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FORMULATION AND EVALUATION OF TOPICAL PREPARATIONS OF REFAXIMIN

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ABSTRACT

The present investigation was aimed to develop and compare the different topical preparations such as Emulgels, ointments and creams of various formulations of Refaximin using suitable excipients. The prepared topical preparations were subjected to various evaluation parameters. Each 3formulations of Emulgels E2, ointments O3, and creams C1 showed better results compared with remaining 2 formulations. From that prepared various different topical preparations due to high antimicrobial activity creams as the best formulation among the all preparations of Refaximin.

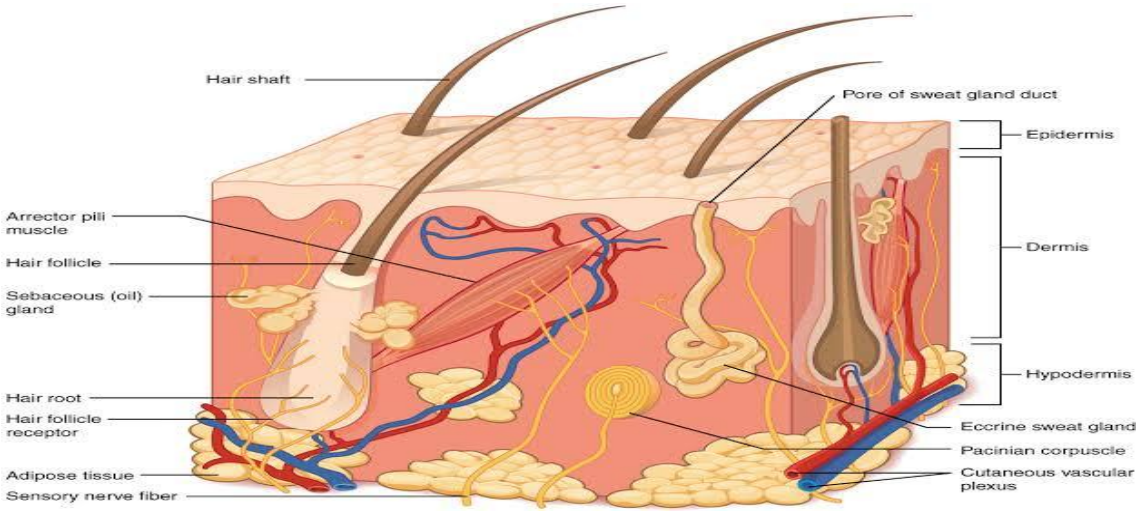
KEYWORDS: Topical preparations emulgel, ointments, creams, refaximin.

INTRODUCTION

Topical delivery is an attractive route for local and systemic treatment. The delivery of drugs on to the skin is recognized as effective means of therapy for local dermatological diseases. It can penetrate deeper into skin and hence give better absorption. Topical application has many advantages over the conventional dosage forms. In general, they are deemed more effective less toxic than conventional formulations due to the bilayer composition & structure. In the formulation of topical dosage forms, attempts are being made to utilize drug carriers that ensure adequate localization or penetration of the drug within or through the skin inorder to enhance the local and minimize systemic effect or to ensure adequate percutaneous absorption. Topical preparation avoids GI irritation, prevent the metabolism of the drug in the liver and increase the bioavailability of the drug. Topical preparation give its action directly at the site of action.

ANATOMY OF THE SKIN

The skin is the largest organ of the body. Its large surface area in direct contact with the environment present tremendous opportunities for drug delivery. The human skin is organized into 2 distinct layers, namely epidermis & dermis. The highly vascular dermis is made up of a connective tissue matrix containing nerves, hair follicles, pilosebaceous units & sweat glands. The epidermis is a vascular & its outermost layer, the stratum corneum consist of keratin rich, dead epidermal cells called corneocytes embedded within a lipid rich matrix. The stratum corneum forms the primary barrier for drug permeation especially to water soluble compound. Consequently, drug delivery across the stratum corneum has become the essence in the design of many dermal delivery systems.



Skin is composed of 2 primary layers

- The epidermis, which provides water proofing & serves as a barrier to infections.
- The dermis, which serves as a location for the appendages of the skin.

EPIDERMIS

The epidermis is composed of the outermost layer of the skin. it forms a protective barrier over the body surface, responsible for keeping water in the body and preventing pathogens from entering and is a stratified squamous epithelium composed of proliferating basal and differentiated supra basal keratinocytes. The epidermis also helps the skin regulate body temperature.

Keratinocytes are the major cells, constituting 95% of the epidermis while merkel cells, melanocytes and langerhans cells are also present. The epidermis can be further subdivided into the following strata or layers(beginning with the outer most layer)

- Stratum corneum
- Stratum lucidum(only in palms and soles)
- Stratum granulosam
- Stratum spinosum
- Stratum germinativum(also called the stratum basal)

The epidermis contains no blood vessels, and cells in the deepest layers are nourished by diffusion from blood capillaries extending to the upper layers of dermis.

BASEMENT MEMBRANE

The epidermis and dermis are separated by thin sheet of fibres called the basement membrane, and is made through action of both tissues. The basement membrane controls the traffic of the cells and molecules between the dermis and epidermis but also serves, through the binding of variety of cytokine and growth factors, has a reservoir for their control release during physiological remodeling or repair processes.

DERMIS

The dermis is the layer of skin beneath the epidermis that consist of tissue and cushions the body from stress and strain. The dermis provides tensile strength and elasticity to the skin through on extracellular matrix composed of collagen fibrils, microfibrils, and elastic fibers, embedded in proteoglycans.

It harbors many mechano receptors (nerve endings) that provide the sense of touch and heat. It also contains the hair follicles, sweat glands, sebaceous glands, apocrine glands, lymphatic vessels and blood vessels. the blood vessels in the dermis provide nourishment and waste removal from its own cells as well as epidermis.

The dermis is tightly connected to the epidermis though a basement membrane and is structurally divided into two areas ; a superfacial area adjacent to the epidermis, called the papillary region, and adeep thicker area known as the reticular region.

PAPILLARY REGION

The papillary region is composed of loose areolar connective tissue. This is named for its finger like projections called papillae that extends towards the epidermis. The papillae provides the dermis with a "bumpy" surface that inter digitaties with the epidermis, strengthening the connection between the two layers of the skin.

RETICULAR REGION

The reticular region lies deep in the papillary region and is usually much thicker. It is composed of dense irregular connective tissue, and receives its name from the dense concentration of collagenious, elastic and reticular fibers that weave throughout it. These protein fibers gives the dermis its properties of strength, extensibility and elasticity. Also located within the reticular region are the roots of the hair, sebaceous glands, sweat glands, nails and blood vessels.

SEMI SOLID DOSAGE FORMS

Semisolids dosage forms are traditionally used for treating topical ailments. Various categories of drugs such as antibacterials, antifungals and antivirals are incorporated into these products which show there activity on the surface layers of tissues or penetrate into internal layers to reach the site of action.

IDEAL PROPERTIES OF SEMISOLID DOSAGE FORMS

- **Physical properties**
- smooth texture
- elegant in appearance
- non dehydrating
- non gritty
- non greasy and non staining

OINTMENTS

Ointments are semi-solid preparations meant for external application to the skin or mucous membrane. They are usually contain a medicament or medicaments dissolved, suspended or emulsified in an ointment base. They may contain a suitable antimicrobial preservative. The ointments are mainly used as protective or emollient for the skin.

Classification of ointments

1. According to their therapeutic properties based on penetration.
2. According to their therapeutic uses.

Ointment classified according to properties based on penetration

1. epidermic ointments
2. endodermic ointments
3. diadermic ointments

ointments classified according to therapeutic uses

1. antibiotic ointments
2. antifungal ointments

3. anti inflammatory ointments
4. antipruritic ointments
5. astringent ointment
6. antieczematous ointments
7. keratolytic ointments
8. counter irritant ointments
9. ointments used for dandruff treatment
10. ointments for psoriasis treatment
11. Parasiticide ointments
12. Protectant ointments

Ointment bases

The ointment bases is that substance or part of an ointment, which serves as carrier or vehicle for the medicament.

Classification of ointment bases

1. Oleaginous bases
2. Absorption bases
3. Emulsion bases
4. Water soluble bases

1. Oleaginous bases: these bases consist of water insoluble, hydrocarbons, vegetable oils, animal fat and waxes. The constituents of hydrocarbon bases are soft paraffin, hard paraffin and liquid paraffin.

(i) peterolatum(soft paraffin): it is a purified mixture of semi-solid hydrocarbons obtained from petroleum. There are two varieties of soft paraffin, one is yellow soft paraffin and other is white soft paraffin. White soft paraffin is prepared by bleaching yellow soft paraffin. Both these soft paraffin have melting points of 38-56◦c. white soft paraffin is used when the medicament is white or colourless. White soft paraffin is never used in the preparation of ophthalmic ointments because the white soft paraffin may contain small traces of bleaching agent which are generally left over after bleaching the yellow soft paraffin. Hence the white soft paraffin may cause irritation to the eye.

(ii) Hard paraffin: it is a purified mixture of liquid hydro carbons and obtained from petroleum. It is colourless or white translucent, odourless, tasteless wax like substance. It is used to harden or soften the ointment base.

(iii) Liquid paraffin: It consist of a mixture of liquid hydrocarbons and obtained from petroleum by distillation. It is also known as white mineral oil or liquid petroleum. It is colourless, odourless, tasteless and transparent oily liquid. It is soluble in ether and chloroform but insoluble in water and alcohol. It is used along with hard paraffin and soft paraffin to get a desired consistency of ointment.

2. **Absorption bases:** these bases are generally anhydrous substances which have the property of absorbing(emulsifying) considerable quantity of water but still retaining their ointment like consistency.

The absorption bases are two types: (i)non emulsified bases, (ii)water in oil emulsion

The non emulsified bases absorb water and aqueous solutions producing water in oil type of emulsion e.g, wool fat, wool alcohol, bees wax and cholesterol. The water in oil emulsions are capable of absorbing more water and have the properties of non emulsified bases e,g, hydrous wool fat (lanolin).

3. emulsion bases: these bases are semi solid or have a cream like consistency. Both o/w and w/o emulsions are used as ointment bases. The oil in water type of emulsion bases are more popular because these can be easily removed from the skin or clothes by washing with water. The w/o type of bases are greasy and sticky.

4. water soluble bases: these are commonly known as "greasless ointment bases". The water soluble bases consists of water soluble ingredients such as, poly ethylene glycol polymers which are popularly known as "carbowaxes". These carbowaxes are water soluble non volatile & inert substances.

PREPARATION OF OINTMENTS

The ointments can be prepared by any one of the following methods

1.Trituration method
2. Fusion method
3. Chemical reaction method
4. Emulsification method

1. Trituration method: it is the most commonly used method for the preparation of ointments. The method is used when the base is soft and the medicament is insoluble in base. So for uniform mixing of medicaments in the base, it becomes necessary to reduce the medicament to the fine powder. The following procedure is used to get a uniform ointment.

(1)Finely powder the solid medicaments.

(2)weigh the required quantity of an ointment base. Triturate the solid medicaments with a small amount of the base on an ointment slab with the help of stainless steel ointment spatula until a homogenous product is formed.

(3) Add remaining quantities of the base until the medicaments is uniformly mixed with it. Incorporate any liquid ingredients if present.

When large quantity of liquid is to be incorporated, pestle and mortar should be used. The pestle and mortar method of trituartion is not preferable as compared to slab method because

(i) The sides of the pestle and mortar will be required to scrap from time totime.

(ii) There are chances of improper mixing of particles of medicament(s) which are usually slip out, from under the pestle during trituration.

2. Fusion method: when an ointment base contains a number of solid ingredients of different melting points, such as white beeswax, stearic acid, hard paraffin and cetyl alcohol, it is necessary to melt them in decreasing

order to their melting point. This means, that the substance with highest melting point should be melted first, then the substance with next melting point and so on. This will avoid the over heating of substances having low melting points. The medicament is incorporated slowly to the melted mass, stirred thoroughly until the mass cools down and homogeneous product is formed.

In case any liquid ingredient or aqueous substance is also to be incorporated, that should be heated to almost the same temperature as the melted base. In case, this precaution is not observed, then upon mixing the two portions, the waxes or the solid will cool down quickly and get separated. This will prevent the uniform mixing of all the ingredients.

After mixing the two portions, the stirring should be down uniformly and thoroughly to make a homogeneous mass. The rapid cooling should be avoided to get a uniform product. The vigorous stirring should be avoided, when the ointment has just begun to thicken. It is necessary otherwise the air will get entrapped in the ointment.

Sometimes, due to rapid cooling of the melted mass, the waxy solids separates out from the ointment and uniform product is not obtained. Inorder to produce a uniform product, it can be remelted over a waterbath and again stirred until cold.

Inorder to remove the dust or foreign particles from the melted base, it is strained through muslin piece. The clarified liquid is collected in an other hot container.

3.chemical reaction method: certain chemical reactions are involved in the preparation of several ointments. For example, iodine ointment. Iodine may be present in free form or in combined form with the ointment base

Ointment containing free iodine: iodine is only slightly soluble in most of the fats and vegetable oils. But it is readily soluble in concentrated potassium iodine solution in water, due to formation of poly iodides. These poly iodides are readily soluble in water, alcohol and glycerine. While selecting liquid to ensure proper distribution of medicament, it is important that the liquid should be non volatile, otherwise the distributed medicament may crystallize when the solvent evaporates. These solutions may be incorporated with the absorption type of ointment bases. For example, strong iodine ointment which is used to treat ringworm infection in cattles.

Ointment containing combined iodine: the fixed oils and many fats obtained from vegetable and animal sources containing unsaturated constituents. The iodine combined with double bonds of unsaturated constituents and thus free iodine is not available. For example,
Oleic acid + iodine → Di- Iodo stearic acid

Since free iodine is not available, these ointments are dark, greenish black in colour. It leaves no stain when rubbed into the skin. Hence they are known as non staining iodine ointment.

4. Emulsification method: in this method, the fats,oils & waxes are melted together on a water bath at temperature of 70◦C. The aqueous solution of all of the heat stable water soluble components is also heated almost at the same temperature as that of the melted bases. The solution is slowely added to the melted bases with continuous stirring until the product cools down & semi solid mass known as ointment is prepared.

It is very important to heat the aqueous liquid to almost the same temperature as that of the melted bases, otherwise high melting point fats & waxes will immediately get solidified when cold aqueous solutions is mixed with it & ointment containing lumps will be formed.

CREAMS

These are viscous semi-solids emulisions which are meant for external use. They usually containg water soluble base due to which they can easily removed from the skin. They are of softer consistency and have light weight in comparison to true ointments when applied to the skin, creams leave no visible evidence of their presence on the skin.

TYPES OF CREAMS: The creams are of 2 types.

1. Aqueous creams: In aqueous creams the emulsions are o/w type. These creams are relatively non greasy. These creams are further divided into 3 types depending on the type of emulsifying agent used for preparing them.

a) Anionic emulsifying wax creams: These creams are prepared by fusion method. The wax and oily ingredients are melted together and cooled to about 60◦C. the water or aqueous solution is warmed to the same temperature and mixed with oily mixture with constant stirring which is continued until cold.

b) Cationic emulsifying wax creams: These are made in the same way as that of anionic emulsifying wax creams. Cetosteryl alcohol & cetrimide is used to prepare creams.

c) Non –ionic emulsifying wax creams: these are prepared in the same way as anionic and cationic creams. These creams are prepared by self emulsifying monostearin, sorbitol ester, macrogel ester non- ionic emulsifying wax, poly sorbate, poly vinyl alcohols and higher fatty alcohols.

2. Oily cream: In oily creams, the emulsions are water in oil type. These creams are greasy. The oily creams are further divided into two types depending on the type of emulsifying agent used for preparing w/o emulsion.

a) Sterol creams: these creams are w/o type emulsions in which woolfat or wool alcohol is used as emulsifying agent.

b) Soap creams: these are w/o emulsions in which the emulsifying agent are triethanolamine soap, calcium soap or borax soap. Emulsions containing soaps of liquid fatty acid are made without heat.

EMULGEL

When gel and emulsion are used in combined form the dosage form are referred as emulgel.

CONSTITUENTS OF EMULGEL

1. Aqueous material: this forms the aqueous phase of the emulsion. Commonly used agents are water, alcohol.
2. Oils: these agents form the oily phase of the emulsion. For externally applied emulsions, mineral oils, either alone are combined with soft or hard paraffins, are widely used both as the vehicle for the drug and for their occlusive and sensory characteristics. Widely used oils in oral preparations are non biodegradable mineral and castor oils that provide a local laxative effect, and fish liver oil or various fixed oils of vegetable origin(example arachis, cotton seed, and maize oil) as nutritional supplements.

Chemicals

CHEMICAL	QUANTITY	DOSAGE FORM
Light liquid paraffin	7.5%	Emulsion and emulgel
Isopropylmyristate	7-7.5%	Emulsion
Isopropyl stearate	7-7.5%	Emulsion
isopropyl palmitate	7-7.5%	Emulsion
Propylene glycol	3-5%	Gel

3. Emulsifiers: emulsifying agents are used both to promote emulsification at the time of manufacture and to control stability during a shelf life that can vary from days for extemporaneously prepared emulsions to months or years for commercial preparations. Example poly ethylene glycol 40 stearate, sorbitan mono oleate (span 80), poly oxyethylene sorbitan mono oleate (tween 80), stearic acid, sodium stearate.
4. Gelling agent: these are the agents used to increase the consistency of any dosage form can also be used as thickening agent.

GELLING AGENT	QUANTITY	DOSAGE FORM
Carbopol-934	1%	Emulgel
Sodium alginate	8%	Emulgel
HPMC-2910	2.5%	Emulgel
HPMC	3.5%	Gel
Sodium CMC	1%	Gel

5. Permeation enhancers; these are agents that partition into and interact with skin constituents to induce a temporary and reversible increase in skin permeability.

PENETRATION ENHANCERS	QUANTITY	DOSAGE FORM
Oleic acid	1%	Gel
Lecithin	5%	Gel
Urea	10%	Gel
Isopropyl myristate	5%	Gel
Linoleic acid	5%	Gel
Clove	8%	Emulgel
Menthol	5%	Emulgel

MECHANISM OF ACTION OF PENETRATION ENHANCERS

Penetration enhancers may act by one or more of three main mechanisms

1. Disruption of the highly ordered structure of stratum corneum lipid.
2. Interaction with intercellular protein.
3. Improved partition of the drug, enhancer of solvent into the stratum corneum
4. The enhancers act by altering one of three pathways. The key to altering the polar pathway is to cause protein conformational change or solvent swelling. The fatty acid enhancers increased the fluidity of the lipid protein portion or the stratum corneum. Some enhancers act on both polar and non polar pathway by altering the multi laminate pathway for penetration. The type of enhancers employed has a significant impact on the design and development of the product.

METHOD OF PREPARATION OF EMULGEL

Preparation of emulgel is a three step process.

Step 1:preparation of emulsion
Step 2: preparation of gel base
Step 3: incorporation of emulsion into gel base with continuous stirring. The flow chart of emulgel preparation is shown in figure.

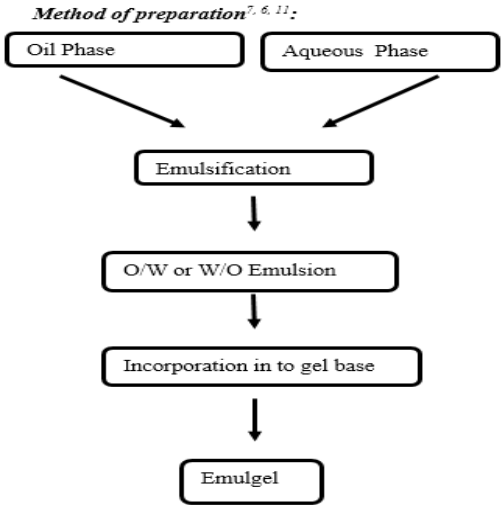


Figure 3: Flowchart of emulgel prepration.

EVALUATION OF SEMISOLID DOSAGE FORMS

1. PHYSICAL EXAMINATION: The prepared emulgel, ointment, cream formulations are inspected visually for their colour homegenity, consistency & phase separation.

2. MEASUREMENT OF pH: The pH of the emulgel formulations was determined by using digital pH meter.1gm of emulgel is dissolved in 100 ml of distilled water & it was placed for 2 hrs. The measurement of pH of each formulation was done in triplicate & average values were calculated.

3. SWELLING INDEX : to determine the swelling index of prepared topical emulgel, 1gm of emulgel is taken on porous aluminium foil & then placed separately in a 50ml beaker containing 10ml 0.1N NaoH. Then samples were removed from beakers at different time intervals & put it on dry place for sometime & re weighed. Swelling index is calculated as follows.

$$\text{Swelling index (sw)\%} = [(Wt-Wo)/Wo] \times 100$$

Where
Swelling index (SW)% = Equilibrium percent swelling
Wo = Original weight of emulgel at zero time
Wt = Weight of swollen emulgel after time t,

4. DRUG CONTENT: Take 1 gm of emulgel. Mix it in suitable solvent. Filter it to obtain clear solution. Determine its absorbance using UV spectrophotometer. Standard plot of drug is prepared in the same solvent. Concentration and drug content can be determined by using the same standard plot by putting the value of absorbance in the standard plot equation.
Drug content = (concentration ×dilution factor× volume taken)× conversion factor

5. IN VITRO RELEASE STUDY: Franze diffusion cell(with effective diffusion area 3.14 cm² and 15.5ml cell volume) was used for the drug release studies. Gellified emulsion (200mg) was applied on to the surface of egg membrane evenly. The egg membrane was calm ped between the donor and the receptor chamber of diffusion cell. The receptor chamber was filled with freshly prepared PBS(pH 5.5) solution to stabilize the drug. The receptor chamber was stirred by magnetic stirrer.

The sample (1.0 ml aliquots) were collected at suitable time interval. Samples were analyzed for drug content by UV spectrophotometer after appropriate dilutions. Cumulative correlations were made to obtain the total amount of drug release at each time interval. The cumulative amount of drug released across the egg membrane was determined as a function of time.

6. MICROBIOLOGICAL ASSAY: Microbiological assay was performed by using plate technique. Previously prepared sabouraud's agar dried plates were used. 3 grams of the gellified emulsion are placed in a ditch cut in the plate. Freshly prepared culture loops are streaked across the agar at a right angle from the ditch to the edge of the plate. After incubation for 18 to 24 hours at 25◦C, the fungal growth was observed and the percentage inhibition was measured as follows.

$$\text{\% inhibition} = L_2/ L_1 \times 100$$

Where
L_1= total length of the streaked culture
L_2= length of inhibition

MATERIALS AND METHODOLOGY

MATERIALS

All the chemicals used in the study were procured from standard source, standard instruments were used and established protocols were followed to conduct the experiments.

S.NO	MATERIAL	SOURCE
1.	Carbopol 936	-
2.	HPMC K 100	-
3.	Liquid paraffin	-
4.	Propylene glycol	-
5.	PEG 600	-
6.	Ethanol	-
7.	Mineral oils	-
8.	Methanol	-
9.	Triethanolamine	-
10.	Stearic acid	-
11.	Lanolin	-
12.	Methyl or propyl paraben	-

INSTRUMENTS

S.NO	INSTRUMENT	SOURCE
1.	Electronic balance	Citizon
2.	Hot air oven	Biotechnic india
3.	Magnetic stirrer	REMI
4.	Refrigerator	SAMSUNG
5.	PH meter	ELICO
6.	Bacteriological incubator	BIOTECHNICES INDIA
7.	UV-spectrophotometer	SHIMAAADZU 1800

METHODS

1. STARDARD GRAPH OF REFAXIMIN

reagents & material:

active pharmaceutical ingredient(API): Refaximin

solvent: Ethanol

Instrumentation

UV-visible spectrophotometer with 10mm matched quartz cells was used. All weighing were done on precision balance.

Selection of analytical wavelength

Stock solution of 1000µg/ml of rifaximin was prepared ethanol. This solution is called as primary stock solution. From this solution 100µg/ml solution was prepared by taking 10ml solution in 100ml of volumetric flask, volume is made upto 100ml with ethanol. This solution is called as secondary stock solution. From secondary stock solution 1ml was diluted to 10ml & scanned with a range of 200-400nm. Maximum absorption was found to be 290nm.

Preparation of standard solutions

Stock solution of 1000µg/ml of rifaximin was prepared in ethanol. This solution is called as primary stock solution. From this solution 100µg/ml solution was prepared by dilution with ethanol. This solution is called as secondary stock solution.

From this secondary solution different concentration like 2µg/ml,4µg/ml,6µg /ml,8µg/ml,10µg/ml were prepared with ethanol. For this solution the absorbance was scanned in the spectrum mode of 290nm. The different absorption was found with different concentration. The graph was plotted by taking concentration on X-axis & absorption on Y-axis.

2. PREPARATION OF REFAXIMIN EMULGEL

1. **Preparation of emulsion**

a) **Preparation of aqueous phase:** The aqueous phase of emulsion was prepared by dissolving Tween80 in purified water.

b) **Preparation of oil phase:** Methyl paraben and propyl paraben were dissolved in propylene glycol where as drug was dissolved in ethanol and both the solutions were mixed with the aqueous phase. Both the oily and aqueous phases were separately heated to 75◦C.Then the oil phase were added to the aqueous phase with continuous stirring until cooled to room temperature.

2. **preparation of gel:** the gel bases were prepared by dispersing different concentrations of polymers in distilled water separately with constant stirring at a moderate speed using mechanical shaker. The pH of all the formulations was adjusted to 6-6.5 using tri-ethanolamine (TEA).

3. **Preparation of emulgel**: The obtained emulsion was mixed with the gel with gentle stirring to obtain emulgel. 4 different formulations were prepared and their composition was given in the table.

Ingredients	E1	E2	E3
Refaximin	1gm	1gm	1gm
Liquid paraffin	6ml	6ml	6ml
Tween80	1ml	1ml	1ml
Propylene glycol	4.5ml	4.5ml	4.5ml
Ethanol	3.4ml	3.4ml	3.4ml
Propyl paraben	0.05gms	0.05gms	0.05gms
Methyl paraben	0.05gms	0.05gms	0.5gms
Distilled water	82ml	82ml	82ml
Cabapol934	2gms	-	1gm
Hpmc k100	-	2gm	1gm

Formulation of emulgel

1. PREPARATION OF REFAXIMIN OINTMENT

Rifaximin ointment was prepared by fusion method. In this method the ingredients are homogeneity according to formula given in table. Up to 4 formulations were prepared.

Ingredients	O1	O2	O3
Refaximin	1gm	1gm	1gm
Peg 6000	20gm	25gm	28gm
Peg 400	40ml	41ml	42ml
Propylene glycol	25ml	25ml	25ml
Tween80	0.5ml	0.5ml	0.5ml
Water	13.5ml	7.5ml	4.5ml

Formulation of ointment

For preparation of ointment both oil phases i.e, PEG 6000 & PEG 400 were heated to 70◦C, then cool upto 40◦C. In the next step rifaximin drug is dissolved in water after wards propylene glycol and tween80 were added in it. In final step both the phase i.e, oil phase and water phase are mixed. The formulations were stored properly in suitable containers.

4. PREPARATION OF REFAXIMIN CREAM

It is o/w type of cream. First the optimized concentration of oil phase stearic acid and lanolin were taken, lanolin is added to get a good consistency. Along with both of this excipients mineral oil was heated as to melt the oil phase, which should not exceed above 70◦C on the other hand the water phases were prepared by dissolving the drug in water and in that tri ethanolamine was added.

Tri ethanolamine was added because stearic acid requires neutralization with alkalies or for formation of the cream. 3 different formulations were prepared and their composition was given in the table.

Ingredients	C1	C2	C3
Refaximin	1gm	1gm	1gm
Mineral oil	10gms	10gms	10gms
Stearic acid	15gms	9gms	12gms
Lanolin	5gms	8gms	7gms
Tri ethanolamine	2ml	2ml	2ml
Distilled water	67ml	70ml	68ml

Formulation of cream

4. EVALUATION TESTS

The prepared emulgel, ointment, cream formulations were evaluated for following tests:

a) **Physical examination:** The prepared emulgel, ointment, cream formulations were inspected visually for their colour, homogeneity, consistency, grittilness & phase separation.

b) **Measurement of pH:** The pH of the prepared formulations were determined by using digital pH meter. 1gm of formulation was dissolved in 100 ml of distilled water & it was kept aside for 2hrs.The measurement of pH of each formulation was done in triplicate & average values were calculated.

c) **Swelling index:** To determine the swelling index of prepared topical formulations, 1gm is taken on porous aluminium foil and then placed separately in a 50 ml beaker containing 10ml 0.1 N NaOH. Then samples were removed from beakers a different time intervals and put it on dry place for sometime after it reweighed. Swelling index is calculated as follows.

Swelling Index(SW) % = [(Wt-Wo)/Wo] ×100

Where;

Swelling index (SW)%=Equilibrium percent swelling

Wo = Original weight of formulation at zero time after time t

Wt = Weight of swollen emulgel/cream/ointment

2. Drug Content Determination: Weigh accurately 1gm of rifaximin topical formulation and it was dissolved in 100ml of 0.1 N NaOH. The volumetric flask kept for 2hours and shaken well in a shaker to mix it properly. The solution was passed through the filter paper and filtered. The absorbance was measured spectrometrically at 375nm after appropriate dilution the drug content was determined using following formula.

Drug content = (concentration× dilution factor × volume taken)×conversion factor

3. In vitro Drug Release Study: The in vitro drug release studies of the emulgel were carried out in modified diffusion cell using dialysis membrane.

The membrane was soaked in phosphate buffer solution(PBS) pH 7.4 for 9-12 hours was to clamped carefully to one end of the hallow glass tube of dialysis cell. Then emulgel (300mg) was spread uniformly on the dialysis membrane. 100ml of phosphate buffer solution pH 7.4 used as dissolution media was added to receptor compartment.

This whole assembly was kept on a magnetic stirrer and the solution

On the receptor side was stirred continuously using a magnetic bead and temperature of the cell was maintained at 37±0.5◦c. Sample (10ml) was withdrawn at suitable time intervals and replaced with equal amounts of fresh dissolution medium. Samples were analysed spectrometrically at 257nm & cumulative percentage drug release was calculated.

4. Antimicrobial test

Nutrient broth preparation

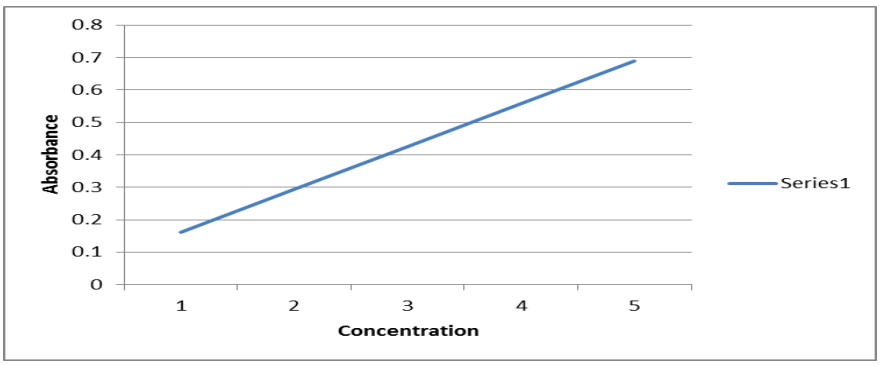
Nutrient agar medium was prepared by adding the required quantity of peptone, agar, sodium chloride, yeast/beef extract taken into the clean & dried conical flask & followed byadding required quantity of distilled water. Mixed well and dissolved by heating with frequent agitation. It was sterilized by autoclaving at 121◦c for 15 min. Then removed and cooled. The medium was then inoculated with microorganism (E. coli & bacillus)and poured into the petri-plates. The petriplates were allowed to solidify and cavities were made to fill the prepared gels. The petriplates were incubated for 24 hrs and results were reported.

The prepared nutrient both is cooled and it is divided into two equal parts one of these portions are added to E.coli and to the another portion is added to bacillus. This both is poured into petriplates upto ¾ th volume and it is allowed to solidified. After solidification the cavities are done in each petriplates. The prepared formulations are filled into the cavities and two standard formulation is also filled. These petriplates are placed in the refrigerator for 15 mints for diffusion. All the petriplates were incubated for 24hrs. the inhibition zones are observed. The zone of inhibition was measured by zone reader.

RESULTS AND DISCUSSION

1) STANDARD GRAPH

Concentration	Absorbance
1	0.162
2	0.294
3	0.426
4	0.559
5	0.690



2) EVALUATION PARAMETERS

i) PHYSICAL EXAMINATION

The prepared rifaximin ointment, cream, emulgel formulations inspected visually for their colour, phase separation, grittleness, homogeneity and consistency. Results have been discussed in table.

S.no	Formulation	Colour	Phase separation	Grittleness	Homogeneity	Consistency
1	C1	White	-	-	+++	+++
2	C2	White	-	-	+++	+++
3	C3	White	-	-	+++	+++
4	E1	White	-	-	+++	+++
5	E2	White	-	-	+++	+++
6	E3	White	-	-	+++	+++
7	O1	White	-	-	+++	+++
8	O2	White	-	-	+++	+++
9	O3	White	-	-	+++	+++

ii) pH MEASUREMENTS

S.no	Formulation	pH
1	C1	5.82
2	C2	6.0
3	C3	6.2
4	E1	6.3
5	E2	6.5
6	E3	6.6
7	O1	6.42
8	O2	6.71
9	O3	6.83

iii) SWELLING INDEX

The swelling index of the different formulations was observed and the data was shown in the table.

Time min	C1	C2	C3	E1	E2	E3	O1	O2	O3
0	1	1	1	1	1	1	1	1	1
15	1.14	1.13	1.22	1.13	1.10	1.15	1.11	1.10	1.13
30	1.18	1.16	1.30	1.19	1.16	1.20	1.22	1.20	1.25
45	1.22	1.20	1.5	1.23	1.18	1.25	1.24	1.22	1.28
60	1.5	1.25	1.9	1.24	1.20	1.27	1.35	1.30	1.6
120	1.3	1.7	1.95	1.26	1.25	1.30	1.46	1.42	1.62

iv) DETERMINATION OF DRUG CONTENT

Drug content of each formulation was determined and the data was shown in table.

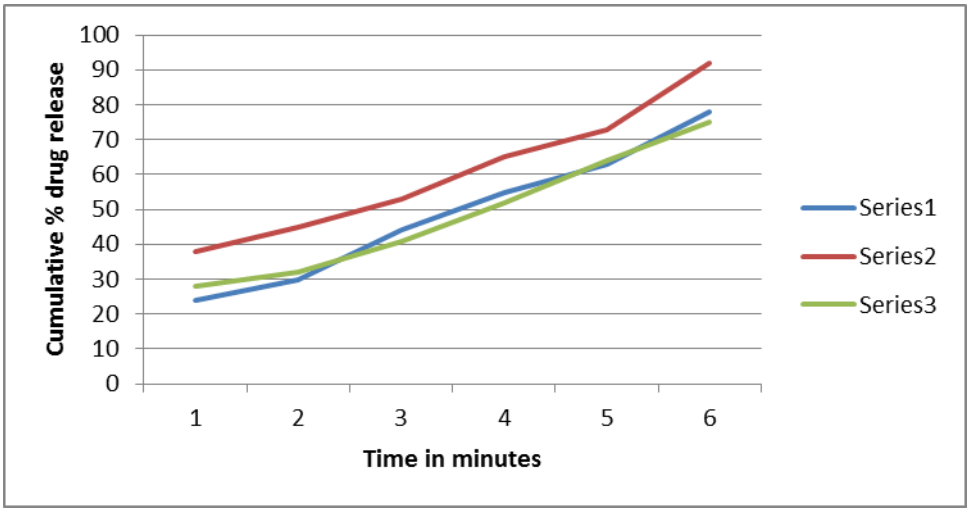
S.NO	FORMULATION	DRUG CONTENT
1	C1	90%
2	C2	82%
3	C3	80%
4	E1	65%
5	E2	70%
6	E3	60%
7	O1	63%
8	O2	60%
9	O3	75%

v) INVITRO DRUG RELEASE

The cumulative % drug release profile of all the formulation batches has been shown in table and the graph is plotted between cumulative % drug release verses time as shown in fig.

a) Emulgel

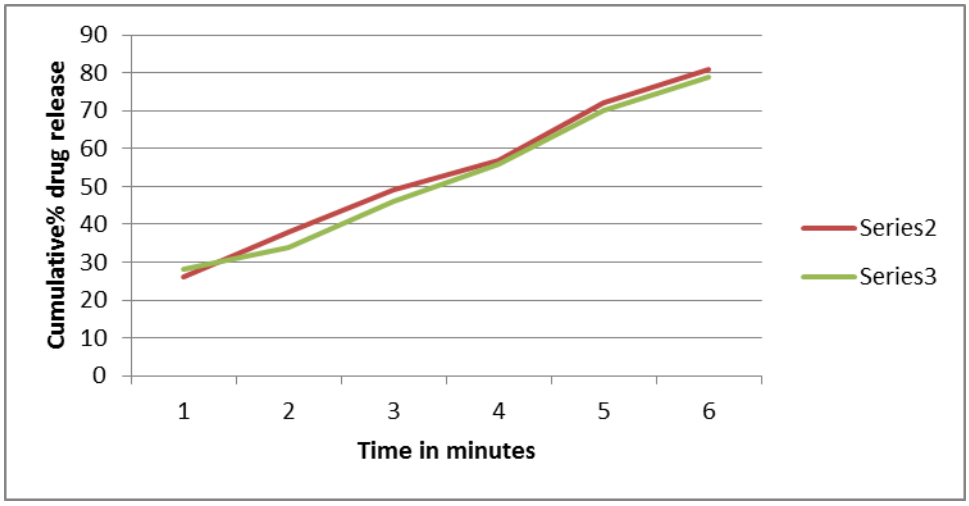
s.no	Formulation	1hr	2hr	3hr	4hr	5hr	6hr
1	E1	24	30	44	55	63	78
2	E2	38	45	53	65	79	92
3	E3	28	32	41	52	64	75



Invitro drug release from emulgel formulations

b) cream

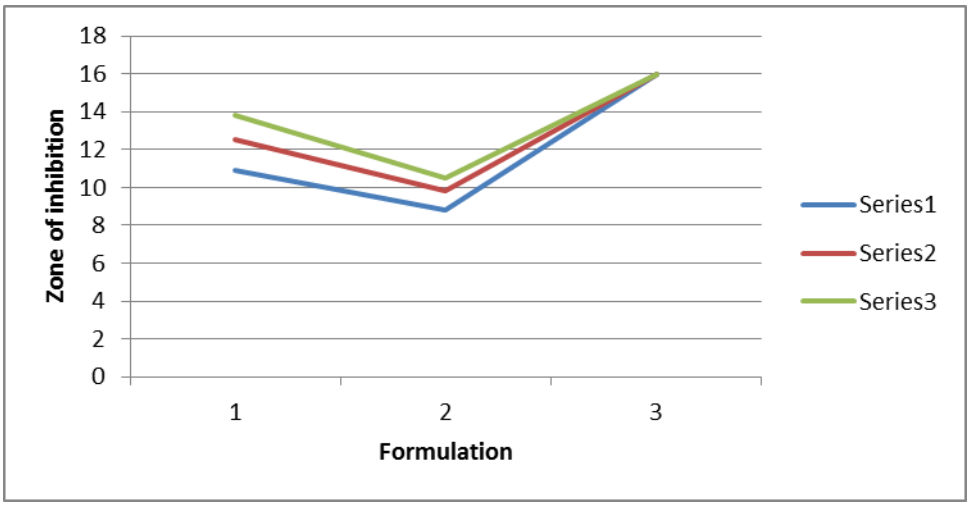
s.no	Formulation	1hr	2hr	3hr	4hr	5hr	6hr
1	C1	32	41	56	68	74	87
2	C2	26	38	49	57	72	81
3	C3	28	34	46	56	70	79



Invitro drug release from cream formulations

c) ointment

s.no	Formulation	1hr	2hr	3hr	4hr	5hr	6hr
1	O1	28	34	46	54	65	82
2	O2	26	32	48	52	61	76
3	O3	30	44	53	62	75	89

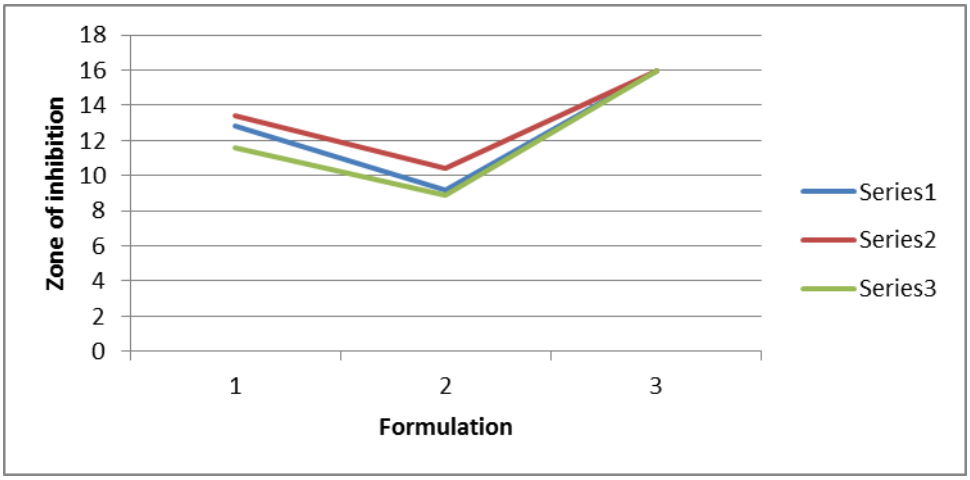


Invitro drug release from ointment formulations

vi) ANTIMICROBIAL TEST: All the prepared formulations were tested for antimicrobial activity by using E.coli and Bacillus. The of inhibition was measured by zone reader and it was mentioned in below table.

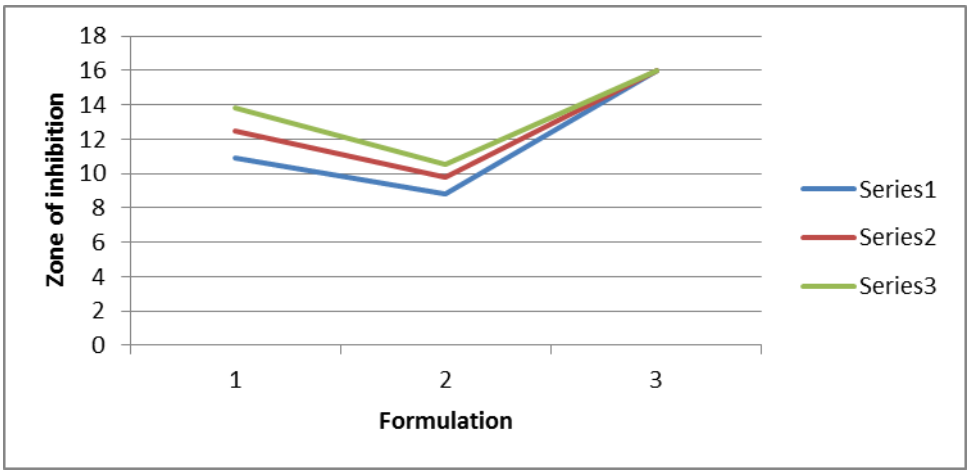
a) Emulgel

Formulation	Zone of inhibition		
	E.coli	B.subtilis	Std(streptomyces)
E2	13.4mm	10.4mm	16mm
E1	12.5mm	9.2mm	16mm
E3	11.6mm	8.9mm	16mm



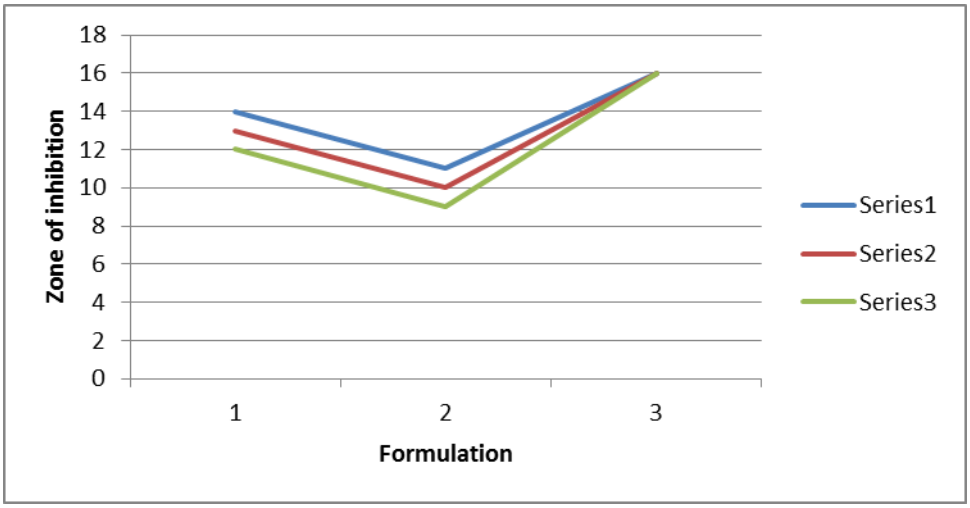
b) ointment

Formulation	Zone of inhibition		
	E.coli	B.subtilis	Std(streptomyces)
O1	10.9mm	8.8mm	16mm
O2	12.5mm	9.8mm	16mm
O3	13.8mm	10.5mm	16mm



c) cream

Formulation	Zone of inhibition		
	E.coli	B.subtilis	Std(streptomyces)
C1	14mm	11mm	16mm
C2	13mm	10mm	16mm
C3	12mm	9mm	16mm



CONCLUSION

- In present study we prepared different topical preparations such as Emulgels, creams and ointments of various formulations of Refaximin.
- From that each formulation of Emulgels, ointments and creams by changing the quality of excipients different 3 formulations were prepared.
- Above prepared all 9 formulations were subjected to evaluation parameters and found to be within limits.
- In Emulgels E2, ointments O3 and creams C1 formulations showed better results when compared with that of remaining formulations.
- Finally we can concluded that creams were the best formulation among the all the different topical preparations of Refaximin.

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