ABSTRACT

Invasive fungal infections can be devastating, especially in immunocompromized patients. Oral administration of fluconazole (FLZ) often produces gastric irritation, heart-burn, vomiting and sometimes patient can develop ulceration resulting in less patient compliance with long term therapy.

The aim of the present study was to formulate topical microsponge-based delivery system containing FLZ for controlled release of the drug and consequently avoiding its oral side effects. Ethyl cellulose (EC) and Eudragit RS 100 based microsponges were prepared using quasi-emulsion solvent diffusion method. The effect of formulation variables such as drug to polymer ratio, polymer type, polyvinyl alcohol (PVA) concentration and type of internal phase on the physical characteristics of the microsponges was examined using $2^4$ factorial design. Results revealed that FLZ loading and microsponge particle size were increased with increasing the polymer fraction. Moreover, EC significantly increased the drug entrapment efficiency (EE%) and the mean particle size. There was a reverse proportionality between the PVA concentration and both the EE% and the mean particle size. Regarding the solvent type, ethanol significantly increased the EE% and the particle size when compared to methylene chloride. A selected FLZ microsponge (F3, containing FLZ and EC in 1:1 ratio and prepared using 0.75% PVA and methylene chloride) was incorporated in carbopol gel formulation and evaluated for its in vitro release characteristics. The developed microsponges were spherical and porous. There was no interaction between drug and polymer molecules. The drug release through cellulose dialysis membrane showed flicker release pattern. Further antifungal activity and in vivo animal studies for the obtained formula are recommended.

Keywords: Fluconazole, microsponge, Controlled release, Porous structure, Particle size, Entrapment efficiency

INTRODUCTION

Microsponges are polymeric delivery systems composed of porous microspheres. They are tiny sponge-like spherical particles that consist of myriad of interconnecting voids within a non collapsible structure having large porous surface. Microsponges are prepared by several methods utilizing emulsion systems as well as by suspension polymerization in a liquid-liquid system. The most common emulsion system used is oil in water with the suspension polymerization in a liquid-liquid system. The most common emulsion system used is oil in water with the microsponges being produced by the emulsion solvent diffusion method.

Microsponge delivery is one of the techniques used to slow down the release of active ingredients from topical formulations; moreover, microsponges may enhance stability, reduce side effect, and modify drug release favorably.

The incidence of mycoses especially superficial fungal infections is increasing and according to a recent report more than 25% of the world’s population is affected. Disease progression is more rapid and severity increased in patients with compromised immune function. Selective attention has been paid to the triazole derivatives due to their broad spectrum antifungal activity and low toxicity.

Fluconazole (FLZ) is a synthetic triazole antifungal drug for the treatment of superficial and systemic fungal infections with possible drawback of itching in topical therapy. FLZ is able to produce a high selective inhibition of the fungal cytochrome P450 system and also an inhibition of the C-14 a esterol demetilation process, avoiding in this way membrane ergosterol synthesis. It has shown activity against yeasts, yeast-like fungi, dimorphic fungi, Candida spp., Blastomyces dermatitidis, Cryptococcus neoformans, Epidermophyton spp., Histoplasma, Microsporum spp., and Trichophyton spp., and it has been extensively used in the treatment of dermatophytoyses by oral administration. Recently, FLZ has been used for the treatment of some Leishmania specimens such as cutaneous leishmaniasis. However, high doses (100-200 mg/day for 2-6 weeks) were used in these studies, which led to potential side effects varying from headache, nausea to liver dysfunction and hepatic failure. Oral administration of FLZ often produces gastric irritation, heart-burn, vomiting and sometimes patient can develop ulceration and there is less patient compliance with long term therapy. Furthermore, oral FLZ is reported to interact with a number of medications including oral hypoglycemics, coumarin-type anticoagulants, cyclosporins, terfenadine, theophylline, phenytoin, rifampin and astemizole.

In order to minimize these adverse effects, topical delivery of FLZ in cutina lipogel gel microemulsion and emulgel has been studied. Topical therapy is desirable since, in addition to targeting the site of infection, it reduces the risk of systemic side effects thus the aim of the present investigation was to design novel microsponges as carriers for topical delivery of FLZ. This investigation consisted of preparation, and evaluation of microsponges and incorporation of the selected microsponges in a gel to obtain cosmetically acceptable product for prolonged duration of time and less side effects. A factorial design was adopted to study the effect of different factors namely polymer type (Eudragit RS100 and ethyl cellulose), type of solvent (ethanol or methylene chloride), drug to polymer ratio (1:1, 1:2) and polyvinyl alcohol (PVA) concentration (0.5% and 0.75%) on entrapment efficiency (EE), and mean particle size.

Table 1: The $2^4$ factorial design for the preparation of fluconazole microsponge systems

<table>
<thead>
<tr>
<th>Variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug:polymer ratio</td>
<td>1:1, 1:2</td>
</tr>
<tr>
<td>Polymer type</td>
<td>Ethyl cellulose, Eudragit RS100</td>
</tr>
<tr>
<td>Solvent type</td>
<td>Ethanol, Methylene chloride</td>
</tr>
<tr>
<td>PVA concentration</td>
<td>0.5%, 0.75%</td>
</tr>
</tbody>
</table>
**Preparation and characterization of FLZ microsponges**

All microsponges were prepared by quasi-emulsion solvent diffusion method with slight modification **1** using 2 factorial design (Table 1). The organic internal phase was consisted of EC or Eu RS100 dissolved in 10 ml (dichloromethane or ethanol). The calculated amount of FLZ was added gradually with stirring. The resulting solution was then poured into 0.5% or 0.75% (w/v) of PVA solution (Table 1). The organic internal phase was consisted of EC or Eu RS100 was obtained from Degussa-Rhom GmbH and Co., Germany, poly vinyl alcohol (PVA) mol wt 88000 was obtained from (Acros Organics New Jersey USA), Dichloromethane, ethanol, potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from (Adwic, El-Nasr Chemical Co., Cairo, Egypt), carbopel 940 was purchased from (Sigma-Aldrich, Buchs). All other chemicals used were of analytical grade.

**Method**

**Preparation and characterization of FLZ microsponges**

**Preparation of the microsponges**

The actual drug content (%) = \(\frac{M_{\text{act}}}{M_{\text{ms}}} \times 100\)

The EE (%) was calculated according to the following equation:

\[
\text{Entrapment efficiency} = \left(\frac{M_{\text{act}}}{M_{\text{the}}}\right) \times 100
\]

Where \(M_{\text{act}}\) is the actual FLZ content in the weighed quantity of the microsponge, \(M_{\text{ms}}\) is the weighed quantity of powder of microsponges, and \(M_{\text{the}}\) is the theoretical amount of FLZ in microsponge calculated from the quantity added during preparation.

All the experiments were performed in triplicate and the mean of the values was reported.

**Particle size studies**

Particle size studies were carried out using laser light scattering technique using Mastersizer 2000 (Malvern Instrumets Ltd, Worcestershire, UK).

**Dissolution behavior of microsponges**

The drug release tests of the microsponges were carried out for 6 h at 100 rpm by the paddle method. The temperature of the dissolution medium was controlled at 32±1 °C. The microspheres equivalent to 50 mg of FLZ were weighed. The dissolution medium was 150 ml of phosphate buffer (pH 5.5) to keep the sink condition for the drug. Three milliliters of the dissolution medium was sampled at certain intervals, and fresh dissolution medium was simultaneously replaced in the apparatus to keep the volume constant. The withdrawn samples were filtered with a membrane filter (0.45 μm), and the filtrate was assayed spectrophotometrically at 260 nm.

**Differential scanning calorimetry**

Thermal analysis of FLZ, EC, physical mixture of FLZ and EC and the selected FLZ microsponge (F3) were scanned at a rate of 10°C/min on a Shimadzu DT-40 Thermal Analyser between 30ºC and 300ºC under dynamic nitrogen atmosphere. The DSC thermograms were recorded.

**Fourier-Transform Infrared Spectra (FT-IR)**

FT-IR spectra of finely powdered pure fluconazole, physical mixture of FLZ and EC as well as the selected FLZ microsponge formulation (F3) were recorded on a FT-IR spectrophotometer (Shimadzu, Kyoto, Japan) by potassium bromide (KBr) disk pellet method.

**Scanning electron microscopy (SEM)**

The morphology of microsponges was examined using a scanning electron microscope (GEOCL 5400, USA) operating at 20 kV. Dried microspheres were coated with gold-palladium alloy for 45 s under an argon atmosphere before observation. SEM photograph was recorded at magnification of ×500 and 1500.
Preparation and characterization of microspongic FLZ gel

Preparation of the topical carbopol gel

The gel was prepared according to Saboji et al.27 Exactly 0.35 g of carbopol 940 was dissolved in 65 ml water using propeller. In another beaker, exactly weighed FLZ microspheres (F3) equivalent to 0.5% FLZ was dissolved in 15 ml ethanol and added to the carbopol solution under continuous stirring; then, 15 ml PEG 400 were added and the carbopol solution was neutralized by slowly adding 5 ml triethanolamine with constant stirring until gel formation.

In vitro release of FLZ microspongic gel

The in vitro release of FLZ microspongic gel was performed by the membrane diffusion technique using SpectraPore dialysis membrane (12,000-14,000 molecular weight cut off, Spectrum Laboratories Inc., Rancho Dominguez, Canada) with an effective diffusion area of 3 cm². Exactly, one gram gel containing the equivalent of 0.5% FLZ in microsponge was transferred into the dialysis membrane which was previously soaked overnight in the dissolution medium (freshly prepared phosphate buffer pH 5.4). The membrane was tied from both ends and attached to the paddle of a USP 32 dissolution test apparatus II (VanKel VK 700, USA). The paddle was suspended in 150 ml of dissolution medium maintained at 32 ± 1°C, and stirred at 100 rpm. Aliquots, each of 5 ml volume were withdrawn periodically at predetermined time interval of 15, 30, 60, 120, 180, 240, 300, 360 min and replaced by an equal volume of the fresh medium to maintain sink conditions. The withdrawn samples were filtered with a membrane filter (0.45 μm), and assayed spectrophotometrically at 260 nm. The experiment was conducted independently in triplicate.

Several mathematical models attempt to correlate dissolution profiles with the mechanisms of drug release from the drug delivery system.28, 29 In this work, zero order, first order, Higuchi, and Korsmeyer-Peppas models were applied to analyze the release profile of FLZ from the prepared microspongic gel.

RESULTS AND DISCUSSION

Preparation and characterization of FLZ microsponges

The prepared microsponge delivery systems were made of EC and Eu RS 100 which are biologically inert, non-irritating, non-mutagenic, non-allergenic, non-toxic and non-biodegradable polymers. As a result, the human body cannot convert them into mutagenic, non-allergenic, non-toxic and non-biodegradable polymers. The prepared microsponge delivery systems were made of EC and Eu RS 100 which are biologically inert, non-irritating, non-mutagenic, non-allergenic, non-toxic and non-biodegradable polymers. As a result, the human body cannot convert them into mutagenic, non-allergenic, non-toxic and non-biodegradable polymers.

The mean particle size of the formulations was between 28-116.5 μm. There was a significant difference between formulations (p < 0.05) in both the PY and the actual FLZ content.

Effect of formulation variables on EE% of FLZ microsponges

Concerning the effect of formulation variables on the EE% of FLZ in the microsponges (Fig. 1), the results of the ANOVA study according to the 2 factorial design revealed that the FLZ loading was increased with increasing the polymer fraction. This result may be attributed to two aspects. First, the increase in the viscosity of the internal phase as a result of the increase in polymer concentration can impede drug mobility in the droplets which was observed as an increase in the EE%. The previous result was in full accordance with the results by Biswal et al. on encapsulation of Losartan potassium using Eu RS 100.22

Second, the entrapment efficiency may be improved simply by the greater proportion of polymer with respect to the amount of drug available.23 Hence, more polymer particles can entrap more drug particles.

Concerning the polymer type EC significantly increased the drug EE% when compared to Eu RS 100. This may be attributed to the increased viscosity of the internal phase containing the EC polymer, reducing the drug mobility outside the formed droplets, and hence entrapping larger amount of FLZ. The viscosity of internal phase of 10 ml methylene chloride containing 1 g Eu RS100 or 1 g EC was determined with rotation viscometer by using small sample adapter, S18 spindle for dispersed phase at the rate of 50 rpm at 25°C. The results showed that the apparent viscosities of the Eu RS100 solution was 4.8 cp compared to 17.6 cps for the EC containing solution. This result is in full accordance with those obtained during the characterization of S- fluorouracil loaded microspheres prepared using EC and Eu RS 100.27

Concerning the PVA concentration, the negative influence of PVA concentration on the EE% of FLZ may be attributed to the nonionic nature of the emulsifier. The molecules may associate away from the oil water interface at higher concentration forming an alternative hydrophobic region which can dissolve some portions of the drug resulting in a reduction of the EE%.24

Regarding the solvent type, ethanol significantly increased the EE% when compared to methylene chloride. This may be due to the higher boiling point of ethanol (78.4°C) compared to methylene chloride (40°C), so ethanol would evaporate more slowly than methylene chloride. The lower organic solvent evaporation rate led to a lower solvent front kinetic energy, which accordingly decreased the rate of diffusion of the solvent from the inner to the outer phase so increasing the chance for entrapping the drug inside the polymer.

Effect of formulation variables on particle size of FLZ microsponges

Fig. 2 shows that changing drug:polymer ratio has a considerable effect on the size of the prepared FLZ microsponges. Increasing the polymer fraction significantly increased the particle size (p<0.0001). This could probably be due to increasing the amount of polymer available per microsponge, hence larger particle size was obtained. Previously published data have shown similar findings.25, 26 Another explanation of this enlargement arise from the more viscous polymer solution resulting from increasing the polymer fraction. This increase in viscosity hindered the breaking of emulsion into smaller droplets resulting in microsponges with larger particle size.29

Concerning the polymer type EC significantly increased the mean particle size when compared to Eu RS100. The mean particle size of microsponges usually increased with increasing viscosity of the dispersed phase as the formed globules can be hardly divided into smaller particles, hence larger droplets were formed and the mean particle size was increased.25 It was found from the previous viscosity measurement that EC was more viscous than Eu RS 100.

Concerning the PVA concentration, there was a reverse proportionality between the PVA concentration and the mean particle size. This may be due to decreasing the surface tension of the continuous phase upon increasing the surfactant concentration with a resultant diminution of the particle size.20, 21

Concerning the solvent type, ethanol significantly increased the particle size. This may be due to the lower boiling point of methylene chloride (40°C) compared to ethanol (78.4°C). Thus, methylene chloride would evaporate more rapidly. The higher organic solvent evaporation rate led to a higher solvent front kinetic energy, which accordingly increased the rate of diffusion of the solvent from the inner to the outer phase (the critical parameter determining the particle size) and resulted in smaller particles.21
Table 3: Production yield, actual drug content, entrapment efficiency, and mean particle size of fluconazole microsponge (n=3)

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Production yield (% ± S.D.)</th>
<th>Theoretical drug content (%)</th>
<th>Actual drug content (% ± S.D.)</th>
<th>Entrapment efficiency (% ± S.D.)</th>
<th>Mean particle size (µm ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>25.0 ± 1.07</td>
<td>50</td>
<td>26.37 ± 0.99</td>
<td>52.74 ± 1.06</td>
<td>39.00 ± 2.70</td>
</tr>
<tr>
<td>F2</td>
<td>9.03 ± 0.92</td>
<td>50</td>
<td>17.94 ± 1.04</td>
<td>35.89 ± 1.26</td>
<td>28.00 ± 1.14</td>
</tr>
<tr>
<td>F3</td>
<td>50.0 ± 0.73</td>
<td>50</td>
<td>37.06 ± 0.85</td>
<td>74.12 ± 0.72</td>
<td>42.50 ± 1.13</td>
</tr>
<tr>
<td>F4</td>
<td>29.2 ± 1.01</td>
<td>50</td>
<td>22.40 ± 1.15</td>
<td>44.80 ± 1.47</td>
<td>35.50 ± 3.70</td>
</tr>
<tr>
<td>F5</td>
<td>9.03 ± 0.92</td>
<td>50</td>
<td>17.94 ± 1.04</td>
<td>35.89 ± 1.26</td>
<td>28.00 ± 1.14</td>
</tr>
<tr>
<td>F6</td>
<td>50.0 ± 0.73</td>
<td>50</td>
<td>37.06 ± 0.85</td>
<td>74.12 ± 0.72</td>
<td>42.50 ± 1.13</td>
</tr>
<tr>
<td>F7</td>
<td>60.0 ± 1.22</td>
<td>33.33</td>
<td>20.93 ± 0.95</td>
<td>62.80 ± 0.66</td>
<td>36.50 ± 3.53</td>
</tr>
<tr>
<td>F8</td>
<td>30.0 ± 1.14</td>
<td>50</td>
<td>21.21 ± 1.10</td>
<td>42.42 ± 1.14</td>
<td>93.00 ± 2.24</td>
</tr>
<tr>
<td>F9</td>
<td>11.1 ± 0.99</td>
<td>50</td>
<td>10.48 ± 0.95</td>
<td>20.96 ± 1.04</td>
<td>61.00 ± 3.54</td>
</tr>
<tr>
<td>F10</td>
<td>50.0 ± 1.02</td>
<td>50</td>
<td>26.44 ± 0.99</td>
<td>52.88 ± 0.92</td>
<td>84.00 ± 1.41</td>
</tr>
<tr>
<td>F11</td>
<td>79.0 ± 1.10</td>
<td>50</td>
<td>15.19 ± 1.12</td>
<td>30.36 ± 1.31</td>
<td>47.50 ± 2.12</td>
</tr>
<tr>
<td>F12</td>
<td>85.0 ± 0.89</td>
<td>33.33</td>
<td>21.26 ± 0.97</td>
<td>63.85 ± 1.01</td>
<td>116.50 ± 4.94</td>
</tr>
<tr>
<td>F13</td>
<td>6.33 ± 1.09</td>
<td>33.33</td>
<td>5.20 ± 1.11</td>
<td>15.60 ± 0.99</td>
<td>56.50 ± 2.12</td>
</tr>
<tr>
<td>F14</td>
<td>40.0 ± 0.99</td>
<td>33.33</td>
<td>10.49 ± 0.87</td>
<td>31.47 ± 0.76</td>
<td>91.50 ± 2.12</td>
</tr>
<tr>
<td>F15</td>
<td>12.1 ± 1.11</td>
<td>33.33</td>
<td>9.98 ± 1.00</td>
<td>29.94 ± 1.13</td>
<td>54.00 ± 5.41</td>
</tr>
<tr>
<td>F16</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Fig. 1: Line plots representing the effect of formulation variables on the fluconazole microsponge entrapment efficiency.

Dissolution behavior of microsponges

The FLZ release from the microsponge formulations are shown in Fig. 3. The figure shows that, generally, the release rate was high during the first two hours then the microsponges were able to sustain the release of FLZ for more than 6 h in most formulations. FLZ release kinetics of microsponges on the basis of the highest $r^2$ can be explained by Higuchi diffusion mode (data not shown). Based on the above characterization F3 (having acceptable yield, particle size, high entrapment efficiency and slow release profile) was chosen as candidate formula and was subjected to further investigations.

Differential scanning calorimetry and fourier-transform infrared spectra (FT-IR)

The DSC thermogram of pure FLZ showed a clear endothermic peak associated with crystal melting at 140.1°C (Fig. 4). However, the DSC
The thermogram of FLZ-loaded EC microsponge (F3) showed a very weak wide melting endotherm between 150°C and 200°C at about 175°C apart from a broad signal around 45–60°C. The amorphous blank polymer did not show any fusion peak or phase transition, however, showed a broad signal around 45–60°C due to a partial loss of residual humidity. The thermal behavior indicated that the polymers probably inhibited the melting of drug crystals close to its reported melting point (140.1°C)\(^{35}\). Our results are in accordance with Maiti et al.\(^{36}\), who formulated oral FLZ microspheres.

The thermal behavior suggested that the drug was able to disperse almost homogenously in the microsponge and it was realized that the degree of crystallinity of FLZ was significantly reduced in the microspheres when compared to its physical mixture with the polymer or the pure FLZ.

The compatibility of FLZ in this formulation was evaluated qualitatively through FTIR analysis. In the FTIR spectrum of pure FLZ (Fig. 5), the frequencies associated with C–O stretching vibration consistent with a tertiary alcohol, aromatic C–F stretching vibrations, aromatic C=C stretching vibrations, and aromatic C–H stretching vibrations were identified at 1140.06, 1275.92, 1619.38, and 3117.11 cm\(^{-1}\), respectively\(^{37}\). Similar vibrational peaks of FLZ were detected in the physical mixture and in the spectrum of FLZ-loaded microsponges with minor differences in frequencies (Fig. 5). This suggested that FLZ was compatible with EC and it was apparently stable in the microsponges.

**Scanning electron microscopy**

The scanning electron photograph of the microsponges (F3) is shown in Fig. 6. It was observed by SEM analysis that the microsponges were finely spherical and uniform. Microscopy studies showed that FLZ microsponges contained pores. The pores were induced by the diffusion of the solvent from the surface of the microparticles\(^{38}\). The appearance of the particles was such that they were termed microsponges. These findings are similar to the results reported previously\(^{39}\).
Fig. 3: Release profile of fluconazole in phosphate buffer pH 5.5 from microsponges containing a) drug-polymer in 1:1 ratio and 0.75% PVA, b) drug-polymer in 1:2 ratio and 0.75% PVA, c) drug-polymer in 1:1 ratio and 0.5% PVA, d) drug-polymer in 1:2 ratio and 0.5% PVA.

Fig. 4: DSC thermogram of 1) ethylcellulose, 2) F3 microsponge formulation, 3) pure fluconazole, 4) fluconazole-ethylcellulose physical mixture
Preparation and characterization of FLZ microspongic gel

The in vitro release profile of FLZ microspongic gel was depicted in Fig. 7. The total amount of drug release was 57.28±5 % observed after 6 h. From the logarithmic plot of the release data log Q versus log t, the diffusion exponent (n) and the kinetic constant (k) have been calculated, as shown in Table 4. The results showed that n value of F3 microsponges loaded gel are less than 0.5. This indicates that the mechanism of drug release is Fickian diffusion, and is controlled by the porosity of the microsponges, as shown by the SEM images. Similar results were obtained by Nokhodchi et al. for microsponges containing benzoyl peroxide.

Fig. 5: FT-IR spectra of 1) pure fluconazole, 2) fluconazole-ethylcellulose physical mixture, 3) F3 microsponge formulation.

Fig. 6: SEM of fluconazole loaded microsponge formulation coaded F3 under, A) x 270, B) x 550, C) x 1500
Table 4: Kinetic treatment of the release data of fluconazole from F3 microsponge loaded carbopol gel

<table>
<thead>
<tr>
<th>Release Order</th>
<th>Correlation Coefficient (r)</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero Order</td>
<td>0.9471</td>
<td>0.0925</td>
<td>26.4988</td>
</tr>
<tr>
<td>First Order</td>
<td>0.9651</td>
<td>0.0007</td>
<td>1.8716</td>
</tr>
<tr>
<td>Diffusion</td>
<td>0.9747</td>
<td>24.5010</td>
<td>-7.9573</td>
</tr>
<tr>
<td>Peppas</td>
<td>0.9801</td>
<td>0.2877*</td>
<td>1.0169**</td>
</tr>
</tbody>
</table>

*n value

** k

Fig. 7: In vitro release profile of fluconazole from microspongy carbopol gel. Each value represents mean± SD (n=3).

CONCLUSION
Microsponge based delivery system has been developed using quasi-emulsion solvent diffusion method to provide a sustained release medication for topical delivery of fluconazole. The drug entrapment efficiency and the size of the prepared microsponges were affected by the drug-polymer ratio, polymer type, solvent type, and the emulsifier concentration. Results of the present study demonstrated that the optimum formulation consisted of the hydrophobic polymer (ethyl cellulose), methylene chloride as a solvent and 0.75% PVA emulsifier. A fickian diffusion which is controlled by the porosity of the microsponges, might be the mechanism of the drug release from the carbopol gel loaded with the selected formulation.

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