An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

FORMULATION AND EVALUATION OF EMUGEL OF AMPHOTERICIN-B ANTIFUNGAL AGENT

Maryann C Uzodinma* and Jesindha Beyatricks

Hillside College of Pharmacy and Research Centre Bangalore, Karnataka, India

*Corresponding author E-mail**: jolyjesi@gmail.com**

ARTICLE INFO

Key Words

Amphotericin B, Sodium CMC; FTIR; Clove Oil; Oleic acid; *In vitro* drug release; Stability; Spread ability

Access this article online

Website: https://www.jgtps.com/

Quick Response Code:

ABSTRACT

Amphotericin B belongs to the class IV of BCS (biopharmaceutical classification system) which has low solubility and low permeability. It was found to be only active by topical application and inactive by oral and intraperitoneal routes of administration. One of the approaches to increase penetrability of the drug thereby without compromising the efficacy is through development of emulgel for topical delivery since Amphotericin B is hydrophobic in nature and emulgel is used to deliver hydrophobic drugs into the skin. **Aim:** The main aim of this research work was to develop and characterize the Amphotericin B emulgels for topical delivery. The effect of various gelling agents and permeation enhancers were also studied. **Methodology:** Oleic acid and Clove oil were used as penetration enhancers. The effects of these enhancers on diffusion of tolnaftate were tested using semi permeable membrane using a 3^2 factorial design. Evaluation of the tolnaftate emulgel was carried for physical appearance, pH values, spread ability, viscosity, drug content determination, in-vitro release study, accelerated stability studies, etc. The results from the FTIR studies showed no incompatibility between the drug-polymer. All the prepared formulations FC1 to FC13 and FO1 to FO13 showed good stabilities, spread ability and in-vitro drug release which complied with the official limits. Factorial design studies showed that the best formulation with clove oil as permeation enhancer gave 75.10% drug release and when oleic acid was used as permeation enhancer gave 76.25% drug release. It could be concluded that prepared topical emulgel, enhanced permeation of tolnaftate and possessed an effective antifungal activity, with avoidance of GIT adverse effect. **Conclusion:** The Preformulating studies involving solubility and melting point of the drug were found to be comparable with the standard. The drug, Amphotericin B was checked for compatibility with selected polymers by FTIR and was found to be compatible. Formulation of emulgel were carried out using Sodium CMC as gelling agent. A 3^2 Factorial Design was carried out to optimize the formulation and to find out the effect of permeation enhancers namely clove oil and oleic acid in formulation. In the present study, 26 formulations of emulgels of Amphotericin B was prepared. The prepared topical emulgels of Amphotericin B were formulated and subjected to physicochemical studies that is; viscosity, spread ability, extrudability and in-vitro release studies. Drug content of all the formulation were found to be in the range of 86-104% and pH was found to be in the range 6-7 for all the formulations. All the formulations showed good spread ability and extrudability. The stability study as per ICH guidelines was performed for the optimized formulation FC8 and FO8 at intermediate testing of 30oC ± 2oC/65 ± 5% RH. No major change in appearance, pH and drug content was seen. Finally, it could be concluded that the prepared emulgel formulation was found to be an effective vehicle for the delivery of the drug. Further studies should be carried out to prove the clinical effectiveness of the formulation.

INTRODUCTION

Amphotericin B is a low-soluble polyene antibiotic which can self-aggregate. The aggregation state can modify its activity and pharmacokinetically characteristics. Despite its high toxicity it is still widely employed for the treatment of systemic fungal infections and parasitic disease and different formulations are marketed. Some of these formulations, such as liposomal formulations, can be considered as classical examples of drug targeting. The pharmacokinetics, toxicity and activity are clearly dependent on the type of amphotericin B formulation. New drug delivery systems such as liposomes, nanospheres and microspheres can result in higher concentrations of Amphotericin B in the liver and spleen, but lower concentrations in kidney and lungs, so decreasing its toxicity. Moreover, the administration of these drug delivery systems can enhance the drug accessibility to organs and tissues (e.g., bone marrow) otherwise inaccessible to the free drug. [1] Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal, and skin as topical routes. Skin is one of the most readily accessible organs on human body for topical administration and is the main route of topical drug delivery system. Topical preparations are applied to the skin for surface, local or systemic effects. In some cases, the base may be used alone for its therapeutic properties, such as emollient, soothing, or protective action.[2] Emulgel is formed When gels and emulsion are mixed. Water phase containing gelling agent will convert an emulsion into an emulgel. Oil in water system is used for encapsulating lipophilic drugs whereas water in oil system is used for hydrophilic drug. Emulgels can be easily washed away whenever needed and shows elegant properties.

It also shows good penetration through the skin. Emulgels with properties such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, water soluble, longer shelf life, biofriendly, transparent and pleasing appearance are used for dermatological purposes. Drug molecules can enter the skin by three routes: through intact stratum corneum, through sweat ducts, or through sebaceous follicle. The surface of the stratum corneum presents more than 99% of the total skin surface available for percutaneous drug absorption. Passage through this outermost layer is the rate limiting step for percutaneous absorption. The major steps involved in percutaneous absorption include the establishment of a concentration gradient, release of drug from the vehicle (partition coefficient), and drug diffusion across the layers of the skin (diffusion coefficient).[3]

Advantages of Emulgel

- Avoidance of first pass metabolism.
- Avoidance of gastrointestinal incompatibility.
- More selective to a specific site.
- Improve patient compliance.
- Suitability for self-medication.
- Drug with short biological half-life and narrow therapeutic window can use this method.
- Ability to stop medication when needed. Convenient and easy to apply.

Disadvantages of emulgel

- Skin irritation on contact dermatitis.
- Possibility of allergenic reactions.
- Poor permeability of some drug through skin.
- Larger particle size drugs are not easy to absorb through the skin.[4]

1.8.4 CONSTITUENTS OF EMULGEL

1**. Aqueous Material:** This forms the aqueous phase of the emulsion. Commonly used agents are water, alcohols.

2. Oils: These agents from the oily phase of the emulsion. Mineral oils, either alone or combined with soft or hard paraffin, are used for externally applied emulsion and also used as a vehicle.

3. Emulsifiers: They are used to maintain stability of a preparation during its shelf life and to cause emulsification during the manufacturing. e.g. Polyethylene glycol 40 stearate, Sorbitan monooleate (Span 80), Polyoxyethylene sorbitan monooleate (Tween 80), Stearic acid, Sodium stearate.

4**. Gelling Agent:** It is used to improve the consistency of any dosage form and also used as a thickening agent. e.g. carbopol 934, carbopol 94

5. Permeation Enhancers: They are used to improve the absorption of drugs by disrupting the skin barrier, fluidize the lipid channels between

corneocytes, altering the partitioning of the drug into skin structures. Commonly used penetration enhancers are oleic acid, lecithin, urea, clove oil, menthol etc[5]

MATERIALS AND METHODS

Materials, Chemicals and Reagents

The following materials used in the study are Pharma grade or the best possible Laboratory Reagent (LR) supplied by the manufacturer.

Amphotericin B was from Amoli organic pvt ltd.

Other ingredients

- Sodium Cmc
- Liquid paraffin
- Tween 80
- Span 80
- Clove oil
- Oleic acid
- Propylene glycol
- Methyl paraben
- Propyl paraben

INSTRUMENTS USED

- UV-Visible spectrophotometrr
- Ftir Spectrophotometer
- Electronic Balance
- Brookfield digital viscometer
- Pen type ph meter
- Franz Diffusion cell
- Magnetic Stirrer
- Electronic weighing Balance
- Mechanical Stirrer

METHODS

- Preformulation tests of Amphotericin B

1. Determination of solubility: Solubility of Amphotericin B was performed in various solvents like water, ethanol, methanol, chloroform, acetone, carbon tetrachloride and ether. One gram of drug was accurately weighed and transferred to a clean and dry test tube followed by addition of the solvents individually and shaken vigorously. The solubility of the drug was checked.

2. Determination of melting points: Melting point of pure Amphotericin B was determined by open capillary method. The capillary tube was closed at one end by fusion method and was filled with the drug by repeated tapings. The capillary tube was place in Thiel's melting point apparatus. The temperature at which the drug started melting was recorded. This was performed thrice and the average value was calculated.

3. Infrared Spectral studies: In this technique, approximately 1mg of the drug was allowed to mix with about 100mg of KBr in the ration 1:100 and then thoroughly grind the mixture in a mortor, press the mixture in a mortor, press the mixture in a pallet die manually and placed it in Fourier transform infrared spectrophotometer.

4.2.2 Determination of λmax by UV Visible spectroscopy

The solution containing 10mcg/ml of drug in methanol was prepared and scanned over the wavelength range of 200-400nm against methanol as a blank using double beam UV spectrophotometer. The plot of absorbance vs wavelength was recorded using double beam spectrophotometer. λmax was obtained 254nm

4.2.3 Calibration curve of Amphotericin B

Preparation of phosphate buffer pH 6.8

Place 50ml of 0.2M potassium dihydrogen phosphate in 200ml volumetric flask, add 22.4ml of 0.2M sodium hydroxide and then add water to volume.

Standard Graph of Amphotericin B

Stock I : 100mg of drug was accurately weighed and dissolved in 25ml of 6. 8pH phosphate buffer in a 100ml volumetric flask and volume was made upto the mark using 6.8 pH phosphate buffer

Stock II: From the above solution of Amphotericin B, 1ml was pipetted out into another 100ml volumetric flask and volume was made upto 100ml with methanol. From the standard solution of stock II, 2, 4, 6, 8 and 10ml were pipetted into 100ml volumetric flasks. The volume was made up with 6.8pH phosphate buffer in order to get 2,4,6,8 and 10 µg/ml of the final solution. Then the absorbance was measured by double beam UV – visible spectrophotometer. This procedure was performed in triplicate to validate the calibration.

4.2.4 Drug –excipient compatibility studies

The FTIR allows identification of functional groups in various chemicals as well as incompatibilities between the drug and excipients. The FTIR study of Amphotericin B(BF) was carried out by KBr pellet method. FTIR spectrum was taken for pure BF and also physical mixture of excipients with drug. In this method 3mg of sample and 300mg of potassium bromide was finely ground using mortar and pestle. A small amount of mixture was placed under hydraulic press compressed at10kg/cm to form a transparent pellet. The pellet was kept in the sample holder and scanned from 4000cm to 500cm -1in Shimadzu FTIR spectrophotometer.

4.2.5 Formulation Development

a. Preparation of stable O/W emulsion of liquid paraffin by using various surfactant and its concentration

O/W type emulsion was prepared by using modified procedure. The aqueous phase of emulsion was prepared by mixing tween 80 in a purified water and the oil phase composed of span 80 in liquid paraffin. Propyl paraben was mixed with propylene glycol and mixed with the aqueous solution contain tween 80. The two emulsion phases were heated separately to 70-80^{o}C, then adding the oil phase, it was mixed with continuous stirring with the aqueous phase until it got cooled to room temperature. Formulations E1-E5 was prepared by using surfactant concentration like 2, 2.5, 3, 3.5 and 4%.[6,7]

Preparation of gel using different polymer

The gel bases were prepared by dispensing carbopol 934 in distilled water with constant stirring at a moderate speed using mechanical shaker. Formulation F1 and F2 was prepared by carbopol 934 as gelling agent. The pH of the formulations was adjusted using triethanol amine to 5.5 to 6.5. In the formulation F3 and F4, the gel was prepared by dispersing HPMC in heated distilled water and the dispersion was cooled and left overnight. Formulation F5 and F6 were prepared by sodium CMC as gelling agent. Sodium CMC was prepared by dispersing SCMC powder in 100ml of heated distilled water and the dispersion was cooled and left overnight.

c. Preparation of Amphotericin B emulgel formulations

1. Preparation of emulsion: The aqueous phase prepared by mixing tween 80 in purified water and oil phase composed of span 80 in liquid paraffin. Drug was dissolved in methanol first and then added to the aqueous phase, propyl paraben and methyl paraben were mixed in propylene glycol and then mixed with the aqueous solution of tween 80. Oleic acid or clove oil were mixed with oil phase. The two emulsion phases were heated separately to 70-80^{0}c, then adding the oil phase, it was mixed with continuous stirring with the aqueous phase until it get cooled to room temperature.[8,9]

2. Preparation of gel: Sodium CMC gel was prepared by dispersing sodium CMC powder in 100ml of heated distilled water and the dispersion was cooled to room temperature and left overnight.

3. Incorporation of emulsion in to the gel: the previously gel base was then mixed with the emulsion in 1:1 weight ratio to prepare final formulation. Different formulations were prepared using varying concentration of gelling agent and permeation enhancer.[10,11]

Physical appearance: The prepared emulgel formulations are inspected visually for their color, omogeneity, consistency and phase 0separation after 24 h of preparation.

2. pH: The pH values of 1% aqueous solutions of the prepared emulgels were measured by a calibrated pH meter.[7]

3. Spreadability: The spread ability of the emulgel formulations was determined 48 hrs after preparation, by measuring the spreading diameter of 0.5 g emulgel which was placed within a circle of 1 cm diameter pre-marked on a glass plate over which a second glass plate (75 gm) was placed. A weight of 425 g was allowed to rest on the upper glass plate for 5 min where no more spreading was expected[8, 9]. The increase in the diameter due to spreading of the gels was noted. The spread ability (g.cm.min-1) was calculated by using the formula:

$$S = m X 1/ t$$

Where: S is spread ability, m is the weight of the upper plate and rested on it (g), l is the diameter of the spreading emulgel (cm), and t is the time taken (min)[10-12].

4. Centrifugation: This parameter could be measured to evaluate physical stability. Emulgel could be centrifuged at an ambient temperature and 6000 RPM for 10 minutes to evaluate the system for creaming or phase separation. System could be observed visually for appearance[13].

5. Drug Content Determination: Take 1gm 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 same solvent. Concentration and drug content can be determined by using the same standard plot by utilizing the value of absorbance.

6. *In-vitro* permeation study: *In-vitro* release study is carried out using a modified Franz diffusion cell. 1g of the formulation was weighed and placed on the dialysis membrane having the surface area of 2.5cm2, which is placed between donor and receptor compartment of the diffusion cell. Phosphate buffer 7.4 was prepared and used as the diffusion media. The temperature of the cell was maintained at 37˚c. This whole setup was stirred using the Teflon coated magnetic

stirrer at 50 rpm. At specified time intervals 5ml of the sample solution was taken and analyzed spectrophotometrically at 281nm. The cumulative % drug release was determined.[12]

7. Kinetic Analysis of i*n-vitro* Release Rates of Amphotericin B Emulgel

The results of *in vitro* release profile obtained for all the formulations were plotted in modes of data treatment as follows,

1. Zero order kinetic model – Cumulative % drug released versus T.
2. First order kinetic model – Log cumulative percent drug remaining versus T.
3. Higuchi's model – Cumulative percent drug released versus square root of T.
4. Korsmeyer equation / Peppa's model – Log cumulative percent drug released versus log T.[13]

8. In vitro antifungal activity: The in vitro antifungal activity of Amphotericin B of the optimized formulation (BC8, BO8) was carried out using *Candida albicans* as a representative fungi, adopting the cup – plate method. Commercial clotrimazole ointment was taken as a reference standard. Clotrimazole is a well known effective antifungal drug and it is available as a topical formulation. Suspension of *Candida albicans* was inoculated in sabouraud dextrose agar medium and then poured into the sterile petridish and allowed to solidify. Wells were done in plate using borer and the formulations were poured into wells. These plates were incubated at 37º C for 24 hours. The standard and test were tested at a concentration of 0.1mg/ml. The mean zone of inhibition was calculated using Hiantibiotic Zone scale for each plate and this value was taken as an indicator for the antifungal activity

9. Stability studies: The selected emulgel was exposed to different temperature conditions in a cyclic pattern that simulate the changes likely to be encountered during its use or in distribution process. During the course of study the selected formulations were filled in aluminium collapsible tube and crimped. They were placed horizontally in the stability chamber and subjected to stability studies at intermediate testing conditions (30°C ± 2°C at 70 ± 5% RH) for 6 months. At specified intervals of time, the samples were withdrawn and evaluated for parameters such as physical appearance, pH, drug content and invitro permeation studies.[14]

RESULT

Preformulation tests of Amphotericin B

Determination of solubility: Amphotericin B found to be practically insoluble in water, slightly soluble in alcohol, free soluble in acetone, chloroform and carbon tetrachloride and sparingly soluble in ether.

Determination of melting point: The melting point was found to be in the range of 142-145°C. The reported melting point is in the range of 142-143°C.

Calibration Curve of Amphotericin B: The calibration curve of Amphotericin B was carried out in 6.8pH phosphate buffer. All the trials were carried out in triplicate. The study of the FTIR spectra of Amphotericin B demonstrated that the characteristic absorption peaks for the aromatic C-H stretching at 3025 cm -1, aromatic C-C stretching at 1487 cm -1 , aliphatic C-H stretching at 2950 and 2830 cm -1 and C-H deformation at 1452 cm-1. This further confirms the purity of Amphotericin B. The major peaks of sorbitan monostearate was found to at 1486, 3020, 2951 cm-1. In the formulation of emulgel (FC 8) peak at 3020 cm-1 was due to presence of sorbitan monostearate, peak at 3025cm-1 and 1487 cm-1 was due to the presence of drug Amphotericin B in the formulation. So from the study it can be concluded that the major peaks of drug (3025cm-1, 1487 cm-1) remains intact and no interaction was found between the drug and sorbitan monostearate. Hence polymer mixture reveals that here is no incompatibility was observed between Amphotericin B

Evaluation studies

a. Evaluation of stable O/W emulsion of Liquid Paraffin by using various surfactants and its concentration

b. Evaluation of gel using different polymers

1. Physical appearance: Gels were prepared by using carbopol, HPMC, Sodium CMC were subjected to physical evaluation. The results are shown in table no

2. Spreadability and Extrudability:

The spread ability and Extrudability of the prepared gel were carried out and the results are shown in table no

c. The prepared emulgel formulated by using 3% emulsifying agent, 3% gelling agent (Sodium CMC) and 7.5% light liquid paraffin was found to be stable. Further studies carried out by using optimized concentration of gelling agent. The effect of permeation enhancer namely oleic acid and clove oil were also studied.

Ingredients	E1	E2	E3	E4	E5
Amphotericin B	250	E1	E1	E1	E1
Light Liquid Paraffin	7.5	250	250	250	250
Span 80	1	1.25	1.5	1.75	2
Tween 80	1	1.25	1.5	1.75	2
Propylene glycol	5	5	5	5	5
Propyl paraben	0.2	0.2	0.2	0.2	0.2
Water	qs	qs	qs	qs	qs

Table 1: Preparation of O/W type stable emulsion using various surfactants concentration

Table Preparation of Emulgel using sodium CMC as gelling agent

Ingredients	F1
Amphotericin B	1.25gm
Light liquid paraffin	7.5%
Span 80	1.5%
Tween 80	1.5%
Propylene glycol	5%
Oleic acid or clove oil	6ml
Sodium CMC	3%
Methyl Paraben	0.02gm
Water	qs

Table Calibration curve of Amphotericin B in 6.8pH phosphate buffer

S.No	Concentration (µg/ml)	Absorbance (Mean nm ±SD)
1	0	0
2	2	0.123 ± 0.002
3	4	0.232 ± 0.001
4	6	0.336 ± 0.003
5	8	0.447 ± 0.005
6	10	0.567 ± 0.07

Fig 5.1 Standard Graph of Amphotericin B in 6.8pH phosphate buffer

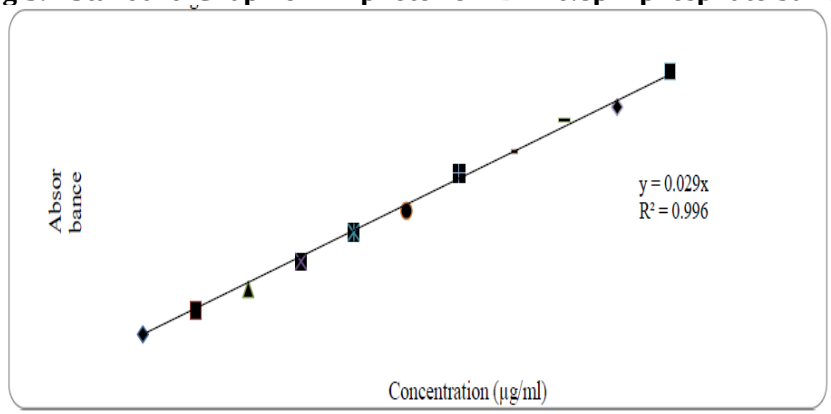
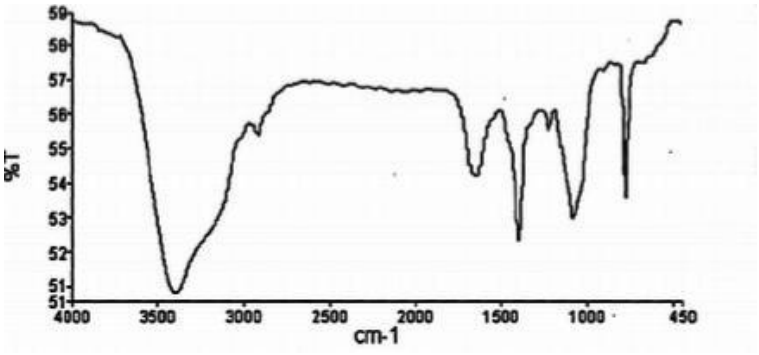


Figure 1.1: Drug and Polymer compatibility studies
Fig 1.2: FTIR spectra Amphotericin B



a. Physical appearance

Table : Data of physical appearance of emulsion

Formulation	Colour	Homogenicity	Phase separation
E1	White	Homogeneous	Seen
E2	White	Homogeneous	Seen
E3	White	Homogeneous	Not Seen
E4	White	Homogeneous	Not Seen
E5	White	Homogeneous	Not Seen

Table Data of Physical appearance of gel using different polymers

Formulation	Colour	Homogenicity	Consistency
G1	White	Homogenicity	Good
G2	White, viscous	Homogenicity	Poor
G3	White, creamy	Homogenicity	Poor
G4	White	Homogenicity	Good
G5	White	Homogenicity	Very Good
G6	White	Homogenicity	Good

Table Data of spread ability and extrudability of gel using different polymers

S.No	Formulation	Spreadability (Mean cm±SD)	Extrudability (Mean cm ±SD)
1	F1	2 ± 0.10	7.8 ± 0.5
2	F2	1.6 ± 0.071	7.3 ± 0.1
3	F3	1.77 ± 0.17	6.8 ± 0.26
4	F4	2.1 ± 0.10	7.7 ± 0.19
5	F5	2.53 ± 0.06	7.5 ± 0.11
6	F6	2 ± 0.10	6.8 ± 0.28

Table Physical appearance of emulgels

Formulation		Colour	Homogeneity	consistency	Phase separation	pH
Clove oil	FC1	Creamy yellowish white	Homogenous	Very good	Not seen	6.2
	FC2			Excellent		6.5
	FC3			Good		6.6
	FC4			Very good		6.8
	FC5			Excellent		6.2
	FC6			Good		6.7
	FC7			Very Good		6.3
	FC8			Excellent		6.7
	FC9			Good		6.5
	FC10			Excellent		6.7

	FC11			Excellent		6.7
	FC12			Excellent		6.7
	FC13			Excellent		6.7
Oleic acid	FO1	White softy cream	homogenous	Very good	Not seen	6.5
	FO2			Excellent		6.2
	FO3			Good		6.6
	FO4			Very good		6.5
	FO5			Excellent		6.6
	FO6			Good		6.9
	FO7			Very Good		6.8
	FO8			Excellent		6.6
	FO9			Good		6.4
	FO10			Excellent		6.6
	FO11			Excellent		6.6
	FO12			Excellent		6.6
	FO13			Excellent		6.6

Table Vicosity of emulgels

RPM	Temperature (°C)	Torque (%)	Viscosity (FC8) (Centipoise)	Viscosity (FO8) Centipoise
1.5	37 ±0.5 °C	60	26089 cp	23079cp

Table : Data of spread ability and extrudability of Amphotericin B emulgel

S.No	Formulation	Spreadability (Mean cm ± SD)	Extrudability ((Mean cm ± SD
1	FC1	3.7 ±0.05	7.8 ± 0.5
2	FC2	3.5 ± 0.1	7.3 ± 0.1
3	FC3	3.1 ± 0.28	6.8 ± 0.26
4	FC4	3.8 ± 0.12	7.7 ± 0.19
5	FC5	3.5 ± 0.31	7.5 ± 0.11
6	FC6	3.2 ± 0.28	6.8 ± 0.28
7	FC7	3.9 ± 0.29	8.0 ± 0.29
8	FC8	4.0 ±0.1	7.4 ± 0.39
9	FC9	3.0±0.29	7.0 ± 0.29
10	FC10	3.5 ± 0.31	7.5 ± 0.11
11	FC11	3.6 ± 0.10	7.4 ± 0.39
12	FC12	3.5 ± 0.31	7.5 ± 0.11
13	FC13	3.5 ± 0.31	7.5 ± 0.11
14	FO1	3.5 ± 0.1	8.1 ± 0.10
15	FO2	3.5 ± 0.1	7.8 ± 0.13
16	FO3	3.76 ± 0.12	7.5 ± 0.17
17	FO4	3.5 ± 0.31	8.3 ± 0.31
18	FO5	3.5 ± 0.31	7.9 ± 0.11
19	FO6	3.2 ± 0.28	7.6 ± 0.48
20	FO7	3.53 ± 0.09	8.4 ± 0.09
21	FO8	3.93 ± 0.29	7.8 ± 0.45
22	FO9	3.0 ± 0.29	7.4 ± 0.54
23	FO10	3.5 ± 0.31	7.9 ± 0.11
24	FO11	3.5 ± 0.31	7.9 ± 0.11
25	FO12	3.5 ± 0.31	7.9 ± 0.11
26	FO13	3.5 ± 0.31	7.9 ± 0.11

Average of three trials

Table: Data of drug content of Amphotericin B emulgel

S.No	Formulation code (clove oil)	Drug content (Mean % ± S D)	Formulation code (Oleic acid)	Drug content (Mean % ± SD)
1	FC1	92.36 ± 0.05	FO1	93.6± 0
2	FC2	90.16 ± 0.95	FO2	92.1 ± 0.3
3	FC3	86.7 ± 0	FO3	91.6 ± 0.1
4	FC4	101.060 ± 0.41	FO4	95.0 ± 0
5	FC5	96.0 ± 0.05	FO5	93.2 ± 0
6	FC6	92.36 ± 0.05	FO6	94.3 ± 0.3
7	FC7	98.53 ± 0.40	FO7	97.2 ± 0
8	FC8	103.26 ± 0.25	FO8	100.4 ± 0.2
9	FC9	96.2 ± 0.2	FO9	93.2 ± 0
10	FC10	103.26 ±0.25	FO10	100.4 ± 0.2
11	FC11	103.26 ±0.25	FO11	100.4 ± 0.2
12	FC12	103.26 ±0.25	FO12	100.4 ± 0.2
13	FC13	103.26 ±0.25	FO13	100.4 ± 0.2

*Average of three trials

Table Data of *in-vitro* drug release of Amphotericin B emulgel with clove oil used as permeation enhancer

Tim (min)	Cumulative % drug release												
	FC1	FC2	FC3	FC4	FC5	FC6	FC7	FC8	FC9	FC10	FC11	FC12	FC13
0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	35.33	36.48	37.96	33.2	32.91	32.6	33.3	40	27.08	32.91	32.91	32.91	32.91
60	42.4	42.06	43.98	38.4	38.75	36.52	40.39	44.48	42.91	38.75	38.75	38.75	38.75
90	48.54	46.78	49.07	44.8	45	40.43	43.92	48.16	50	45	45	45	45
120	53.5	49.35	55.09	52.4	50	45.65	47.05	53.35	57.5	50	50	50	50
150	60.08	54.07	58.79	57.2	55.4	51.73	53.72	60.4	62.08	55.4	55.4	55.4	55.4
180	65.24	59.65	62.96	63.2	60.83	57.39	59.6	66.63	65.83	60.89	60.86	60.86	60.86
210	67.57	63.5	65.74	69.2	65.83	64.34	66.27	69.93	66.27	66.27	65.83	65.86	65.86
240	71.06	69.52	69.44	72.04	71.25	70.86	72.91	75.10	72.26	71.26	71.25	71.25	71.25

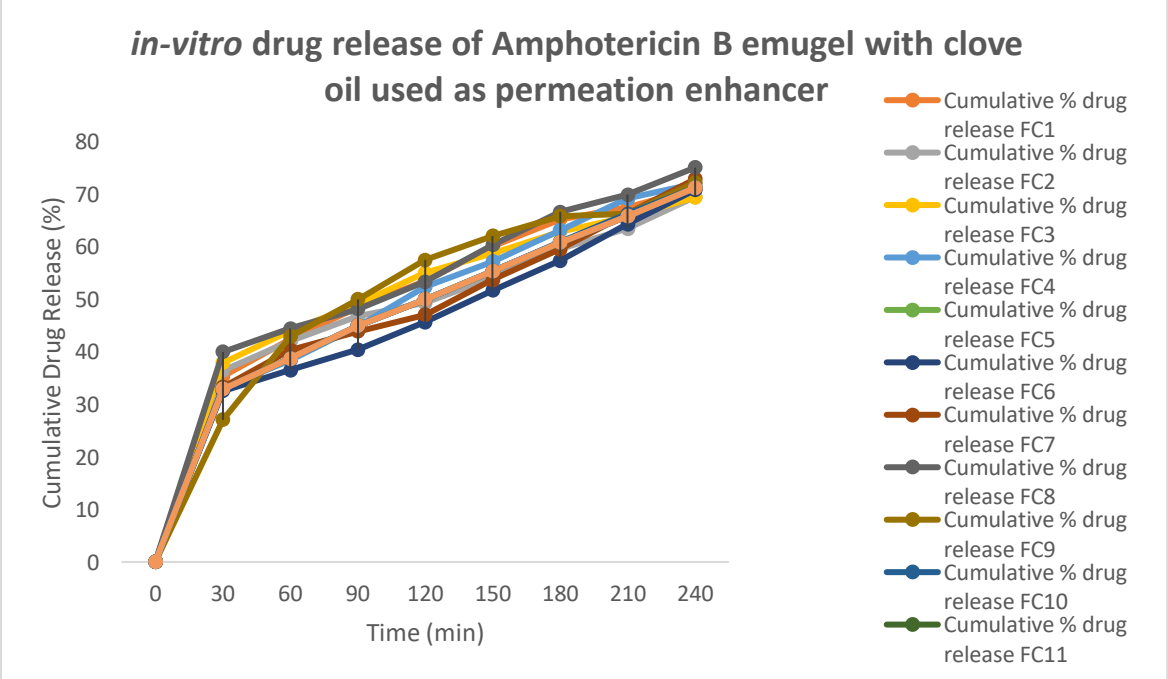


Figure 1.4 Invitro drug release of Amphotericin B emulgel with clove oil used as permeation enhancer

Table : Data of *invitro* reelase bifanazole emulgel with oleic acid used a s penetration enhancer

Tim (min)	Cumulative % drug release												
	FO1	FO2	FO3	FO4	FO5	FO6	FO7	FO8	FO9	FO10	FO11	FO12	FO13
0	0	0	0	0	0	0	0	0	0	0	0	0	0
30	23.04	35.93	26.66	26.38	19.31	27.2	17.7	28.3	22.6	19.31	19.31	19.31	19.31
60	35.65	42.42	41.33	44.25	36.95	32.8	26.63	33.25	31.11	36.95	36.95	36.95	36.95
90	44.78	48.48	48.8	47.68	44.78	40.4	45.66	43.75	39.65	44.78	44.78	44.78	44.78
120	50.43	52.34	56.66	51.91	53.47	47.2	55.69	50.4	47.58	53.47	53.47	53.47	53.47
150	56.1	54.34	60	57.02	60.00	54.00	62.57	55.41	58.84	60.00	60.00	60.00	60.00
180	61.17	64.4	64.88	64.8	66.52	59.2	64.69	61.66	65.24	66.52	66.52	66.52	66.52
210	68.69	69.26	70.66	69.78	69.58	66	68.92	68.75	69.58	69.58	69.58	69.58	69.58
240	75.24	74.45	75.05	75.5	74.2	71.21	75.74	76.25	74.2	74.2	74.2	74.2	74.2

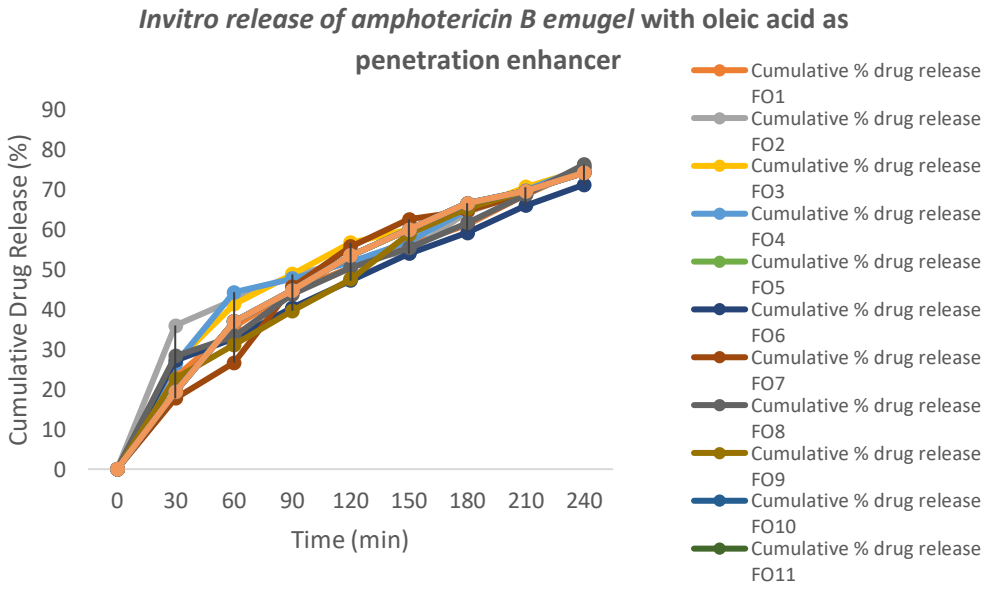


Figure 1.5*: In vitro* release of Amphotericin B emugel with oleic acid used as penetration enhancer

Table Data of stability study of formulation FC8, FO8

FORMULATION		Initial	30 days	60 days	90 days
FC 8	Physical appearance	++	++	+	+
	pH evaluation	6.7	6.7	6.6	6.5
	% drug content	103.26 ±0.25	103..26 ± 0.25	100.76 ± 0.45	98.56 ± 0.34
FO 8	Physical appearance	++	++	+	+
	pH evaluation	6.6	6.6	6.4	6.3
	% drug content	100.4 ± 0.2	100.4 ± 0.2	97.9 ± 0.45	96.56 ± 0.34

++No change in colour, + Slight change in colour

5.1.6 Evaluation of emulgel: Emulgel prepared by using 3^2 factorial designs subjected to various evaluation studies.

Physical appearance

The prepared emulgel using clove oil and oleic acid were used as permeation enhancers subjected to physical evaluation. The colour, homogenicity, consistency, phase separation and pH were shown in table no . The pH values of the prepared formulation of Amphotericin B emulgel were in the range of 6-7.

Viscosity

The viscosity of the optimize formulation of emulgel was shown in table no . Viscosity of FC8 and FO8 was found to be 26089 and 23079 cp respectively

Spreadability and Extrudability studies: The spread ability and extrudability of prepared emulgel was carried out and shown in the table no . . Spreadability of emulgel using clove oil as a permeation enhancer found to be in the range of 3-4cm. In case of oleic acid as a permeation enhancer found to be in the range of 3-4cm.

Drug content study: The drug content in the formulation varied from 86.7%- 103.3 % which indicates that the drug is stable in each of the formulation.

5. *In-vitro* drug release study

***In-vitro* drug release of Amphotericin B emulgel with clove oil used as a penetration enhancer**

The *in-vitro* drug release of Amphotericin B emulgel with clove oil is used as penetration enhancer was carried out and the value are shown in table no

b. *In-vitro* drug release of Amphotericin B emulgel with oleic acid used as a permeation enhancer

In-vitro drug release of Amphotericin B emulgel with oleic acid as a permeation enhancer was carried out and the values are shown in the table no -----

6. *In vitro* antifungal activity: The antifungal activity of the optimized gel was quite effective for inhibition of fungi with desired concentration. The optimized gel was compared with control giving more efficacy than control which was depicted in fig. The zone of inhibition was compared with reference standard *candida albicans* which was given . The zone of inhibition mean for optimized formulation was found to be 23.5mm comparing with clotrimazole 18mm.Hence the optimized formulation FC8, FO8 is most effective than clotrimazole.

7. Stability Studies: From freeze-thaw and thermal cycling test it was concluded that there was no phase separation observed in all the batches of Amphotericin B formulations. The stability study of optimized formulation BC8, BO8 was stored for three months. The variations of drug content, viscosity, spread ability and %cumulative drug release were within the limit which was depicted in Table

CONCLUSION

Amphotericin B is an antifungal agent structurally related to other drugs in this group Iv Bcs classification. It possesses a broad spectrum of activity in vitro against dermatophytes, moulds, yeasts, dimorphic fungi and some Gram-positive bacteria. Both non-comparative and comparative clinical trials have clearly demonstrated the efficacy and safety of various formulations of Amphotericin B 1% (cream, gel, solution and powder) applied once daily in the treatment of superficial fungal infections of the skin such as dermatophytoses, cutaneous candidiasis and pityriasis versicolor. In comparative studies Amphotericin B was significantly superior to placebo and at least as effective as alternative imidazole antifungal drugs including clotrimazole, econazole, miconazole, oxiconazole and sulconazole. Preliminary studies in other superficial skin and nail infections/dermatoses suggest that Amphotericin B may be useful for treating onychomycose.

Thus, Amphotericin B is an effective and well-tolerated treatment for superficial fungal infections of the skin. Compared with the majority of topical antifungal drugs, which need to be applied at least twice daily, Amphotericin B offers the convenience of once daily administration, which may improve patient compliance. Formulation of emulgel was carried out using Sodium CMC as gelling agent. A 32 Factorial Design was carried out to optimize the formulation and to find out the effect of permeation enhancers namely clove oil and oleic acid in formulation. In the present study, 26 formulations of emulgels of Amphotericin B was prepared. The prepared topical emulgels of Amphotericin B were formulated and subjected to physicochemical studies that is; viscosity, spread ability, extrudability and *in-vitro* release studies. Drug content of all the formulation were found to be in the range of 86-104% and pH was found to be in the range 6-7 for all the formulations. All the formulations showed good spread ability and extrudability. The stability study as per ICH guidelines was performed for

the optimized formulation FC8 and FO8 at intermediate testing of 30oC ± 2oC/65 ± 5% RH. No major change in appearance, pH and drug content was seen. Finally, it could be concluded that the prepared emulgel formulation was found to be an effective vehicle for the delivery of the drug. Further studies should be carried out to prove the clinical effectiveness of the formulation.

REFERENCES

1. 1.John D. Cleary, Stanley W. Chapman, Edwin Swiatlo, Robert Kramer, High purity *amphotericin B*, Journal of Antimicrobial Chemotherapy, Volume 60, Issue 6, December 2007, Pages 1331–1340
2. Debojit Bhowmik, Harish Gopinath, B. Pragati Kumar, Recent Advances In Novel Topical Drug Delivery System: The pharmajournal.com.2012; vol1(9):12-30
3. Chen HY, Fang JY. Therapeutic patents for topical and transdermal drug delivery systems.
4. Expert Opinion on Therapeutic Patents 2000;vol10:1035-43
5. Paul A.J. Kolarsick Bs , Maria Ann kolarsick MSN, and Carolyn Goodwin,Journal of the Dermatology Nurses Association. 2011;vol3:203-213
6. Goyal S., Sharma P., Ramchandani U., Shrivastava S. K., Dubey P. K. Novel Anti- inflammatory topical gels. International Journal of Pharmaceutical and Biological Archives. 2011; vol 2(4): 1087-1094.
7. Bharadwraj, Gupta, Sharma, Topical Gel: A novel approach for drug delivery. Journal of Chemical,
8. Thomas, S Kuppuswamy, Anwara Aliyar Sahib, Ashinaa, A Review on Emulgel as a Current Trend in Topical Drug Delivery System;International journal of pharmacy and pharmaceutical research 2017; vole 9(3):274-281.
9. Arpan A. Emulgel: A topical preparation for hydrophobic drugs. AAPS Journal. 2013; 2(5):370-376.
10. Mitkari B V., Formulation and Evluation of topical liposomal gel for Fluconazole, Indian Journa of Pharmaceutical Science, 2010, 44(4): 324-325.
11. . Dodov Glaves Dodov, Maja Simonoska. 5-Flurouracil in topicalliposome gels for anticancer treatment – Formulation and evaluation. Act a Pharm, 2003; (53): 241-250
12. Lubna A Sabri, Halah T. Sulaiman, Yehia I. Khalil. An investigation release and rheological properties of Miconazole Nitrate from emulgel. Iraqi Journal of Pharmaceutical Science. 2009;18(2):26-31.
13. Dadwal Meenakshi. Emulgel; a novel approach to topical drug delivery. International Journal of Universal Pharmacy and Biosciences. 2013;4(1): 847-856.
14. Vijay kumar, Sheefali Mahant, Rekha Rao, Sonja Nanda, Emulgel based topical delivery system for Loratidine, ADMET. 2014: 2(4):254-271.
15. Nair R, Sevukarajan M, Mohammed B, and Kumar J. Formulation of microemulsion based vaginal gel in-vitro and in-vivo evaluation. Der Pharmacia Lettre, 2010; 2: 99-105.
16. Kalpesh Ashara, Moinuddin Soniwala, Ketan Shah. Emulgel: A novel drug delivery system. Journal of Pakistan Association of Dermatologists, 2016; 26 (3):244-249.
17. Krishnaveni Manubolu, Sujatha Byna, Yanadaiah P, Sreenivasulu Munna, Venkata Anudeep padavala. A study on the effect of penetration enhancer on ketoprofen emulgel. International Journal of Research in Pharmacy and Life Sciences, 2015; 3(2): 346–351.
18. .Suvakanta Dash, Padala Narasimha Murthy, Lilakanta Nath and Prasanta Chowdhury. Kinetic modeling on drug release from controlled drug delivery systems- A review. Acta Polonaise Pharmaceutica- Drug Research. 2010; 67(3): 217-223.
19. Vintiloiu, Leroux. Organogels and their use in drug delivery. J.Control. Release, 125, 2008, 179-192.
20. Mona , solid lipid nanoparticles and nanostructured lipid carriers of tolnaftate. Design, optimi.Mona M. zation and in-vitro evaluation. International Journal of Pharmacy and Pharmaceutical Sciences. 2016; 8(1): 380-384.