Novel Vesicular Carrier for Enhanced Transdermal Delivery of Tramadol Hydrochloride Transfersomal Gel

ABSTRACT

The main aim of current probe is to formulate and evaluate transfersomal gel for effective transdermal delivery of Tramadol Hcl. It was investigated by encapsulating the drug in various formulations which composed of various ratios of phosphatidyl choline, propylene glycol and ethanol prepared by lipid film hydration by conventional rotary evaporation method. The shapes of most Tramadol Hcl-containing Transfersomes were found to be spherical from SEM analysis. The percentage entrapment efficiency of optimised formulation T5 were found to be 92.71±0.56. The prepared formulations had been characterised for the loaded drug amount and vesicle size. The prepared vesicular systems were incorporated into 1% carbopol 934 gel. In vitro skin permeation studies were carried out by cellophane membrane using a Franz diffusion cell. Transfersome gel was found to increase the skin permeation and deposition showing a controlled effect.

KEYWORDS Tramadol hydrochloride, Transfersomes, transdermal delivery, entrapment efficiency, in vitro drug permeation studies

INTRODUCTION

Nano vesicular Tramadol Hcl delivery system has the ability to improve the pharmacokinetics and increase biodistribution of therapeutic agents to target organ, resulting in improved efficacy and drug toxicity is reduced as a consequence of preferential accumulation at target sites and lower concentration in healthy tissues. Hence nano vesicular systems have been promoted as a means of sustained or controlled release of drugs.1

Transdermal route offer a number of potential advantages over conventional route like the avoidance of first pass metabolism, predictable and extended duration of activity.2 However, the major limitation of transdermal route is the permeability of the skin, it is permeable to small molecules and lipophilic drugs and it is highly impermeable to macro molecules and hydrophilic drugs. The main barrier and rate limiting steps for diffusion of drugs across the skin is provided by the outer most layer of the skin, the stratum corneum. Hence new classes of highly deformable (elastic or ultraflexible) liposomes also called Transfersomes have been developed. Transfersomes are promising nano carriers for non-invasive transdermal delivery.4

Cevc’s group introduced Transfersomes, which are the first generation of elastic vesicles. Transfersomes improved in vitro skin permeation of various drugs, penetrated intact skin in vivo and efficiently transferred therapeutic amounts of drugs.5 Transfersomes are prepared from phospholipids and edge activators such as phosphatidyl choline and propylene glycol. An edge activator is often a single chain with a high radius of curvature that destabilises the lipid bilayer of the vesicles and increases the deformability of the bilayer. Transfersomes are colloidal carriers which are easily accumulated into the leaky synovial tissue which leads to peripheral targeting. Transfersomes also act as depot resulting in controlled drug delivery system.6

Tramadol Hcl is freely water soluble. It is a synthetic centrally acting amnocylohexal analgesic that acts as an opioid agonist with selectively for the μ-receptor it inhibits reuptake of nor-epinephrine and serotonin.4,9

Shastrulagari S. Shivani1*, Kumar M Srujan2

1Department of Pharmaceutics, Samskruti College of Pharmacy, Hyderabad, Telangana, India
2Department of Pharmaceutics, Institute of Pharmaceutical and Research Center, Bhagwant University, Ajmer, Rajasthan, India

Received Date: 09 January 2016 – Accepted Date: 19 February 2016 – Published Online: 03 March 2016


E-mail: Pharma.shivani45@gmail.com

Statement of originality of work: The manuscript has been read and approved by all the authors, the requirements for authorship have been met, and that each author believes that the manuscript represents honest and original work.

Sources of funding: None.

Acknowledgement: The author wish to thank the management of Samskruti College of Pharmacy and Comprime Labs Pvt Ltd for providing the necessary facilities for carrying out the research work. Also want to thank my parents, brothers for their support and encouragement.

Competing interest / Conflict of interest: The author(s) have no competing interests for financial support, publication of this research, patents, and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript.

Disclaimer: Any views expressed in this paper are those of the authors and do not reflect the official policy or position of the Department of Defense.
Hcl is used as an effective agent for moderate to severe chronic pain. Transdermal delivery system is a desirable alternative for route of administration of Tramadol Hcl for patients with chronic pain. Thus the study encompasses the ability of lipid vesicles to deliver Tramadol Hcl across the skin in order to evaluate potential of drug delivery as well as overcome its side effects.

**MATERIALS AND METHODS**

**Materials**

Drug Tramadol Hcl was supplied by Dr. Reddy’s Laboratories Ltd, Hyderabad. Soya phosphatidyl choline (SPC) was obtained from Bright laboratories. Propylene glycol and Ethanol were purchased from SD Fine chemicals Mumbai. Carbopol-934 from research lab fine chem industry (Mumbai) India. Other chemicals and reagents were of analytical grade.

**Methods**

Compatibility study of drug and polymer using FTIR

The compatibility between the pure drug and excipients was detected by Fourier transform infrared spectroscopy (FTIR) of the pure drug Tramadol Hcl and mixture of drug with excipients was taken using the Shimadzu (IR Tracer-100) FTIR. The sample was prepared with potassium bromide and data were collected over a spectral range of 500–8000 cm⁻¹.

Preparation of tramadol Hcl-loaded transfersomes

Transfersomes formulations were prepared by using conventional rotary evaporation method using drug, soya lecithin and surfactant (propylene glycol). The amount of drug is kept constant in all the formulations. Different formulations were prepared by using different ratio of surfactant. The accurately weighed amounts of phospholipid, surfactant and drug were taken and this lipid mixture was dissolved in small quantity of ethanol. The organic solvent was removed by rotary evaporation under reduced pressure at 45°C final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the phosphate buffer (pH 6.8) by rotation at 60 rpm for 1 hr at room temperature, which resulted in multilamellar vesicles. These were further size reduced by using probe sonicator to form small unilamellar vesicles.

Preparation of topical transfersome gel

As a vehicle for incorporation of Transfersomes for topical delivery, carbopol gels were prepared. Transfersomes aqueous dispersion was utilised for the formulation of topical gel. Gel polymer such as carbopol-934 was utilised to prepare Transfersomes gel. One gram of carbopol-934 powder was dispersed into vigorously stirred (stirred by magnetic stirrer RemiSMLH) distilled water (preferably 88 ml) (taking care to avoid the formation of in dispersible lumps) and allowed to hydrate for 24 hrs. Later 10 ml of Propylene glycol was added. The dispersion was neutralised with the drop wise addition of 10% Hcl hydroxide, mixing was continued until a transparent gel was appeared. Then the amount of base was adjusted to achieve a gel with pH 6.8. It can be measured by using pH meter (Lab India Sab 5000).

**CHARACTERIZATION OF TRANSFERSOMES**

**Determination of entrapment efficiency percentage**

The amount of Tramadol Hcl entrapped in Transfersomes suspension was estimated by centrifugation method. Five millilitres of Transfersomes suspension was taken and placed in centrifugation tube which is centrifuged at 14,000 rpm for 30 minutes. Supernatant was withdrawn and diluted with pH 6.8 phosphate buffer before performing absorbance measurement using UV spectrophotometer (Shimadzu) at 254 nm. This gives the total amount of unentrapped drug. Entrapment efficiency is expressed as the percent of drug trapped.

\[
\% \text{ Entrapment} = \frac{\text{Total drug} - \text{Unentrapped drug}}{\text{Total drug}} \times 100
\]

**Vesicle shape**

Transfersomes vesicles can be visualised by SEM and optical microscope. The morphological characterization of Transfersomes vesicle such as shape and surface feature were projected by using optical microscope and SEM.

**Vesicle size, size distribution and zeta potential**

Vesicle size, size distribution and zeta potential were determined by Malvern Zetasizer DTS version 5.03 (Malvern, UK). Zeta potential was analysed to measure the permeation of Transfersome by studying its colloidal property and stability of the vesicle.

**%Drug content**

One gram of Transfersomes gel formulation was taken and the vesicles were lysed with 25 ml of methanol by sonication [citizen, India] for 15 min. Later this solution was placed in centrifugation tube and centrifuged at 14,000 rpm for 30 min. The clear solution was diluted to 100 ml with methanol. Then 10 ml of solution was diluted to 100 ml with saline phosphate buffer pH 6.8. Aliquots were withdrawn and drug content was calculated for Tramadol Hcl by using UV spectrophotometer at 254 nm.

\[
\% \text{Drug content} = \frac{\text{Amount of drug taken}}{\text{Amount of drug obtained after centrifugation}} \times 100
\]
In vitro drug release studies

Modified Franz diffusion cell with a receiver compartment volume of 30 ml and effective diffusion area of 2 cm² was used for this study. In vitro drug release study was performed by using cellophane membrane in phosphate buffer solution (pH 6.8). To perform in vitro drug release study, cellophane membrane was mounted horizontally on the receptor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2 cm² and capacity of receptor compartment was 30 ml. The receptor compartment was filled with 18 ml of phosphate buffer (pH 6.8) maintained at 37±0.5°C and stirred by a magnetic bar at 100 rpm. Transfersome gel formulation equivalent to 5 mg drug was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 6.8) to maintain sink conditions. The samples were analysed spectrophotometrically at 254 nm.

Stability studies of transfersomes

After measuring the initial percentage entrapment of the drug in the optimized formulation, the three batches of the same formulation were stored in sealed glass ampoules (one each) at refrigeration temperature (4±2°C), room temperature (25±2°C) and body temperature (37±2°C) for a period of at least 3 months. The percentage entrapment of the drug and % drug content was determined in the formulations after 15, 30, 45 and 90 days to know the amount of drug leaked out. The percent drug lost was calculated taking the initial entrapment of drug as 100%.

RESULTS AND DISCUSSION

Transfersomes were used for non-invasive delivery of drugs into or across the skin. Transfersomes are also known as elastic liposomes or flexible vesicles which have better penetration ability than conventional liposomes. So in the present vocation deformable lipid vesicles Transfersomes were formulated. The proposed system is more stable, having higher entrapment efficiency, can be used as self penetration enhancer, easy to scale up and better for dermal delivery.

Compatibility studies

Compatibility studies were carried out for any interference of drug-polymer, drug-excipients used in the formulation, which reveals that there is no interaction between drug and polymer used in the formulation. The data are given in Fig. 1 and Table 1.

Vesicle shape and size

The shape and morphology of the Transfersome were determined by SEM, show the spherical shape and nano size range of vesicle. Demonstrating unilamellar struc-

Table 1 FTIR Spectra data for final formulation F(5).

<table>
<thead>
<tr>
<th>Functional groups in stretching region</th>
<th>Frequency (cm⁻¹)</th>
<th>Bond</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>O-H</td>
<td>Stretch</td>
</tr>
<tr>
<td></td>
<td>3635.94</td>
<td>O-H</td>
<td>Stretch</td>
</tr>
<tr>
<td></td>
<td>3331.18</td>
<td>N-H</td>
<td>Stretch</td>
</tr>
<tr>
<td></td>
<td>3070.78</td>
<td>O-H</td>
<td>Stretch</td>
</tr>
<tr>
<td></td>
<td>3043.77</td>
<td>=C-H</td>
<td>Stretch</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Functional groups in bending region</th>
<th>Frequency (cm⁻¹)</th>
<th>Bond</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1629.90</td>
<td>N-H</td>
<td>Bend</td>
</tr>
<tr>
<td></td>
<td>950.94</td>
<td>=C-H</td>
<td>Bend</td>
</tr>
<tr>
<td></td>
<td>1357.93</td>
<td>C-H</td>
<td>Rock</td>
</tr>
</tbody>
</table>

Fig. 1 FTIR spectra of optimised transfersome formulation.
% Entrapment efficiency

The % entrapment efficiency of deformable vesicles formulation were found to be in the range of 54.18–92.71%.

% Drug content

The % drug content results obtained shows (93.7–97.8%) in all the formulations that there is no degradation of the drug in the process. The data are shown in Table 2.

In vitro drug release

Comparison of results obtained from diffusion studies for all formulations have been done. It was found that optimised formulation T5 shows higher drug release rate than other formulations. This result is probably caused by the release of drug absorbed on the Transfersome surface or precipitated from the superficial lipid layer. Prolonged release in the later stage can be attributed to the slow diffusion of the drug from the lipid vesicles. The data are shown in Table 3 and Fig. 5.
Table 3  *In vitro* drug release of transfersome gel (T1 to T9).

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
<th>T9</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>4.90</td>
<td>5.62</td>
<td>5.07</td>
<td>5.54</td>
<td>5.91</td>
<td>3.66</td>
<td>4.19</td>
<td>3.99</td>
<td>3.99</td>
</tr>
<tr>
<td>0.5</td>
<td>7.25</td>
<td>8.18</td>
<td>7.30</td>
<td>8.63</td>
<td>8.36</td>
<td>5.51</td>
<td>6.36</td>
<td>5.74</td>
<td>5.74</td>
</tr>
<tr>
<td>1</td>
<td>13.05</td>
<td>13.06</td>
<td>10.33</td>
<td>12.19</td>
<td>13.43</td>
<td>9.37</td>
<td>10.98</td>
<td>7.93</td>
<td>7.93</td>
</tr>
<tr>
<td>2</td>
<td>20.20</td>
<td>20.10</td>
<td>15.14</td>
<td>17.79</td>
<td>22.61</td>
<td>14.48</td>
<td>18.75</td>
<td>12.16</td>
<td>12.16</td>
</tr>
<tr>
<td>4</td>
<td>27.64</td>
<td>28.31</td>
<td>19.48</td>
<td>23.74</td>
<td>34.63</td>
<td>21.91</td>
<td>28.96</td>
<td>18.41</td>
<td>17.41</td>
</tr>
<tr>
<td>6</td>
<td>41.76</td>
<td>39.25</td>
<td>26.37</td>
<td>31.90</td>
<td>44.44</td>
<td>27.97</td>
<td>42.40</td>
<td>26.13</td>
<td>23.13</td>
</tr>
<tr>
<td>8</td>
<td>50.06</td>
<td>48.88</td>
<td>33.60</td>
<td>40.19</td>
<td>53.30</td>
<td>36.12</td>
<td>52.70</td>
<td>34.97</td>
<td>34.97</td>
</tr>
<tr>
<td>10</td>
<td>59.69</td>
<td>61.35</td>
<td>48.39</td>
<td>48.77</td>
<td>59.25</td>
<td>46.89</td>
<td>58.95</td>
<td>49.78</td>
<td>45.78</td>
</tr>
<tr>
<td>24</td>
<td>75.71</td>
<td>88.18</td>
<td>64.48</td>
<td>69.06</td>
<td>97.65</td>
<td>58.75</td>
<td>79.10</td>
<td>59.45</td>
<td>55.45</td>
</tr>
</tbody>
</table>

**Fig. 5**  *In vitro* drug release study for transfersomal gel formulation T1–T9.

Table 4  % Entrapment efficiency and % drug content after stability studies.

<table>
<thead>
<tr>
<th>Number of days</th>
<th>% Entrapment efficiency at temperatures</th>
<th>% Drug content at temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4±2°C</td>
<td>25±2°C</td>
</tr>
<tr>
<td>15</td>
<td>91.8</td>
<td>91.63</td>
</tr>
<tr>
<td>30</td>
<td>90.6</td>
<td>90.42</td>
</tr>
<tr>
<td>45</td>
<td>90.27</td>
<td>88.67</td>
</tr>
<tr>
<td>90</td>
<td>89.93</td>
<td>85.42</td>
</tr>
</tbody>
</table>

**Stability studies**

It is clear from the results obtained that the Transfersomes have shown the minimum drug lost at refrigerated condition, and fairly high retention of drug inside the vesicles was observed. At this low temperature condition % remaining drug entrapped and drug content was good over a period of months. While, storage at higher temperatures 25±2°C and 37±2°C leads to less % remaining drug entrapped and drug content over a period of 3 months, respectively. The higher amount of drug leakage at elevated temperature may be due to the degradation of lipids constituting bilayer with the increase in temperature, there is also increase in the fluidity of lipid bilayer, due to phase transition phenomenon. So it can be inferred from the above discussion that the Transfersomes formulation should be stored at lower temperature to minimize the drug loss and increase the stability of drug. The data are shown in Table 4.

**CONCLUSION**

Finally, it may be concluded from the study that Transfersomes can increase the transdermal flux, prolong the release and improve the site specificity of bioactive molecules. Transfersomes may be used as alternative carriers for...
transdermal drug delivery systems because they interact with solid gel phase sc lipids and thus leading to disruption and fluidization of the sc lipid. Tramadol HCl was successfully incorporated into Transfersomes. From this study, it can be concluded that Transfersomes loaded with Tramadol HCl showed a prolonged action.

REFERENCES