ejpmr, 2022, 9(6), 259-265

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT AND EVALUATION OF LOTION OF AMPHOTERICIN-B

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Article Received on 25/03/2022 **Article Revised on 15/04/2022** **Article Accepted on 05/05/2022**

ABSTRACT

The main aim of this innovative research was to develop an effective and safe topical drug delivery formulation (Lotion) of Amphotericin-B to reduce the dose concentration of the active drug, to improve patient compliance, to promote rare side effects and increase the local fast absorption and action. Amphotericin B (AmB) is a polyene macrolide class of antifungal agent and it is the drug of choice for systemic fungal infection, but unfortunately, oral bioavailability of this drug is negligible due to its low aqueous solubility. **Methods:** Lotion formulations of Amphotericin-B were prepared using cetyl alcohol, steric acid glycerine with different penetration enhancer with their different concentrations. Two different formulations of Amphotericin-B were prepared and evaluated with respect to their colour, viscosity parameter, determination of pH, drug content, in vitro drug release studies, antifungal studies, and stability studies. **Results:** FT-IR and DSC study results that there were no any significant interaction and reactions between the drug, excipients. All the formulations of Amphotericin-B show acceptable standard physical properties. The drug content and percentage yield were higher for F1 formulation among all formulation F1 shows better drug release. Stability study of the best formulation F1 (Coconut oil) shows that there was no difference in drug content and in vitro drug release studies. **Conclusion:** From the above observation results that this F1 formulation (Coconut oil) may be more encouraging topical substitute for the healing of fungal infections in the skin.

KEYWORDS: Amphotericin-B, Lotion, Skin, solubility, Polene, Fungal, Stability study.

INTRODUCTION

Lotion is a low-viscosity topical preparation intended for application to the skin. By contrast, cream and lotions have higher viscosity, typically due to lower water content. Lotions are applied to external skin with bare hands, a brush, a clean cloth, or cotton wool.

Human fungal infections have increased dramatically in incidence and severity in recent years, due mainly to advances in surgery, cancer treatment, and critical care accompanied by increases in the use of broad-spectrum antimicrobials and the HIV epidemic. These changes have resulted in increased numbers of patients at risk for fungal infections. Pharmacotherapy of fungal disease has been revolutionized by the introduction of the relatively nontoxic oral azole drugs and the echinocandins. Combination therapy is being reconsidered, and new formulations of old agents are becoming available.

Amphotericin B is selective in its fungicidal effect because it exploits the difference in lipid composition of fungal and mammalian cell membranes. Ergosterol, a cell membrane sterol, is found in the cell membrane of fungi, whereas the predominant sterol of bacteria and human cells is cholesterol. Amphotericin B binds to ergosterol and alters the permeability of the cell by forming amphotericin B-associated pores in the cell membrane. As suggested by its chemistry, Amphotericin B combines avidly with lipids (ergosterol) along the double bond-rich side of its structure and associates with water molecules along the hydroxyl-rich side. This amphipathic characteristic facilitates pore formation by multiple amphotericin molecules, with the lipophilic portions around the outside of the pore and the hydrophilic regions lining the inside. The pore allows the leakage of intracellular ions and macromolecules, eventually leading to cell death. Some binding to human membrane sterols does occur, probably accounting for the drug's prominent toxicity. Resistance to amphotericin B occurs if ergosterol binding is impaired, either by decreasing the membrane concentration of ergosterol or by modifying the sterol target molecule to reduce its affinity for the drug.

PREPARATION OF LOTION

Lotions are generally prepared at the industrial scale under room/ Standard temperature. However few of

polymers need special treatment before processing. Lotions are also prepared by following methods.

1. Trituration Method
2. Fusion Method
3. Chemical process/ reaction
4. Homogenizer Method

MATERIALS AND METHODS

All the Chemical/ingredients were collected according to the formula the given above table required amount. Active ingredient (Amphotericin-B) weighted and dissolved in DMSO than added remaining ingredients and homogenized at 2000 rpm for about 15 minutes. Add given oils as penetration enhancer, methyl paraben, propyl paraben were added to it with maintaining 60°C than a homogeneous mixture clear and transparent lotion formulations. All the samples were allowed to equilibrate for 24 hrs at room temperature prior to performing evaluation test. Evaluation of physicochemical parameters of prepared Amphotericin-B lotion Drug-excipients compatibility studies Fourier transfer infrared spectrophotometer (FTIR). The drug, polymer, and excipients interactions are studied using the FTIR method. In general, drug and excipients must be coinciding with each other which produce a stable, safe, and efficacious product. IR spectral analysis of pure drug and polymers carried out. Pure drug that gives peak and patterns were compared with the peaks and patterns with the combination of polymer and drug.

RESULTS AND DISCUSSION

Drug-excipients compatibility studies

The IR studies of clear Amphotericin-B formulation comprises greater proportion of the polymers that are conducted to know about the bond between the used polymers and the drug.

The IR spectrum of pure Amphotericin-B and Amphotericin-B lotion formulations that used greater proportion of polymer that gives comparable basic patterns and peaks. Outcome status that no notable drug and polymer interactions.

Visual inspection

Visual determination is done to examine the physical properties and color of the developed formulation.

Determination of pH The pH value of all developed lotion was in the range of 6.6–7.1. This is sufficient for appealing to skin and avoid the chances of irritation.

Spreadability

The study has a few major elements that show the lotion character that emerges out from the tube. Spreadability test is carried for all the formulations.

Determination of drug content

The drug content of the formulated lotion was estimated. The drug content manifests that the drug was distributed equally throughout the lotion.

Percentage drug content and viscosity

Percentage yield of a topical lotion consisting of Amphotericin-B was in the range of 80.27–85.58%. This was identified that the percentage yield of F1 formulation showed an increase in percentage drug content than the other preparation due to use of coconut oil as penetration enhancer.

In vitro drug release

The drug release profile of Amphotericin-B topical lotion formulations was accomplished by diffusion cell. As an outcome of the *in vitro* release studies of all formulations are given in Table 3, and the statistically represented is shown in Figure.

Amphotericin-B Formulation Table

Ingredients (mg)	F1	F2
Amphotericin-B	2	2
Stearic Acid	10	10
Cetyl Alcohol	4	4
DMSO	25	25
Coconut Oil	4	-
Lemmon Oil	-	4
Glycerin	3	3
Methyl Paraben	2	2
Propyl Paraben	0.5	0.5
Triethanolamine	QS	QS
Water Upto 100ml	QS	QS

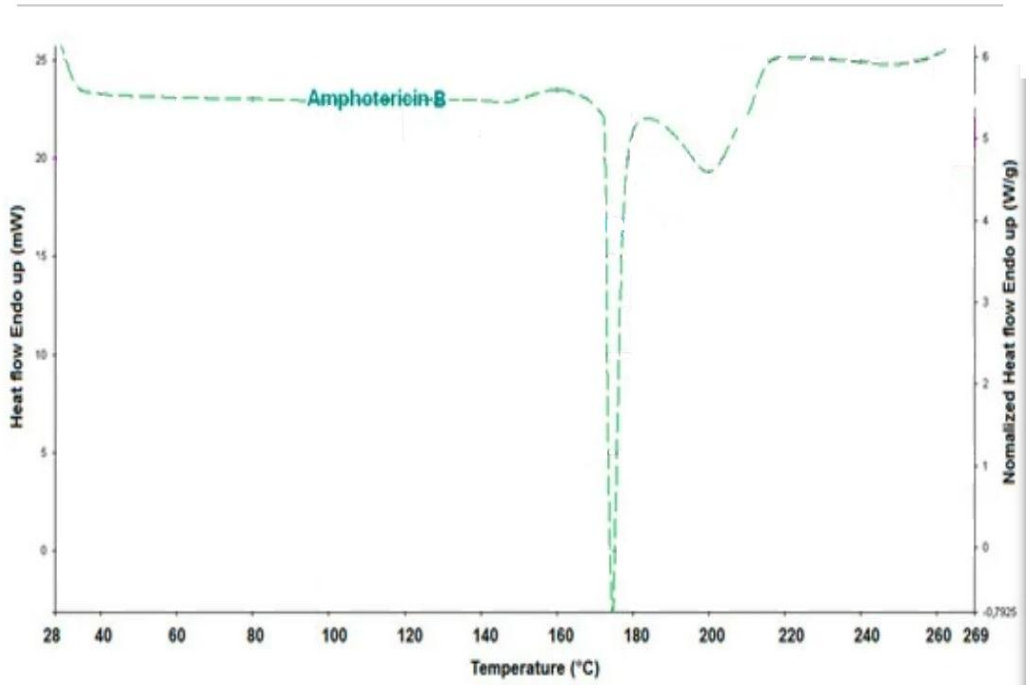

Figure: DSC Thermogram of Amphotericin-B.

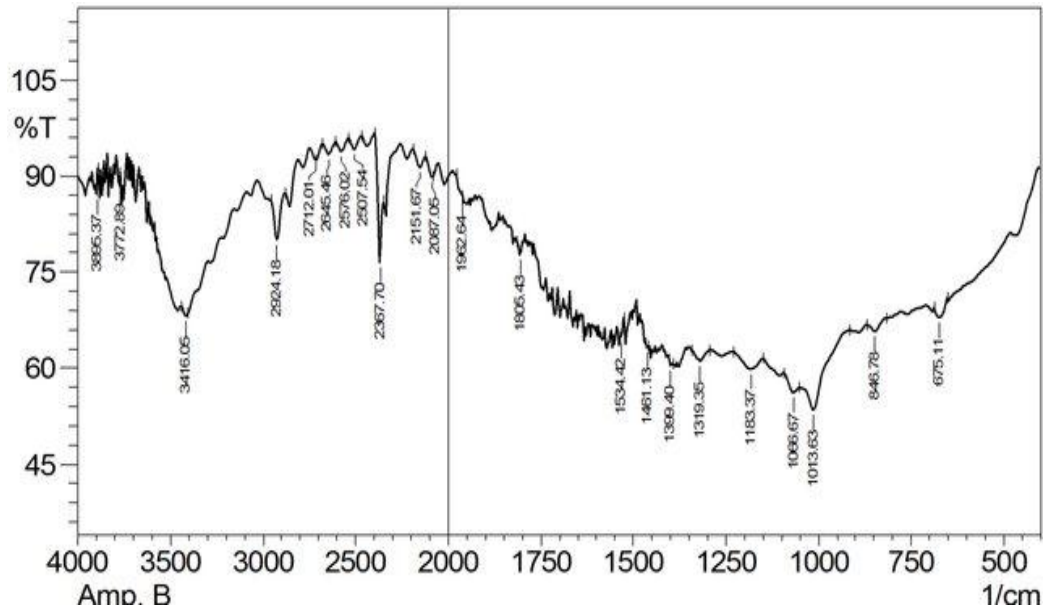

Figure: IR Spectra of Amphotericin-B.

- **Preparation of Calibration curve of Amphotericin-B in DMSO**

Table 5.4 Preparation of Calibration curve of Amphotericin-B in DMSO.

Concentration	Absorbance (415 nm)
0.0	0
2.0	0.173±0.001
4.0	0.321±0.001
6.0	0.472±0.004
8.0	0.621±0.003
10.0	0.772±0.004
12.0	0.941±0.002

All values are expressed as mean (± SD), $n = 3$

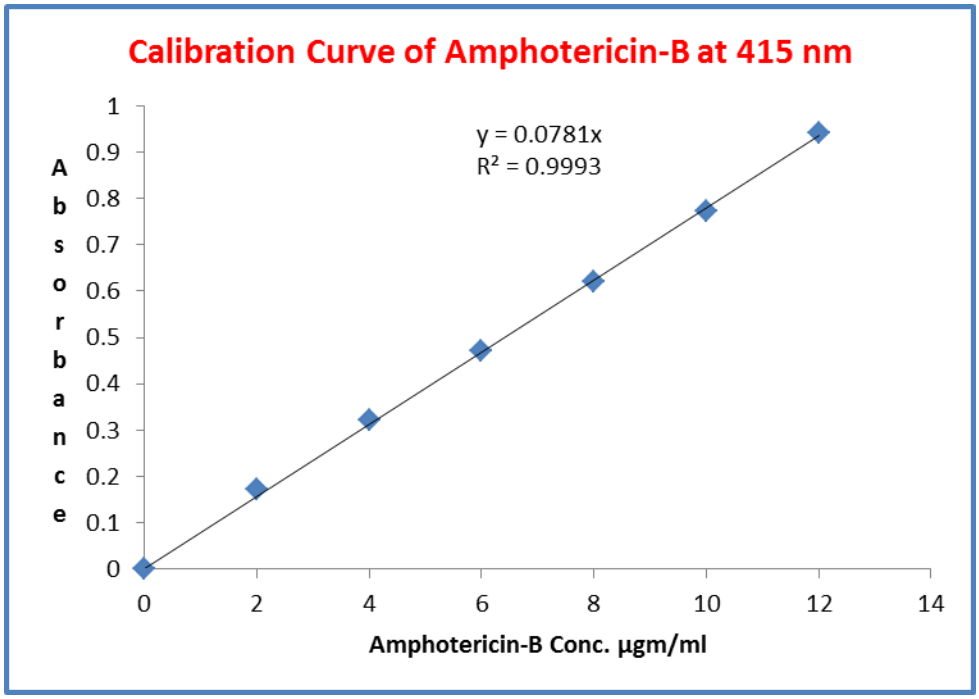

Figure 5.3: Calibration curve of Amphotericin-B in DMSO.

Table: Characterization of formulation of Amphotericin-B Lotion

a) Drug release profile of Formulation F1

i) Amphotericin-B+ Coconut

Time (minutes)	Absorbance at 415nm	Concentration (µg/ml)	Amount of drug release(mg)	Percentage drug release*
0	0.121	0	0	0
30	0.221	4.551	0.91	9.1
60	0.475	10.859	2.178	21.78
90	0.611	19.975	3.995	39.95
120	0.735	27.156	5.431	54.31
150	0.765	35.591	7.118	71.18
180	0.785	42.794	8.558	85.58

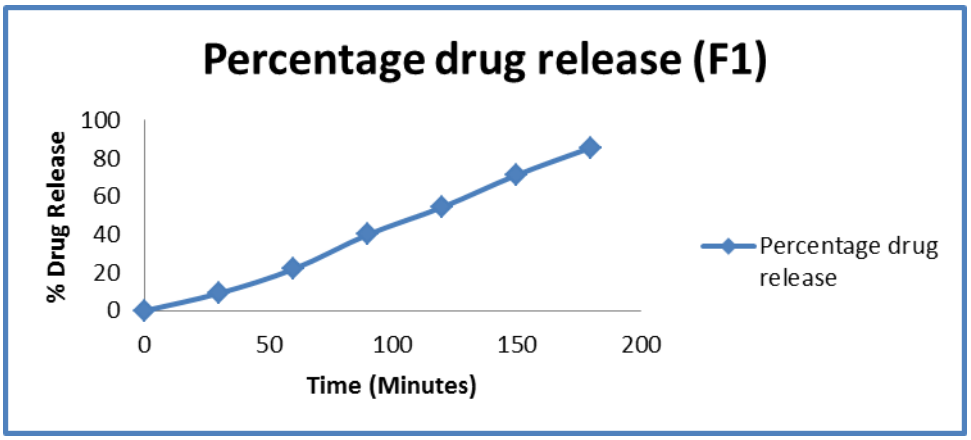

Figure: %Drug release of Formulation F1.

b) Drug release profile of Formulation F2

i) Amphotericin-B+ Lemon Grass oil

Time (minutes)	Absorbance at 415nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
0	0.125	0	0	0
30	0.162	3.522	.7044	7.044
60	0.312	9.609	1.921	19.21
90	0.396	18.337	3.667	36.67
120	0.443	25.304	5.060	50.60
150	0.503	32.539	6.578	65.78
180	0.532	40.135	8.027	80.27

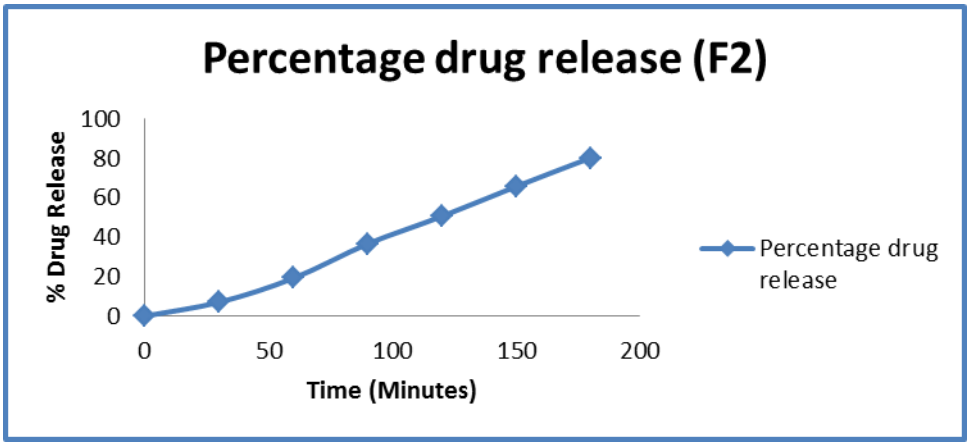

Figure 5.8: % drug release of Formulation F2.

Table: Release kinetics and release mechanism of from various Formulations.

Model	F1	F2
Zero Order	0.925	0.898
First Order	0.941	0.923
Higuchi	0.933	0.899
Korsmeyer Peppas	0.969	0.960

Table: Drug content of formulation F1 (Amphotericin-B with coconut oil)

Table: Physical Evaluation of formulation F1 (Amphotericin-B with coconut oil).

Parameters	Room Temperature	37±5^{0}C	4-5^{0}C
Visual appearance InitialFinal	TransparentTransparent	Transparent Transparent	Transparent Transparent
pH InitialFinal	6.9 7.1	6.9 7.0	6.9 6.9
Viscosity (cps) Initial Final	43,000 43,000	43,000 43,500	43,000 43,000
Phase separation	Not found	Not found	Not found
Leakage	Not found	Not found	Not found
Nature InitialFinal	SmoothSmooth	SmoothSmooth	SmoothSmooth

Chemical evaluation

The drug content of the formulation was estimated over a period of 3 months. The results were tabulated as follows.

Table: Drug content of formulation F1 (Amphotericin-B with coconut oil).

Storage condition	Withdrawal period (monthly)			
	0	1	2	3
4-5^{0}C	85.58	85.54	85.44	85.36
Room Temperature	85.58	85.50	85.48	85.40
37±5^{0}C	85.55	85.55	85.48	85.24

DISCUSSION

The imidazole derivative of Amphotericin-B is one of the best drugs used for the treatment of fungal infections. In this study, the topical lotion preparation of Amphotericin-B was formulated for efficient that absorption of the drug across the skin. Advanced formulations of Amphotericin-B were analyzed for physiochemical parameters such as viscosity, drug content, and *in vitro* drug release studies.

CONCLUSION

By analysing the above results, concluded that our drug Amphotericin-B was incorporated with success into the topical lotion development among all the designed formulation, the formulation F1 shows better Spreadability, drug content, viscosity, and drug release studies. Therefore, this was concluded that our formulation would be very effective and safe topical alternative for the treatment of skin fungal infections.

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