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Novel Innovative Strategy of Hydriogels of Amphotericin –B Conjugated Aloevera 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 aloevera 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 AmB-conjugated hydrogels significantly exhibits the antifungal activity. The results of the present study indicated that the AmB-conjugated hydrogels are suitable carriers for poorly water soluble drugs and for enhancement of therapeutic efficacy of antifungal drugs. The effect of aloevera gel on, appearance, viscosity, spredability, extrudability, drug content uniformity, in-vitro drug diffusion study of Amphotericin B were investigated. At the end of 4 hrs, F4 released 75.23% when compared with all the other 3 formulations which reveals that increase in the gel concentration will prolong the release of drug which is a favourable condition for wound healing.

KEYWORDS: Amphotericin B, Antifungal activity, Aloevera, Therapeutic efficacy, Hydrogels

I. INTRODUCTION

Hydrogels are defined as two or multiple component systems which consisting of three dimensional network of polymer chains and water that fills the space between macromolecules. Depends upon properties of the polymer used, as well as on the basis of nature and density of the network joints, such structures in an equilibrium can contain various amounts of water; typically in the swollen state, the mass fraction of water in a hydrogel is much higher than the mass fraction of polymer. In practice, to achieve high degrees of swelling index of polymer, it is common to use synthetic polymers that are water-soluble when in non-cross-linked form.

Hydrogels of natural polymers, especially polysaccharides, have been used recently because of their unique advantages. Polysaccharides are, in general, non-toxic, biodegradable and abundant. Natural polymers are biocompatible and enhance the drug release efficacy with reduced toxicity and improved patient compliance with *in vivo* degradation at a well-defined rate.

Amphotericin is a macrocyclic, polyene, anti-fungal antibiotic. Amphotericin B is used intravenously for serious systemic fungal infections.

Aloe Vera belonging to liliaceae family is a perennial succulent plant. This plant has been known as “the healing plant”. The aloe Vera has been used for traditional medical purposes in several cultures for millennia, it has been demonstrated that aloe Vera has growth promoting activities.

So an attempt was made to formulate amphotericin hydrogel based topical dosage form for wound healing using a natural gelling agent aloe Vera.

A. RELATED WORK

KirtKumari*et al.*, (2013), Developed Mometasone furoate hydrogel, a potent corticosteroid used in the treatment of skin disorders like scalp dermatitis. Hydrogels were prepared using various polymers like carbopol-940, HPC, MC, polaxamer-407, sodium alginate, HPMC along with 0.1% (w/w) Mometasonefuroate. The formulated gel was evaluated for drug content, pH, viscosity, spreadability and in vitro drug release. Comparison of data obtained from successful gel formulation with marketed product. Spreadability of Carbopol gel containing Mometasonefuroate (C1) was 26.76g.cm/sec as compared to 21.61g.cm/sec of marketed gel, indicating good spreadability of the prepared gel

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(C1). The percent drug release was 74.58 and 73.50 from C1 and marketed gel respectively. Stability studies under room temperature showed satisfactory results.

Anisha Singh*et al.*, *(2015)* explained thatPolymers play a vital role in pharmaceutical development. Efforts have been continuously made to search a polymer that act in a controlled and desired way. Hydrogel development has solved many such issues. This article deals with the fundamental and some recent advances made in the fabrication and design criteria of hydrogel based drug delivery.

OjhaKyathi*et al.*, (2013) Studied controlled release hydrogel using natural polymers like aloevera and starch. Different formulations of Ascorbic acid hydrogels were formulated using aloevera gel powder as a drug carrier and a nutrient fortifying excipient starch in different concentrations. Formulated hydrogels were evaluated for different parameters like swelling studies and in-vitro drug release. Maximum swelling % was seen at pH 1.4 and least in distilled water. appreciable swelling was seen at pH 5.4 to 7.4. The in vitro release studies showed that the drug was released at a pre-determined rate over a controlled period of time hence it can be used in sustained drug delivery. The materials used in this study are bio available and biocompatible hence will not impart any toxicity and side-effects.

II. MATERIALS AND METHODOLOGY

A. DRUGS AND CHEMICALS

Amphotericin B was purchased from Bharath serums and vaccines Ltd, Mumbai and other chemicals used were poly ethylene glycol (fisher scientific Ltd), glycerine (fisher scientific Ltd), methyl paraben (oxford laboratory, Mumbai), formaldehyde (SDFC chemicals Ltd, Mumbai), dimethyl sulfoxide (SDFC chemicals Ltd).

B.METHODOLOGY

Aloe vera gel is prepared by collecting the thick 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 gelly is collected with a spoon , minced and passed through musclin cloth to get uniformity of the gel or homogenized in a mixer. It is stored in a air tight container by adding preservative. And the collected gel is evaluated for various properties.

C. FORMULATION DESIGN:

S.no	Ingredients	F1	F2	F3	F4
1	Aloevera gel (ml)	5	10	15	20
2	Amphotericin B (mg)	5	10	15	20
3	DMSO (ml)	1.5	2.5	3.75	5
4	Formaldehyde (ml)	1	2	2.5	3
5	Ethanol (ml)	2	4	6	8
6	Poly ethylene glycol (ml)	1	1	1	1
7	Glycerine (ml)	1	1	1	1
8	Methyl paraben (mg)	0.2	0.2	0.2	0.2

Table 1: Formulation design

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III. RESULTS

A. CALIBRATION CURVE OF AMPHOTERICIN B :

Concentration(µg/ml)	Absorbance
5	0.037
10	0.111
15	0.163
20	0.228
25	0.282

Table 2: Calibration curve of Amphotericin B in phosphate buffer pH 7.4 (λ_{max}=416nm)

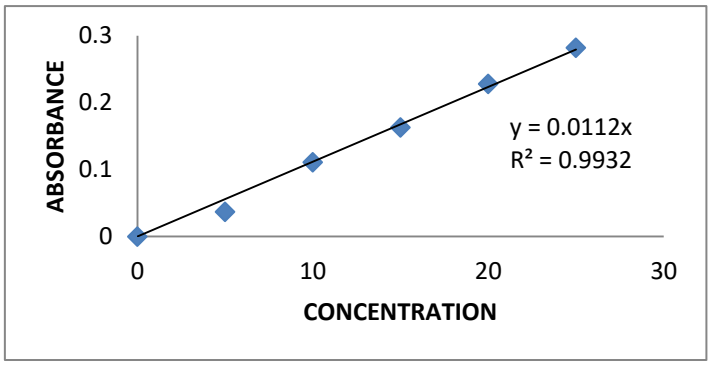

Fig 1: calibration curve in phosphate buffer of amphotericin B

B. COMPATABILITY STUDIES:

An I.R study was carried out to check the compatibility between the selected excipient and Amphotericin. The spectra obtained for I.R studies at wavelength from 4000 cm^{-1} to 400 cm^{-1}

After interpretation through the above spectra it was confirmed that there are no major shifting as well as no loss of functional peaks between the spectra of drug, gel. From the I.R studies it was concluded that, the selected excipient are compatible with the selected drug amphotericin B.

IV. EVALUATION OF HYDROGELS

A. PHYSICAL EVALUATION

All the prepared Hydrogel formulations were physically evaluated found to be translucent in nature with ethanolic odour, smooth feel on application, and the results were tabulated in table no: 9

Formulation	Physical appearence	Feel on application
F1	Opaque	Smooth
F2	Transculent	Smooth
F3	Light yellowish colour	Smooth
F4	Light yellowish colour	smooth

Table 3: physical evaluation

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B. 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:4

Formulation	**Homogeneity**
F1	Poor
F2	Good
F3	Good
F4	good

Table 4: homogeneity

C. SPREADABILITY

The spreadability test was performed with the mentioned method All the prepared Hydrogels using different concentrations were spreadable on the skin surface. The addition of aloevera gel instead of synthetic gelling agent to all the prepared formulae improved the physical characteristics concerning spreadability, consistency and skin feel. Also, its addition helped the dissolution of the drug and prevented its precipitation upon storage and it revealed that increasing the concentration of any of the gelling agents was always associated with a decrease in the spreadability and results were tabulated in table no:6

Formulation	Spreadability (g.cm/sec)
F1	25.09
F2	21.61
F3	19.15
F4	15.51

Table 5: preadability of the formulation

D. 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:6

formulation	Extrudability grades
F1	+
F2	++
F3	+++
F4	+++

Table 6: Extrudability of formulation

E. VISCOSITY

Viscosity of the prepared hydrogel formulation were determined using brooke field viscometer using spindle S-06 and it was found to be increase in the polymer concentration results in increase in viscosity and the results were tabulated in table no:7

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Formulation	Viscosity (cps)
F1	16600
F2	19950
F3	25870
F4	33800

Table 7: Viscosity of the formulation

F. DRUG CONTENT

The drug content was estimated by the mentioned method and it was found to be that Amphotericin B drug content is within the permissible limits (>97%). This indicates that the drug was uniformly distributed throughout the formulations and the results were tabulated in the table no: 8

formulation	Drug content (%)
F1	97
F2	97.48
F3	98.52
F4	99.83

Table 8: Drug content of the formulation

G. pH

The pH of the different hydrogel formulations was found out using pH meter and pH of gel was determined after diluting and dispersing it in distilled water (10% w/v). all the developed formulations was in accordance with that of human skin pH enduring them more acceptable to avoid the risk of irritation upon application and the results were tabulated in table no:9

Formulation	**pH**
F1	6.57
F2	6.49
F3	6.81
F4	6.92

Table 9: $_{p}$H of the formulation

H. *IN-VITRO* DRUG DIFFUSION STUDY:

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. Diffusion cell was assembled on magnetic stirrer along with diffusion membrane, which separates donor and receptor compartments. Gel (2g) was kept on membrane in donor compartment. The contents were stirred using magnetic stirrer at 50 rpm and aliquots each of 5 ml were withdrawn from the release medium at time intervals of 10, 20, 30, 60, 90, 120, 180, 240 minutes. Withdrawn samples were replaced by equal volumes of same fresh medium. Absorbance of these samples was measured spectrophot-ometrically 416 nm by UV-Visible spectrophotometer. And it was found to be that at the end of 240 min formulation F4 was showing 75.23% of drug release when compared with F1(89.12%), F2 (85.31%), F3 (79.27%). It shows that as the concentration of the polymer increases the release of drug is delayed form the hydrogel which is a favourable condition for wound healing purposes. And the values are tabulated in table no:10

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Cumulative % of drug release from formulations

Time(mins)	Cumulative % of drug release from hydrogel formulations (%)			
	F1	F2	F3	F4
0	0	0	0	0
30	10.31	8.42	6.21	4.23
60	19.42	16.89	12.56	10.78
90	35.21	30.76	25.78	22.31
120	48.90	45.34	39.02	37.83
150	56.21	50.21	48.92	43.12
180	71.23	64.78	61.23	54.71
210	79.68	72.31	71.45	61.23
240	89.21	85.31	79.27	75.23

Table 10: cumulative % of drug release from formulations

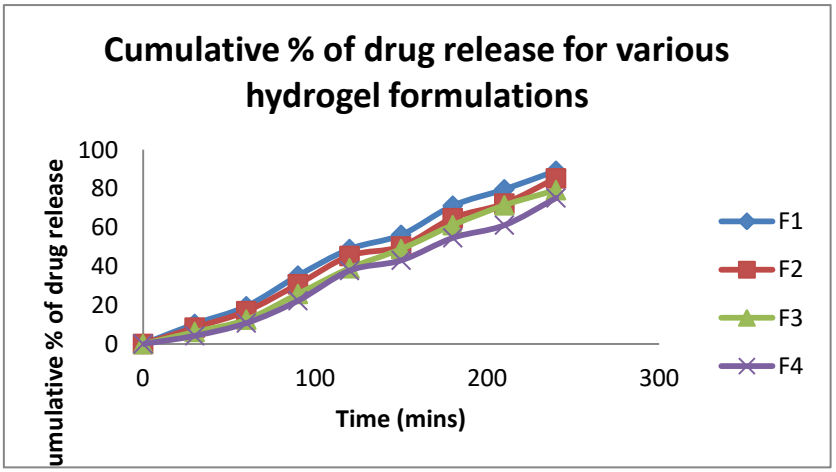

Fig 2: Cumulative % of drug release for various hydrogel formulations

V. CONCLUSION

In present work, aloevera gel has been evaluated as a as a gelling agent to develop Amphotericin B hydrogel. The effect of aloevera gel on, appearance, viscosity, spredability, extrudability, drug content uniformity, in-vitro drug diffusion study of Amphotericin B were investigated. Amphotericin B was used as the model drug for the present work because of its topical wound healing activity.

The compatability studies were performed by using FTIR. The spectra of pure drug, pure polymer were examined. The study revealed the absence of significant interactions between drug and polymer.

Different hydrogel formulations were prepared using different concentrations of Aloevera gel with Amphotericin B and all the formulations were evaluated.

The result of present study showed the effects of different concentration of aloevera gel on physical and cumulative % of drug release. At the end of 4 hrs, F4 released 75.23% when compared with all the other 3 formulations which reveals that increase in the gel concentration will prolong the release of drug which is a favourable condition for wound healing.

The evaluation of hydrogel revealed the gelling capacity of aloevera gel. Therefore it is concluded that aloevera gel can also be used as gelling agent to produce hydrogels with good physical properties and drug release profiles.

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