MICONAZOLE LOADED NOVEL PHYTOSOMAL TOPICAL GELS

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ABSTRACT
Phytosomes are phospholipid vesicles advocated for enhanced delivery of polar biomolecules. Phosphatidylcholine-complexes of Mitomycin, Clarithromycin, silybin, rutin, 10-hydroxycamptothecin, oxymatrine, luteolin have been successfully formulated and evaluated demonstrating the merit of the novel formulation. The aim of the present work was to formulate and evaluate miconazole loaded topical phytosomal gels. This study attempts the use of phytosome as carrier for drug loading of Miconazole(MCZ). Schrebera swietenioides root bark has high polyphenol content and can form phytosomal complexes with soyalecithin. Schrebera root bark extract (SRE) was used to formulate miconazole loaded phytosomal complexes (MPC’s). Schrebera swietenioides (Oleaceae) root bark methanolic extract (SRE) was prepared by soxhlation. Miconazole nitrate (MCZ) and SRE were complexed using soyalecithin in definite drug: lipid ratios by ethanol method. All Miconazole loaded phytosomal complexes were evaluated and characterized for entrapment efficiency, in vitro release, vesicle size, vesicle stability and SEM. Blank phytosomes (SRE-soyalecithin complex) and drug-loaded phytosomes (MCZ loaded SRE phytosomes) were included for comparison. Further, the optimised MPC’s were formulated as gels using varying polymers in differing concentrations and were assessed for their homogeneity, pH, drug content and in vitro permeation using Franz diffusion cell. The optimized formulation F4 was subjected to stability studies as per ICH guidelines. Microscopical examination of MPC’s revealed spherical shape and uniform size. MP1B and MP6B were optimized based on their in vitro drug release profile. The evaluation of prepared phytosomal gels of MP1B and MP6B...
indicated F4 to be better with 93.3 % drug content and 92.54% cumulative drug release of MCZ in 12hrs. Miconazole was successfully loaded into the SRE-phytosomal complex. The results demonstrated that other poorly soluble drugs can be further be explored for better therapeutic benefit.

KEYWORDS: Miconazole, Schrebera swietenioides, phytosomal complex, topical phytosomal gel.

INTRODUCTION
Phytosome is a vesicular drug delivery system in which polar phytoconstituents of plant extract are surrounded and bound by lipid. Phytosomes offer protection to the encapsulated drugs from destruction by digestive secretions and gut bacteria, show better absorption, better bioavailability and hence improved therapeutic efficacy than conventional formulations. Natural phospholipids like soyalecithin can incorporate in their vesicles, both hydrophilic and lipophilic drugs either in the central aqueous core, in between the hydrophobic lamellar layers or by binding to the polar heads of the phospholipid by covalent or hydrogen bonding depending on the polarity of the drug. Entrapment efficiency of the polar compound of interest is predetermined during formulation of phytosomes.[1] Few drugs, phytochemicals or plant extracts have been successfully complexed and encapsulated using phytosome technology. Phosphatidylcholine-complexes of Mitomycin, Clarithromycin, silybin, curcumin, rutin, 10-hydroxycamptothecin etc have been successfully formulated and evaluated demonstrating the merit of the novel formulation.[2] Slight modification of the conventional methods of preparation of phytosomes might result in additional incorporation of poorly water soluble drugs.

Miconazole is an imidazole antifungal agent used in clinical treatment of both superficial and systemic fungal infections. It acts by inhibiting ergosterol synthesis. It is effective against dermatophytes, Malassezia spp, and Candida spp. Commercial formulations of Miconazole are available as creams and gels. Although used as a nitrate salt, it is insoluble in water and has an octanol–water partition coefficient of about 6.25 emphasizing the lipophilic character of the nitrate salt. Hydrophobicity limits its bioavailability and antifungal effects.[3] Stratum corneum of human skin is a potential barrier to penetration of drugs through the skin. Antifungal drugs should reach effective therapeutic levels in viable epidermis after dermal administration. Poor dissolution and lack of absorption of miconazole nitrate (MCZ) limit it’s candidature for oral administration. Poor skin-penetration capability presents a problem
in the treatment of deep cutaneous infections by topical application. Various approaches have been used to enhance it’s permeability. MCZ- cyclodextrin complexes,[4] chewing gum for buccal delivery,[5] topical liposomes,[6,7] niosomes,[8] and cubosomal formulations[9] are reported to increase bioavailability and drug permeation into deeper layers of the skin.

The aim of the present study was to formulate and evaluate miconazole loaded topical phytosomal gel for controlled drug release.

MATERIALS AND METHODS
Miconazole nitrate (MCZ) was kindly gifted by NMR Labs, Hyderabad. Schrebera swietenioides root bark was procured from Tirupathi, Andhra Pradesh and was authenticated by Dr.K.Madhava Chetty Associate Professor, Sri Venkateswara University, Tirupathi, Andhra Pradesh. Methanolic extract was prepared in house by soxhlation. Soyalecithin was procured from Lobachemie, Mumbai. All other chemicals and solvents used for study were obtained from S.D. Fine chemicals, Mumbai and were of analytical grade.

Preparation of MCZ loaded phytosomal complexes of SRE (MPC’s).

Ethanol method[10]
Weighed quantity of SRE was dissolved in suitable volume of methanol and taken in a round-bottom flask containing ethanol as a reaction medium. Soyalecithin was dissolved in Dichloromethane and was added to the contents of the flask. The contents were mixed thoroughly by stirring and sonication. Ethanol was evaporated under vacumm at 40 °C, the residue was placed in desiccator overnight, then crushed in the mortar and sieved with a 100 mesh. To facilitate loading, methanolic MCZ was added to the contents of RBF (Method A), to the concentrated solution (Method B) or sprinkled on to the SRE – phospholipid complex ( Method C). The resultant MCZ loaded SRE-phospholipid complex was transferred into an air tight glass bottle, flushed and stored at room temperature. The effect of method of drug loading on entrapment efficiency and in vitro dissolution profile was evaluated.

Drug: Phospholipid ratio
SRE and MCZ were together considered as an entity. The amount of soyalecithin to be taken for phytosome formulation was varied in the proportion of 1:1, 1:2, 1:4, and 1:6.
Table 1: Formulations of Miconazole loaded phytosomal complexes

<table>
<thead>
<tr>
<th>Formulation code/ Method</th>
<th>Drug:lipid</th>
<th>Formulation code/ Method</th>
<th>Drug:lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP1A</td>
<td>1:1</td>
<td>MP4A</td>
<td>1:4</td>
</tr>
<tr>
<td>MP1B</td>
<td>1:1</td>
<td>MP4B</td>
<td>1:4</td>
</tr>
<tr>
<td>MP1C</td>
<td>1:1</td>
<td>MP4C</td>
<td>1:4</td>
</tr>
<tr>
<td>MP2A</td>
<td>1:2</td>
<td>MP6A</td>
<td>1:6</td>
</tr>
<tr>
<td>MP2B</td>
<td>1:2</td>
<td>MP6B</td>
<td>1:6</td>
</tr>
<tr>
<td>MP2C</td>
<td>1:2</td>
<td>MP6C</td>
<td>1:6</td>
</tr>
</tbody>
</table>

**Analysis**
Spectrophotometric analysis using a Double beam UV 3000+ spectrophotometer, Lab India was used for determination of absorption maxima of SRE and MCZ. The absorption maxima was determined to be 240 and 220nm respectively.

**Evaluation and characterization**\(^{[11,12]}\)

**Determination of percent drug entrapment**
Unentrapped drug was separated using the centrifugation method. Centrifugation was done at 12000 rpm for 45 min and supernatant was collected and analyzed to determine the concentration of unentrapped drug.

Drug entrapment was calculated using the formula,

$$\text{Entrapment efficiency (\%) } = \frac{\text{Wt. of total drug } - \text{wt. of free drug}}{\text{Wt. of total drug}} \times 100$$

**In-vitro dissolution study**
The prepared drug loaded phytosomal complexes were filled in zero size capsules. In-vitro dissolution studies for all the prepared formulations were carried out using type-II dissolution apparatus at 50rpm in 900 ml of phosphate buffer pH 6.8 as a dissolution media, maintained at 37±5\(^\circ\) C. 5ml aliquots were withdrawn once in 2hrs and an equal volume of fresh media was replaced after each sampling to maintain constant volume. The samples were spectrophotometrically analyzed at wavelength maxima of 220nm.

**Visual examination**
The complex was suspended in buffer and a drop was placed on a slide. Microscopic view of the complex was observed under 10X10 magnification.
Scanning Electron microscopy
SEM gives a three dimensional image of the vesicles. The samples were examined at a suitable accelerating voltage of 15.0KV.

Vesicle size and Zeta potential
The vesicle size and zeta potential were determined by using Horiba SZ 100Z Zetasizer.

FTIR studies
The compatibility between the drug and the selected lipid and other excipients were evaluated using FTIR. The FTIR of pure drug MCZ and formulation was determined to detect any major interference.

Preparation of phytosomal gels
Phytosomal gels were prepared using the optimized MCZ loaded phytosomal complexes. Different gels were formulated by cold mechanical method as per the composition given in Table 2. The required quantities of polymer HPMC K15, HPMC K100, HPMC E50 and Carbopol 934 were weighed. Weighed polymers were added slowly in the beaker containing distilled water with continuous stirring at 400-600 rpm. The mixture was stirred continuously for 1h until a clear gel was formed. Accurately weighed MPC’s containing MCZ equivalent to 2gm were dissolved in sufficient volume of methanol and the methanolic solution of drug was added slowly with stirring (400-600 rpm) in the previously prepared polymer gel. Triethanolamine (0.5%) was added to neutralize the pH. Methyl paraben was added as a preservative. The final quantity was made up to 100gm with distilled water. The prepared gel was kept for 24h and were filled into glass vials and stored in a refrigerator at a temperature of 4 to 8°C.

Evaluation of Phytosomal gels
All the formulated gels were evaluated for homogeneity, pH, drug content, viscosity and invitro drug diffusion studies.

Homogeneity
All developed gels were tested for homogeneity by visual inspection. They were tested for their appearance and presence of any aggregates.
Measurement of pH
The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values were calculated.

Drug content
1 g of the prepared gel was mixed with 100ml of suitable solvent. Aliquots of different concentration were prepared by suitable dilutions after filtering the stock solution and absorbance was measured. Drug content was calculated using the equation \( y = 0.09x - 0.006 \) which was obtained by linear regression analysis of calibration curve.

Viscosity study
The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer at 5, 10, 50 and 100 rotations per minute.

In vitro Diffusion studies
The diffusion studies of the prepared gels were carried out in Franz diffusion cell using Gelatin membrane of pore size 3µ and thickness 250µm. Gel sample (0.5g) was taken on gelatin membrane and the diffusion studies were carried out at 37 ± 1° using 250 ml of phosphate buffer (pH 6.8) as the dissolution medium. 5ml aliquots were withdrawn periodically at 1, 2, 3, 4, 5, 6, 7 and 8 h and each time was replaced with equal volume of fresh dissolution medium after each sample withdrawal. The samples were analyzed for drug content at 220nm using phosphate buffer pH 6.8 as blank.

Stability studies of Phytosomal gels
Optimized gel formulation of Miconazole was kept at a temperature of 40° C and 40° C for 90 days and evaluated for change in pH, viscosity and drug content.

RESULTS AND DISCUSSION
Microscopical examination
Examination by light microscopy showed clearly that in all formulations of MPC’s there was vesicle formation indicating that all the three methods A,B and C in all drug: phospholipid ratios of 1:1, 1:2, 1:4 and 1:6 favourably resulted in the formation of phytosomal complexes. Fig.1 showed spherical shape of the vesicles which were found to be
more diffuse and showed no aggregation in formulations of 1:1 and 1:6. Therefore, these two were selected as suitable candidates for further studies.

![Image](image1.png)

**Figure 1.** Image of Miconazole phytosomal complexes as viewed under 10x10 magnification.

**Determination of percentage Drug Entrapment Efficiency**

The percentage drug entrapment efficiency was determined by ultracentrifugation technique.

![Graph](graph1.png)

**Figure 2**  % Drug Entrapment Efficiency of MPC’s - MP1A to MP6C.

The % Drug Entrapment Efficiency of MP formulations varied in the range of 54.3 % (MP1B) to 70.7 % (MP6C). In all formulations, method of loading of the drug into the phytosomal complex caused a variation of 2.3 to 4.7% in drug entrapment and the results indicated that Method C facilitated slightly higher entrapment of drug. Also, as seen in Fig.2 entrapment of MCZ increased significantly by 16.4% as the drug: phospholipid ratio was increased from 1:1 to 1:6, the increase in lipid providing a greater hydrophobic matrix for entrapment of MCZ.
In vitro drug dissolution studies

In vitro drug dissolution studies of all MPC’s was carried out using USP dissolution apparatus in PB pH 6.8 for 12 hrs.

![Figure 3 Invitro drug release profiles of Miconazole loaded Phytosomal complexes.](image)

Drug release data of all formulations indicated in Fig.3 revealed that drug release was high in formulations developed by Method B. The drug release of MP1B, MP2B, MP4B and MP6B were determined to be 95.10%, 96.51%, 90.14% and 86.10% respectively. All the formulations showed an initial burst release in the first two hours of 27.5% in MP1B and 20.0% in MP6B formulations. MP6B formulation exhibited a controlled release of MCZ for 12 hrs. Increase in drug lipid ratio resulted in controlled delivery of drug due to increased thickness of the diffusion matrix. Optimization of method of loading drug and drug: soyalecithin ratio was based on in %vitro drug release data. Of all MP formulations, MP1B and MP6B were optimized for formulation of miconazole loaded topical phytosomal gel as in the former drug release was complete in 12h and the latter exhibited controlled release.

Surface morphology

The morphology of the drug loaded phytosomal complex was determined by SEM (Scanning electron microscopy). The samples of blank SRE and MP1B phytosome when examined indicated that the formulation contained spherical vesicles, uniform in size with absence of aggregation. The drug loading of MCZ in the blank SRE Phytosomes can be clearly observed in Fig.5 (SEM of MCZ loaded SRE phytosome).
FTIR studies

FTIR spectroscopy reveals any interaction between various functional groups present in drug and excipients. In the present study, the compatibility between the drug, SRE, lipid and other excipients were evaluated using FTIR peak matching method. The FTIR spectra Fig. 6, 7 and 8 of MCZ, SRE and MP1B respectively were compared. All characteristic peaks of MCZ and SRE of C-Cl, C=C, C-O, C-N, -O-H and C=O were retained in the formulation and were observed at 638.46cm⁻¹, 1612.54cm⁻¹, 1089.82cm⁻¹, 1375.58cm⁻¹, 3400cm⁻¹ and 1734.06cm⁻¹ respectively. No physico-chemical interaction between MCZ, SRE and Soyalecithin was detected and MCZ and SRE were present in the pure form in the formulation.
Figure: 6 FTIR spectrum of Miconazole (pure drug).

Figure: 7 FTIR Spectrum of SRE (Plant extract)

Figure: 8 FTIR Spectrum of Miconazole phytosomes
Vesicle size
The vesicle size and zeta potential were determined by using Zetasizer (Horiba instruments). Their sizes vary between 50 nm to a few hundred µm. Smaller vesicles with a mean size of around 120nm were found to penetrate the skin better than larger vesicles close to 1micron.

The results of particle size analysis by laser diffraction as shown in Fig.9 revealed that particle sizes varied from <50 nm to 246.98 nm and the mean vesicle size in the MP1B was 191.9nm (SD ± 67.3nm) and hence it can be mentioned that the formulation would exhibit a better penetrating ability when formulated into a gel. About more than 55% particles were found to have diameter of 216 nm.

Zeta potential
Zeta potential was determined by using a Horiba SZ 100Z particle size analyzer with the measurement mode of zeta potential. The result in zeta potential of -25mV as shown in Fig.10 indicates the stability of the complex.

![Particle size distribution profile of MP1B](image-url)
Evaluation of Phytosomal gels

Phytosomal gel formulations of MP1B and MP6B were developed using different polymers in varying concentrations as shown in Table 2.

Determination of pH and percentage drug content

The results indicated that the pH of all formulations was in the range of 6.7 to 6.91.

Table 2: Formulations of phytosomal topical gels loaded with MP1B F1-F8 and MP6B F9-F10 (2% MCZ).

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Polymer</th>
<th>pH</th>
<th>Drug content</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>HPMC K15 -1%</td>
<td>6.87</td>
<td>90.22</td>
</tr>
<tr>
<td>F2</td>
<td>HPMC K100 - 1%</td>
<td>6.73</td>
<td>90.0</td>
</tr>
<tr>
<td>F3</td>
<td>HPMC E50 -1%</td>
<td>6.79</td>
<td>89.7</td>
</tr>
<tr>
<td>F4</td>
<td>Carbopol 934 0.15%</td>
<td>6.9</td>
<td>93.3</td>
</tr>
<tr>
<td>F5</td>
<td>HPMC K15 1.2%</td>
<td>6.88</td>
<td>91.1</td>
</tr>
<tr>
<td>F6</td>
<td>HPMC K100 – 1.2%</td>
<td>6.74</td>
<td>91.5</td>
</tr>
<tr>
<td>F7</td>
<td>HPMC E50 – 1.2%</td>
<td>6.7</td>
<td>90.2</td>
</tr>
<tr>
<td>F8</td>
<td>Carbopol 934-0.2%</td>
<td>6.91</td>
<td>94.2</td>
</tr>
<tr>
<td>F9</td>
<td>HPMC K100- 1%</td>
<td>6.74</td>
<td>91.3</td>
</tr>
<tr>
<td>F10</td>
<td>Carbopol 934 -0.15%</td>
<td>6.91</td>
<td>95.11</td>
</tr>
</tbody>
</table>
The percentage drug content in all MP1B formulations varied between 90.0% in F2 and 94.2% in F8 formulations. However, F8 exhibited stickiness and insufficient spreadability. F4 with drug content of 93.3% was found to be a suitable formulation. F10 of MP6B formulation showed drug content of 95.11%.

**Viscosity studies**
Results indicated that the gels exhibited a pseudoplastic flow.

**Drug diffusion studies**
The evaluation of topical gels of MP1B and MP6B has shown F2 with 57.8% and F4 with 61.97% drug release in 8 hours as formulations with a suitable matrix for controlled release.

![Figure: 11 In vitro diffusion profile of Miconazole loaded Phytosomal gels F1 to F10](image_url)

F9 and F10 showed 44.4 and 47.73% drug release in 8 hours.

From the release data shown in fig. 12, it is evident that F4 and F10 showed 92.54% and 69.73% compared to the marketed formulation (DK-Gel) which showed 98.5% drug release in 12 hours.
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Table 3: Comparative entrapment and drug release profile of SRE and MCZ in MP1B and F4

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MP1B</th>
<th>F4</th>
<th>DK-Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SRE</td>
<td>MCZ</td>
<td>SRE</td>
</tr>
<tr>
<td>% Entrapment</td>
<td>73.8</td>
<td>54.3</td>
<td>96.2</td>
</tr>
<tr>
<td>% Cumulative drug release in 12h</td>
<td>100.00±1.53</td>
<td>95.10±2.01</td>
<td>98.46±2.53</td>
</tr>
</tbody>
</table>

The results as shown in Table 3 indicated that 73.8% w/w and 54.3% w/w of SRE and MCZ were entrapped into the vesicle. This is very relevant to the study as it indicated that 54.3% miconazole was loaded into SRE-Phytosome which was selected as novel phytosomal carrier. As expected, the drug release of SRE was enhanced when complexed with Soyalecithin and miconazole exhibited a controlled delivery.

Release kinetics of F4 formulation
The release kinetics of the optimized formulation F4 were studied and the data presented in Table 4.
Table 4: Correlation coefficient values for release kinetics of sustained release of MCZ Phytosomal gel F4

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero-order</th>
<th>First order</th>
<th>Higuchi</th>
<th>Hixson</th>
<th>Korse Mayer Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td>F4</td>
<td>0.995</td>
<td>0.9874</td>
<td>0.9228</td>
<td>0.9914</td>
<td>0.93</td>
</tr>
</tbody>
</table>

Stability studies

F4 was stored at temperatures of 4°C and 40°C and these formulations when subjected to evaluation indicated that the one stored at 4°C was much stable. F4 stored at 40°C indicated slight changes in pH, drug content and viscosity as indicated in Table 5.

Table 5: Stability studies of F4 stored at 4°C±2°C/40°C±2°C

<table>
<thead>
<tr>
<th>Parameter</th>
<th>After 3 months (stored at 4°C)</th>
<th>After 3 months (stored at 40°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug content</td>
<td>Initial 93.3%</td>
<td>Final 92.26%</td>
</tr>
<tr>
<td></td>
<td>Final 90.1%</td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>Initial 6.9</td>
<td>Final 7.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Final 7.2</td>
</tr>
<tr>
<td>Viscosity at 5rpm</td>
<td>Initial 6031</td>
<td>Final 6621</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Final 6645</td>
</tr>
</tbody>
</table>

CONCLUSIONS

This study was conducted to investigate the feasibility of using phytosome as a novel carrier for transdermal drug delivery system of Miconazole. Schrebera root bark rich in polyphenols was selected and the methanolic extract was used to formulate phytosomal complex and miconazole was entrapped into it. Topically applied phytosomes can increase residence time of drug in the stratum corneum and epidermis, while reducing the systemic absorption of the drug. The collective data clearly indicated that the work carried out has achieved its objective. The study of optimized miconazole loaded phytosomal complexes revealed that miconazole was loaded in the phytosomes together with the plant extract (SRE) with comparative drug entrapment and release profiles. Topical phytosomal gels formulated exhibited controlled delivery. It can be concluded that phytosomal complexes of lecithin and polar biomolecules could serve as vehicles for loading miconazole and other such poorly water soluble drugs for controlled delivery.

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