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FORMULATION AND EVALUATION OF CLOTRIMAZOLE AND ICHTHAMMOL OINTMENT

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ABSTRACT

We intended to develop a desired ointment for treatment of fungal infection like eczema itching, pruritis. Main objective of this study is to formulate the ointment with different ointment bases having good consistency, better diffusion, and antifungal and antiseptic properties. Combination of clotrimazole and ichthammol is good for treatment of infection caused by *coryne* bacteria and also used for skin infection as clotrimazole and ichthammol having antifungal and antiseptic properties respectively. To assess the efficacy of formulations assay, drug release, uniformity, viscosity, diffusivity, rheology, stability, spread ability, permeability and other physical characteristics were evaluated. Two Formulations, formulation A and B were prepared with same active ingredients except different bases. Formulation A contains white soft paraffin and cetostearyl alcohol while B contains hard paraffin, cetostearyl alcohol, Light liquid paraffin and microcrystalline wax. Formulation B was found better than formulation A in all aspects like spreadability, viscosity, consistency, stability, diffusibility etc.

KEY WORDS Ointment, permeability, diffusivity, eczema, rheology.

INTRODUCTION

The delivery of drug through the skin has long been a promising concept because of the ease of access, large surface area, vast exposure to the circulatory and lymphatic networks, and noninvasive nature of the treatment. This is true whether the bioavailability desired is systemic or local. Formulations

suitable for skin delivery are ointment, cream, gels etc. Ointments are homogeneous, semi-

solid preparations intended for external application to the skin or mucous membranes. They are used as emollients or for the application of active ingredients to the skin for protective, therapeutic, or prophylactic purposes and where a degree of occlusion is desired.¹

Eczematous diseases are very common with an estimated prevalence of more than 10% in general population. According to statistics, 15-25% of all dermatological patients suffer from eczema. Surveys have shown that the prevalence of eczema is increasing. Eczema is

a common chronic or relapsing dermatitis characterized by intense pruritus. It occurs primarily in infants and children with a personal or family history of atopy. 9 to 12% of all children are affected with the disease^{2, 3} and 60 to 70% of those with mild to severe dermatitis continue to experience symptoms into adulthood.⁴ A significant number of patients, who have outgrown the typical manifestations of this disease develop irritant dermatitis which may be chronic in nature and may also interfere with the ability to work, especially in wet conditions or those involving chemicals.⁵ Pruritis is one of the most common symptoms of eczema. The itch-scratch cycle increases the damage to the epidermal barrier, thereby increasing water loss and drying, which creates a suitable environment for skin pathogens to cause infection and flaring of symptoms. Approximately 15-25 % of all dermatological patients suffer from atopic dermatitis and children's are more prone to this disease. Atopic dermatitis^{2, 3} is a chronic, relapsing noncontiguous disease and highly pruritic condition. While developing ideal topical dosage form some factors must be considered like flux of the drug across the skin, retention of the dosage form on the skin surface, reservoir capacity of the dosage form and patient acceptability⁴. Pruritis is one in which itch-scratch cycle increases the damage to epidermal barrier, thereby increasing water loss and drying, which creates a suitable environment for skin pathogens to cause infection and flaring of symptoms. Although some commercial ointments with different clinical properties are available, it is necessary to develop multifunctional ointment suitable to the disease stages of atopic dermatitis and eczema. Thus combination in topical treatment with antifungal and antiseptic agent has been recommended. The different topical formulations were developed in this study using a combination of clotrimazole and ichthammol as an ointment. The main aim is to formulate the two different ointment formulations (formulation A and B) with same active ingredient (clotrimazole,

ichthammol) but by using different bases. Formulation code A contains white soft paraffin 25% w/w and Cetostearyl alcohol 20% w/w and formulation code B contains hard paraffin 1.5% w/w, microcrystalline 2.5% w/w, light liquid paraffin 26% w/w and Cetostearyl alcohol 15% w/w. This antifungal topical ointment is free from steroids and is used to cure the cracks on heels, candidiasis, psoriasis, ringworm, pityriasis versicolor, help to regenerate the macerated skin. Acceptability and clinical efficacy of such preparations required them to pass optimal mechanical properties (ease of removal from the container, spreadability on the substrate), rheological properties (viscosity, elasticity, thixotropy, flowability), and other properties such as bioadhesion, desired drug release and absorption⁶.

MATERIALS

Clotrimazole, ichthammol, boric acid, zinc oxide, menthol, cetostearyl alcohol, hard paraffin, white soft paraffin, microcrystalline wax, light liquid, methyl paraben, propyl paraben, triethanolamine, propylene, demineralized water were provided by ANAGHA PHARMACEUTICALS, INDIA.

INSTRUMENTS

- Brookfield viscometer.
- pH meter: 361- micro pH syatem.
- Water bath: Electro lab.
- Microscope: Roslane meditech.
- UV- Visible spectrophotometer.
- Stability chamber: sainath boilers and pneumatics.
- Digital weight box: alcoset.
- Homogeniser: silverson
- Desicator: silverson.

All other apparatus like pipette, burette, glass beaker, glass slide, petridish, volumetric flask, sieves were used.

METHODS

The composition for Formulation A and B are depicted in table 1

Table 1
Formula for ointment formulation

Sr. no	Name of ingredients	Formulation code	
		A	B
		% W/W of Formulation	
1	Clotrimazole	0.5 gm	0.5 gm
2	Ichthammol	0.20 gm	0.20 gm
3	Boric acid	1.0 gm	1.0 gm
4	Zinc oxide	3.0 gm	3.0 gm
5	Menthol	1.0 gm	1.0 gm
6	Hard paraffin	----	1.5 gm
7	Microcrystalline wax	----	2.5 gm
8	Light liquid paraffin	----	26 gm
9	White soft paraffin	25 gm	----
10	Cetostearyl alcohol	20 gm	15 gm
11	Sodium lauryl sulphate	1.0 gm	1.0 gm
12	Methyl paraben	0.1 gm	0.1 gm
13	Propyl paraben	0.1 gm	0.1 gm
14	Triethanolamine	0.5 gm	0.5 gm
15	Propylene glycol	10 gm	10 gm
16	Purified water	q.s.	q.s.

FORMULATION CODE A

Water phase was prepared by heating Zinc oxide, boric oxide sodium lauryl sulphate, methyl paraben, and triethanolamine in a vessel till temperature attains 75⁰ C to 80⁰C. Oil phase was prepared by heating soft paraffin and cetostearyl alcohol in a stainless steel vessel till temperature of oil phase attains 75⁰ C to 80⁰ C. Both water phase and oil phase were mixed by passing them through 40# and 150# double cone sandwich stainless steel filter respectively into ointment manufacturing vessel under vaccum. The mass was stirred and cooled for 1.5 hours. Active ingredients like propylene glycol, menthol, clotrimazole and ichtammol were made into homogenous slurry by stirring it for 30 minutes.

The slurry was transferred to ointment manufacturing vessel and homogenization was continued for 1.5 hours. Then it was cooled and again stirred till ointment is obtained. Temperature was maintained to 35⁰ C to 37⁰C.

FORMULATION CODE B

The procedure for preparing water phase preparation, water and oil phase mixing, active material is same as formulation A except oil phase preparation.

OIL PHASE PREPARATION

The required quantities of hard paraffin, microcrystalline wax, cetostearyl alcohol, light liquid paraffin were added in stainless steel vessel. This was considered as oil phase. The oil phase was heated till the temperature of oil phase attains 75° C to 80° C.

EVALUATION PARAMETERS

Description

1 gm of ointment was taken to a clean and dry petridish and observed visually.

Identification: - The ointment was subjected to identification tests for active ingredients. 10 gm of ointment equivalent to 50mg was taken in separating funnel. Water was added and extracted with chloroform and anhydrous sodium sulphate. The layer was collected. The residue obtained was used for the identification tests of clotrimazole and ichthammol.

a) Clotrimazole : The residue was diluted with methanol and methyl orange solution was added. Yellow colour was developed⁷.

b) Ichthammol : The residue was treated with 2M hydrochloric acid. The gas evolved turns lead acetate paper black. Solution was filtered. Residue was taken in water and 2M hydrochloric acid and barium chloride solution was added. A white precipitate was produced.

c) Menthol : To weighed quantity of ointment little quantity of sulphuric acid and vanillin

was added. Orange yellow colour was produced.

d) Zinc oxide : The residue obtained shows yellow colour when hot and white color when cool.

E) Boric acid : To the weighed quantity of ointment methanol and few drops of sulphuric acid were added. It was warmed, shaken and ignited. The flame shows green border⁷.

EVALUATION OF OINTMENT

1. Uniformity of weight

Ten tubes were filled randomly and weighed. Ointment was removed from each tube and each empty tube was washed with methanol. The empty tubes were dried and their weight was taken. The difference between two weights was calculated as net weight of the ointment of tube. The average of net weight of ointment of ten tubes was noted.

2. Globule diameter : The average globule diameter was calculated with help of microscope.

3. pH: The pH of ointment solution was measured with the help pH meter.

4. Loss on drying : Loss on drying was determined by placing ointment in petridish on water bath and dried for 105°C.⁸.

$$\text{Percentage loss on drying} = \frac{100 \times (Wt - MW)}{Wt}$$

5. Spreadability : Spreadability of the formulation was determined by an apparatus suggested by Muttimer *et al.*, it consist of a wooden block having a pulley at one end with fixed glass slide on block. An excess of ointment (3gm) placed on ground plate. The ointment was sandwiched between this plate and another glass plate having the dimension of fixed ground plate and provided with the hook. A 1kg

weight was placed on the top of the two plates for 5 minute to expel air and to provide a uniform film of the ointment between the plates. Excess of ointment was scrapped off from the edges. The top plate was then subjected to pull of 240gms. With the help of string attached to the hook and time required by the top plate to cover a distance of 10cm. was noted. A shorter interval

indicates better spreadability⁹. Spreadability is measured as $S = m \times l/t$

6. Consistency or hardness of ointment : It was measured by Penetrometer. Three containers were filled carefully and completely, without forming air bubbles and stored at $25 \pm 0.5^\circ\text{C}$ for 24 hrs. Three samples were stored at $25 \pm 0.5^\circ\text{C}$ and with shear for 5min. Three samples were melted and carefully and completely filled three containers, without forming air bubbles stored at $25 \pm 0.5^\circ\text{C}$ for 24 hrs. Test samples were placed on Penetrometer. Temperature of penetrating object was adjusted at $25 \pm 0.5^\circ\text{C}$ and position was also adjusted such that its tip just touches the surface of sample. Penetrating object was released for 5sec. Depth of penetration was measured. Same was repeated with remaining containers¹⁰.

7. Viscosity of ointment : The viscosity was determined by CAP- 2000 Brookfield viscometer. Test sample was taken in a clean and dry 250 ml beaker and the viscosity of the test sample was determined by standard operating procedure of Viscometer by using spindle nos. 1 to 4. Each spindle was used for finding the viscosity of the sample at speeds of 0.3, 0.6, 1.5, 3, 6, 12, 30 and 60r.p.m. respectively. Their rheological characteristics were also tested at 25°C using Brookfield viscometer¹¹.

8. Microbiological studies : The antibacterial activity of various ointment formulations of clotrimazole and ichthammol against various strain of anaerobic and aerobic microorganism were evaluated by standard cup plate method and the inhibition zone diameters were measured with the help of zone reader. *Bacillus subtilis*, *staphylococcus aureus*, *Escherichia coli* (aerobic organism) and *bacterioids fragilis* (anaerobic organism) were used for testing of antibacterial activity. Nutrient agar media was used for aerobic bacterial culture and blood agar media was used for Bacterial fragilis and incubated at temperature $37^\circ\text{C} \pm 2^\circ\text{C}$ for 48 hrs¹².

9. Diffusion study : In modified kiescary chein diffusion cell, 2 gm of ointment was kept in donor

compartment. The entire surface of cellophane membrane was in contact with the receptor compartment containing 22 ml of phosphate buffer pH 7.4. The receptor compartment was continuously stirred (100rpm) using the magnetic stirrer. The temperature was maintained $37 \pm 1^\circ\text{C}$. The surface area available for diffusion was calculated and found to be 3.14 cm^2 . The study was carried out for 5 hrs and the sample was withdrawn at 30 minute time interval and same volume was replaced with free phosphate buffer. The content of clotrimazole and ichthammol from withdrawn sample was measured after suitable dilution. The experiment was carried out in triplicate and average values are reported.

Diffusibility gives the amount of ointment diffused with the body surface. When a drug system applied topically, the drug diffuses out of its vehicle onto the surface tissues of the skin. The transient diffusion that occurs shortly after the application of a substance to the surface of the skin is potentially far greater through the appendages than through the matrix of the stratum corneum. After steady state diffusion has been established, the dominant diffusion mode is probably no longer intra appendage, but occurs through the matrix of stratum corneum. Once a substance passes through the stratum corneum, there is apparently no significant further hindrance to penetration of the remaining epidermal layer and corneum. There is then a ready entry into the circulation via the capillaries.

The diffusibility experiment was carried out by preparing agar nutrient medium of any concentration. It was poured into petridish. A hole was made at the centre and ointment was placed in it. The time taken for the ointment to get diffused was noted.

10. Stability studies : The international conference on harmonization (ICH) harmonized tripartite guidelines on stability testing of new drug substance and product was issued on 27, 1993. The formulated clotrimazole and ichthammol ointment were filled in the collapsible tubes and stored at different temperature condition viz. $25^\circ\text{C} \pm 2^\circ\text{C}/60\% \text{ RH} \pm$

5%RH, 30°C± 2°C/65% RH± 5%RH, 40°C± 2°C/75% RH± 5%RH for a period of three months and studied for appearance, pH, extrudability, viscosity, spreadability and assay of the drug¹³.

The formulation code A and B were found to be in light brown in colour and mentholated odour. Both the formulations met required specification when identified for its active ingredients. The specifications are given in table 2.

RESULT AND DISCUSSION

Table 2

Identification of active ingredients for formulation A and B

Sr no	Identification test	Specification
1	Clotrimazole	Yellow colour produced.
2	Ichthammol	A white precipitate is produced.
3	Menthol	Violet colour is produced.
4	Zinc oxide	White colour produced.
5	Boric acid	Flame has green border.

All the parameters like uniformity of weight, pH, globule diameter, loss on drying, drug content were found to comply with standard. The results are shown in table 3.

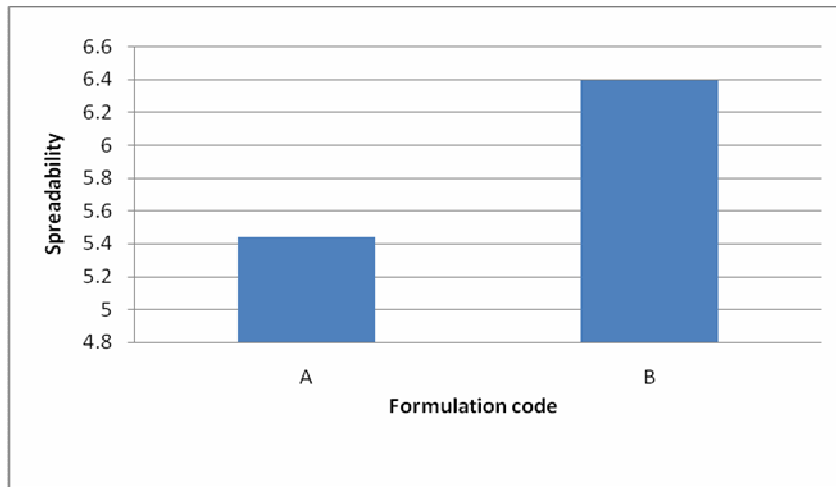
Table 3

Evaluation of different parameters clotrimazole and ichthammol ointment

Evaluation parameters	Formulation code A	Formulation code B
Description	Colour-light brown Odour- mentholated	Colour-light brown Odour-mentholated, soft semisolid
Uniformity of weight	Comply with standard	Comply with standard
Globule diameter	4.28mm	4.42mm
pH (10%w/v solution)	5.8	6.7
Loss on drying	37%w/w	41%w/w
Hardness or consistency	157mm	205mm
Viscosity	209cps	182cps
Spreadability	5.44cm.gm/sec	6.4cm.gm/sec

The consistency of formulation code B was better (205mm) than formulation code A (157mm). The formulation code A was found to be more viscous (209cps) than formulation B (182cps). Because of this, the formulation code B has good spreadability character (6.4cm.gm/sec) than formulation code A (5.44cm.gm/sec) and it is graphically shown in graph 1.

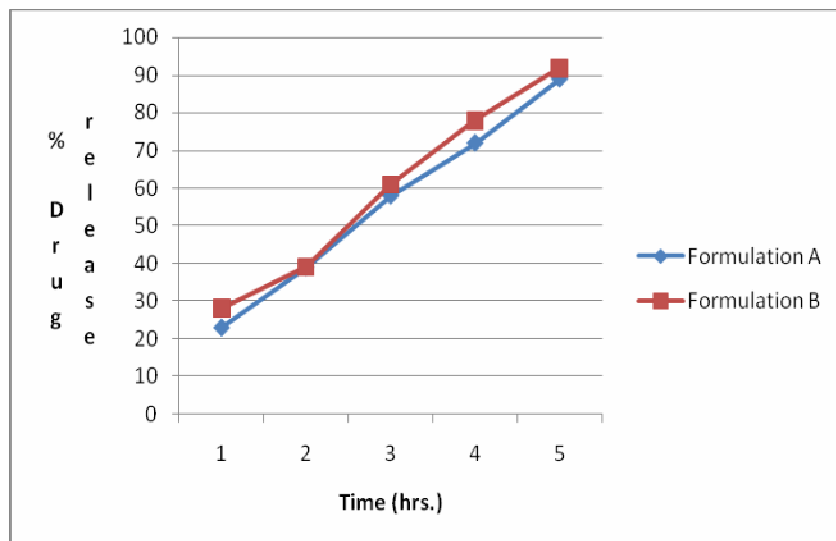
Graph 1 Spreadability of formulation A and B



The amount of ointment diffused with body surface and percentage drug release was determined. From the results it was found that formulation code B has diffusion power in particular interval of time diffusing 0.7cm length after 60 min than formulation code A which diffuse only 0.5 cm after 60 min and drug release for formulation

B after 5th hour was found to be 92% and drug release for formulation A after 5th hour was found to be 89% .The results are depicted in graph 2.

Graph 2 Diffusion study of formulation A and B



The antimicrobial activity of clotrimazole and ichthammol in formulation code A and code B were determined. The zones of inhibition of various strains of aerobic and anaerobic organisms are

depicted in table 4. The zone of inhibition for formulation code B was found to be nearer to standard than code A.

Table 4
Antimicrobial activity of clotrimazole and ichthammol ointment.

Formulation code	Diameter of Zone of inhibition (mm)			
	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Bacteroid fragilis</i>
A	31.58	31.35	31.33	30.17
B	36.40	34.22	32.19	35.14
Standard	38.24	35.58	34.08	37.32

The developed ointment formulations were subjected to stability study as per ICH guidelines for the period of 3 weeks i.e. 25° C/ 60 %RH, 30 °C/ 65%RH and 40° C/ 75 %RH. The results are discussed in table 5.

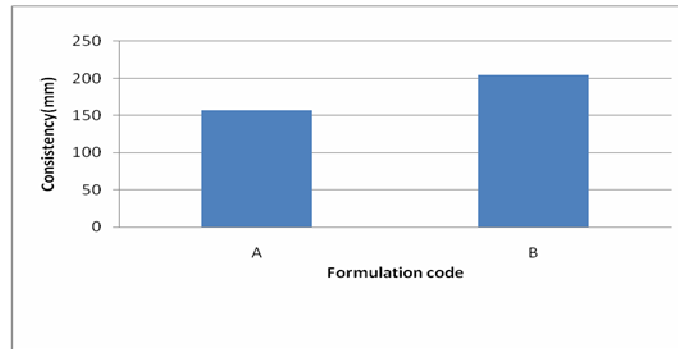
Table 5 Stability parameters

Parameters (after 3 rd week)	Formulation A			Formulation B		
	25 ^o C	30 ^o C	40 ^o C	25 ^o C	30 ^o C	40 ^o C
pH	5.7	5.7	5.5	6.6	6.5	6.4
Spreadability	5.3	5.8	6.7	6.4	6.7	7.6
Consistency	156	154	151	204	203	201
Globule diameter(mm)	4.24	4.22	4.15	4.39	4.36	4.26

From the data it is clearly evident all the physicochemical characteristics of both the formulation were found satisfactory and there were no significance changes in evaluation parameters. The formulation code B containing hard paraffin, microcrystalline wax, cetostearyl alcohol, light liquid paraffin was found to be more satisfactory than formulation code A. From all above results it is concluded that the formulation code B exhibited stable and good physical properties like consistency and viscosity and it is shown in Graph 2 and 3 respectively

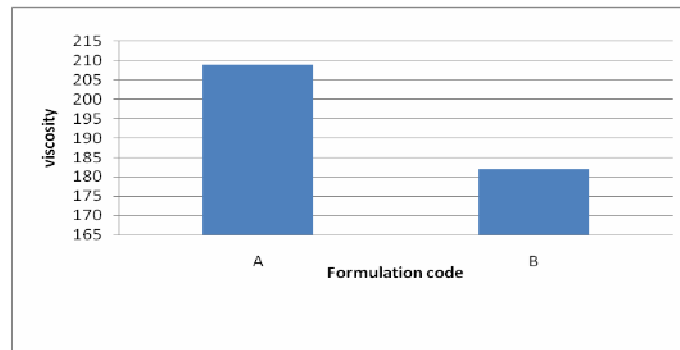
Graph 2

Consistency of formulation A and B



Graph 3

Comparison of viscosity of formulation A and B

**CONCLUSION:**

A combinational therapy is the need of hour to treat eczema and pruritis. This can be achieved by clotrimazole and ichthammol (an antifungal and antiseptic). In this study, ointment was formulated with different bases like white soft paraffin, cetostearyl alcohol hard paraffin, light liquid paraffin and microcrystalline wax. From the study, it can be

concluded that formulation containing hard paraffin, cetostearyl alcohol, Light liquid paraffin and microcrystalline wax was better than formulation with white soft paraffin and cetostearyl alcohol alone. By combining these drugs with appropriate ointment bases (as formulation B) a better therapy and patient compliance can be attained.

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