FORMULATION AND EVALUATION OF MEFENAMIC ACID LOADED TRANSFEROSOME FOR TOPICAL DELIVERY
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KEYWORDS:
Transferosome,
Mefenamic acid, Soya Lecithin, Surfacants.

ABSTRACT
The present study aimed to prepare and evaluate Mefenamic acid loaded transfersomes for transdermal drug delivery. Transferosomes were prepared by modified hand shaking, lipid film hydration method by varying the ratios of soya lecithin and surfactants in the organic phase. Four surfactants i.e. Span 20, span 80, tween 20, tween 80 were selected for the present study. Drug concentration was kept constant. With each surfactant 4 formulations were prepared. The prepared formulations were evaluated for vesicle size, morphology, drug content, entrapment efficiency and in vitro skin permeation.
INTRODUCTION:
Transdermal drug delivery systems (TDDS) offer a number of potential advantages over conventional methods such as injectables and oral delivery. However, the major limitation of TDDS is the permeability of the skin; it is permeable to small molecules and lipophilic drugs and highly impermeable to macromolecules and hydrophilic drugs. The main barrier and rate-limiting step for diffusion of drugs across the skin is provided by the outermost layer of the skin, the stratum corneum. Several strategies have been developed to overcome the skin’s resistance, including the use of prodrugs, ion pairs, liposomes, microneedles, ultrasound, and iontophoresis.[1-3]

Various types of liposomes (LPs) exist, such as traditional liposomes, niosomes, ethosomes, and transfersomes. Various LPs have been extensively investigated for improving skin permeation enhancement. Liposomes are promising carriers for enhancing skin permeation because they have high membrane fluidity. Among the various types of liposomes, Transfersomes are the first generation of elastic vesicles. Transfersomes are prepared from phospholipids and edge activators. An edge activator is often a single-chain surfactant with a high radius of curvature that destabilizes the lipid bilayers of the vesicles and increases the deformability of the bilayers. Sodium cholate, sodium deoxycholate, Span 60, Span 65,Span 80, Tween 20, Tween 60, Tween 80, and dipotassium glycyrrhizinate were employed as edge activators. Compared with subcutaneous administration, transfersomes improved in vitro skin permeation of various drugs, penetrated intact skin in vivo, and efficiently transferred therapeutic amounts of drugs.

The high and self-optimizing deformability of typical composite transfersomes membrane, which are adaptable to ambient tress allow the ultra deformable transfersomes to change its membrane composition locally and reversibly, when it is pressed against or attracted into narrow pore. The transfersomes components that sustain strong membrane deformation preferentially accumulate, while the less adaptable molecules are diluted at sites of great stress. This dramatically lowers the energetic cost of membrane deformation and permits the resulting, highly flexible particles, first to enter and then to pass through the pores rapidly and efficiently. This behaviour is not limited to one type of pore and has been observed in natural barriers such as in intact skin (Bain et al, Cevc et al., 1996).[4-8]

Salient features and limitations of transfersomes [9-15]
Transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility as shown in fig 1.
Transfersomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss. This high deformability gives better penetration of intact vesicles.

They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anaesthetic, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin. They are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes. They have high entrapment efficiency, in case of lipophilic drug near to 90%. They protect the encapsulated drug from metabolic degradation. They act as depot, releasing their contents slowly and gradually. They can be used for both systemic as well as topical delivery of drug. Easy to scale up, as procedure is simple, do not involve lengthy procedure and unnecessary use or pharmaceutically unacceptable additives (jain., 2001).

Mefenamic acid (MA) is a potent nonsteroidal antiinflammatory drug (NSAID) of the enolic acid class, which shows preferential inhibition of cyclooxygenase-2 (COX-2) and inhibits the prostaglandin synthesis. It is highly prescribed in the treatment of rheumatoid arthritis, osteoarthritis and other joint disorders. However, its oral bioavailability is very low in addition to the side effects.[16,17]

**MATERIALS AND METHODS**

**Materials**

Mefenamic acid and Alpha soya phosphatidyl choline were supplied as gift samples from Chemdyes Corporation, Ahmedabaad. All other chemicals were of analytical grade and procured from the authentic sources.
Preparation of Mefenamic acid loaded Transfersomes [18-21]

The transfersome were prepared by modified hand shaking, lipid film hydration technique. The composition of formulation is in (Table 1). Drug, lecithin (PC) and surfactant were dissolved in chloroform: ethanol mixture. Organic solvent was removed by evaporation while hand shaking. A thin lipid film was formed inside the flask wall with rotation. The thin film was kept overnight for complete evaporation of solvent. The film was then hydrated with phosphate buffer (pH 7.4) with gentle shaking for 15 minute at corresponding temperature. The transfersomal suspension further hydrated up to 1 hour at 2-80 C and it is stored in refrigerator.

Table-1 Formulation variables used in the preparation of Mefenamic acid Transfersomes

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Surfactant</th>
<th>PC: Surfactant ratio</th>
<th>Chloroform: Ethanol (ml)</th>
<th>Mefenamic acid (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>Span 20</td>
<td>1:1</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F2</td>
<td>Span 20</td>
<td>1:2</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F3</td>
<td>Span 20</td>
<td>2:1</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F4</td>
<td>Span 20</td>
<td>2:2</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F5</td>
<td>Span 80</td>
<td>1:1</td>
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<td>Span 80</td>
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<td>F7</td>
<td>Span 80</td>
<td>2:1</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F8</td>
<td>Span 80</td>
<td>2:2</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F9</td>
<td>Tween 20</td>
<td>1:1</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F10</td>
<td>Tween 20</td>
<td>1:2</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F11</td>
<td>Tween 20</td>
<td>2:1</td>
<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F12</td>
<td>Tween 20</td>
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<td>6:4</td>
<td>250</td>
</tr>
<tr>
<td>F13</td>
<td>Tween 80</td>
<td>1:1</td>
<td>6:4</td>
<td>250</td>
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<tr>
<td>F14</td>
<td>Tween 80</td>
<td>1:2</td>
<td>6:4</td>
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<td>Tween 80</td>
<td>2:1</td>
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<td>250</td>
</tr>
<tr>
<td>F16</td>
<td>Tween 80</td>
<td>2:2</td>
<td>6:4</td>
<td>250</td>
</tr>
</tbody>
</table>

CHARACTERIZATION OF DICLOFENAC SODIUM TRANSFEROSOMES

The transfersomal suspension obtained for all the formulations (F1- F16) was then characterized for morphological analysis of particle by Scanning Electron Microscopy, particle size distribution and zeta potential to ensure that they were within Nano /micron size range and possessed optimum stability respectively. Further, they were evaluated for following parameters like entrapment efficiency, drug content and in vitro diffusion studies.[22]

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EVALUATION OF DICLOFENAC SODIUM TRANSFEROSOMES

Morphological Study using SEM
Scanning electron micrographs (the outer macroscopic structure) for the prepared mefenamic acid loaded transferosome was taken using Scanning Electron Microscope (JEOL, Japan) at accelerating voltage at 3-5 kV to study surface topography.

Drug Content
1 ml of Mefenamic acid loaded transferosomal suspension was taken and diluted with 10ml 7.4ph phosphate buffer. It was ultracentrifuged at 15000 rpm for 40 minutes. The pellet formed after centrifugation was disrupted with 10 ml methanol to come out the drug from vesicles. 1ml of this solution was taken and suitable dilutions were made and analysed by UV spectrophotometer at 285 nm which gives the concentration of entrapped drug. The concentration of drug in supernatant and pellet collectively gives the amount of drug present in 1ml of suspension. % drug content was calculated by dividing with theoretical drug content present in 1ml of suspension.[23,24]

\[
\text{Practical drug content} = \frac{\text{Drug content}}{100} 
\]

Entrapment Efficiency:
1 ml of Mefenamic acid loaded transferosomal suspension was taken and diluted with 10ml 7.4ph phosphate buffer. It was ultracentrifuged at 15000 rpm for 40 minutes at. After centrifugation pellet was formed at the bottom of centrifuge tube. 1 ml of supernatant was collected and suitable dilutions were made and analyzed by UV spectrophotometer at 285 nm. % entrapment efficiency was calculated by the following formula.[25]

\[
\text{% entrapment efficiency} = \frac{\text{Total drug added-unentrapped drug}}{\text{Total drug added}} \times 100
\]

In vitro Drug Diffusion Study
Diffusion studies were carried out using franz diffusion cell by using dialysis membrane. 1 ml of transferosomal suspension was taken in donor compartment and 25 ml of 7.4ph phosphate buffer was taken in receiver compartment. Allquotes of 1ml of samples were withdrawn at definite time intervals from the sampling port and replaced with the buffer to maintain sink conditions. The samples were analysed by UV spectrophotometer at 285nm. The % of drug release in a time period of 12 hours was reported.[27,28]
RESULTS AND DISCUSSION:

Morphological Study using SEM

![SEM analysis image of formulation F7](image)

**fig 2: SEM analysis image of formulation F7**

The SEM photographs of microsponge are shown in fig 3 and the particles are found to be spherical in shape.

**Drug Content of the Formulations:**

The drug content for all the 16 formulations was evaluated and it varied between 88.26% to 98.8%. Among all the formulations the transferosomes prepared by span 80 (F7) 2:1 ratio was superior with highest drug content of 98.8%. Followed by span 20 (F3) formulation with 97.25% followed by span 80 (F6) with drug content of 96.5% respectively.

![Drug Content Graph](image)

**Entrapment Efficiency:**

The Entrapment Efficiency for all the 16 formulations was evaluated and it varied between 35.7% to 82.45%. Among all the 16 formulations the highest entrapment efficiency was found to be for span 80 (F7) 2:1 ratio with entrapment of 82.45% followed by span 20 (F3) 2:1 ratio with the
entrapment efficiency of 78.6% followed by span 80 (F6) 2:1 ratio with entrapment efficiency of 65.5% respectively.

**In vitro Drug Diffusion Study**

In vitro drug diffusion studies were performed using Franz diffusion cell to determine the sustained release nature of the formulations. The diffusion study was continued up to 12 hours. For F7 formulation, the drug release was found to be sustained with the release of 87.6 % for span 80 2:1 ratio, 84.5 % for span 80, 1:2 ratio (F6) formulation and 80.5% for span 20 2:1 ratio (F3) formulation.

**F1 – F8 Formulations**
CONCLUSION:

The mefenamic acid loaded transferosomes were successfully prepared by modified hand shaking, lipid film hydration technique using lecithin and different grades of spans and tweens as nonionic surfactants. The presence of lecithin and nonionic surfactants made the transferosomes more stable. Transferosomes formulated with span 80 have shown the best entrapment efficiency compared with the those prepared with other grades like span 20, tween 80 and tween 20.

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