DEVELOPMENT AND CHARACTERIZATION OF KETOROLAC TROMETHAMINE ENCAPSULATED TRANSETHOSOMES FOR ENHANCED TRANSDERMAL DELIVERY

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ABSTRACT

Objective: The aim of this current research is to study a novel carrier, transethosome, for its enhanced transdermal delivery of ketorolac tromethamine (KT). Methods: Transethosomes (TEls) are elastic vesicles composed of phospholipid, ethanol and edge activator (surfactant). TELs were prepared by cold method and characterized by particle size, entrapment efficiency, transmission electron microscopy (TEM), ex-vivo skin corrosive test, in-vitro dissolution, ex-vivo permeation studies and in-vivo study. Results: Microscopic examination of transethosomes showed soft, malleable and spherical vesicles with a smooth surface. Vesicle size and % EE of the optimized transethosomal formulation was found to be 180 ± 70 nm and 80.08 ± 4.5% respectively. Zeta potential of the optimized transethosomal formulation was found to be -46.19± 13.3 mv. The ex-vivo study showed transdermal flux of transethosomal gel (47.43± 0.2 µg/cm²/h) 3fold higher than that obtained after application of plain drug gel (15.05±0.09 µg/cm²/h), and 2.7fold higher than that of the hydroethanolic solution of drug (17.33± 0.15µg/cm²/h).

Conclusions: Data obtained from this experimental work concludes that transethosomal formulation is safe, very effective and promising drug carrier for transdermal delivery of drug.

Keywords: ketorolac tromethamine, transdermal delivery, transethosomes, permeation studies, in- vivo study.
INTRODUCTION
Nanocarriers improve the pharmacokinetics and increase the biodistribution of drugs to the target organ, resulting in improved efficacy. Toxicity of drugs is reduced due to preferential accumulation of drug at target sites than in healthy tissues. Hence nanocarriers provide a means for sustained and controlled release of drugs.

Osteoarthritis (OA) is one of the most common forms of arthritis and it is a chronic condition characterized by the breakdown of the joint’s cartilages. OA is a group of mechanical abnormalities involving degradation of joints including articular cartilage and subcondral bone. Its main symptom is pain, causing loss of mobility and often stiffness.

Ketorolac tromethamine (KT) is a non-steroidal anti-inflammatory drug (NSAID) and analgesic. It is used in treatment of OA and acute pain including pain after surgery. Its oral route has been associated with number of gastrointestinal disorders \[^1\]. Thus transdermal is only an alternative route for delivering KT so as to overcome its side effects.

The major disadvantage of transdermal delivery is low skin permeability which limits number of drugs that can be delivered in this manner \[^2\]. Liposomes do not penetrate deep into the skin but remains confined to the upper layer of the stratum corneum \[^2,3\]. New classes of lipid carriers such as transfersomes and ethosomes were developed. Transfersomes are elastic carriers containing edge activators such as Tween 20, Tween 60, Tween 80, Span 60, Span 65, Span 80, dipotassium glycyrrhizinate, sodium cholate or sodium deoxycholate. Edge activators destabilize the lipid bilayer of transfersomes and increases its flexibility \[^4,5\]. Ethosomes are ultradeformable carriers containing high concentration of ethanol. Enhanced skin permeation of drugs incorporated into these carriers is due to the interdigitation effect of ethanol on the lipid bilayer of the carriers and increases the fluidity of lipids in the stratum corneum \[^6\]. Hence a new carrier which incorporates the advantages of both transfersomes and ethosomes is desirable. It will enhance transdermal permeation of drugs to dermal layer through stratum corneum due to its ultradeformable nature. Transethosomes are elastic vesicles which contains high concentration of ethanol along with edge activator \[^7\].

MATERIALS AND METHODS
Materials
Ketorolac Tromethamine was a gift sample obtained from Dr. Reddy’s Laboratory, Mumbai. Phosphatidyl choline (Phospholipon 90G) was a gift sample from Lipoid, Germany. Sodium
deoxycholate (SDC) was obtained from S.D Fine chemicals. All other chemicals used were of analytical grade. After getting approval from ethical committee, Albino Wistar rats were obtained from Bharat Serum and Vaccines Pvt. Ltd. (Mumbai, India).

METHODS

Transethosomes preparation
TELs were prepared by cold method which is used to prepare ethosomes \[8\]. Composition of all TELs formulations are shown in Table 1. Phospholipon 90G was dissolved in ethanol in a conical flask. This mixture was heated to 30\(^\circ\)C. Ketorolac tromethamine and sodium deoxycholate was dissolved in water and heated to 30\(^\circ\)C in a separate vessel. This aqueous phase was then added to the alcoholic phase slowly in a fine stream with constant stirring (Mechanical stirrer, Remi, Mumbai) at 700 rpm in a closed vessel. Stirring was continued for additional 5 min. The system was kept at 30\(^\circ\)C throughout the preparation. Finally preparation was sonicated using probe sonicator (Oscar, Japan) for 4 min.

Characterization of transethosomes

Visualization by transmission electron microscopy (TEM)
Shape and morphology of the TELs was investigated using transmission electron microscopy. TELs were negatively stained with 2% w/v aqueous solution of phosphotungstic acid on a carbon-coated copper grid. The grid was examined under transmission electron microscope (Philips CM 200) with resolution of 2.4\(\text{Å}\) at accelerating voltage of 200 kV.

Determination of entrapment efficiency
Entrapment efficiency of TELs was determined by ultracentrifugation method \[8\]. TELs were separated by ultracentrifugation at 15,000 rpm for 60 minutes at a temperature of 4\(^\circ\)C. The sediment and supernatant liquid were separated, the amount of drug in the sediment was determined by rupturing the vesicles using methanol and the amount of drug was quantified spectrophotometrically at 322 nm. Entrapment efficiency was determined by the following equation;

\[
\text{\% Entrapment efficiency} = \frac{\text{Amount entrapped KT}}{\text{Total KT added}} \times 100
\]

Determination of vesicle size and zeta potential: The particle size and zeta potential of freshly prepared TELs was determined by Nanoparticle tracking analysis (NTA 2.3) using
Nanosight NS500 with automated sample introduction, computer controlled motorized stage with CCD camera and red (638nm) laser[9].

**Preparation of TELs gel**
Carbopol Ultrez 10 (0.75%) was soaked in minimum amount of water for an hour. TELs suspension was then added to the swollen polymer under stirring. Stirring was maintained at 700 rpm in a closed vessel and the temperature was maintained at 30°C until homogeneous TEL gel was obtained. The pH was then adjusted to neutral using triethanolamine and stirred slowly till a clear gel was obtained.

**Characterization of TELs gel**

**Assay:** 1g of gel equivalent to 5mg of KT was weighed and dissolved in 100ml methanol. The drug content was determined using UV spectrophotometer at 322nm.

**Spreadability:** 0.5gm gel was placed within a circle of 1cm diameter pre-marked on a glass plate over which a second glass plate was placed. A weight of 500gm was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the gel was noted.

**pH:** The pH was determined using a digital pen pH meter, standardized using pH 4.0 and 7.0 standard buffers.

**Viscosity:** Brookfield CAP 2000 viscometer was used to determine the viscosity of KT loaded TELs gel using spindle no 32 at 25°C.

**Invitro drug release**
*Invitro* drug release was evaluated using Franz diffusion cell. A cellophane dialysis membrane with molecular weight cut-off of 12,000 daltons (Hi-media) was hydrated with phosphate buffer saline pH 7.4 (PBS 7.4) overnight. The donor medium consisted of 2 ml of the TELs suspension or 1 gm of TELs gel equivalent to 5mg of KT. The receptor compartment was filled with 13 ml of PBS 7.4 and stirred with a magnetic bar at 100 rpm and the temperature of the system was maintained at 32 ± 1°C to mimic human skin. The available diffusion area was 2.61 cm². 1ml aliquot was withdrawn at predetermined time intervals and was immediately replaced with an equal volume of fresh buffer. All samples were analyzed for KT content by UV spectrophotometry at 322 nm.
Exvivo skin permeation study
Porcine ear skin obtained from a slaughter house was used as a model membrane for the skin permeation study because of its similarity to human skin in lipid content and permeability. The skin sample was mounted between the donor and receptor compartments of the diffusion cell. The receptor compartment was filled with 2 ml of TELs suspension or 1 gm of TELs gel equivalent to 5mg of KT. The receptor chamber was filled with PBS of pH 7.4 and stirred with a magnetic bar at 100 rpm and the temperature was maintained at 32 ± 1°C. 1 ml of aliquot from the receptor compartment was withdrawn at predetermined time intervals and was replaced with equal volume of fresh PBS. The concentration of the drug in the samples was analyzed spectrometrically by UV, and the cumulative amount of drug was plotted as function of time.

Permeation data analysis
The flux ($J, \mu g \text{ cm}^{-2} \text{ hr}^{-1}$) was calculated from the slope of linear portion of the plot divided by the skin surface area $^{[10]}$. The steady state permeability coefficient (Kp) of the drug through porcine skin was calculated by using the following equation:

$$K_p = \frac{J}{C_0}$$

Where; J is the flux and C0 is the concentration of KT in the gel. The penetration enhancing activity of the enhancer may be calculated in terms of enhancement ratio (ER), using the following equation:

$$ER = \frac{\text{Drug permeability coefficient (Treated)}}{\text{Drug permeability coefficient (Control)}}$$

Skin deposition studies: At the end of 24 hrs of the permeation experiment, the surface of the skin was washed five times with 30% methanol to remove excess of KT from the surface of the skin. The skin was cut into small pieces which were further homogenized with 5 ml of 30% of methanol. The resulting solution was then centrifuged for 10 min at 5,000 rpm, the supernatent was then seperated to determine the KT content by UV spectrophotometer at 322nm $^{[11]}$.

Ex-vivo skin corrosion studies
Corrosive potential of TELs gel was determined by Corrositex® test $^{[12]}$. Corrosive substances destroy the epidermal proteins and cause color shift in the underlying chemical detection liquid. Corrosive potential of TELs gel was determined on porcine ear skin using 37% nitric acid and 0.9%w/v NaCl solutions as positive and negative controls respectively.
Skin samples were prepared and clamped on Franz diffusion cell. 200μl of 37% nitric acid solution, 0.9% NaCl solution or TELs gel was deposited onto the epidermis of the porcine skin. After 15 min, the sample was removed and the epidermis was further washed with 2 ml of distilled water to remove the residual sample. 1ml of Sulforhodamine B (skin proteins labeling dye) was deposited onto the epidermis. After 15 min, 1ml the dye was removed and the epidermis was washed with 1 ml of distilled water. The experiment was conducted in triplicate. The absorbance of the washing water was measured with a spectrophotometer at 313 nm. The corrosive factor was calculated with the Eq. as shown below:

\[ F = \frac{(\text{sample Abs} - \text{0.9% NaCl solution Abs})}{\text{0.9% NaCl solution Abs}} \]

If F>0, then the sample is non-corrosive.
If F<0, then the sample is corrosive.

**Bilayer fluidity of stratum corneum (SC) using differential scanning calorimetry (DSC)**

Thermal analysis of the skin was done using the same skin sample which was used for permeation analysis. The skin sample of 2 mg was weighed into an aluminum crimp pan. Untreated skin served as a control. The samples were heated from 30 to 300°C at a heating rate of 10°C/min. DSC measurements were collected under a nitrogen atmosphere with a flow rate of 100 ml/min.

**In-vivo anti inflammatory activity**

The anti-inflammatory activity of the gel was carried out by carrageenan induced paw edema method [13] to compare the activity of marketed gel and the KT loaded TELs gel. After getting approval from ethical committee, male albino rats of Wister strain weighing 150-200gms was randomly divided into 3 groups of six rats each. TELs gel and marketed gel were applied on the subplantar region of the left hind paw of first and second groups, respectively. Third group was untreated and served as control. 1 h post transdermal application, paw edema was induced by subplantar injection of 0.1 ml of a 1% w/v freshly prepared carrageenan in normal saline into the left hind paw of each rat. The paw volume up to the ankle joint was measured before and at different time intervals after the carrageenan injection using graduated plethysmograph (INCO, India). Percentage reduction in edema was calculated using the following formula:

\[ \% \text{ Inhibition of paw edema} = \frac{(V_t - V_0) \text{ control} - (V_t - V_0) \text{ treated}}{(V_t - V_0) \text{ control}} \]
Where; \( V_t \) is the paw volume at time ‘t’
\( V_0 \) is the initial paw volume (before carrageenan treatment)
\((V_t - V_0)\) control is edema produced in control group
\((V_t - V_0)\) treated is edema produced in treated group

RESULTS AND DISCUSSIONS]

Fig. 1: TEM images of TELs

Fig. 2: Particle size of optimized formulation (F-9) batch
Fig. 3: Zeta potential of optimized formulation (F-9) batch

Fig. 4: *In-vitro* release of KT from (A) Plain drug solution (B) Hydroethanolic drug solution (C) TELs dispersion (D) TELs gel

Fig. 5: *Ex-vivo* release profile of KT from (A) Plain drug solution (B) Hydroethanolic drug solution (C) TELs gel
Fig. 6: % skin deposition of KT (%) of TELs gel and hydroethanolic drug solution

Fig. 7: DSC thermogram of [A] Untreated porcine skin [B] Treated porcine skin with TELs gel

Fig. 8: Percentage (%) swelling inhibition (%) produced by KT loaded TELs gel, and Std marketed gel in carrageenan induced rat paw
Table 1: Composition of transethosomal formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Drug (mg)</th>
<th>Phospholipon 90G (%)</th>
<th>Ethanol (%)</th>
<th>Sodium deoxycholate (mg)</th>
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<tr>
<td>F-1</td>
<td>50</td>
<td>1</td>
<td>10</td>
<td>20</td>
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<td>F-2</td>
<td>50</td>
<td>1</td>
<td>10</td>
<td>60</td>
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<td>F-3</td>
<td>50</td>
<td>1</td>
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<td>F-5</td>
<td>50</td>
<td>2</td>
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<td>40</td>
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<td>F-6</td>
<td>50</td>
<td>3</td>
<td>10</td>
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<td>F-9</td>
<td>50</td>
<td>3</td>
<td>30</td>
<td>60</td>
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Table 2: Entrapment efficiency and particles size of TELs formulations

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Size (nm)</th>
<th>% Entrapment efficiency n=3</th>
</tr>
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<tbody>
<tr>
<td>F-1</td>
<td>142.8±30</td>
<td>43.22±3.22</td>
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<tr>
<td>F-2</td>
<td>150.9±39</td>
<td>45.59±1.07</td>
</tr>
<tr>
<td>F-3</td>
<td>121.1±45</td>
<td>48.87±2.56</td>
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<tr>
<td>F-4</td>
<td>130.2±80</td>
<td>50.99±1.83</td>
</tr>
<tr>
<td>F-5</td>
<td>155.8±49</td>
<td>60.01±1.52</td>
</tr>
<tr>
<td>F-6</td>
<td>206.0±64</td>
<td>68.13±2.24</td>
</tr>
<tr>
<td>F-7</td>
<td>215.6±60</td>
<td>70.01±2.29</td>
</tr>
<tr>
<td>F-8</td>
<td>175.2±21</td>
<td>75.54±3.98</td>
</tr>
<tr>
<td>F-9</td>
<td>180.6±70</td>
<td>82.08±4.5</td>
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</table>

Table 3: Permeability coefficient, transdermal flux and ER of the formulation

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Permeability coefficient ((x 10^{-3}) (\text{cm h}^{-1}))</th>
<th>Flux ((\mu g \text{h}^{-1} \text{cm}^{-2}))</th>
<th>ER*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transethosomal gel</td>
<td>9.486</td>
<td>47.432</td>
<td>3.150</td>
</tr>
<tr>
<td>Hydroethanolic drug solution</td>
<td>3.466</td>
<td>17.333</td>
<td>1.151</td>
</tr>
<tr>
<td>Plain drug solution</td>
<td>3.010</td>
<td>15.053</td>
<td>-</td>
</tr>
</tbody>
</table>

**ER* Enhancement Ratio**

Table 4: Corrosive factor of the formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Corrosive Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>37% Nitric acid solution (positive control)</td>
<td>-0.6547±0.005</td>
</tr>
<tr>
<td>Transethosomal gel</td>
<td>1.2047 ± 0.004</td>
</tr>
</tbody>
</table>

Visualization by transmission electron microscopy (TEM)

TEM images showed that TELs has a unilamellar vesicular structure as shown in Fig. 1, and this confirms the existence of vesicular structure at higher concentration of ethanol and edge activator.
Determination of entrapment efficiency

Entrapment efficiency of TELs formulations ranged from 43.22% to 82.088% as shown in Table 2. Increasing the concentration of ethanol increases the entrapment efficiency owing to increase in fluidity of the membranes. However, further increase in the ethanol concentration made the carrier membrane leakier leading to decrease in entrapment of KT\textsuperscript{14}. Increasing the concentration of phospholipids and edge activator also increases the entrapment efficiency. The batch which showed good entrapment efficiency and smaller particle size was then selected for further studies (F-9).

Determination of vesicle size and zeta potential

The average particle size of all formulations ranged from 121.1-215.6 nm as shown in Table 2. The vesicular size of the transethosomes decreased with increase in ethanol concentration and increased with increase in phospholipid and edge activator concentration\textsuperscript{7}. The particle size of an optimized batch was found to be 180 ±70 nm as shown in Fig. 2. Ethanol causes a modification in net charge of the system and confers it some degree of stearic stabilization that may lead to decrease in mean vesicle size\textsuperscript{15}. The zeta potential of optimized batch was found to be -46.19 ± 13.3 mv as shown in Fig. 3. Zeta potential of TELs showed negative values, which is due to the presence of ethanol and sodium deoxycholate\textsuperscript{6}. The charge of the transethosomal vesicles is an important parameter that can influence both vesicular properties such as stability, as well as skin-vesicle interactions.

Characterization of TELs gel

Assay: All hydrogels have a mild acidic pH which is acceptable for topical preparations. The ketorolac tromethamine content of transethosomal gel was found to be 97.52 % ± 0.17.

Spreadability: The diameter of transethosomal gel was found to be 5.633 ± 0.18 cm which indicates good spreadability.

pH: pH of transethosomal was found to be 6.9 which are within acceptable limits.

Viscosity: The viscosity of the plain gel and KT loaded TELs gel was found to be 14100 cps, 11500 cps respectively.

In-vitro Diffusion Studies

Encapsulation of KT into TELs led to significant prolongation of its release across the artificial membrane in comparison with the hydroethanolic and plain drug solution. From the Fig. 4, it was observed that the sustained effect was in the order: TELs dispersion > TELs gel.
> hydroethanolic drug solution > plain drug solution. Higher permeation of TELs gel might be due to combination of both ethanol and sodium deoxycholate an edge activator. The release profile of KT loaded TELs enriched gel indicated slow release as compared to TELs dispersion. This may be due to the fact that drug diffuses from the TELs carrier followed by diffusion from the gel matrix which results in sustained release effects.

**Ex vivo skin permeation analysis**

Ethanol has permeation enhancement properties. Permeation of TELs is much enhanced as compared to simple hydroethanolic drug solution. Synergistic mechanism is observed between phospholipid vesicles, ethanol and skin lipids. Ethanol fluidizes the stratum corneum lipids which enhances drug permeation. Ethanol also increases the lipid fluidity of lipid carriers thus making them flexible. These flexible vesicles squeeze themselves intact through the disturbed stratum corneum to the deeper layers of skin with consequent release of the drug by fusion of TELs vesicles with skin lipids [16]. The order of permeation profile observed from Fig. 5 is as follows: TELs gel > hydroethanolic drug solution > plain drug solution.

Higher flux and higher release was observed for TELs gel in comparison to hydroethanolic and plain drug solution because of the synergistic mechanisms of ethanol, phospholipid vesicles, edge activator and skin lipids interaction, which promoted the passage of KT through porcine skin.

The percent cumulative permeation in 24hr was found to be 70.593% for TELs gel which was found to be higher than that of hydroethanolic and plain drug gel.

As shown in Table 3 enhancement ratio of the TELs gel was found to be 3 fold higher as compared to hydroethanolic solution and. The transdermal flux of TELs gel was found to be 3fold higher than that obtained after application of plain drug gel, and 2.7fold higher than that of the hydroethanolic solution of drug.

**Drug Deposition Studies**

As shown in Fig. 6, deposition of KT on porcine skin from TELs gel was found to be 10.813% which was much higher than the hydroethanolic solution which was 5.1698% and plain drug solution which was found to be 1.067 %. Higher skin deposition of transethosomes is due to the combined effect of phospholipids, edge activator and ethanol on skin and thus providing a mode for sustained delivery of drug for a greater period of time [17].
**Exvivo corrosive studies**

The experiment was conducted in triplicate. The results are shown in Table 4. As observed, TELs dispersion was found to be non-corrosive as compared to the positive control.

**Bilayer fluidity of stratum corneum (SC) using differential scanning calorimetry (DSC)**

Differential scanning calorimetry is an invaluable tool for a detailed thermodynamic characterisation of macromolecule and their interactions with skin. Porcine stratum corneum samples were also investigated by DSC techniques. The DSC profiles are compared in Fig 7. Treatment with TELs showed pronounced shift to lower temperature as compared to untreated skin. The maximal thermal transition temperature is shifted from about 60°C to 54°C after treatment with TELs. The data suggest that incorporation TELs into stratum corneum results in increase in the fluidity of SC [18].

**Pharmacodynamics Study**

Application of TELs gel and the marketed gel resulted in 65% and 58% inhibition of edema after inducing carrageenan at 3hrs as shown in Fig. 8. After 3 hrs there was decrease in the percent swelling inhibition. There is a significant difference between the tested groups and the control as determined by a one way ANOVA with P < 0.05.

**CONCLUSION**

The results obtained from this study indicates, new phospholipid carrier tranethosomes which consists of high concentration of ethanol and edge activator enhances the permeation of ketorolac tromethamine due to its better penetration as compared to hydroethanolic drug solution and plain drug solution. *In-vivo* studies showed better anti-inflammatory activity due elastic nature of the carriers as compared to the marketed formulation. Thus, the developed tranethosomal formulation could be a potential carrier for ketorolac tromethamine and other similar drugs especially due to their simple production and ease of scale-up.

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