

## Design, Development and Evaluation of Bigel-Based Drug Delivery of Amphotericin-B

Neha Yadav<sup>1\*</sup>, Kavita Vijay Singh<sup>2</sup>, Mayank Bansal<sup>3</sup>, Manoj Gupta<sup>4</sup>

<sup>1</sup>Research Scholar, Department of Pharmaceutics, Jaipur College of Pharmacy, Jaipur, Rajasthan

<sup>2</sup>Associate Professor, Jaipur College of Pharmacy, Jaipur, Rajasthan

<sup>3</sup>Professor & Principal, Jaipur College of Pharmacy, Jaipur, Rajasthan

<sup>4</sup>Assistant Professor, Jaipur College of Pharmacy, Jaipur, Rajasthan

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Corresponding author: Neha Yadav

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### Abstract

Topical administration is applied to deliver a drug instantaneously at the point of application, so enough drug is depleted into the systemic circulation to cause medicinal effects. To develop an effective drug absorption through an intact skin, several topical preparations are used one of that is "Gels". Gels basically used for the purpose of topical dosage form a lot which is to deliver drug across a localized area of the skin. The main aim of present study was to develop a recent advanced Bigel topical drug delivery of Amphotericin-B to improve patient compliance, to avoid the side effects, first pass metabolism and increase local onset absorption and action. polyene antifungals, amphotericin B associates with ergosterol, the main component of fungal cell membranes, forming a transmembrane channel that acts as monovalent ion (K<sup>+</sup>, Na<sup>+</sup>, H<sup>+</sup> and Cl<sup>-</sup>) leakage, which is the primary effect resulting in fungal cell death.

**Methods:** Topical Bigel formulations development of Amphotericin-B was prepared by using Different-different concentration of polymers to enhance the stability and viscosity. Nine different formulations of Amphotericin-B were prepared and evaluated standard parameters with their colour, Spreadability, viscosity, determination of pH, drug content of formulations, in vitro drug release studies, and stability studies.

**Results:** DSC of the drug samples represents the purity of drug. FT-IR study show that there were no any interaction between the both drugs, Polymers, and other excipients. All the designed and developed formulations of Amphotericin-B show acceptable standard physical properties. The drug content and percentage yield were higher for BG1 formulation among all formulation. BG1 shows the best drug release. Stability study of the best formulations BG1 with Carbopol polymer was found with best results.

**Conclusion:** From the above observation results concluded that formulation BG1 may be more effective topical formulation for the healing and treating of fungal infections.

**Keywords:** Amphotericin-B, Bigel, Zeta potential, Lanosterol, Ergosterol, Cell membrane, Fungal Infection.

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### Introduction

Bi-gels are the advanced developed semi solid formulation combination of

organogel and hydrogel with better applications. The main objective of this

formulation is specially focuses on application and use of bi-gels as drug delivery vehicles by transdermal route to enhance drug bioavailability and patient suitability. It contains two different phases which are the polar and nonpolar due to which, it possess some significant features such as ability to deliver the hydrophilic and hydrophobic drugs which also have improved better permeability of drugs, better spreading ability, and easy water wash ability. [1-8]

Bigels have both organogels and hydrogels they can enhanced hydration of stratum corneum and also had an ability to manipulate the drug release rate from the delivered dosage from. It interacts with ergosterol and forms a transmembrane ion channel in the fungal membrane. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in the increased cellular permeability causing leakage of cellular contents responsible for cell death. [9-13]

#### PREPARATION OF GELS:

Gels are generally prepared at the industrial scale under room temperature. However few of polymers such-Synthetic and Natural need special treatment before processing. Gels are also prepared by following methods. [14-15]

1. Thermal changes
2. Flocculation
3. Chemical process/ reaction

#### Materials and Methods

Amphotericin-B was received as gift sample from Cipla Ltd, Mumbai, India. All Other required Chemicals used in the formulation development were of the standard analytical grade received from various pharma companies and Manufacturers. Amphotericin-B bigel formulations were prepared by using different polymers with their different-different concentrations. A bigel was obtained when the heated organogel was added to the hydrogel under continuous stirring (500 rpm) to obtain a homogeneous mixture and with cooling to ambient temperatures. Nine bigel formulations were prepared by using various ratios of hydrogel and Organogel. The prepared bigels were inspected/evaluated visually for their color, homogeneity, consistency, and phase separation. Various preparations of bigel are shown in Table 1. They all were kept in the dark and cool place. Evaluation of physicochemical parameters of prepared bigel drug-excipients compatibility studies by Fourier transfer infrared spectrophotometer (FTIR). The drug, polymer, and excipients interactions are studied using the FTIR method. In general, drug and excipients must be compatible with each other which produce a stable, safe, and effective formulation. 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. [16-19]

**Table 1: Hydrogel**

<b>Ingredients (mg)</b>	<b>DCH1</b>	<b>DCH2</b>	<b>DCH3</b>
Amphotericin-B	200mg	200mg	200mg
Carbopol	100mg	-	-
HPMC	-	100mg	-
Guar Gum	-	-	100mg
Propylene Glycol	500mg	500mg	500mg
DMSO	1000ml	1000ml	1000ml
Triethanolamine	10mg	10mg	10mg
Methyl Paraben	10mg	10mg	10mg
Propyl Paraben	0.25mg	0.25mg	0.25mg

Water	8180ml	8180ml	8180ml
Total	≈10gm	≈10gm	≈10gm

Active Drug (Amphotericin-B) and all the ingredients were collected according to the formula the given above table. Dissolve 200mg Amphotericin-B in 1000ml of ethanol. Solution of Drug ethanol, water and Propylene glycol prepared and tagged with Beaker-A. Add 100 mg cabolpol/HPMC/Guar Gum in solution-A

with constant stirring at 500 rpm for about 2 hours. Propylene glycol, methyl paraben, propyl paraben and Triethanolamine were added to it with maintaining 25°C. Final weight was made with water. All the samples were allowed to equilibrate for 24 h at room temperature prior to performing evaluation test.

**Table 2: Organogel**

Ingredients (mg)	DCO1	DCO2	DCO3
Amphotericin-B	200mg	200mg	200mg
Coconut Oil	2000mg	-	-
Lemon Oil	-	2000mg	-
Almond Oil	-	-	2000mg
DMSO	3000mg	3000mg	3000mg
Span-60	4780mg	4780mg	4780mg
Triethanolamine	10mg	10mg	10mg
Methyl Paraben	10mg	10mg	10mg
Propyl Paraben	0.25mg	0.25mg	0.25mg
Total	≈10gm	≈10gm	≈10gm

All the Chemical/ingredients were collected according to the formula the given above table. Add 200mg Amphotericin-B in 500mg DMSO and dissolve completely and tagged name label-A. Now add Span-60 in solution –A. Required amount of coconut oil/ Lemon oil and Almond oil added in solution-A

and continuously stirring at 500 rpm for about 1 hour. Propylene glycol, methyl paraben, propyl paraben and Triethanolamine were added to it with maintaining 60°C. Final weight was made with Oil. All the samples were allowed to equilibrate for 24 h at room temperature prior to performing evaluation test.

#### Preparation of Bigel of Amphotericin:

**Table 3: Bigel formulation**

Formulation	BG1	BG2	BG3	BG4	BG5	BG6	BG7	BG8	BG9
Ratio	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1	1:1
Hydrogel+Organogel Code	DCH1+ DCO1	DCH1+ DCO2	DCH1+ DCO3	DCH2+ DCO1	DCH2+ DCO2	DCH2+ DCO3	DCH3+ DCO1	DCH3+ DCO2	DCH3+ DCO3

## Results and Discussion

### Standard curve of Amphotericin-B

**Table 4: Absorbance Amphotericin-B**

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 ( $\pm$  SD),  $n = 3$

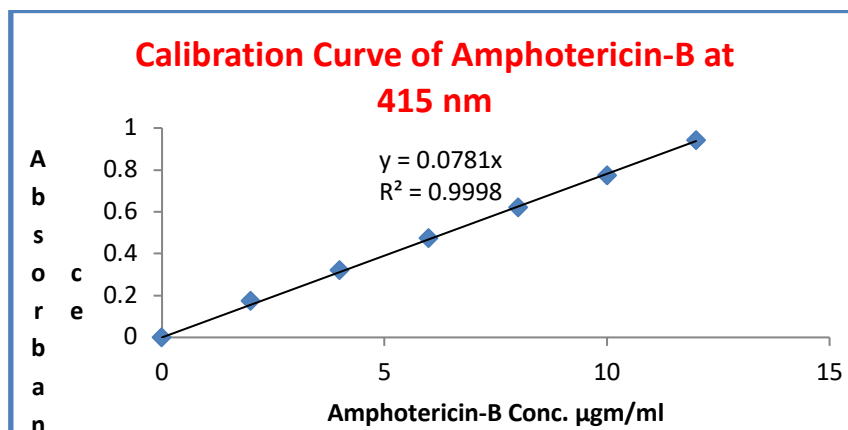


Fig 1: Calibration Curve Amphotericin-B

### PHYSICAL EVALUATIONS

**pH:** pH of prepared formulation was evaluated by Digital pH meter. The pH of prepared formulations observed in range 7.0 to 7.6.

**Viscosity measurements:** The readings taken over a period of 60 seconds at 6 to 10 rpm were averaged to obtain viscosity. The viscosity of prepared various gel

formulations was resulted as 36050±25 to 51250±20CPS.

**Extrudability:** The extrudability of the gel formulations were checked as per the procedure. Extrudability of carbopol and HPMC gels were excellent than Guar gum gel and the results were shown in Table

Table 5: Bigel formulation

Formulation	Extrudability
BG1	++++
BG2	++++
BG3	+
BG4	++
BG5	++
BG6	++
BG7	++
BG8	+
BG9	++

**Determination of Spreadability:** The Spreadability of gels was determined as per the procedure. From Spreadability data is observed that the formulation with carbopol-934 showed maximum (8cm),

whereas the formulations with carbopol-940, HPMC and Guar gum were showed significant Spreadability. The results were tabulated in Table.

**Table 6: Spreadability**

Formulation	Time taken (minutes)	Spreadability (cm)
BG1	30	8.0
BG2	30	7.8
BG3	30	5.4
BG4	30	4.7
BG5	30	5.5
BG6	30	6.3
BG7	30	5.4
BG8	30	5.6
BG9	30	5.2

**Skin irritation test:** The primary skin irritation test was performed on healthy albino rabbits, weighing between 2.0-3.5 kg. The gel formulation film was prepared and used as test patches, while adhesive tape (USP) was used as control. The test was conducted on unbraided skin of the rabbits. The control and test patches were placed on the left and right dorsal surfaces of the rabbits respectively. The patches were removed after 24 hours with the help of alcohol swab and the skin was examined for erythema and edema.

**Stability studies for the formulation BG1 (carbopol-934 with coconut oil):** Stability study for the best formulation was done as per the procedure. The gel

**Antifungal sensitivity:** The antifungal sensitivity test is employed on to the all the fungi colony of Tinea Versicolor under present study. For this experiment 6 mm diameter wells, stock of bigel applied on it. A SDA plate is seeded with Tinea Versicolor with the help of spread plate technique and left for 5 minutes then incubated for 24 hours at 37°C. After incubation, plates were observed to see the sensitivity of formulation towards test at particular concentration in the form zone of inhibition.

was both physically and chemically stable at  $4\pm 3^{\circ}\text{C}$ , Room temperature and  $40\pm 2^{\circ}\text{C}$ . The results were tabulated in Table

**Table 7: Stability studies**

Parameters	Room Temp. ( $25\pm 2^{\circ}\text{C}$ )	$40\pm 2^{\circ}\text{C}$	$4\pm 3^{\circ}\text{C}$
<b>Visual appearance</b>			
Initial	Transparent	Transparent	Transparent
Final	Transparent	Transparent	Transparent
<b>pH</b>			
Initial	6.9	6.9	6.9
Final	7.1	7.0	6.9
<b>Viscosity (cps)</b>			
Initial	43,000	43,000	43,000
Final	43,000	43,500	43,000
<b>Extrudability</b>			
Initial	+++	+++	+++
Final	+++	+++	+++
<b>Phase separation</b>	Not found	Not found	Not found

<b>Leakage</b>	Not found	Not found	Not found
<b>Nature</b> Initial Final	Smooth Smooth	Smooth Smooth	Smooth Smooth

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

**Table 8: Drug content of formulation BG1 (Carbopol-934 with coconut oil)**

Storage condition	Withdrawal period (monthly)			
	0	1	2	3
4±3°C	101.72	101.54	100.04	99.36
Room Temp. (25±2°C)	101.72	100.86	99.48	98.93
40±2°C	101.72	100.55	99.08	98.24

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

#### **In vitro drug release**

The drug release profile of Bigel formulations was accomplished by Franz

#### **Drug release profile of Formulation BG1**

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

**Table 9: BG1 In-Vitro Release**

Time (minutes)	Absorbance at 415nm	Concentration (µg/ml)	Amount of drug release(mg)	Percentage drug release*
30	0.215	10.651	2.130	21.30
60	0.471	16.879	3.374	33.74
90	0.601	29.475	5.894	58.94
120	0.715	35.856	7.170	71.70
150	0.757	38.591	7.718	77.18
180	0.771	42.894	8.578	85.78

#### **Drug release profile of Formulation BG2**

**Table 10: BG2 In-Vitro Release**

Time (Minutes)	Absorbance at 415nm	Concentration (µg/ml)	Amount of drug release (mg)	Percentage drug release*
30	0.162	09.522	1.904	19.04
60	0.312	15.609	3.121	31.21
90	0.396	28.337	5.667	56.67
120	0.443	34.304	6.860	68.60
150	0.503	37.539	7.507	75.07
180	0.532	41.135	8.227	82.27

**Table 11: Release kinetics and release mechanism of from various Formulations**

Model	BG1	BG2	BG3	BG4	BG5	BG6	BG7	BG8	BG9
Zero Order	0.998	0.981	0.995	0.981	0.995	0.985	0.991	0.984	0.985
First Order	0.961	0.963	0.959	0.962	0.979	0.988	0.975	0.989	0.973
Higuchi	0.963	0.979	0.972	0.989	0.972	0.974	0.969	0.978	0.966
Korsm.Peppas	0.999	0.991	0.997	0.994	0.989	0.992	0.989	0.994	0.988

## Discussion

In this study, the topical bigel preparation of Amphotericin-B were formulated for effective absorption of the drug across the skin. Advanced formulations of both drugs were analyzed for physiochemical parameters such as viscosity, Spreadability, drug content, and in vitro drug release studies.

## Conclusion

By analysing the above results, concluded that drugs Amphotericin-B were incorporated with success into the topical gel development among all the designed formulation, the formulation BG1 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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