.

Propyl Parabene (PP)		E/M combination + PP	
Peaks	Groups	Peaks	Groups
2889.37	Alkanes	2970.38	Alkanes
2571.11	Aldehydes	2609.38	Aldehydes
2285.65	Alkynes	1919.17	Alkynes
1580.50	Aromatics	1735.50	Aldehydes and saturated aliphatics

4.3 Post formulation characteristics.

4.3.1 Organoleptic characteristics

Formulations G1 to G8 were observed for clarity, homogeneity, phase separation, grittiness and pH. All test were performed at room temperature and pressure.

Table 12: Organoleptic Characteristics of topical gel per formulation codes.

Topical gel	Clarity	Homogeneity	Phase separation.	Grittiness /presence of lumps	Ph
G1	+	++	+	+	5.3
G2	++	++	+	+	4.5
G3	+	++	+	+	5.3
G4	++	++	+	+	5.3
G5	+	++	+	+	5.2
G6	++	++	+	+	4.5
G7	++	++	+	+	5.1
G8	++	++	+	+	4.7

Key: Color: turbid (+), clear (++) , very clear (glassy) (+++).

Homogeneity: excellent (+++), good (++) , satisfactory (+).

Phase separation and grittiness: yes (++) , none (+).

pH limits: (4 - 6.5)

4.4. Viscosity of the topical gels.

Table 13: Viscosity of topical gels formulations

Formulation code	G1	G2	G3	G4	G5	G6	G7	G8
Viscosity (mpa.s)	1300	9350	3000	9360	1320	9360	1410	9360
%viscosity	13	93.50	30	93.6	13.2	93.6	14.1	93.60

NB: Viscosity for accuracy, the percentage should lie between 10 and 90% but not above 100%.

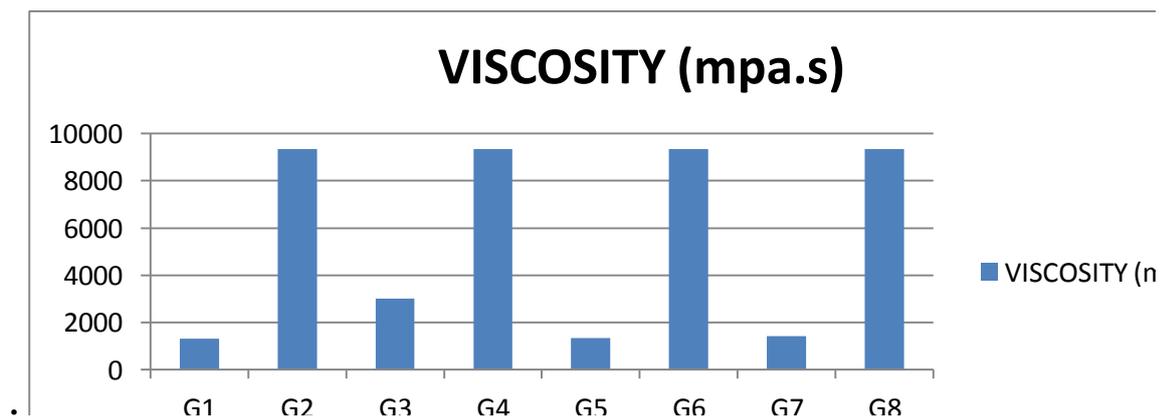


Chart 1: Compared viscosity of the gel formulation.

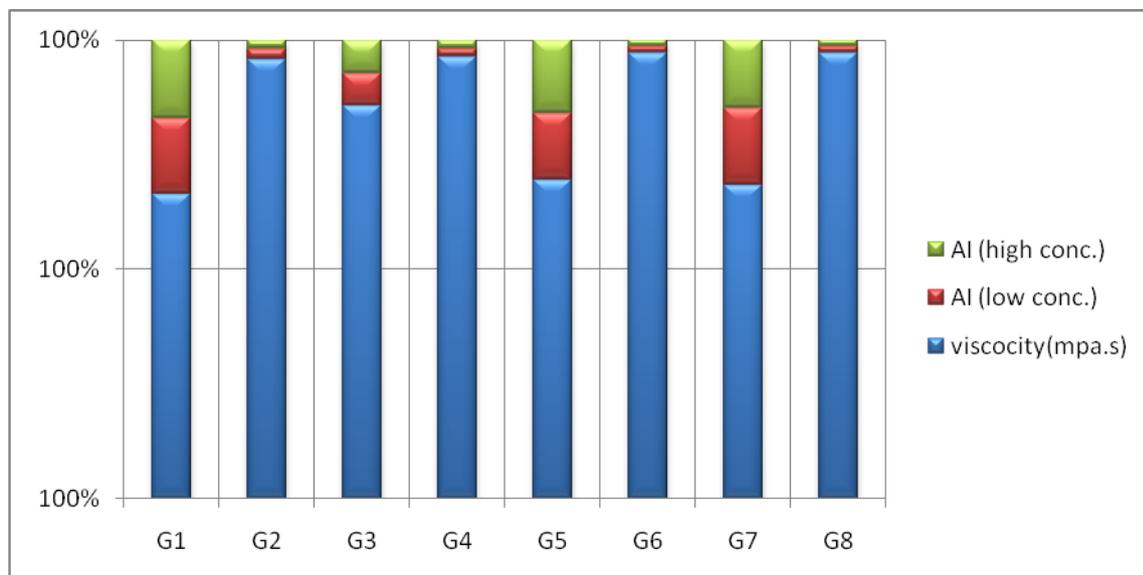


Chart 2. Compared Viscosity in relation to Microbial activity index (AI)

4.5. Micro-bioassay plates: Zones of inhibition.



Figure 19: G1, G4, G2, G5 plate's zone of inhibition



Figure 20: Plate G3 zone of inhibition



Fig 21: Plate G6 zone of inhibition.



Figure 22: Plates G7 zones of inhibition



Fig 23: Plate G8 zone of inhibition

Table 13: Microbial activity index (AI)

Test organism: <i>Bacillus Subtilis</i>								
Gel formulation	G1	G2	G3	G4	G5	G6	G7	G8
%bioassay	63.698	59.388	63.155	57.198	61.059	49.158	64.047	49.4389
AI (low conc.)	0.858	0.834	0.866	0.656	0.765	0.4848	0.945	0.510
1.5mg/ml								
AI (high conc.)	0.891	0.702	0.854	0.723	0.842	0.552	0.836	0.543
3.0 mg/ml								

4.6 Drug content determination:

Table 14: Metronidazole drug content in the gel: by UV spectrometry at 282nm.

Sample code /conc.	G1	G2	G3	G4	G5	G6	G7	G8	Standard
(0.003g/ml)									
Absorbance at 282 nm	0.535	0.385	0.550	0.565	0.499	0.487	0.467	0.499	0.498
	0.534	0.383	0.551	0.566	0.499	0.487	0.468	0.498	0.497
	0.533	0.385	0.552	0.568	0.500	0.487	0.467	0.499	0.498
Average	0.534	0.384	0.551	0.566	0.499	0.487	0.467	0.499	0.498
SD ±	0.001	0.0012	0.01	0.0015	0.0006	0	0.0006	0.0006	0.0006
% Label claim	106.94	77.10	110.34	113.35	99.93	97.53	93.52	99.93	n/a

Compendia reference: - 90-110%.

4.7 Stability studies:

Physical stability studies:

The samples were observed after 2 months, a descriptive result was noted as follows: There was color change, phase separation and odor from formulation G1 – G8, and this demonstrated their stability at room temperature and pressure conditions.

CHAPTER FIVE

DISCUSSION

5.0 Discussion

Gels consists of organic macromolecules or inorganic particles in small amounts of entangled polymers, interpenetrated by relatively large volumes of water, hydro-alcoholic or organic liquid. Topical drug delivery are mainly used for producing a local action rather than a systemic action. In the presented study the topical gel of Erythromycin/Metronidazole was formulated using carbopol 940. This polymer possesses excellent formulating properties at 1% in other studies, when analyzed for organoleptic properties, rheological properties and in-vitro release studies.

The concentration of the polymer was varied, carbopol 940 at (0.5% and 1.5%) and HPMC (thickening agent) at (1%, 1.5% and 2%) were used in the formulations as described in [section 3.8.1, Table 5] and analyzes on there rheological properties and organoleptic properties at the above concentrations .This were prepared with water, ethanol and methanol as a diluent. The trial formulations with water showed phase separation in the initial stages of formulation, this observation were seen to have been contributed by the re-crystallization of erythromycin once water was added into the hydrated polymer during the gel formulation. This procedure was again repeated with methanol only and phase separation was also observed in the formulations that contained added HPMC. The HPMC added had separated from the matrix film with some gritty particle presumably Metronidazole due to its insolubility in the alcohol. From this descriptive observation a ratio concept was put in place to vary the amount of water and alcohol in the solvent system, which after many dilution ratios been prepared for the formulation, the descriptive results indicated 40% water and 60 % methanol ratio, presented the better homogeneous gel formulation compared to the other diluents under trial, this diluent ratio was recommended and used in the compounding of the samples as per the formulation codes shown in [Table 4, section 3.8].

The pre-formulation analysis was done for compatibility studies using FT-IR spectroscopy. The IR absorption of the functional groups vary and give characteristic IR absorption at specific narrow frequency ranges depending on the compound and the major peaks presented by the API's as indicated in [figure 7 and figure 8]. Prominent peaks produced by

Erythromycin and Metronidazole that were most characteristic, showed that the functional groups at distinct IR absorption were visible for the individual API's as presented in [Table 6], this was likewise for the combined form of both the API's as IR spectra in [figure 9] showed the prominent peak were visible indicating that there was no chemical interaction between the API's when combined together.

When the combined API's were incorporated with carbopol polymer, the observation from the prominent peaks obtained from carbopol alone and the mixture of both the API's with carbopol [figure 10 and figure 11] showed functional groups were still been absorbed at the some IR and could be confirmed by the peaks presented for both carbopol and the mixture as showed in [Table 8], indicated no distinct interaction. Similarly this was repeated with HPMC as showed in [figure 12 and figure 13] prominent peaks were observed indicating no interference of the functional groups and no incompatibility in the mixture as showed in [Table 8]. For the preservative in the formulation that were used the compatibility studies were also done for both methyl parabene and propyl parabene incorporated into the combined mixture of the API's to check for any interference/incompatibility when used, the IR absorption as shown in [fig 15, 16 and fig 17, 18] were observed and indicated in [Table 9 and 10] showed no interaction as all the prominent peaks were observed respectively for both the API's in the mixture.

The Formulation could know commence as per the formulation codes in [Table 5]. Analysis was conducted on the organoleptic properties of the gel formulation and all adhered to the limits set under the target product profile as indicated in [Table 3].The in process control that could be monitored was the pH of the diluents that were been incorporated into the gel formulation, this was to enhance of gelling of carbopol which would be at an acidic pH range and also to have achieved the required pH of our product to be within 4.2- 6.5 pH.

The conditions under which the organoleptic properties done were as described in [Table 12] by visual examination on a dark back ground, the clarity of the formulated gel were observed. Formulations G2, G4 ,G6 ,G7 and G8 descriptive results showed all were clear with no turbidity, this formulations contained 1.5 % carbopol. Formulation G1, G3 and G5 descriptive results had observed turbidity, this formulations contained carbopol at 0.5% in all there formulation. From this descriptive observation we could conclude that the amount of carbopol as it was increased the clearer the mixture. The homogeneity and phase separation

of the formulations G1 to G8 were observed over a period of 2 month and in all the formulations descriptive result showed consistency in the pharmaceutical formulations.

In any gel formulation presence of lumps or grittiness is a sign of instability in the formulation, all gel formulations were placed under a light microscope and observed for aggregates, descriptive result observed showed absences of lumps in all the formulation this was also used as an indicator of stability within the formulation.

As a topical formulation that would be applied on the skin, the pH was of paramount importance to be within the some limit as that on the skin, the pH of the product was monitored at three consecutive duration and average indicated showing the pH of all the formulations been within the limits set in the target product profile in [Table 3] for all the formulations.

Viscosity as a measure of rheological properties of the gel was done using an NJS- 5S rotating viscometer and the viscosity was performed at month two from formulation, but it's a requirement as an in-process control parameter that should be observed during formulation. As per [Table 13] formulations G1, G3, G5 and G7 were within the limits set as per the viscometer used to be between 10% and 90% or 1000mpa.s and 9000 mpa.s and formulations contained carbopol at 0.5%. The addition of HPMC as a thickening agent did not improve much on the viscosity of the gel as from the descriptive results observed from the percentage difference between formulation G1 that had only carbopol 940 and formulations G3, G5 and G7 that contained different ratios of HPMC .Formulation G2, G4, G6 and G8 were outside the limit set of between 10%-90% but not outside the limits of 100% or 10000mpa.s. From [Chart 1] showed that the formulation containing 1.5% carbopol 940 had a high viscosity and most preferred elegance appearance from a cosmetic perspective. From [Chart 2] a relationship pattern was derived for the compared viscosity of the gel in relation to the microbial activity index of both low and high concentrations used and the pattern showed, that the gel formulation that had viscosity within the 10%-90% limit, G1, G3, G5 and G7 had higher microbial activity index a descriptive indication of the availability of the antibiotic been greater in the above formulations for the inhibition of microbial growth.

Micro bio-assay was done for formulations G1 to G8 using *Bacillus Subtillus* as standard bacteria. A reference standard of erythromycin ethyl succinate and sample at 1.5mg/ml and 3.0mg/ml concentration respectively were used and observed after 72hr of incubation.

Descriptive results indicated formulation G1 to G8 presented zones of inhibition close to the observed reference standard [Figure18-22].The potential percentage micro-bioassay calculated was at an average for Erythromycin but not within the limits as indicated in [Table 14] as per the method used for analysis. The activity index descriptive results indicated the concentration in the matrix of the formulation was evenly spread for microbial inhibition. Formulations G1, G3, G5 and G7 showed better microbial activity index result compared to the other formulations.

The percentage label claim for Metronidazole was determined by UV spectroscopy analysis as shown in [Table 15]. Formulations G5, G6, G7 and G8 were within pharmacopeia limit of between 90-110%.

In-vitro drug release studies was a challenge and could not be performed due to lack of the Franz diffusion cell, and also a validated analytical procedure for simultaneous determination by HPLC for combined Erythromycin/ Metronidazole using other C18 column other than xtera, such as hypersil OD and hypersil cyano, due to the sensitivity of erythromycin chromospheres. More research on the some should be done to enhance further research on the above formulation.

Stability studies conducted after 2 month at room temperature was observed as follows and descriptive results showed no color change, no phase separation, and no odour from the formulations, this demonstrated stability at room condition of temperature and pressure for the formulations.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.0 CONCLUSION

The presented work envisages the feasibility of the use of the methanol 60%/water 40% system as recommended diluents for the formulations G1 to G8 from the solubility studies done. The active drugs employed Erythromycin and Metronidazole both showed good solubility and also the polymer used in the methanol water system. The use of HPMC as thickening agent effect was observed to have no descriptive variation on the rheology of the formulations. Carbopol 940 use at 0.5% in the gel formulations showed descriptive results on the physical appearance, it was seen to be more free flowing as compared to 1.5% carbopol 940. Topical Erythromycin/Metronidazole gel containing the combined dosage was an attempt to utilize the immense potential of a unit combined dosage in the gel formulation as a carrier to increase their potential and efficacy at the site of local action. In this contribution, we developed and evaluated the gel containing Erythromycin /Metronidazole with the different ratios of carbopol 940 and HPMC to obtain the optimized Formulation which is suited for application as a skin delivery system. A number of problems associated with drug molecules such as bioavailability, degradation, stability were overcome by the methanol water system employed in the formulation as it created a balance within the matrix of the gel as showed from the descriptive stability studies after 2 months. Compatibility studies showed consistency in the functional groups IR peaks, by this it indicated no chemical reaction took place to reduce the activity of the API's. The method of preparation used proved to favor both the active drugs and the polymer carbopol 940 and HPMC, so the major objective has been achieved successfully in the formulation part.

6.1 RECOMMENDATIONS

From the practical aspect further test such as extrudability, spreadability need to be performed this was not possible at the time of this research due to lack of equipments.

Stability studies needed to be done at accelerated and long term condition to obtain more data on stability of the formulation but due to time within which project was done and also the equipment accessibility it was a challenge.

In vitro drug release needed to be done but due to lack of instruments, franc cell and xtera column. But an attempt was made by using the HPLC method using different C18 of hypersil OD and hypersil cyano column and varying the mobile phase of (acetonitrile: water: 0.2M phosphate buffer pH 7.0) but no conclusive result were obtained.

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