TRANSFERSOMES LOADED TRANSDERMAL DRUG DELIVERY SYSTEM OF METHOTREXATE FOR RHEUMATOID ARTHRITIS

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ABSTRACT
Methotrexate is a disease-modifying antirheumatic drug used in the treatment of rheumatoid arthritis. Transfersomes are highly deformable vesicles and known to have considerable potential as drug carriers for transdermal drug delivery system. The purpose of this study was to develop transfersomes loaded transdermal patch of methotrexate for rheumatoid arthritis. The transfersomes were prepared by thin film hydration technique using phosphatidylcholine 95% & sodium cholate. Process parameters like speed of rotation (80 RPM) and vacuum (350-400 mm Hg) and formulation parameters like solvent system (chloroform : methanol), volume of solvent (15 ml), drug to lipid ratio (1:10) and lipid to surfactant ratio (6:4) were optimized. The optimized transfersomes formulation was evaluated for globule size (130 ± 3 nm), zeta potential (-29.3 ± 2.4 mV), drug entrapment (49.3 ± 1.8%) and drug release (94.9 ± 2.5% in 6 hrs). The lyophilized transfersomes were added to an acrylic adhesive DURO TAK® 87-4098 to obtain a new hybrid transdermal patch. Patches were optimized on the basis of parameters like thickness, folding endurance, tensile strength, moisture content & in vitro drug release. Thus, the prepared transfersomes loaded patch could prove to be a promising topical drug delivery system in the treatment of RA.

KEYWORDS: Transfersomes, Methotrexate, Acrylic Adhesive, DURO TAK®87-4098.

INTRODUCTION
Rheumatoid arthritis (RA) is an autoimmune disease that causes chronic inflammation of the joints. The joint inflammation of rheumatoid arthritis causes swelling, pain, stiffness, and
redness in the joints.\textsuperscript{[1]} The exact cause of rheumatoid arthritis is unknown, but it is thought to be due to a combination of genetic, environmental, and hormonal factors, something seems to trigger the immune system to attack the joints and sometimes other organs. Some theories suggest that a virus or bacteria may alter the immune system, causing it to attack the joints. Once the immune system is triggered, immune cells migrate from the blood into the joints and joint-lining tissue, called synovium. There the immune cells produce inflammatory substances that cause irritation, wearing down of cartilage (cushioning material at the end of bones), and swelling and inflammation of the joint lining.\textsuperscript{[1, 2]}

Methotrexate (MTX) is a disease-modifying antirheumatic drug (DMARD). DMARDs are also called immunosuppressive drugs or slow-acting antirheumatic drugs (SAARDs). It is known to interfere with the way cells utilize essential nutrients. As a result, methotrexate inhibits the activity of the immune system, consequently reducing inflammation. As a cytotoxic drug it may slow the rapid growth of cells in the synovial membrane that lines the joints. It can reduce inflammation and slow the progression of the disease.\textsuperscript{[3]}

Currently marketed formulations of methotrexate for RA include conventional tablets and intramuscular, subcutaneous & intraarticular injection. The problem with conventional formulation is that its solubility is low and hence it is less bioavailable and affected by gastric environment and hepatic metabolism while in injection clearance of methotrexate is very fast from joint.\textsuperscript{[4]}

Transfersome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer. Due to their high deformability, transfersomes penetrate the epidermal barrier to a greater extent as intact vesicles. This could lead to reduced dose frequency compared to the conventional oral dosage forms. Transfersomes can also significantly enhance the accumulation of drug at the site of administration as a result of high substantively of transfersomes with biological membrane.\textsuperscript{[5, 6]}

Transfersomal patch enhances the drug release potential of transdermal delivery systems and also increase the rate of skin permeation of the drug.\textsuperscript{[7]} Hence, an attempt was made to load transfersomes with methotrexate and formulate them into an adhesive transdermal patch.
MATERIALS AND METHODS
Methotrexate was supplied as gift by Intas pharmaceutical Ltd., Ahmedabad, Gujarat. DURO TAK® 87-4098 was as gifted by Henkel, USA. Phospholipon 90G was supplied as gift by Lipoid, Germany. Sodium Cholate was purchased from Chemdyes Corporation, Ahmedabad, India. Other excipients used to prepare transfersomes loaded patch were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

PREPARATION OF TRANSFERSOMES
Transfersomes containing Phospholipon 90G (PC) and sodium cholate (SC) were prepared by a thin film hydration technique. Appropriate amounts of PC and SC were dissolved in a 15 ml amount of chloroform and methanol (6:4). Film formations were done using Rota evaporator at 80 rpm, 350-400 mm Hg pressure, at 45°c for 45 minutes in round bottom flask (RBF). MTX was dissolved in 2 ml of phosphate buffer pH 7.4 and volume made up to 15 ml with distilled water. Hydration of the film was done with preheated drug solution for 1 hour. The formed transfersomal suspension was sonicated using probe sonicator to obtain appropriate size and allowed to swell for 24 hr at 4°c. Unentrapped drug was separated from transfersomal suspension by centrifugation at 25000 rpm for 30 min at 4°c.

OPTIMIZATION OF DRUG: LIPID & PC: SC RATIO BY 3-LEVEL FACTORIAL DESIGN[8-10]
Batches were prepared with different drug: lipid and PC: SC ratio mentioned in Table 1. Both the drug: lipid and lipid: surfactant ratio affects the drug entrapment as well as size of particle. Hence optimization was done on the basis of drug entrapment and particle size. Further optimization was done by 3- level factorial design with 2 factors using Design expert 8.0.7.1 software (Stat Ease, Inc. Minneapolis, MN). Independent variables with their levels and dependent variables are listed in Table 2. The polynomial equation generated from this experimental design is described as:

\[ Y_1 = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \]  

(1)

Where \( Y_1 \) is the dependent variable while \( b_0 \) is the intercept; \( b_1 \) to \( b_{22} \) are the regression coefficients; and \( X_1 \) and \( X_2 \) are the independent variables, levels of which were selected from the preliminary experiments.
Dependent Variables  | Independent Variables
--- | ---
X1. Milimoles of Phospholipon 90G  | Y1. Drug entrapment (%)  
X2. Milimoles of Sodium Cholate  | Y2. Particle size (mm)  

**EVALUATION OF TRANSFEROSOMES**

**Particle Size and Zeta Potential of Transfersomes**

Mean particle size was determined by dynamic light scattering at 25°C with a Malvern 4700 system using a 25 mW He–Ne laser (NEC, Tokyo) and the Automeasure version 3.2 software. Surface charge measurement was done using a Malvern 4700 system analyzer. The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. It is a physical property which is exhibited by any particle in a suspension.

**Entrapment Efficiency**

The percentage of drug encapsulated was determined by UV visible spectrophotometric method. Transfersomal suspension was centrifuged at 25000 rpm at 4°C for 30min for removal of free drug. Supernatant was collected and estimated spectrophotometrically using UV visible spectrophotometer at 302 nm for free drug. The percent drug entrapment (PDE) was calculated through the following relationship:

\[
\% \text{ DE} = \frac{(\text{Total drug} - \text{Free drug})}{\text{Total drug}} \times 100
\]

**In Vitro Drug Release Studies**

Drug release from transfersomes was studied by dialysis method. Transfersomal suspension was placed in an activated dialysis bag. Two ends of the dialysis sac were tightly bound with threads. The sac was hung inside a beaker so that the portion of the dialysis sac with the formulation remains dipped into the buffer solution. The beaker was kept on a magnetic stirrer and stirring was maintained at 25 rpm at 37°C with a thermostatic control. Samples were collected at different time interval for 6 hours and assayed spectrophotometrically for drug content at 302nm for methotrexate.

**FREEZE DRYING OF TRANSFEROSOMES**

Optimized transfersomal batch was lyophilized using mannitol as a cryoprotectant. Four different concentrations (2%, 4%, 6%, 8% w/w) of mannitol were used. The batches were kept for freeze drying in lyophilizer at controlled vacuum and temperature for 18 hours to completely remove the solvent and obtain free flowing powdered formulation. The freeze
dried product was appropriately sealed in air tight vials to avoid the reabsorption of moisture from the atmosphere.

**PREPARATION OF ACRYLIC ADHESIVE PATCH**

Duro Tak® 87-4098 was diluted with ethyl acetate to give the concentrations of 10% to 40% of Duro Tak® 87-4098. Lyophilized transfersomes were suspended in the diluted Duro Tak® 87-4098 solutions of different concentration and these suspensions were spread over previously prepared backing layer of polyvinyl alcohol (2% w/v PVA) and dried in oven at 45°C for 1 hr.

**EVALUATION OF TRANSDERMAL PATCH**

**Thickness and Weight Variation**

The thickness of the patches was assessed at three different points using micrometer screw gauze. Three patches were weighed individually and average weight of three patches was determined.

**Folding Endurance**

Patch was repeatedly folded at the same place till it broke. The number of times the film could be folded at the same place without breaking gives the value of the folding endurance.

**Percentage Moisture Content**

The prepared patches were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature for 24 hrs. After 24 hrs the patches were reweighed and the percentage moisture content was determined.

**Percentage Moisture Uptake**

The weighed patches were kept in a desiccator at room temperature for 24 hrs containing saturated solution of potassium chloride in order to maintain 84% RH. After 24 hrs the patches were reweighed and the percentage moisture uptake was determined.

**In Vitro Drug Release Studies**

In-vitro drug release studies from the patches were studied using Franz diffusion cell. Receptor compartment of diffusion cell was completely filled with phosphate buffer pH 7.4. Patch of 2 cm² area was placed on the surface of dialysis membrane in the donor compartment. Franz diffusion cell was kept on a magnetic stirrer and stirring was maintained.
at 25 rpm at 37\(^0\)C with a thermostatic control. Samples were collected at a different time interval for 72 hrs and assayed spectrophotometrically for drug content at 302 nm.

**Tensile Strength**

Tensile strength of patch was determined using texture analyzer. Patch was cut and fixed to assembly. The weight required to break the patch was noted. Tensile strength is the ratio of maximum stress applied to a point at which the patch specimen breaks and can be computed from the applied force at rupture to the cross sectional area of the fractured patch.

**Adhesion Study**

Adhesive property was measured by texture analyzer. Patches were cut into diameter of 2 cm and applied to adherent plate and pulled from the substrate at an angle of 180\(^\circ\) at a rate of 30mm/s.

**RESULTS AND DISCUSSION**

**OPTIMIZATION OF DRUG: LIPID & PC: SC RATIO**

Table 1 shows the results of preliminary studies of optimization of drug: lipid and PC: SC ratio. It was observed that as the D:L ratio increased from 1:5 to 1:10, the drug entrapment increased. Further increase in ratio upto 1:15 and 1: 20 did not give increase in drug entrapment. This may be due to increasing the concentration of methotrexate in hydration medium that forces the drug into the aqueous core up to certain limit after that core become saturated so entrapment does not increase. \(^{[11]}\)

Also it was observed that if the D: L is kept constant, the drug entrapment increased as the ratio of PC: SC was varied from 8:2 to 7:3 to 6:4. This may be due to the interaction between sodium cholate and methotrexate when complex gets inserted in to the aqueous core.\(^{[7, 8]}\)

The particle size was found to increase as the D: L ratio increased from 1:5 to 1:20. This may be due to increasing the amount of lipid molecules. At constant ratio of D: L the particle size does not show significant change so size was not so much affected by surfactant concentration.\(^{[6]}\) The zeta potential of all the batches was in the range of -28.3 mV to -38.9 mV indicating that all the preparations would remain stable and not aggregate.

Based on the above results, the formulation showing highest drug entrapment (D: L, 1: 10 and PC: SC, 6: 4) was taken for further optimization using 3- level factorial design with 2 factors using Design expert 8.0.7.1 software (Stat Ease, Inc. Minneapolis, MN).
The mathematical equation for the responses was found to be

\[ Y_1 = 36.90 - 2.20 \times X_1 + 3.47 \times X_2 - 2.53 \times X_1 \times X_2 + 1.94 \times X_1^2 + 1.18 \times X_2^2 \]
\[ Y_2 = 162.56 + 30.85 \times X_1 + 42.57 \times X_2 + 71.40 \times X_1 \times X_2 - 6.58 \times X_1^2 + 50.17 \times X_2^2 \]

Where, \( Y_1 \) and \( Y_2 \) are percentage drug entrapment and particle size respectively.
\( X_1 \) and \( X_2 \) are milimoles of phospholipon 90G and milimoles of sodium cholate respectively.

The results of optimization are recorded in table 2. The results show that batch B3 showed highest drug entrapment of 49.36 ± 1.8 % and a particle size of 130 ± 3 nm, hence batch B3 with PC: SC ratio of 6: 4 and D: L ratio of 1: 10 was taken as the optimized batch for further studies.

**TABLE 1: Optimization of drug: lipid & PC: SC ratio**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Drug : Lipid</th>
<th>Lipid: Surfactant</th>
<th>Particle size (nm)</th>
<th>Zeta Potential (mv)</th>
<th>%DE ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1 : 5</td>
<td>8 : 2</td>
<td>108±3</td>
<td>-37.9±1.2</td>
<td>31.45±1.5</td>
</tr>
<tr>
<td>F2</td>
<td>7 : 3</td>
<td>116±2</td>
<td>-31.4±0.8</td>
<td>34.77±2.0</td>
<td></td>
</tr>
<tr>
<td>F3</td>
<td>6 : 4</td>
<td>126±2</td>
<td>-28.7±0.6</td>
<td>46.78±0.6</td>
<td></td>
</tr>
<tr>
<td>F4</td>
<td>8 : 2</td>
<td>113±4</td>
<td>-29.6±1.0</td>
<td>34.26±2.4</td>
<td></td>
</tr>
<tr>
<td>F5</td>
<td>7 : 3</td>
<td>119±5</td>
<td>-30.2±0.5</td>
<td>37.66±1.5</td>
<td></td>
</tr>
<tr>
<td>F6</td>
<td>6 : 4</td>
<td>130±3</td>
<td>-29.3±0.4</td>
<td>51.18±1.8</td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>1 : 10</td>
<td>8 : 2</td>
<td>119±2</td>
<td>-28.6±1.3</td>
<td>33.28±1.5</td>
</tr>
<tr>
<td>F8</td>
<td>1 : 15</td>
<td>7 : 3</td>
<td>135±3</td>
<td>-33.1±1.4</td>
<td>35.88±2.3</td>
</tr>
<tr>
<td>F9</td>
<td>6 : 4</td>
<td>146±3</td>
<td>-34.7±0.7</td>
<td>45.24±0.8</td>
<td></td>
</tr>
<tr>
<td>F10</td>
<td>8 : 2</td>
<td>126±6</td>
<td>-38.9±0.9</td>
<td>32.0±1.0</td>
<td></td>
</tr>
<tr>
<td>F11</td>
<td>7 : 3</td>
<td>132±6</td>
<td>-31.4±1.5</td>
<td>33.79±0.6</td>
<td></td>
</tr>
<tr>
<td>F12</td>
<td>6 : 4</td>
<td>149±2</td>
<td>-30.5±1.0</td>
<td>40.12±1.2</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 2: Matrix of 3-level factorial design for optimizing formulation variables**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Std</th>
<th>Run</th>
<th>Factor A Milimoles of PC</th>
<th>Factor B Milimoles of SC</th>
<th>%DE ± SD</th>
<th>Particle size ±SD (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>6</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>37.18±0.5</td>
<td>177±4</td>
</tr>
<tr>
<td>B2</td>
<td>9</td>
<td>2</td>
<td>8</td>
<td>4</td>
<td>39.16±1.0</td>
<td>348±3</td>
</tr>
<tr>
<td>B3</td>
<td>7</td>
<td>3</td>
<td>6</td>
<td>4</td>
<td>49.36±1.8</td>
<td>130±3</td>
</tr>
<tr>
<td>B4</td>
<td>4</td>
<td>4</td>
<td>6</td>
<td>3</td>
<td>40.11±1.2</td>
<td>125±2</td>
</tr>
<tr>
<td>B5</td>
<td>8</td>
<td>5</td>
<td>7</td>
<td>4</td>
<td>40.01±0.8</td>
<td>265±5</td>
</tr>
<tr>
<td>B6</td>
<td>2</td>
<td>6</td>
<td>7</td>
<td>2</td>
<td>35.76±1.5</td>
<td>150±3</td>
</tr>
<tr>
<td>B7</td>
<td>1</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>36.03±1.5</td>
<td>211±3</td>
</tr>
<tr>
<td>B8</td>
<td>5</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>37.29±0.8</td>
<td>172±4</td>
</tr>
<tr>
<td>B9</td>
<td>3</td>
<td>9</td>
<td>8</td>
<td>2</td>
<td>35.94±1.0</td>
<td>135±5</td>
</tr>
</tbody>
</table>
EVALUATION OF TRANSFERSOMES

Particle Size, zeta potential (shown in fig 1 & 2 respectively) & percent drug entrapment of optimized batch (i.e batch B3) were found to be $130 \pm 3$ nm, $-29.3 \pm 2.4$ mV & $49.3 \pm 1.8\%$ respectively.

Drug release from optimized batch of transfersomes was studied by dialysis method. The transfersomes were found to release $94.9 \pm 2.5\%$ of drug in 6 hours.

FREEZE DRYING OF TRANSFERSOMES

Freeze drying of transfersomes using 8% w/w mannitol gave a free flowing, non-sticky product. However the particle size was found to increase to $353 \pm 6$ nm after freeze drying.

EVALUATION OF TRANSFERSOMES LOADED PATCHES

Transfersomes loaded adhesive patches were prepared by suspending transfersomes in solutions of different concentrations of Duro Tak® 87-4098. These suspensions were spread over previously prepared backing layer of polyvinyl alcohol (PVA, 2%w/v) and dried in oven at 45°C for 1 hr. The dried patches were then evaluated for thickness, weight, moisture content, moisture uptake, folding endurance and drug release in 72 hours. The results are recorded in table 3.

The thickness of the patches ranged from $0.128\pm0.018$ to $0.153\pm0.028$ mm. The minimum standard deviation values assured that the process used for preparing the patches is capable of giving reproducible results. This fact is further confirmed by weight uniformity study. T3 (25% w/v) shows highest folding capacity. The moisture content was low for the prepared formulations which help them to remain stable and free from being a completely dried and
brittle film. This revealed that the prepared patch was having capability to withstand the mechanical pressure along with good flexibility. The moisture uptake of the formulations was also low, which could protect the formulation from microbial contamination and reduce bulkiness.

**Drug Release study from Patches**

Formulation T1 to T3 was subjected to *in vitro* drug release study by Franz diffusion cell. T4 was not studied because of less folding endurance. From the figure 3, it is clearly observed that drug release was in controlled manner. Samples were collected at a different time interval for 72 hrs. Batch T1 and T3 showed 84.56 ± 1.7% and 82.76 ± 1.5% drug release after 72 hr respectively. But batch T3 has more folding endurance than T1 so the T3 was optimized.

Tensile strength and adhesion of the optimized patch was found to be 34.08 ± 1.6 gm/cm² and 0.388 ± 0.08 N/cm respectively.

**Table 3: Evaluation of TransfersomesLoaded Patches**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Concentration of acrylic adhesive (% w/v)</th>
<th>Thickness ± SD (mm)</th>
<th>Weight ± SD (mg/4cm²)</th>
<th>Folding endurance ± SD</th>
<th>% Moisture Content ± SD</th>
<th>% Moisture uptake ± SD</th>
<th>% drug release at 72 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>15</td>
<td>0.128±0.018</td>
<td>33.45±1.76</td>
<td>235 ± 9</td>
<td>0.85±0.01</td>
<td>0.67±0.02</td>
<td>84.56 ± 1.7%</td>
</tr>
<tr>
<td>T2</td>
<td>20</td>
<td>0.135±0.022</td>
<td>36.14±1.49</td>
<td>264 ± 10</td>
<td>1.02±0.02</td>
<td>0.69±0.04</td>
<td>79.48 ± 1.2%</td>
</tr>
<tr>
<td>T3</td>
<td>25</td>
<td>0.141±0.017</td>
<td>38.71±1.58</td>
<td>305 ± 6</td>
<td>0.89±0.03</td>
<td>0.67±0.01</td>
<td>82.76% ± 1.5%</td>
</tr>
<tr>
<td>T4</td>
<td>30</td>
<td>0.153±0.028</td>
<td>42.68±1.45</td>
<td>205 ± 5</td>
<td>1.09±0.02</td>
<td>0.70±0.01</td>
<td>-</td>
</tr>
</tbody>
</table>

![Fig 3: In Vitro Drug Release from Transfersomal Patch](image)
CONCLUSIONS
Transfersome is a highly adaptable and stress-responsive, complex aggregate that enables the formulation to penetrate the epidermal barrier to a greater extent due to its high deformability compared to the conventional topical dosage forms.

Methotrexate is a disease-modifying antirheumatic drug (DMARD) which is effectively used to treat RA. The problem with conventional formulation is that its solubility is low and hence it is less bioavailable and affected by gastric environment and hepatic metabolism while in injection clearance of methotrexate is very fast from joint.

So, the present investigation was aimed at development of transfersomes loaded transdermal patch of methotrexate with an objective of delivering the poorly permeable drug through the skin at controlled rate for prolonged time.

The optimized formulation was evaluated for globule size and size distribution, zeta potential, %drug entrapment and drug release. The globule size and zeta potential of the optimized formulation were found to be 130 ± 3 nm and -29.3 ± 2.4 mV respectively. Percent drug entrapment was 49.3 ± 1.8%. In vitro drug release studies showed a release of 94.9 ± 2.5% of methotrexate after 6 hours. Transfersomes were freeze dried using 8% w/w mannitol as cryoprotectant.

DURO-Tak® 87-4098 in the concentration of 25% w/v was found to give optimized patch which had a weight of 38.71±1.58 mg, thickness 0.141±0.017 mm, tensile strength 34.08 (gm/cm²), folding endurance of 305±6, % moisture absorption 0.67±0.01 % and % moisture content 0.89±0.03 % and drug release 82.76 ± 1.5% at the end of 72 hrs. Tensile strength and adhesion were found to be 34.08 ± 1.6 gm/cm² and 0.388 ± 0.08 N/cm respectively. Hence, the prepared transfersomes loaded patch could prove to be a promising topical drug delivery system in the treatment of RA.

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