ISSN: 0976-822X

Available online on http://www.ijcpr.com

International Journal of Current Pharmaceutical Review and Research 2022; 14(1); 72-81

Original Research Article

# DESIGN, DEVELOPMENT AND EVALUATION OF BIGEL BASED DELIVERY OF AMPHOTERICIN-B AND MICONAZOLE IN THE TREATMENT OF FUNGAL

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**Received: 01-12-2021 / Revised: 13-01-2022 / Accepted: 25-03-2022**
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**Conflict of interest: Nil**

**Abstract**

The main aim of present study was to develop a recent advanced Bigel topical drug delivery of Amphotericin-B and Miconazole 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+, Na+, H+ and Cl−) leakage, which is the primary effect resulting in fungal cell death. Miconazole interfare with 14-α sterol demethylase, a cytochrome P-450 enzyme essential for conversion of lanosterol to ergosterol. These turn in inhibition in synthesis of ergosterol and also enhance cellular permeability of fungus due to reduced amounts of ergosterol present in the fungal cell membrane. **Methods:** Topical Bigel formulations development of Amphotericin-B and Miconazole were prepared by using Different-different concentration of polymers to enhance the stability and viscosity. Nine different formulations of Amphotericin-B and Miconazole 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 and Miconazole 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, Miconazole, 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. Miconazole interfare with 14-α sterol demethylase, a cytochrome P-450 enzyme essential for conversion of lanosterol to ergosterol. These turn in inhibition in synthesis of ergosterol and also enhance cellular permeability of fungus due to reduced amounts of ergosterol present in the fungal cell 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 Rajasthan antibiotics and Miconazole was received 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 and Miconazole 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]

Active Drug (Miconazole) and all the ingredients were collected according to the formula the given above table. Dissolve 200mg Miconazole 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: Hydrogel**

| Ingredients (mg) | DCH1 | DCH2 | DCH3 |
|---|---|---|---|
| Miconazole | 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 |

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.

**Table: 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 |

**Preparation of Bigel of Amphotericin-B and Miconazole:**
**Table: 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: 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 (± SD), *n* = 3

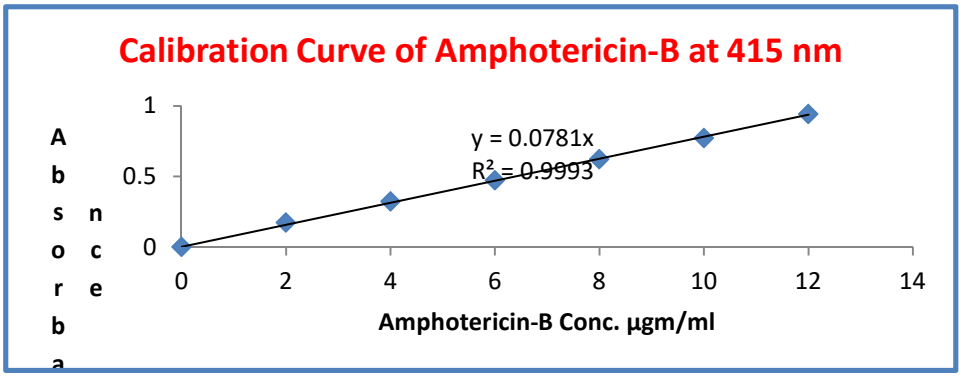


**Fig: Calibration Curve Amphotericin-B**

### Standard curve of Miconazole

**Table: Absorbance Amphotericin-B**

| Concentration | Absorbance (272 nm) |
|---|---|
| 0.0 | 0 |
| 2.0 | 0.123±0.002 |
| 4.0 | 0.258±0.003 |
| 6.0 | 0.388±0.001 |
| 8.0 | 0.525±0.002 |
| 10.0 | 0.651±0.003 |
| 12.0 | 0.789±0.001 |

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

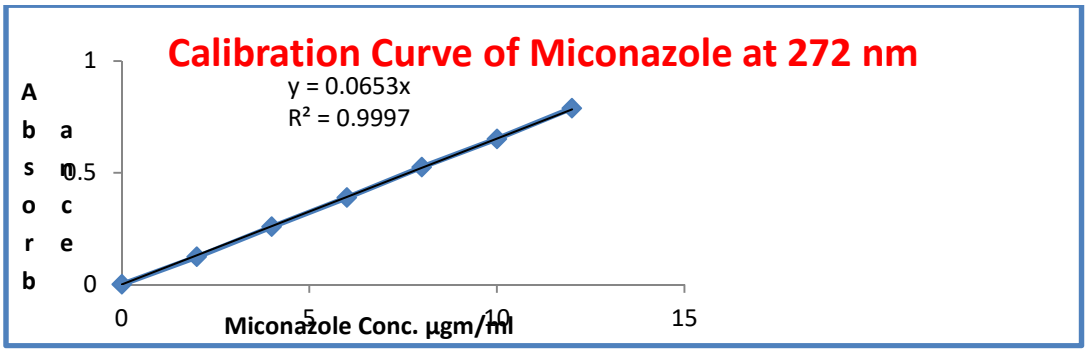


**Fig: Calibration Curve Miconazole**

## PHYSICAL EVALUATIONS

**a) pH:**

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

b) **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

| 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), where as the formulations with carbopol-940, HPMC and Guar gum were showed significant spreadability. The results were tabulated in Table.

| 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 unbraidedskin 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.

**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$^{o}$C. After incubation, plates were observed to see the sensitivity of formulation towards test at particular concentration in the form zone of inhibition.

**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 was both physically and chemically stable at 4±3$^{0}$c, Room temperature and 40±2$^{0}$c. The results were tabulated in Table .

| Parameters | Room Temp. (25±2$^{0}$C) | 40±2$^{0}$C | 4±3$^{0}$C |
|---|---|---|---|
| **Visual appearance** | | | |
| Initial | Transparent | Transparent | Transparent |
| Final | Transparent | Transparent | Transparent |
| **pH** | | | |
| Initial | 6.9 | 6.9 | 6.9 |
| Final | 7.1 | 7.0 | 6.9 |
| **Viscosity (cps)** | | | |
| Initial | 43,000 | 43,000 | 43,000 |
| Final | 43,000 | 43,500 | 43,000 |
| **Extrudability** | | | |
| Initial | +++ | +++ | +++ |
| Final | +++ | +++ | +++ |
| **Phase separation** | Not found | Not found | Not found |
| **Leakage** | Not found | Not found | Not found |
| **Nature** | | | |
| Initial | Smooth | Smooth | Smooth |
| Final | 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.

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

| Storage condition | Withdrawal period (monthly) | | | |
|---|---|---|---|---|
| | 0 | 1 | 2 | 3 |
| 4±3$^{0}$C | 101.72 | 101.54 | 100.04 | 99.36 |

| | | | | |
|---|---|---|---|---|
| **Room Temp. (25±2$^0$C)** | 101.72 | 100.86 | 99.48 | 98.93 |
| **40±2$^0$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**

**a) Drug release profile of Formulation BG1**

The drug release profile of Bigel formulations was accomplished by Franz 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

**i) Amphotericin-B**

Table-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 |

**ii) Miconazole**

| Time (minutes) | Absorbance at 272nm | Concentration (µg/ml) | Amount of drug release (mg) | Percentage drug release* |
|---|---|---|---|---|
| 30 | 0.186 | 09.016 | 1.803 | 18.03 |
| 60 | 0.365 | 14.699 | 2.939 | 29.39 |
| 90 | 0.481 | 27.086 | 5.417 | 54.17 |
| 120 | 0.52 | 33.888 | 6.777 | 67.77 |
| 150 | 0.617 | 37.884 | 7.576 | 75.76 |
| 180 | 0.676 | 40.098 | 8.019 | 80.19 |

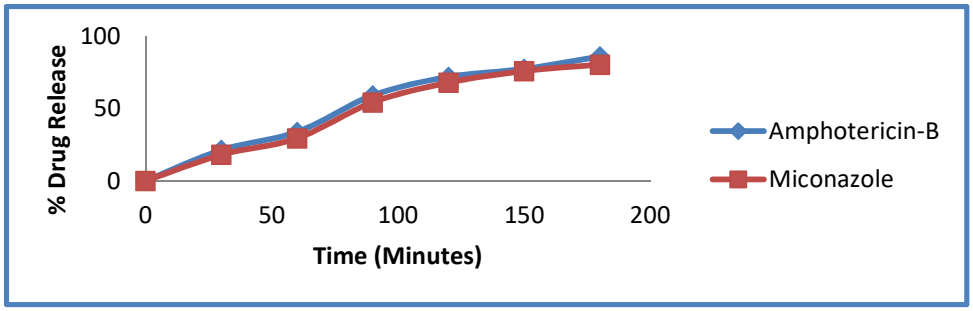


Fig. In-Vitro Release for Formulation BG1

**b) Drug release profile of Formulation BG2**

**i) Amphotericin-B**

Table-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 |

**ii) Miconazole**

| Time (minutes) | Absorbance at 272nm | Concentration (µg/ml) | Amount of drug release (mg) | Percentage drug release* |
|---|---|---|---|---|
| 30 | 0.136 | 08.451 | 1.690 | 16.90 |
| 60 | 0.269 | 13.724 | 2.744 | 27.44 |
| 90 | 0.313 | 26.629 | 5.325 | 53.25 |
| 120 | 0.361 | 32.617 | 6.523 | 65.23 |
| 150 | 0.414 | 36.707 | 7.341 | 73.41 |
| 180 | 0.474 | 39.942 | 7.988 | 79.88 |

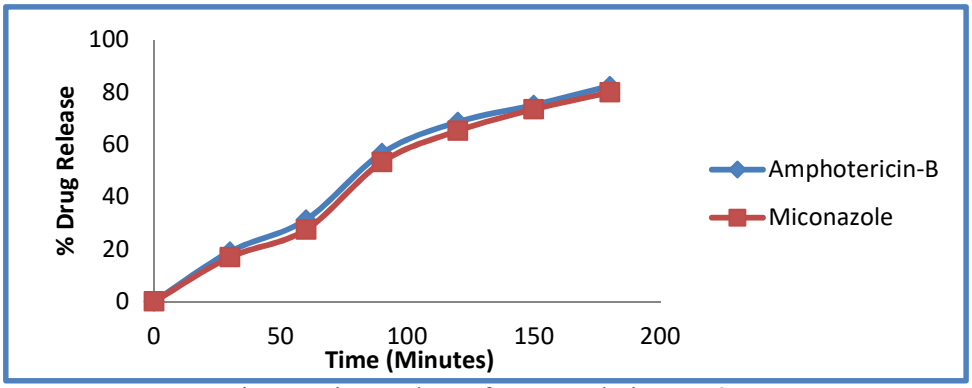


Fig. In-Vitro Release for Formulation BG2

**Table: 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 |
| Korsmeyer Peppas | 0.999 | 0.991 | 0.997 | 0.994 | 0.989 | 0.992 | 0.989 | 0.994 | 0.988 |

## DISCUSSION

The imidazole derivative Miconazole and Polyene antibiotic are the best drugs of choice for the treatment of fungal infections. In this study, the topical bigel preparation of Amphotericin-B and Miconazole 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 and Miconazole 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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