Sorbitan Monostearate Based Organogels for Topical Delivery of Clotrimazole

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ABSTRACT
Organogel is a semi solid preparation in which the apolar phase gets immobilized within spaces of the three dimensional network structure formed due to physical interactions between the self assembled structures of gelators. The current study describes the development of Sorbitan monostearate (Span-60) based organogels using sunflower oil (SO) as the apolar solvent. Different compositions of organogels were prepared by varying the concentrations of the organogelator Span 60. Tween 20 was added to improve the gel stability. The formulated organogels were characterized by visual inspection, gel-sol transition studies, pH, microscopic analysis, drug content analysis, spreadability and rheological studies. Clotrimazole an anti-fungal drug was incorporated within gels and their in vitro release behaviour and antifungal efficiency against Candida albicans were studied. The microscopy of the organogels suggested that a three-dimensional network of needle aggregates of gelator is responsible for immobilising the solvent. The gel-sol transition study indicated that as the concentration of the gelator was increased, there was a subsequent increase in the transition temperature. The drug release profile of all the formulations were subjected to various kinetic models and the mechanism of drug release was found to be non-fickian diffusion controlled process. As the concentration of Span 60 increased, there was a proportionate decrease in drug release pattern. The pH range of the organogels was simulated to the skin pH conditions and all the formulations were able to restrict the growth of Candida albicans efficiently as comparable to the marketed product. Based on the results, the developed organogels can be used as an efficient drug carrier for the topical delivery of Clotrimazole.

Keywords: Organogel, Clotrimazole, Sorbitan monostearate, Anti-fungal, Topical delivery.

INTRODUCTION
The word “gel” has been derived from the Greek word, gelatus, which means “to immobilise”. In general, gels contain two components, one of which is a liquid and the other, a solid. The solid components form a three-dimensional networked structure, which helps in immobilising the liquid component. The solid components are often regarded as gelators. Depending on the polarity of the liquid component, the gels may be regarded either as hydrogels (Polar phase) or organogels (Apolar phase)¹. The immobilization of the liquid within the three-dimensional network structure has been attributed to the surface active phenomena amongst the solid and the liquid phases. As the name organogel suggests, organogels contain apolar solvents (e.g. kerosene oil, sunflower oil, mustard oil, mineral oil) as the continuous phase¹. Organogels may be developed by two mechanisms, namely, fluid-fibre mechanism and solid-fibre mechanism. The fluid fibre mechanism involves the development of reverse-miscellar structures when water is added to a solution of surfactant in apolar solvent. The reverse miscellar structures undergo modification when further amount of water is added to the system, and in turn, undergoes physical interaction amongst each other to give rise to a three-dimensional network structure. Since, the three dimensional structures which are formed are made up of basic structures which contain a polar liquid phase, the mechanism of formation is regarded as fluid-filled fibre mechanism. The networked structures formed in turn, help in immobilising the apolar solvent. On the other hand, solid-fibre mechanism deals with the dissolution of solid organogelators in hot apolar solvent to give rise to a homogeneous solution. The solution, so obtained, is cooled down to room temperature. This results in the change in the solubility parameter of the
organogelator, thereby, resulting in the precipitation of the organogelators. The precipitated organogelators start forming fibre-like structures that undergo interaction amongst each other to form a three-dimensional networked structure. The fibres, which are formed, are basically made up of organogelator molecules and do not contain any liquid phase. Hence, these types of organogels are said to be formed by solid-fibre mechanism. Similar to the fluid-fibre mechanism, the three-dimensional structure formed by the solid organogelators help in immobilising the liquid apolar phase.

Span 60 (Sorbitan monostearate) a hydrophobic non-ionic surfactant has long been used in various cosmetic, food and pharmaceutical applications due to its ability to act as a structuring agent. It has been found that the Span 60 based matrices have the ability to modulate the release property of the bioactive agents (e.g. oligopeptides, polypeptides, cyclosporin, and salicylic acid). The matrices of the Span 60 based formulations are formed by solid-fibre mechanism. The gel structures of these organogels are also found to be thermodynamically stable for a prolonged period of time. Sorbitan monostearate gels a number of organic solvents such as hexadecane, isopropyl myristate, and a range of vegetable oils.

Clotrimazole is a synthetic imidazole derivative having a broad spectrum of fungicidal activity, being effective against both dermatophytes and yeast-like fungi. It is known to be potent and a well tolerated topical anti fungal agent. It inhibits biosynthesis of the sterol ergostol, an important component of fungal cell membranes. Its action leads to increased membrane permeability and apparent disruption of enzyme systems bound to the membrane. The biological half-life of Clotrimazole is about 2 h. Oral clotrimazole is used to treat and prevent yeast infections of the mouth and throat.

In the present study Clotrimazole organogels has been prepared by solid fibre mechanism using a low molecular weight organogelator such as Span 60. This mechanism involves the dissolution of the solid organogelator in an organic solvent at high temperature and subsequent cooling at room temperature. During the cooling process the organogelator precipitates as solid elongated fibres which entangle together to form a three dimensional structure that immobilizes the organic solvent. Polysorbate 20 (Tween 20) was added to improve gel stability.

EXPERIMENTAL

Materials

Clotrimazole was purchased from Yarrow Chem, Mumbai, India. Span-60 (Sorbitan monostearate) was purchased from Rolex Laboratory, Mumbai, India. Tween 20 was purchased from Merck India, Mumbai. Edible refined sunflower oil (SO) was purchased from the local market.

Methods

A. Preparation of organogel

Accurate amount of Clotrimazole was dissolved in the sunflower oil (SO). To this varying proportions of Sorbitan monostearate (Span 60) and Tween 20 (2%w/w) were added. The mixture was heated on a water-bath at 60°C until a homogeneous clear solution was obtained and then allowed to cool by standing at room temperature so as to allow gel formation. The samples were regarded as organogels, if upon cooling, the solution mixture failed to flow when the culture bottles were inverted.

The proportion of Span-60 for all the formulations was selected after determination of critical gelation concentration (CGC). The CGC of the organogelator was found to be 17% (w/w). The organogels used for further analysis have been tabulated in Table 1.

B. Evaluation studies

Physical appearance

The prepared organogels formulations were observed for their colour, homogeneity, consistency, appearance and texture.

pH determination

The pH of the organogels was measured using a Digital pH analyser (ELICO INDIA -Model LI 613).

Drug-polymer compatibility studies

Compatibility studies of drug, Span 60 and the mixture of both drug and Span 60 were carried out using Fourier Transform Infrared Spectrophotometer (Shimadzu FT-IR 8400-S) in the range of 400-4000cm-1 by KBr disc method.

Rheology

The rheological properties of the organogels were determined using a Brookfield's viscometer (Model DV II+). The viscosity values were determined at 5, 10, 20, 50 and 100 rpm at 25°C using spindle No. 1. All the determinations were made in triplicate and the
results obtained are expressed as mean values.

**Spreadability**
The spreadability of each sample was evaluated in triplicate by using a fabricated spreadability apparatus which consisted of two glass plates. 0.5 g of sample was placed on the lower plate and the upper plate was placed on the top of the sample. Force was generated by adding increasing weight slowly at 1 min. interval into the pan connected to the upper plate. Each sample was tested at least three times and exerted weight and the mean values of spread surface area on the lower plate were calculated.

Spreadability is measured as:

\[
S = M \times \frac{L}{T}
\]

Where, \(M\) = weight to be taken, \(L\) = length of the slide, \(T\) = time taken

**Drug content determination**
Amount equivalent to 25 mg of drug was taken and dissolved in 25 ml of methanol. Further dilutions were made using citro-phosphate buffer pH 5.5. The drug content was estimated using UV-Visible spectrophotometer (UV-1601, Shimadzu) at 260nm.

**Microscopic study**
The microstructure of the organogels were analysed under compound light microscope (CH20i, Olympus India Pvt. Ltd., India).

**Gel-sol transition temperature (T_{gel-sol})**
Gel-sol transition temperature was found out by incubating the organogels in a constant temperature water-bath, at temperatures ranging from 25 ºC to 60 ºC. The temperature of the water bath was increased with an increment step of 5 ºC and the gels were kept at the corresponding temperature for 5 minutes. In order to determine any induced flow, the organogels were analysed by inverting test-tube method before further increasing the temperature of the water bath. The temperature, at which the gels started to flow, when the test tubes were inverted, was noted as the gel-sol transition temperature.

**Extrudability**
The organogels were filled into collapsible tubes, crimped and the extrudability of the formulation from the packed material was tested.

**Microbiological analysis**
It was performed by Cup Plate method. Micro organism used for the study was fungal strains of *Candida albicans* and the media used was Sabouraud Dextrose Agar media. Organisms were swabbed on the solidified media and bores of 1cm diameter were punched into it. A standard solution of Clotrimazole was used as the reference. All the formulations were placed in their respective bores and the petri plates were incubated at 25ºC for 24 hrs. After incubation, the plates were observed for growth inhibition and reported with reference to standard.

**In-vitro drug release study**
In-vitro dissolution studies were carried out using Keshary Chien cell. Accurately weighed quantity of the organogel was introduced into the donor compartment. The receptor compartment contained 13ml of citro-phosphate buffer pH 5.5. Both the compartments were separated by a treated cellophane membrane. The receptor fluid was maintained at 37±0.5ºC and stirred at 50 rpm. At specified intervals of time, 1 ml of the sample was withdrawn and analysed for drug content at 260nm against a blank. The *in vitro* release studies were also carried out for the marketed Clotrimazole cream (CANDID 1%w/w) in order to compare the release profile of the drug with the prepared organogels. The data obtained was analyzed by applying various drug release kinetic models, so as to find out best fit model.

**Stability studies**
The selected formulations were stored at ambient humidity conditions between 2-8ºC, room temperature and at 40ºC for a period of one month. The samples were withdrawn at frequent intervals and evaluated for the parameters viz. pH, appearance, drug content and *in vitro* drug release.

**RESULT AND DISCUSSION**
All the formulations were found to be opaque in nature and creamish yellow in colour due to the slight yellow colour of the sunflower oil. They were smooth,uniform and contained no lumps. They were easily and uniformly extrudable from the collapsible tube. The pH of the organogels was measured at room temperature by using a digital pH meter. The pH of all the formulations was found to be in the range of 6.2-6.7, indicating their utility in the formulation of the transdermal or topical products. The results are tabulated in Table 2. The IR spectra of physical mixture (Clotrimazole : Span 60) 1:1 have shown the characteristic peaks of pure drug indicating that there were no interactions between the drug and Span 60 (Figures 1, 2, 3).
The Rheological studies indicated that as the rpm increases the viscosity decreases, confirming the shear thinning nature of the formulations. The viscosity of all the formulations increased with the increase in gelator concentration. As the concentration of the Span 60 increased, the spreadability of the respective formulation also increased. Since the spreadability value is more it would properly spread over the skin. The drug content of all the formulations was in the range of 96.2-99.25%.

The process of gelation was monitored under a compound light microscope as the hot Span 60 solution in sunflower oil is cooled at room temperature. The micrographs revealed the presence of needle shaped crystals of Span-60 in sunflower oil. As the concentration of the gelator was increased, these crystals aggregated to form fibre-like structures which could immobilize the SO (Figure 4). The organogels were subjected to increasing temperatures starting from 25°C. An increment of 5°C was made after 5 min incubation at the previous temperature. The samples were considered to have undergone gel-sol transition when they started to flow when the culture bottles were inverted. The gel-sol transition temperature of all samples was found to be in the range of 47-57°C (Figure 5).

With respect to the microbiological study conducted the zone of inhibition values were: F1: 38mm, F2: 34mm, F3: 32mm, F4: 30 mm, F5: 30 mm, Marketed product: 42mm. All the formulations were able to restrict the growth of Candida albicans efficiently (Figure 7).

In-vitro drug release studies of the formulation F1 showed 100% drug release at the end of 6th hr. The amount of drug released from F2, F3, F4 and F5 were found to be 94%, 92%, 89% and 84% respectively at the end of 6 hr while the marketed product showed a 90% drug release at the end of 8 hr (Figure 6). The release kinetics best fit model indicated that formulations F1 and F2 followed zero order release while F3, F4, F5 and Marketed follow Peppas model, with highest R² values 0.991 and 0.9884. The results are tabulated in Table 3.

### Table 1: Formulation of Clotrimazole organogels

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
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<tbody>
<tr>
<td>Clotrimazole (%w/w)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td>Span 60 (%w/w)</td>
<td>17</td>
<td>19</td>
<td>20</td>
<td>21</td>
<td></td>
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<tr>
<td>Tween 20 (%w/w)</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Sunflower oil (q.s)</td>
<td>50 g</td>
<td>50 g</td>
<td>50 g</td>
<td>50 g</td>
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### Table 2: Evaluation Tests

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>pH</th>
<th>Drug content (%)</th>
<th>T_{gel-sol} (°C)</th>
<th>Spreadability (g/sec/cm)</th>
<th>Viscosity (cps)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100 rpm</td>
</tr>
<tr>
<td>F1</td>
<td>6.4</td>
<td>97.3</td>
<td>47</td>
<td>3</td>
<td>37.85</td>
</tr>
<tr>
<td>F2</td>
<td>6.2</td>
<td>96.2</td>
<td>50</td>
<td>3.2</td>
<td>49.5</td>
</tr>
<tr>
<td>F3</td>
<td>6.3</td>
<td>98.7</td>
<td>50</td>
<td>3.3</td>
<td>67.7</td>
</tr>
<tr>
<td>F4</td>
<td>6.7</td>
<td>97.12</td>
<td>55</td>
<td>3.6</td>
<td>64.7</td>
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<tr>
<td>F5</td>
<td>6.5</td>
<td>99.25</td>
<td>57</td>
<td>3.8</td>
<td>68.8</td>
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<tr>
<td>Marketed Product</td>
<td>6.3</td>
<td>98.12</td>
<td>-</td>
<td>2.64</td>
<td>220.6</td>
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</table>

### Table 3: Kinetic models for the prepared formulations

<table>
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<tr>
<th>Formula</th>
<th>R</th>
<th>K</th>
<th>R</th>
<th>K</th>
<th>R</th>
<th>K</th>
<th>R</th>
<th>K</th>
<th>N value</th>
<th>Best fit model</th>
</tr>
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<tbody>
<tr>
<td>F1</td>
<td>0.9950</td>
<td>12.6714</td>
<td>0.8328</td>
<td>-0.3656</td>
<td>0.9171</td>
<td>29.4571</td>
<td>0.9327</td>
<td>-0.0764</td>
<td>0.9839</td>
<td>12.4626</td>
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<td>F2</td>
<td>0.9908</td>
<td>11.3826</td>
<td>0.8701</td>
<td>-0.2523</td>
<td>0.8959</td>
<td>26.2725</td>
<td>0.9296</td>
<td>-0.0611</td>
<td>0.9890</td>
<td>10.2062</td>
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<tr>
<td>F3</td>
<td>0.9887</td>
<td>10.6135</td>
<td>0.8719</td>
<td>-0.2110</td>
<td>0.8879</td>
<td>24.4243</td>
<td>0.9280</td>
<td>-0.0537</td>
<td>0.9910</td>
<td>8.5664</td>
</tr>
<tr>
<td>F4</td>
<td>0.9684</td>
<td>9.8653</td>
<td>0.8639</td>
<td>-0.1874</td>
<td>0.8484</td>
<td>22.4480</td>
<td>0.9081</td>
<td>-0.0488</td>
<td>0.9884</td>
<td>6.1943</td>
</tr>
<tr>
<td>F5</td>
<td>0.9860</td>
<td>9.5908</td>
<td>0.9060</td>
<td>-0.1657</td>
<td>0.8802</td>
<td>22.0127</td>
<td>0.9415</td>
<td>-0.0450</td>
<td>0.9943</td>
<td>7.1331</td>
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<tr>
<td>Marketed Product</td>
<td>0.9792</td>
<td>10.4667</td>
<td>0.8915</td>
<td>-0.2043</td>
<td>0.8627</td>
<td>23.8557</td>
<td>0.9306</td>
<td>-0.0527</td>
<td>0.9995</td>
<td>4.7332</td>
</tr>
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</table>
Fig. 1: I.R Spectrum of Clotrimazole

Fig. 2: I.R Spectrum of Span 60

Fig. 3: I.R Spectrum of Clotrimazole + Span 60
Fig. 4: Micrograph of organogel

Fig. 5: Organogel on cooling and heating

Fig. 6: Comparative in-vitro drug release
CONCLUSION
The study reports the successful development of stable, shear thinning, non-irritant Span-60 and sunflower oil based organogels of Clotrimazole. Microscopic studies indicated that small needle-shaped clusters aggregated to form fibres, which underwent interaction amongst each other to form a networked structure. The in-vitro and microbiological studies indicated sustained drug release profile and antifungal activity as comparable to marketed product. Hence the organogels developed may be tried as a matrix for controlled drug delivery.

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