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ADVANCED INNOVATIVE HYDROGEL OF AMPHOTERICIN B WITH CONJUGATED ALOE VERA GEL FOR FUNGAL INFECTION

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ABSTRACT
The present work is focused on the development and design of novel Amphotericin-B conjugated along with aloe Vera as Hydrogels to enhance the anti-fungal activity. The Amphotericin -B conjugated Hydrogels are highly soluble in different aqueous solutions because of forming network with biocompatible and biodegradable excipients. The antifungal effect of Am B-conjugated hydrogels significantly exhibits the antifungal activity. The results of the present study indicated that the Am B- conjugated hydrogels are suitable carriers for poorly water-soluble drugs and for enhancement of therapeutic efficacy of antifungal drugs. The effect of aloe Vera gel on appearance, viscosity spreadability, extrudability, drug content uniformity, The Drug content of F3 was found to be 95.38%. *In-vitro* drug diffusion study of Amphotericin B was investigated. At the end of 7 hrs, Drug released 85.66% when compared with all the other 3 formulations which reveal that increase in the gel concentration will prolong the release of drug which is a favourable condition for fungal infections.

KEYWORDS: Amphotericin B, Hydrogel, Aloe Vera, Anti fungal activity, *In-vitro* release.

INTRODUCTION
Topical delivery is used for many types of category via, skin like as i.e. anti-fungal, non-steroidal anti-inflammatory drugs, antiviral, anti-acne, etc. Human skin is well-organized membrane i.e. Stratum corneum, the outermost layer of epidermis is formed by dead and keratinized cells. And it is an excellent barrier to penetration of drugs through the skin.

Topical delivery administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal, and skin as topical routes. Efforts to cure diseases have been leading in the discovery of various drugs, medicine and delivery systems. Route of administration depends on type and severity of disease. Topical route is most preferred for skin disorders.[1]

Advantages
• Controlled delivery resulting in more reliable and predictable blood levels.
• Avoid GIT absorption problems for drugs.
• Self-medication is possible.
• Reduces frequency of dosing.
• Topical patches over a controlled, steady delivery of medication over long periods of time.
• Topical patches have fewer side effects than oral medications or supplements.
• Topical patches are easier to use and remember.
• Topical patches over an alternative to people who cannot, or prefer not to take medications or supplements orally.
• Topical patches are cost-effective.
• People prefer topical patches.

Disadvantages
• Daily dose of more than 10mg is not possible.
• Barrier function changes from person to person and within the same person. Heat, cold, sweating prevents the patch from sticking to the surface of the skin for more than one day.
• TDS cannot deliver ionic drugs.
• TDS cannot achieve high drug levels in blood/plasma.
• It cannot develop for drugs of large molecular size.
• TDS cannot deliver drugs in a pulsatile fashion.
• TDS cannot develop if drug or formulation causes irritation to skin.[2]

Liposomes
Liposomal hydrogels have advantage over other conventional formulation such as creams, ointments and gels. They enhance the skin retention of drugs, a higher drug concentrations in the skin and at the same time slow

down the systemic absorption of drugs. They also act as a drug depot and provide a sustained localized drug delivery and liposomal hydrogels deliver adequate amount of drugs for their therapeutic activity.[3] Liposomes are vesicles made up of phospholipid bilayers. These phospholipid bilayers surround an aqueous core. Liposomal size is directly related to the method of preparation and can range from 50 nm to several microns. They form spontaneously when these lipids are dispersed in aqueous media. Vesicles can be constructed full of natural constituents such that the vesicle membrane forms a bilayer structure which is principal identical to the lipid portion of natural cell membrane. Vesicles can be composed even of entirely artificial components, chosen for their improved chemical properties. Moreover, liposomes may entrap both hydrophilic and lipophilic molecules; and be used as drug carrier for both types of drug molecules.[4]

Hydrogel

The term hydrogel describes as a three-dimensional cross linked polymeric network obtained from synthetic or natural polymers which has the capacity to hold water within its porous structure. The water holding capacity of the hydrogels arise mainly due to the presence of hydrophilic groups, viz. amino, carboxyl and hydroxyl groups, in the polymer chains. These polymeric materials do not dissolve in water at physiological temperature and pH but swell considerably in an aqueous medium.[5] Hydrogels have been widely used as a drug carrier due to its ease in manufacturing and self-application in clinical and fundamental applications. Applications of hydrogels in the biomedical field include contact lenses, artificial corneas, wound dressing and coating for sutures, catheters, and electrode sensors.[6]

Fungal infections

Fungal infections are termed mycoses and can be divided into superficial infections (affecting skin, nails, hairs or mucous membranes) and systemic infections (affecting deeper tissues and organs). Studies have shown that there is an increase in systemic fungal infections, not only by known pathogenic fungi but also by fungi previously thought to be innocuous. These last are termed opportunistic infections. The commonest systemic fungal infection is systemic candidiasis, blastomycos, histoplasmosis, coccidiomycosis and paracoccidiomycosis.[7] Topical application of drug helps in delivering drug directly to the site of action. Local infection can be treated by the formation of transparent, water vapours and air permeable film over the skin surfaces by the application of formulations like gels, from which drug releases continuously to the skin site and the disease of the patient would be treated. Antifungal therapy is clearly beneficial for treatment of infections as well as preventing disease progression. For skin therapy and soft tissue infections topical application of antifungal agent is useful tool. It has several potential merits compared with systemic therapy.[8]

MATERIALS AND METHODS

Material

Amphotericin B was procured from Bharath serums and vaccines Ltd, Mumbai, DMSO was procured from Thomas baker, Mumbai, Formaldehyde was procured from Thomas baker, Mumbai. Triethanolamine was procured from Merk speacialities, pvt. Ltd. worli, Mumbai Ethanol was procured from Changshu hongsneng fine chemical Co.ltd, Polyethylene glycol was procured from Oxford lab fine chem., Mumbai, Glycerine was procured from Thomas baker, Mumbai, Methyl paraben was procured from Loba chemicals, colaba, Mumbai, HPMC was procured from HI MEDIA Bangalore, Karnataka. All the chemicals and reagents used were of analytical grade.

Aloe Vera extract

Aloe Vera is prepared by collecting the succulent leaves and washed with water and mild chlorine solution to remove organic matter and finally cut transversely into pieces and upper green colour layer is removed. The inside thick mucilaginous epidermal jelly is collected with a spoon, minced and passed through Muslin cloth to get uniformity of the gel or homogenized in a mixer. It is stored in air tight container by adding preservative. And the collected gel is evaluated for various properties.

METHOD OF PREPARATION

The hydrogel of Amphotericin B was prepared by hydration method.

Weighed amount of drug and polyethylene glycol is dissolved in beaker 1 and Add weighed amount of formaldehyde, DMSO and ethanol in beaker 2 both the beakers are mixed together up to 80°C and cooled By continuous stirring HPMC is added gradually. The aloe Vera gel and glycerine is added as an emollient. The required amount of water is added, triethonol amine is added drop wise and lastly methylparaben is added as a preservative. The composition of Amphotericin B hydrogel was given in Table 1.

Table 1: composition of Amphotericin B hydrogel.

SI.NO	INGREDIENTS	F1	F2	F3	F4
1	Amphotericin B(mg)	10	10	10	10
2	Aloevera gel(ml)	10	10	10	10
3	HPMC(mg)	0.25	0.50	0.75	1.0
4	DMSO(ml)	1	3	5	7
5	Formaldehyde (ml)	2	2.5	3	3.5
6	Ethanol(ml)	4	6	8	10
7	Polyethylene glycol(ml)	1	1	1	1

8	Triethanol amine(ml)	0.1	0.1	0.1	0.1
9	Glycerine(ml)	1	2	3	4
10	Methyl paraben(mg)	0.2	0.2	0.2	0.2
11	Water	q.s	q.s	q.s	q.s

Characterization of hydrogel

The formulated gels were examined for their physical properties, rheological properties and antifungal activity. Skin irritation test was carried out only on all formulations.

Homogeneity

The gels were examined for their physical properties like colour, clarity and phase separation by visual inspection. They are tested for the presence of any aggregates.[9]

pH measurement

The pH of gel formulations were determined by using digital pH meter. 1gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation is done in triplicate and average values are calculated and reported.[10]

Extrudability

The gel formulations were filled in collapsible tubes. After being set in the containers, the extrudability of gel formulations was determined in terms of weight required in grams to extrude 0.5 cm. Ribbon of gel in 10 sec.[11]

Drug content

1 g gel was dissolved in 100 ml of phosphate buffer pH 7.4. Suitable dilutions were made using phosphate buffer pH 7.4. Absorbance was measured at 378 λmax nm using UV spectrophotometer.[12]

Then the absorbance measured by UV spectrophotometer against blank at λ max and the drug content was calculated

Amount of drug =concentration from the standard graph × DF/1000

Where DF = dilution factor

Spreadability

To determine the spreadability of MBHs were transferred to the centre of a glass plate (10 cm x 10 cm) and this glass plate was compressed under another glass plate of the same size. Thus, the gel was spread out in between the plates. After one minute, the weight was removed and the diameter of the spread area (cm) was measured. The measurement was performed in triplicate.[13]

Viscosity

Viscosity of hydrogel based hydrogel was determined using a viscometer (Brookfield. USA) at 50 rpm with spindle # LV4 at room temperature.[14]

Centrifuge

The centrifugation test to find the stability of formulation by analysing the phase separation by using remi centrifuge instruments at 5000 rpm for 15min. formulation do not undergo phase separation were taken to next stability testing methods.[15]

Skin irritancy

Skin irritancy test was conducted two healthy mice.1grm of gel was applied on area 2cm and observed for any lesions/redness.[16]

***In-vitro* drug diffusion study**

In-vitro drug release studies were carried out using Franz diffusion cell. 0.5 g of gel was applied on cellophane membrane as donor compartment. Phosphate buffer pH 7.4 was placed in the receptor compartment as the dissolution medium. The whole assembly was place on magnetic stirrer with thermostat maintained at 37°c. Samples were collected regular time interval and sink conditions were maintained by replacing with new buffer solution. Collected samples are analysed at 408 λ_{max} nm using UV spectrophotometer.[17]

RESULT AND DISCUSSION

Table No 2 shows the physical and chemical state for formula.

Table 2: physical and chemical appearance of Amphotericin B.

Sl.No	Properties	Observed
1)	colour	Deep yellow prisms or needles.
2)	appearance	Crystalline powder
3)	taste	Bitter taste of the bile salt.
4)	odour	Odourless
5)	Melting point	>170°C (dec)
6)	Identification of λ_{max}	415nm
7)	Solubility	soluble in DMSO and dimethyformamide

Table no. 3: Homogeneity, Extrudability, Spreadability pH, viscosity, and Drug content of Amphotericin B hydrogel.

Formulation	Homogeneity	Extrudability grade	Spreadability (g.cm/sec ±SD)*	pH	Viscosity(cps)	Drug content (%±SD)*
F1	Poor	+	28.52	6.1	94.16	95.16
F2	Good	++	46.61	6.4	95.22	95.22
F3	Excellent	++++	233.3	6.7	97.20	95.38
F4	Good	+++	116.6	6.3	95.30	95.3

Homogeneity

All the formulated hydrogels were tested for homogeneity by visual inspection by setting in the container for their appearance and presence of any aggregate and all the formulations were found to be in homogeneous in nature and the results were tabulated in table no: 3.

Extrudability

The extrudability test was performed and the quantity of gel extruded were weighed from each from each formulation and % of gel extruded was calculated grades were allotted and it was found to be all the formulations F3 & F4 exhibiting good extrudability when compared with other formulations and the results were tabulated in table no:3.

Spreadability

The spreadability of each formulation was determined and it is found within the range of 46.61 to 233.3 gm/sec. (table no 3).

pH

The pH of amphotericin B hydrogel was found within the range of pH of skin and would not cause any irritation to skin. Thus prepared amphotericin B hydrogel formulations are suitable for topical application. The pH was found between 6-7. (Table no 3).

Viscosity

Viscosity measurement of all the formulation revealed optimum consistency and the results are shown in table no.3. The viscosity was found to be in the range of 95.16-95.30 mPas.

Drug Content

Uniformity content 0f Amphotericin B Hydrogel was confirmed to assure uniformity in dosages. The results were reported in table. The drug content of all the formulation was found between 95.05 to 95.25%.(table no 3)

Skin irritancy

Figure 1: Skin irritancy test on mice.

Skin irritancy was conducted and observed that the optimum formulation 3. Do not show any lesions or irritation/ redness.

***In vitro* drug release**

Table no. 4: *In vitro* drug release profile of Amphotericin B hydrogel.

Time (hr)	%CDR			
	F1	F2	F3	F4
0	0	0	0	0
1	6.58	10.54	9.88	11.85
2	13.17	21.08	19.76	23.71
3	25.03	35.57	36.89	31.62
4	32.94	40.84	47.43	39.53
5	44.80	48.75	67.20	46.12
6	46.12	63.25	80.38	60.62
7	63.25	79.07	85.66	81.70

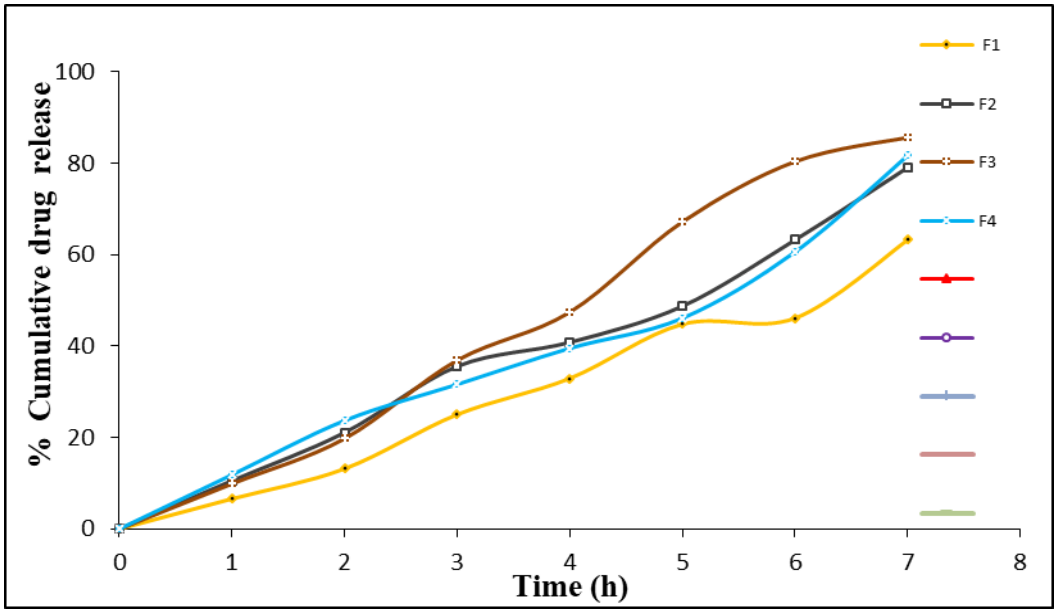

Figure 2: *In vitro* drug release of Amphotericin B hydrogel formulations.

Drug diffusion rate from different gel formulations with different polymer ratios were studied by Franz diffusion cell using cellophane membrane as a barrier maintained at 37±2°C. The percentage of the drug released after 7 hours formulation F3 was showing 85.66% of drug release when compared with F1(63.25%), F2 (79.07%), F4 (81.70%). And the values are tabulated in table no: 4.

CONCLUSION

Amphotericin B is used to treat fungal diseases. The hydrogel of Amphotericin B was formulated to improve the topical absorption of the drug. In the current work, an attempt was made to formulate and evaluate hydrogel for topical delivery using Amphotericin B, Aloe vera gel, polyethylene glycol, HPMC, DMSO, ethanol, Triethanolamine, glycerine, methyl paraben. The hydrogel were prepared by hydration method. The ratio of surfactant and co-surfactant were Selected. Four different batches of hydrogel were prepared using different concentrations of surfactants and co-surfactants. The hydrogel formulations were characterized for physical evaluation, viscosity, pH, centrifuge, drug content, Spreadability, skin irritancy, *in vitro* drug release, the drug content of F3 formulation was found to be 95.38%. The *in- vitro* drug release from the F3 (hydrogel) formulation was found to 85.66% at the end of 7hrs. From the above studies it was concluded that the formulation can be used as possible alternative to traditional topical formulations of amphotericin B hydrogel for the therapy of wound healing, with controlled drug delivery.

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