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Research Article

FORMULATION AND EVALUATION OF NANO-EMULSION GEL OF AMPHOTERICIN B FOR TREATMENT OF SKIN INFECTIONS

Arvind Kaur *[1], Shaveta Sharma [1], Mahavir Singh [2], Peeyush Kaushik [1], Abhilash Kutlehria [1]
[1]Chandigarh College of Pharmacy Landran, Mohali, Punjab, India
[2]Pharmacovigilance Analyst, Parexel International, DLF Building, IT Park, Chandigarh, India
*Corresponding Author Email: arvindkaur504@gmail.com

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ABSTRACT

Amphotericin B is a polyene antifungal agent with activity in vitro against a wide variety of fungal pathogens. Amphotericin B exerts its antifungal effect by disruption of fungal cell wall synthesis because of its ability to bind to sterols, primarily Ergosterol, which leads to the formation of pores that allow leakage of cellular components. This affinity may also account for its toxic effects against select mammalian cells. Amphotericin B is generally considered cidal against susceptible fungi at clinically relevant concentrations. The gel was formulated by changing the polymer ratio. FT-IR study confirmed the purity of drug and revealed no interaction between the drug and excipients. Gel formulations were characterized for drug content, pH determination, drug content, in-vitro drug release and particle size. Among the nine formulations, F9 was selected as the best formulation as its %CDR after 08 hours was 93.92%. The drug content of the F9 formulation was 98.12%. Gel formulation F9 was found to be stable at 30 ±2°C and 65 ± 5 RH. It was found that at 40 ± 2°C and 75 ± 5 RH the gel formulation was not stable and %CDR was decreased.

Keywords: Nanoemulsion, microemulsion, compatibility studies and drug release.

INTRODUCTION

Nanoemulsion is a heterogeneous system and it require the large energy input while nanoemulsions consist of two immiscible phases, one phase is oil phase are formed either with (sometime spontaneously) or other is aqueous phase, while the droplet is of sub without high energy input. Emulsions have smaller micron size range of 5-200nm[1]. A gel is defined as as a semisolid system in which a liquid phase is constrained within a polymer matrix by employing high degree of physical and chemical cross linking. Amphotericin B is a broad-spectrum fungicidal antibiotic which is used mainly in the treatment of systemic fungal infections. However, the therapeutic efficacy is limited due to its poor aqueous solubility, instability in gastric acid and its serious side effects[2]. The conventional AmB formulation used clinically is a micellar suspension of AmB with a bile salt like sodium deoxycholate. Therefore, clinical utility of the micellar formulation of AmB is complicated by frequent and severe side effects including fever, chills, nausea, vomiting, anemia and nephrotoxicity. The novel lipid-containing formulations of AmB (i.e. liposomes and micro emulsions) have been developed in an attempt to decrease its nephrotoxicity and increase its potential therapeutic effect. But, disadvantage of these liposomal colloidal carriers is the physical and chemical instability in aqueous dispersion. Interestingly, topical application of antifungal drug offers several advantages by targeted drug delivery to the infected site so as to maximize local side effects without concurrent systemic toxicity like nephrotoxicity[3].

The primary aim of present study was to provide sustained release and localized action of Amphotericin B and increased bioavailability by formulating nanoemulsion Gel of Amphotericin B.

MATERIALS AND METHODS

Table 1: Material and source

Material	Source
Amphotericin B	Health biotech limited, Baddi, Himachal Pradesh
Sesame oil	Medlab supply
Soyabean oil	Medlab supply
Cholesterol	Sigma aldrich
Carbopol	Bodar industries

Preparation of Amphotericin B loaded nanoemulsion[4]

The nanoemulsions of Amphotericin B were prepared by hot homogenization method. The composition was prepared by two phase that is oil phase and water phase. The oil phase was composed of 1000 mg of Sesame oil or soya bean oil, 300 mg of Tween 80 (as hydrophilic surfactant 100 mg (0.5%w/v) of cholesterol and 100 mg of α-tocopherol (antioxidant). The aqueous phase (AP) was composed of glycerol (2.25%; isotonizer) and purified water. Both oil and aqueous phases were heated at 70^0C and then the aqueous phase was slowly added in to the oil phase and homogenized using and Ultra-Tur-RAX T25at 8000 rpm for 5 min. The nanoemulsion was immediately introduced in to probe Sonicator (20% amplitude) for 5 min, using a high intensity ultrasonic processor. The blank nanoemulsion is prepared. The AmB (10 mg) was incorporated in to the blank nanoemulsion and the mixture was homogenized by using a magnetic stirrer at 600 rpm for 1 min. Next, the pH was adjusted to 7.0-7.5 with Hydrochloric acid at room temperature and the final volume was completed with sterile water.

Formulation of Nanoemulsion Gel Loaded Amphotericin B

Carbopol Gel Preparation method[5]

The Carbopol polymer is add in to the distilled water and then homogenized vigorously. Kept overnight after adding two or three drops of Triethanolamine (cross-linking agent) with slow stirring and final pH was adjusted by 7.4 and the Amphotericin B loaded nanoemulsion was incorporated in to the Carbopol gel.

RESULTS AND DISCUSSION

Drug-excipient compatibility studies[6]

The infrared spectrum for the pure Amphotericin B sample was recorded in the wavelength region between 4000 - 400 cm-1. In the same way infrared spectrum for the drug-polymers combination was recorded. The FTIR spectra of drug and drug-polymer combination are shown in Figure 6 and Figure 8 respectively. Compatibility studies were done by comparing the peaks of pure drug FTIR spectra with that of the peaks of drug-excipient combination spectra. There was no significant change in the peaks of these two spectra which indicated that the drug was compatible with the polymers used in the formulation.

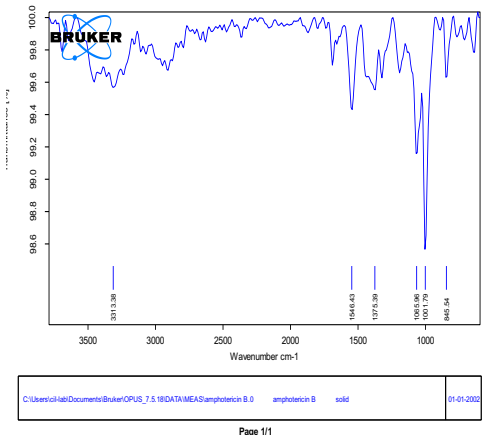

Figure 1: FTIR spectra of Amphotericin B

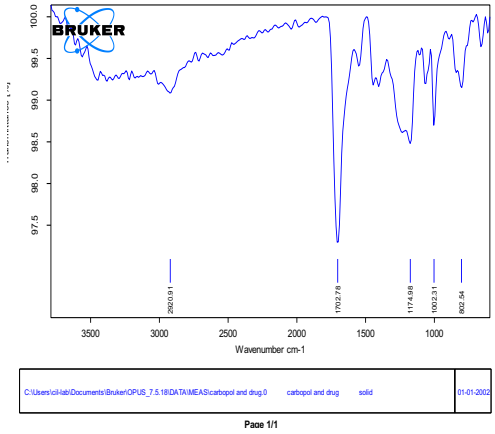

Figure 2: FTIR spectra of Drug and polymer

CHARACTERIZATION OF NANOEMULSION[7]

Microscopical examination

The nano emulsion preparations were observed under microscope and the shape was found to be spherical.

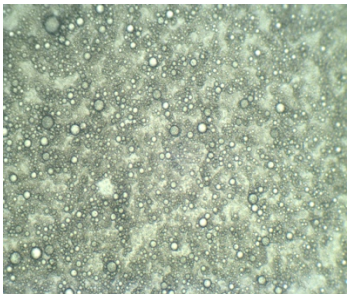

Figure 3: Microscopic image of nanoemulsions

Drug Content (Nanoemulsion)

Table 2: Drug content (Nanoemulsion)

Formulation Code	Drug Content (%)
Formulation 1	94.22
Formulation 2	95.55
Formulation 3	95.30
Formulation 4	93.01
Formulation 5	96.61
Formulation 6	94.80
Formulation 7	98.32
Formulation 8	97.95
Formulation 9	98.93

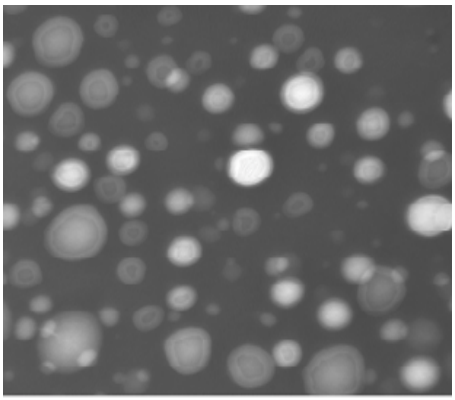

Figure 4: Transmission Electron Microscopy

Table 3: *In-Vitro* release studies (Nanoemulsion)

Time (h)	F1 (%DR)	F2 (%DR)	F3 (%DR)	F4 (%DR)	F5 (%DR)	F6 (%DR)	F7 (%DR)	F8 (%DR)	F9 (%DR)
1	3.17	3.09	3.21	3.86	3.97	4.04	4.11	6.50	6.84
2	5.20	5.68	5.94	6.93	6.75	7.02	9.87	12.86	15.86
3	6.40	7.06	9.23	9.23	10.57	11.18	20.05	25.29	29.29
4	10.37	11.35	12.25	18.84	15.68	17.90	25.67	33.40	37.57
5	20.51	24.43	25.18	22.65	25.53	23.69	39.89	52.58	55.52
6	38.89	44.30	47.34	32.86	36.57	34.16	59.86	65.90	73.17
7	51.65	53.15	56.76	46.91	42.78	44.67	72.98	74.99	77.89
8	60.49	63.98	68.80	53.29	55.61	59.10	84.27	85.80	82.29

Characterization of gel[8]

Drug content: The drug content of nano-emulsion loaded gel was measured by using phosphate buffer (7.4) with 2% DMSO as solvent. (Table 4, 5)

pH: The pH of gel was measured by digital pH meter at 25°C ± 1°C. The pH was recorded in triplicate. (Table 6)

Stability Studies[9]

Stability studies were carried for the most satisfactory formulation at 30 ± 2° and 40 ± 2° for 1 month and 65 ± 5° and 75 ± 5° RH for 2 months. At the end of 2 months, sample were evaluated. Drug content study showed that, there was no major change in the content drug of F_9 (from 93.92 to 93.09%) at 30 ± 2°RH and decrease at 40 ± 2° and 75 ± 5° RH (from 93.92 to7%). There was no significant change in the in vitro drug diffusion study F_9 (from 93.92% to 93.19%) at 30 ± 2° at 65 ± 5° RH. However, after stability at 40 ± 2° at 75 ± 5° RH showed decrease in the in vitro diffusion study of F_9 (from 93.92% to 3.54%). This may be due to effect of temperature with possible degradation of drug. (Table 7-10)

Table 4: Drug content (Amphotericin B Gel)

Formulation code	Drug Content (%)
Formulation 7	97.63
Formulation 8	96.92
Formulation 9	98.12

Table 5: *In vitro* release studies (Amphotericin B Gel)

Time (h)	Formulation 7 (%DR)	Formulation 8 (%DR)	Formulation 9 (%DR)
1	4.86	7.29	5.84
2	8.34	18.72	14.87
3	18.93	29.63	32.56
4	22.69	37.52	48.76
5	41.58	51.58	62.53
6	58.14	69.01	78.72
7	77.73	74.36	86.42
8	88.92	90.39	93.92

Table 7: Drug Content (After 01 month)

Formulation	Drug Content %	
	30 ± 2° at 65 ± 5° RH	40 ± 2° at 75 ± 5° RH
F9	97.50	72

Table 8: In vitro release studies (After 01 month)

Time (in hours)	% Drug Release	
	30 ± 2° at 65 ± 5° RH	40 ± 2° at 75 ± 5° RH
0	0	0
1	4.74	2.49
2	11.77	9.67
3	28.83	18.85
4	45.15	22.55
5	62.42	31.85
6	78.25	43.36
7	88.49	53.04
8	93.09	61.1

Table 9: Drug content (After 02 months)

Formulation	Drug Content %	
	30 ± 2° at 65 ± 5° RH	40 ± 2° at 75 ± 5° RH
F_9	97	7

Table 10: In vitro studies (After 02 months)

Time (in hours)	% Drug Release	
	30 ± 2° at 65 ± 5° RH	40 ± 2° at 75 ± 5° RH
0	0	0
1	6.78	0
2	13.12	0
3	30.49	0.14
4	48.67	0.21
5	63.58	0.74
6	79.04	1.83
7	87.05	2.75
8	93.19	3.54

Table 6: pH determination

Formulation	pH
Formulation 7	6.8
Formulation 8	7.0
Formulation 9	6.4

CONCLUSION

Amphotericin B is an antifungal drug which is used for treatment of serious fungal infections and leishmaniasis e.g. aspergillosis, blastomycosis, candidiasis, coccidioidomycosis, and cryptococcosis. Amphotericin B binds with Ergosterol (component of fungal cell membranes) and cause rapid leakage of monovalent ions (K+, Na+, H+ and Cl−) and subsequent fungal cell death. Amphotericin B showed maximum UV-absorption at a wavelength of 381nm. The value of correlation coefficient was found to be 0.9998 in phosphate buffer pH 7.4 with 2% DMSO which showed linear relationship between concentration and absorbance. Hence Beer's law was obeyed. Preformulation study for drug-polymer compatibility by FT-IR showed no interaction between drug and selected excipients. Various formulations of Amphotericin B nanoemulsion were prepared by using Carbopol 940 polymer and then the selected formulations were incorporated in the Gel formulation. The formulations were evaluated for physicochemical parameters, drug content, *in-vitro* release studies, particle size analysis, pH determination and stability studies. All these studies showed best results for formulation F9. Stability studies carried out for formulation F9 showed a large decrease in the drug content after a period of 02 months at 40 ± 2° at 75 ± 5° Relative Humidity.

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