Formulation and Characterization of Nanotransfersome of Famotidine in Transdermal Patch

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ABSTRACT
Transfersomes are highly efficient and extremely deformable lipid vesicles. The aim of the present study was to investigate transfersomes as a transdermal delivery system for delivery of famotidine to overcome the problems associated with its oral delivery. The oral bioavailability of famotidine has been reported to be low and highly variable. Famotidine undergoes first-pass metabolism resulting in about 50% bioavailability. It shows poor aqueous solubility, gastric degradation and is incompletely absorbed from gastrointestinal tract. Here, we made an attempt to deliver famotidine through transdermal route. Transfersome were made using phospholipon-90G and Span-80. A 3² Factorial design was applied. Optimal permeation flux was achieved as 22.4 μgm/cm² hour and drug entrapment efficiency of 61% and average particle size of 215 nm was obtained. Vesicle morphology was characterized using transmission electron microscopy. Ex vivo study using porcine ear skin indicated an excellent drug release profile.

KEYWORDS: Transfersome; Famotidine; Liposome; Transdermal patch; Permeation study.

Introduction
Transdermal drug delivery has progressed from basic transdermal gels and patches to vesicle loaded drug delivery systems i.e., liposomes and modified liposomes loaded transdermal systems. One type of modified liposome includes ultra-deformable liposomes. Transfersomes or ultra-deformable liposomes are made up of phosphatidyl choline and Edge activators. An edge activator is a surfactant which enhances the deformability of the vesicles. Sodium cholate, Sodium deoxycholate, Span 60, Span 80, Span 85, Tween 80 and Dipotassium glycyrrhizinate are examples of some commonly used edge activators (Mohammed I et al., 2012). Transfersomes or ultradeformable liposomes have advantage over conventional liposomes as they overcome the skin barrier by squeezing themselves along the intracellular sealing lipids of the stratum corneum. They permeate the skin intact without fragmentation of vesicles (Cevc et al., 2002). Phosphatidyl choline-span 80 elastic vesicles were recently reported to be more effective in enhancing skin penetration of drugs like dexamethasone, diclofenac, zidovudine as compared to phosphatidyl choline-cholesterol rigid vesicles (Jain, 2005).

Famotidine is a potent H₂ receptor antagonist used for active and maintenance therapy of ulcers and various types of hypersecretory disorders, Zollinger Ellison Syndrome and gastroesophageal reflux disorder. The drug not only decreases both basal and food-stimulated acid secretion by 90% or more but also promotes healing of duodenal ulcer (Maheshwari R et al., 2009). The oral bioavailability of Famotidine has been reported to be low to variable. It was reported that Famotidine undergoes first-pass metabolism resulting in bioavailability of around 50 % (McQuaid et al., 2012). Famotidine is incompletely absorbed from gastrointestinal tract, its bioavailability of 40-45% and half-life of 2.5-3.5 hours following oral administration favours the development of a sustained release formulation (Alagusundaram, 2011).

Fig. 1. Diagram representing deformable nature of transfersome.
The objective of the study was to develop famotidine loaded transfersome which were further incorporated in a transdermal patch for treatment of chronic conditions like Zollinger-Ellison syndrome and gastroesophageal reflux disorder.

Materials and Methods

Famotidine was a kind gift sample from SMS Pharma (Hyderabad, India). Phospholipon 90 G was obtained as a gift sample from Lipoid GmbH (Germany). Span 80 was purchased from Molychem (Mumbai, India). Dialysis Membrane was purchased from Himedia (Mumbai, India).

Drug excipient interaction study by FTIR

Compatibility of excipients with drug was studied using FTIR. The samples were scanned over a wave number range of 4000 to 500 cm⁻¹.

Drug excipient interaction study by DSC

The Samples were heated from 35 °C - 400 °C at a heating rate of 10°C/min under a stream of nitrogen at a flow rate of 20 ml/min. Alumina was used as standard. The DSC analysis of Famotidine, physical mixture and drug loaded transfersomal formulation was carried out. The melting point and peak maxima are reported in the DSC graphs.

Method of preparation of ultradeformable vesicles

Rotary evaporation-sonication method described by Ceve et al. was used for preparation of Transfersome. Weighed quantity of Drug, Edge activator and Lipid were dissolved in methanol. The organic solvent was removed under vacuum using Rotary vacuum evaporator to deposit a thin dry film. The deposited film was hydrated with phosphate buffer pH 7.4 for one hour at room temperature. The transfersomal dispersion was then probe sonicated for 10 minutes to obtain unilamellar vesicles.

Fabrication of patch

Polyvinyl Alcohol solution in water was made by heating the polymer solution till the entire polymer dissolved. The solution was brought down to room temperature. The dispersion of transfersome was added to the Polyvinyl Alcohol solution and stirred with help of magnetic stirrer. Plasticizer was added and solution was stirred for 1 hour. The resulting solution was poured on a glass mould and allowed to dry at room temperature for 24 hours. Propylene glycol served as plasticizer as well as penetration enhancer. Different concentrations of polymer were tried and amount of polymer was fixed at 4 % w/v in final patch. The concentration of polymer was kept constant throughout the study.

Experimental design

Traditional methods of formulation by changing one variable at a time are very time consuming hence it becomes essential to use tools to minimize time and maximize utility. Hence the Software Design Expert (Version 9.0.2, Stat-Ease Inc, Minneapolis, MN) was used to optimize and evaluate main effects, interaction effects of the formulation. A 3² Design was used, the design contained two factors i.e., amount of surfactant and amount of lipid which were varied at three levels i.e., high, medium and low to obtain 9 formulations (Shaji et al., 2014). The Responses chosen were Percentage drug entrapment efficiency and Flux. Analysis of variance (ANOVA) and all statistical analyses were also performed using the same software. The coded values are given in Table 1 and design layout is given in Table 2.

TABLE 1

<table>
<thead>
<tr>
<th>Level of factor</th>
<th>Coded values</th>
<th>Amount of surfactant (mg)</th>
<th>Amount of lipid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>+1</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>Medium</td>
<td>0</td>
<td>40</td>
<td>120</td>
</tr>
<tr>
<td>Low</td>
<td>-1</td>
<td>20</td>
<td>60</td>
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</tbody>
</table>

TABLE 2

Full factorial design layout

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Lipid</th>
<th>Surfactant</th>
<th>Lipid (mg)</th>
<th>Surfactant (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>0</td>
<td>+1</td>
<td>120</td>
<td>60</td>
</tr>
<tr>
<td>T2</td>
<td>0</td>
<td>-1</td>
<td>120</td>
<td>20</td>
</tr>
<tr>
<td>T3</td>
<td>+1</td>
<td>0</td>
<td>180</td>
<td>40</td>
</tr>
<tr>
<td>T4</td>
<td>+1</td>
<td>+1</td>
<td>180</td>
<td>60</td>
</tr>
<tr>
<td>T5</td>
<td>-1</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>T6</td>
<td>+1</td>
<td>-1</td>
<td>180</td>
<td>20</td>
</tr>
<tr>
<td>T7</td>
<td>-1</td>
<td>-1</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>T8</td>
<td>0</td>
<td>0</td>
<td>120</td>
<td>40</td>
</tr>
<tr>
<td>T9</td>
<td>-1</td>
<td>+1</td>
<td>60</td>
<td>60</td>
</tr>
</tbody>
</table>

Determination of drug entrapment efficiency of ultradeformable vesicles

Entrapment of drug was determined using centrifugation method. Transfersomal dispersion were subjected to centrifugation at 20,000 rpm at 2-4 °C for 30 minutes. The vesicles were broken using methanol and then sonicated using bath sonicator. The pellet was analyzed for the drug content after suitable dilution with buffer by measuring absorbance at 283 nm using UV Spectrophotometer. Encapsulation efficiency was calculated according to equation below (Sharma A, 2012)

\[
\text{Percentage entrainment (EE %) } = \frac{\text{Amount of Entrapped drug} \times 100}{\text{Total drug added}}
\]

Determination of EE % was conducted for various transfersomal formulations and the effect of variables was studied.

Permeation study (In vitro Diffusion Study)

Franz diffusion cell was used to perform diffusion study. The receptor compartment was filled with phosphate buffer pH 7.4 and maintained at 37 °C. Dialysis membrane was used for the study Magnetic stirer was placed in receptor compartment and driven at 100 rpm. At regular time intervals aliquots were removed from receptor. The samples were analysed spectro-photometrically for drug content. Percentage
cumulative release was determined. Flux was determined using the formula (Ghada and Zaafarany, 2010)

\[
\text{Flux} = \frac{\text{Amount of permeated drug}}{\text{Time} \times \text{area of release membrane}}
\]

**Ex vivo skin permeation study**

Porcine skin has similarity to human skin in lipid content and permeability hence it was used for ex vivo study. Porcine ear skin was obtained from local slaughter house immediately after sacrificing the animal. Then the hair was removed from the upper portion of skin surface using an animal hair clipper and the fatty layer adhering to the dermis side was removed by surgical scalpel. Finally, the skin was rinsed with deionized water and packed in an aluminum foil. The skin samples were stored at –20 °C and used within a week (Malakar., 2012). The skin was soaked in phosphate buffer overnight before the diffusion study. Skin was mounted between two half cells and diffusion study was performed.

**Vesicle morphology**

Transmission electron microscopy was used for studying morphology of the nanotransfersome. Copper grids having a thin layer of carbon was loaded with the transfersomal dispersion; they were stained with 1 % w/v aqueous solution of phosphotungstic acid (PTA) and allowed to dry under IR lamp. After the sample was dried thoroughly, the images were captured on a Transmission Electron Microscope (Rahul et al., 2012).

**Vesicle size and vesicle size distribution**

Examination of the transfersome vesicle size before sonication was performed by optical microscopy using a stage eyepiece micrometer calibrated using a micrometer scale (Gupta A et al. 2012). Dynamic light scattering using Sympatec Particle size analyzer was employed for determination of size of vesicle after sonication.

**Evaluation of Patch**

**Thickness**

The thickness of each patch was measured at the different sites using screw gauge and the average thickness was calculated. Percentage deviation from mean thickness was determined.

**Film folding endurance**

This was determined by repeatedly folding the patches until it shows any crack or break. The number of times the film could be folded without breaking/cracking gave the value of folding endurance (Gavali P, 2010).

**Moisture content**

The film was weighed and kept in a desiccator containing calcium chloride at 40 °C in a drier for at least 24 hrs or more until it showed a constant weight. The moisture content was the difference between the constant weight taken and the initial weight and was reported in terms of percentage (by weight) moisture content (Madhulatha, 2013).

Percentage of moisture content = \[
\frac{\text{Initial weight} – \text{Final weight}}{\text{Final weight}} \times 100
\]

**Percentage moisture uptake**

The weighed films were kept in a desiccator at room temperature for 24 hours and then exposed to 84% RH using a saturated solution of potassium chloride. The films were weighed repeatedly until they showed a constant weight. Values for the percentage of moisture uptake were calculated using the formula

Percentage of moisture uptake = \[
\frac{\text{Final weight} – \text{Initial weight}}{\text{Initial weight}} \times 100
\]

**Results**

**Drug excipient interaction study by FTIR**

Drug shows peak at 3504 cm\(^{-1}\) which corresponds to NH\(_2\) stretching vibration and 1639 cm\(^{-1}\) which depicts NH\(_2\) Bending vibrations. Peak at 3105 cm\(^{-1}\) denotes C-H stretching of aromatic group. On combining drug with excipients there was no disappearance of any peak. The final formulation containing drug along with all the excipients showed the major peaks. The IR study concluded that there was no incompatibility between famotidine and any of the excipients.
Drug excipient interaction study by DSC

The DSC of famotidine shows sharp peak at 167.9 °C. The thermogram of physical mixture shows 3 peaks of individual components at 167.2 °C, 175 °C, 223.5 °C corresponding to Drug, Phospholipon 90 G and Span 80 respectively. Hence we can say that in physical mixture there is no interaction of drug with the excipients as sharp peak of drug is visible. DSC of drug loaded transfersomal dispersion shows a broad peak at 77.9 °C representing that the lipid has interacted with the drug to a large extent indicating enhanced entrapment of famotidine (ChandaHarika., 2011). The DSC results show that there is enhanced entrapment of drug in the lipid bilayer Fig. 3.

Experimental design

The $3^2$ Factorial design was used and ANOVA was applied. The responses obtained are represented in Table 3. For percentage entrapment efficiency polynomial equation in terms of coded factors was obtained as

\[
\text{Entrapment efficiency} = + 48.22 + 12.83 \times A \quad - 3.17 \times B
\]

TABLE 3

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Lipid (mg)</th>
<th>Surfactant (mg)</th>
<th>% entrapment efficiency</th>
<th>Flux (microgram/ cm sq × hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>120</td>
<td>60</td>
<td>46</td>
<td>22.2</td>
</tr>
<tr>
<td>T2</td>
<td>120</td>
<td>20</td>
<td>51</td>
<td>21.2</td>
</tr>
<tr>
<td>T3</td>
<td>180</td>
<td>40</td>
<td>61</td>
<td>22.4</td>
</tr>
<tr>
<td>T4</td>
<td>180</td>
<td>60</td>
<td>57</td>
<td>23.1</td>
</tr>
<tr>
<td>T5</td>
<td>60</td>
<td>40</td>
<td>36</td>
<td>20</td>
</tr>
<tr>
<td>T6</td>
<td>180</td>
<td>20</td>
<td>64</td>
<td>22</td>
</tr>
<tr>
<td>T7</td>
<td>60</td>
<td>20</td>
<td>38</td>
<td>19</td>
</tr>
<tr>
<td>T8</td>
<td>120</td>
<td>40</td>
<td>50</td>
<td>21.8</td>
</tr>
<tr>
<td>T9</td>
<td>60</td>
<td>60</td>
<td>31</td>
<td>20.7</td>
</tr>
</tbody>
</table>

$R^2$ was found to be 0.9932 which implies that 99.32% of the variation in the responses was attributed to independent variables. The Model F-value of 435.46 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. The signal to noise ratio of 50.51 implies that the model can be used to navigate the design space.

In case of Flux the experimental data was fitted into a polynomial equation and equation in terms of coded factor for optimum flux was found to be

\[
\text{Flux} = + 21.76 + 1.30 \times A + 0.63 \times B - 0.15 \times AB - 0.53 \times A^2 - 0.033 \times B^2
\]

The $R^2$ was 0.9934. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 27.68 indicates an adequate signal hence this model can be used to navigate the design space.
Fig. 4. Contour plot of percentage entrapment efficiency.

Fig. 5. 3D surface graph of percentage entrapment efficiency.

Fig. 6. Contour plot of Flux.
Fig. 7. 3D surface graph of flux.

Fig. 8. Drug Release in Batches T1 – T8.
Vesicle morphology

The Vesicles were found to be spherical in shape.

Vesicle size and vesicle size distribution

Vesicles size before sonication was measured using optical microscopy using a stage eyepiece micrometer calibrated using a micrometer scale and was found to be 5 µm. After sonication the particle size was estimated using Nano Phox particle size analyser. The instrument uses principle of dynamic light scattering. The graph of percentage cumulative distribution VS Particle size (nm) was plotted. Mean size of particles was found to be 215 nm and polydispersity index was found to be 0.31. Low polydispersity index indicated particle size uniformity within the formulation.

Evaluation of patch

Evaluation of patch are given in Table 4.

DISCUSSION

It was found using exhaustive grid search that formulation T3 was the optimized formulation having permeation flux of 22.4 µgm/cm² hour and drug entrapment efficiency of 61 %. Mean particle size for this formulation was 215 nm which was also beneficial as small particle size helps in penetration of drug whereas liposomes of larger sizes penetrate into the deeper layers of the skin with difficulty (Seyed et al., 2011). A polydispersity index of 0.3 indicated uniform size distribution of particles. The morphology of the Transfersome was determined by TEM and demonstrates the spherical shape and nano size range of vesicle. The patch formulated was also uniform and had good physical characteristics. In vitro and ex vivo diffusion study indicated sustained drug release over a period of 24 hours.
TABLE 4
Evaluation of patch.

<table>
<thead>
<tr>
<th>Formulation No.</th>
<th>Mean Thickness (mm)</th>
<th>Folding endurance</th>
<th>Percentage Moisture content</th>
<th>Percentage Moisture uptake</th>
<th>Tensile Strength (kg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>0.154 ± 0.011402</td>
<td>More than 300</td>
<td>10.3</td>
<td>4.2</td>
<td>0.54</td>
</tr>
<tr>
<td>P2</td>
<td>0.13 ± 0.007071</td>
<td>More than 300</td>
<td>9.5</td>
<td>3.9</td>
<td>0.47</td>
</tr>
<tr>
<td>P3</td>
<td>0.152 ± 0.008367</td>
<td>More than 300</td>
<td>9.9</td>
<td>3.7</td>
<td>0.45</td>
</tr>
<tr>
<td>P4</td>
<td>0.138 ± 0.008367</td>
<td>More than 300</td>
<td>10</td>
<td>4.9</td>
<td>0.57</td>
</tr>
<tr>
<td>P5</td>
<td>0.166 ± 0.011402</td>
<td>More than 300</td>
<td>11</td>
<td>5</td>
<td>0.51</td>
</tr>
<tr>
<td>P6</td>
<td>0.13 ± 0.01</td>
<td>More than 300</td>
<td>10.8</td>
<td>4.5</td>
<td>0.53</td>
</tr>
<tr>
<td>P7</td>
<td>0.15 ± 0.007071</td>
<td>More than 300</td>
<td>10.6</td>
<td>4.3</td>
<td>0.58</td>
</tr>
<tr>
<td>P8</td>
<td>0.134 ± 0.008914</td>
<td>More than 300</td>
<td>10.3</td>
<td>4.8</td>
<td>0.48</td>
</tr>
<tr>
<td>P9</td>
<td>0.136 ± 0.011402</td>
<td>More than 300</td>
<td>10.5</td>
<td>4.9</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Transdermal patches loaded with ultra deformable liposomes showed enhanced delivery as compared to the plain drug patch. Since in case of transfersome they are highly elastic vesicles and can squeeze through the stratum corneum intact. Transfersomes are hence ideal candidates for delivery through transdermal route. The study suggests that incorporation of transfersome of Famotidine in patch is an excellent approach for delivery of the drug.

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References


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