FORMULATION AND EVALUATION OF RESERVOIR TYPE TRANSDERMAL PATCHES OF 18-B-GLYCRRHETIC ACID WITH PIPERINE AS BIOENHANCER

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ABSTRACT
A reservoir-type transdermal delivery system (TDS) of 18 β-Glycyrrhetic acid (GA) with Piperine as bioenhancer was prepared using 2*3 factorial design allowing for independent variables like penetration enhancers, formulation matrix and rate controlling membranes. The formulations were evaluated for drug content, in vitro and ex vivo studies. Cellophane membrane and Fresh abdominal skin of goat were used in in vitro and ex vivo permeation experiments with Franz diffusion cell respectively. In conclusion, current reservoir transdermal patch containing 5% menthol as a permeation enhancer, 42% ethanol, 2% carbopol 934 gel base (50 g) with 0.5% piperine as bioenhancer provided an improved sustained release of phytopharmaceuticals through transdermal administration. The 18 β-Glycyrrhetic acid patch was demonstrated the feasibility for future biopharmaceutical study in rats and clinical trials.

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INTRODUCTION
18-β-glycyrrhetic acid is a pentacyclic triterpenoid derivative of the beta-amyrin type obtained from the hydrolysis of glycyrrhizic acid, which was obtained from the herb liquorice. The structure of glycyrrhetic acid is similar to that of cortisone. Both molecules are flat and similar at position 3 and 11. This might be the basis for licorice’s anti-inflammatory action. It is solid off-white powder, molecular formula C_{39}H_{31}O_{7} molar mass 470.68 g mol\(^{-1}\), soluble in ethanol and chloroform [1]. 100μM 18-β-Glycyrrhetic Acid suppresses LPS-induced TNF-α production and NF-κB activation in mouse macrophages [2]. The liposomal gel with GA 0.9% showed a stronger anti-inflammatory activity than triamcinolone acetone and eazonazole nitrate cream [3]. 18-β-Glycyrrhetic acid has anti-inflammatory effects in rats and mice. The acute intraperitoneal LD (50) for 18-β-Glycyrrhetic Acid in mice was 308 mg/kg and the oral LD (50) was > 610 mg/kg. The oral LD (50) in rats was reported to be 610 mg/kg. Higher LD (50) values were generally reported for salts [4].

![Reservoir Type Transdermal Patch](image)

Fig. 1 Reservoir type transdermal patch

Pharmacokinetic parameters of 18 β-Glycyrrhetinic acid after single administration at a oral dose of 15 mg·kg\(^{-1}\) in rats (n = 6) are AUC\(_{0-\infty}\) 9.79 ± 0.98 μg·h·mL\(^{-1}\), AUC\(_{0-\infty}\) 10.30 ± 0.75 μg·h·mL\(^{-1}\), Cmax 2.09 ± 0.41 μg·mL\(^{-1}\), t\(_{\text{max}}\) 1.58 ± 0.38 h, t\(_{\frac{1}{2}}\) 2.95 ± 0.61 h. The half-life was 2.95 h shows poor bioavailability [5]. 18-β-Glycyrrhetic acid (extract of liquorice) as anti-inflammatory agent in an amount from 0.1% to 10% by weight [6].

Piperine is a pungent alkaloid present in Piper nigrum Linn, was reported to have antidiarrhoeal, antiinflammatory, immune-enhancing, anticonvulsant and antioxidant activity. Piperine inhibited gastric emptying of solids/liquids in rats and gastrointestinal transits in mice in a dose- and time-dependent manner. Piperine inhibited CYP3A4 activity in humans. Piperine may work as a drug bioavailability enhancer, which is evident by the increase in the Cmax and AUC of phenytoin, propranolol, and theophylline by coadministration of piperine (20 mg/kg) [7]. Transcutaneous permeation of Repaglinide (antidiabetic drug) in rats was enhanced by 8-fold from transdermal formulations containing piperine (0.008 % w/v) [8]. Piperine enhances bioavailability by inhibiting various metabolizing enzymes as well as transdermal permeation of aceclofenac via partial extraction of stratum corneum (SC) lipid and interaction with SC keratin. This provides scientific basis for use of Piperine to enhance the therapeutic efficacy of the concurrently administered drugs [9,10]. The objective of the present study was to design and evaluate reservoir type transdermal patch of 18-β-Glycyrrhetic acid with Piperine as bioenhancer to avoid the hepatic first pass metabolism and improve the therapeutic efficacy of the drug.

MATERIALS AND METHODS

Materials
Pure 18-β-glycyrrhetic acid and Piperine were purchased from Yucca Enterprises, Mumbai-37. The Carbopol 934, Benzyl alcohol, Menthol, Alcohol, Triethanolamine and polysobutylene were procured from Chemdyes corporation, Ahmedabad. Ethylene vinyl acetate (EVA) membranes with 9% VA content (EVA9%; 3M CoTran 9702) and 19% VA content (EVA19%; 3M CoTran 9715), backing layer (a polyester film laminate; 3M Scotchpak Backing 1006) and release liner (polyester film; 3M Scotchpak 1022 Release Liner) were gift samples from 3M Pharmaceuticals, USA. All other chemicals and solvents used were of analytical grade.

Development of reservoir type transdermal patches of 18-β-glycyrrhetic acid by heat seal method
4 g of Carbopol 934 was dispersed in100 ml of distilled water and kept overnight to get a smooth gel. Benzyl alcohol as preservative was added into gel base. Menthol, 18-β-glycyrrhetic acid and Piperine were dissolved in ethanol and poured into the Carbopol 934 gel base with continuous stirring. Triethanolamine was added drop wise to the formulation for adjustment of required skin pH (6.8-7) mentioned in Table 1.

Accurately weighed quantity of the 1g gel (9 mg 18 β-glycyrrhetic acid) was placed on a sheet of backing layer (polyester film) covering 2 cm x 2 cm areas. A rate controlling membrane (EVA with 9% or 19% vinyl acetate) was placed over the gel and the edges of 2 cm x 2 cm area was heat-sealed to obtain a leak proof device mentioned in Table 1. To ensure intimate contact of the patch to the skin, a pressure sensitive adhesive, polyisobutylene (PIB), was applied onto rate controlling membrane (3 ml; 10% w/v in petroleum ether). A release liner (3M Scotchpak 1022 Release Liner) was placed over the adhesive coated rate controlling membrane.
Table 1 Formulation of reservoir type transdermal patches of 18-β-glycyrrhetic acid

<table>
<thead>
<tr>
<th>Formulation of gel base</th>
<th>Formulations</th>
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<tbody>
<tr>
<td>Sr. No.</td>
<td>Ingredients</td>
</tr>
<tr>
<td>1</td>
<td>Carbopol 934</td>
</tr>
<tr>
<td>2</td>
<td>Distilled water</td>
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</table>

Formulation of medicated gel

<table>
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<tr>
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<tbody>
<tr>
<td>1 Gel base</td>
</tr>
<tr>
<td>2 Benzyl alcohol</td>
</tr>
<tr>
<td>3 18 β-glycyrrhetic acid</td>
</tr>
<tr>
<td>4 Piperine</td>
</tr>
<tr>
<td>5 Menthol</td>
</tr>
<tr>
<td>6 Alcohol</td>
</tr>
<tr>
<td>7 Triethanolamine</td>
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</table>

Table 2 Formulation of reservoir type transdermal patches of 18-β-glycyrrhetic acid show bioenhancer property of piperine

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<td>7 Triethanolamine</td>
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Formulation of Reservoir TDDS

<table>
<thead>
<tr>
<th>Formulations</th>
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<tbody>
<tr>
<td>1 EVA with % VA</td>
</tr>
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</table>

Preformulation studies

Description

18-β-glycyrrhetic acid was physically examined for colour and odour etc.

Solubility

Solubility of 18-β-glycyrrhetic acid was determined in water, ethanol, chloroform, phosphate buffer 7.4, DMSO.

Drug-polymer interaction study

The FTIR spectra (Spectrometer Model 2500) was taken and analyzed for any interaction between the drug and the polymers showed in Fig. 2,3 and 4.

Evaluation of Transdermal patches

Drug content uniformity

The patch (4 cm2) was added to a beaker containing 100 ml of phosphate buffered saline pH 7.4 (PBS). The medium was stirred (500 rpm) with magnetic bead for 24 hours. The contents were filtered using what man filter paper and 0.5 ml filtrate was extracted with 5 ml chloroform. Chloroform layer evaporated on water bath. Then reconstituted in 5 ml methanol and analysed by HPTLC using toluene-ethyl acetate-glacial acetic acid 12.5:7.5:0.5 as mobile phase for the drug content against the reference solution consisting of placebo patch. The plates were scanned and the compound was quantified at the wavelength of maximum absorption of 260 nm for glycyrrhetic acid [11,12].
In vitro permeation studies
The patch was placed on cellophane membrane (cellulose acetate membrane) and attached to the Franz diffusion cell such that the cell’s drug releasing surface towards the receptor compartment which was filled with 50 ml of Phosphate buffer pH 7.4 at 37± .5°C. The elution medium was stirred magnetically. The aliquots (5ml) were withdrawn at predetermined time intervals and replaced with same volume of Phosphate buffer pH 7.4[11]. The aliquot (5ml) was extracted with 5 ml chloroform. Chloroform layer evaporated on water bath. Then reconstituted in 5 ml methanol. The samples were analyzed for drug content using HPTLC at 260 nm showed in Fig. 5,6,7 and Table 3,4[12].

Ex vivo permeation studies using goat skin
Preparation of goat skin
Fresh abdominal skin of goat were collected from slaughterhouse and used in the permeation experiments. Abdominal skin hairs were removed and the skin was hydrated in normal saline solution. The adipose tissue layer of the skin was removed by rubbing with a cotton swab. Skin was kept in isopropyl alcohol solution and stored at 0-40°C. The transdermal permeation was performed in Franz Diffusion cell. The cells were filled with freshly prepared phosphate buffer pH 7.4. While placing the patch, the donor compartment contains patch on stratum corneum side of skin and dermis side was facing receptor Compartment. Receptor compartment contains phosphate buffer pH 7.4 and samples were withdrawn at regular time intervals and replaced the same with receptor fluid. The samples were analyzed HPTLC at 260 nm showed in Fig. 8 and Table 5[13].

Mechanism of drug release
Various models were tested for explaining the kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi and Korsmeyer-Peppas release model showed in Fig. 9,10,11,12 and Table 6.

• Zero order release rate kinetics
To study the zero–order release kinetics the release rate data are fitted to the following equation.

\[ F = K_0 t \]

Where ‘F’ is the drug release, ‘K_0’ is the release rate constant and‘t’ is the release time.
The plot of percentage drug release versus time is linear.

• First order release rate kinetics
The release rate date are fitted to the following equation

\[ \log (100 – F) = K t \]

A plot of log % drug release versus time is linear.

• Higuchi release model
To study the Higuchi release kinetics, the release rate data were fitted to the following equation,

\[ F = K t^{1/2} \]

Where, ‘k’ is the Higuchi constant.
In Higuchi model, a plot of percentage drug release versus square root of time is linear.

• Korsmeyer and Peppas release model
The release rate data were fitted to the following equation,

\[ \frac{M_t}{M_\infty} = k.t^n \]

Where, M is the fraction of drug released, ‘K’ is the release constant,’t’ is the release time ,’n’ is diffusion exponent.
The value of n gives an indication of the release mechanism: when n = 1, the release rate is independent of time (zero-order) (case II transport), n = 0.5 stands for Fickian diffusion and when 0.5 < n < 1.0, diffusion and non-Fickian transport (swellable and cylinder Matrix) are implicated. Lastly, when n > 1.0, super case II transport is apparent. n is the slope value of log mt/mT vs. the log time curve[14,15,16].

Permeation data analysis
The flux (μg cm\(^{-2}\). hr\(^{-1}\)) of GA was calculated from the slope of the plot of the cumulative amount of GA permeated per cm\(^2\) of skin at steady state against the time using linear regression analysis. The steady state permeability coefficient (Kp) of the drug through goat skin was calculated by using the following equation and showed in Table 7.

\[ K_p = \frac{J}{C} \]

Where. J is the flux and C is the concentration of GA in the patch.
The penetration enhancing effect of penetration enhancer was calculated in terms of enhancement ratio (ER). It was calculated by using the following equation showed in Table 7[17].

\[ ER(\%) = \frac{K_p \text{ with penetrating enhancer}}{K_p \text{ without penetrating enhancer}} \times 100 \]

**Stability study**

The stability study was conducted according to ICH guidelines by storing the prepared patch (F1) at 4°C, 40 °C and 60 °C kept in refrigerator, stability chamber and incubator for period of three months. The sample was withdrawn at 15, 30, 45, 60, 75, 90th day and analyzed for physical appearance, drug content, \textit{in-vitro} diffusion studies (showed in Fig. 13)[18].

**RESULT AND DISCUSSION**

**Preformulation Studies**

It showed white colored crystals 18-β-glycyrrhetic acid was poorly soluble in water, buffer solution pH 7.4 while soluble in ethanol, chloroform, DMSO. Interaction of drug with polymers was confirmed by carrying out FTIR interactions studies. It shows that there are no interactions found between the drug and polymers.
Drug content uniformity

Drug content was observed for all the formulations F1 to F12 which were 99.01± 0.06 % to 99.17 ± 0.06%. The results indicated that the film preparation was capable of yielding uniform drug content due to the homogenous dispersion of the drug into gel base.

In vitro release of 18 β-Glycyrrhetic acid

The release of a drug from a transdermal drug delivery system occurs by diffusion. Transport of GA from the polymeric rate controlling membrane (EVA) into the in vitro study medium depending upon % of Carbopol Gel base, % of penetrating enhancer menthol, % of vinyl acetate in EVA as well as % of bioavailability enhancer Piperine. The results of release profile indicated that as the % of Carbopol Gel base increased in patch, the drug release from the patches is decreased (F5>F1, F6>F2 F7>F3 F8>F3).

Concentration of menthol increased from 2% to 5% in the formulation; the in vitro release rate increased (F3> F1, F4> F2, F7> F5, F8> F6).

Hydrophobic nature of EVA polymer retards the drug release but the percentage of vinyl acetate in EVA membrane helps in the release of drug from membrane due to pore forming property. EVA with 19% VA membrane showed greater drug release (F2> F1, F4> F3, F6> F5, F8> F7) as compared to EVA with 9% VA.

0.25 %, 0.5% and 1 % piperine in the formulations F10,F4,F11 increased bioavailability of GA 30%,55.44% and 55.44%. However, increasing the concentration of piperine to 1% w/v did not further enhance the permeation of GA.

The enhancement in the permeation of GA (95.5% in F4) in the presence of piperine:methanol (0.5%:5%) mixture suggested its better performance as compared to that of 0.5 % piperine ( 50.31 % in F12) as well as 5% methanol (40.11 % in F9) alone. It is worthy to note that the piperine:methanol (0.5%:5%) mixture in F4 formulation was significantly more effective for ex vivo analysis of 18 β-Glycyrrhetic acid.

Fig.5 HPTLC of S = Standard (Glycyrrhetic acid + Piperine) and Test = T1,T3,T5(In vitro release of F4 patch after 2, 6, 10 hr)

Fig. 6 In vitro drug release of 18 β-glycyrrhetic acid
Table 3 In vitro drug release of 18 β-glycyrrhetic acid

<table>
<thead>
<tr>
<th>Formulations</th>
<th>In vitro % Cumulative release of 18 β-Glycyrrhenic acid</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.5 hr</td>
</tr>
<tr>
<td>F1</td>
<td>2.5</td>
</tr>
<tr>
<td>F2</td>
<td>3.9</td>
</tr>
<tr>
<td>F3</td>
<td>4.2</td>
</tr>
<tr>
<td>F4</td>
<td>5.1</td>
</tr>
<tr>
<td>F5</td>
<td>1.4</td>
</tr>
<tr>
<td>F6</td>
<td>3.1</td>
</tr>
<tr>
<td>F7</td>
<td>2.3</td>
</tr>
<tr>
<td>F8</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Fig 7. In vitro drug release show bioenhancer property of piperine

Table 4 In vitro drug release show bioenhancer property of piperine

<table>
<thead>
<tr>
<th>Formulations</th>
<th>In vitro % Cumulative release of 18 β-Glycyrrhelic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 hr</td>
</tr>
<tr>
<td>F9</td>
<td>1.2</td>
</tr>
<tr>
<td>F10</td>
<td>4.7</td>
</tr>
<tr>
<td>F11</td>
<td>4.9</td>
</tr>
<tr>
<td>F12</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Ex vivo release of 18 β-glycyrrhetic acid

The prepared F4 transdermal patch of 18 β-Glycyrrhetic acid was evaluated for ex vivo release pattern. The mechanism of permeation enhancement of menthol could involve its distribution preferentially into the intercellular spaces of stratum corneum and the possible reversible disruption of the intercellular lipid domain [19]. Bioenhancer, piperine increase the bioavailability of drug by biphasic mechanism involving partial extraction of stratum corneum (SC) lipid and interaction with SC keratin [20]. Ex vivo study using fresh abdominal skin of goat as permeability membrane confirms the release of 18 β-Glycyrrhelic acid in patch as controlled delivery over 10h as 91.58 %.
Kinetic modeling of ex vivo drug release

Release of the drug from transdermal patch is controlled by the chemical properties of the drug and delivery form, as well as physiological and physicochemical properties of the biological membrane. The release profile for formulation F4 was fitted to zero order kinetics (cumulative % drug release vs. time plot) in Fig.5, first order kinetics (log% remaining to be released vs. time plot) in Fig.6, Higuchi model (cumulative % release vs. square root of time plot) in Fig.7 and Korsmeyer-Peppas model in Fig.8 (log cumulative % release vs. log time plot). The rate constants were calculated from the slope of the respective plots. The regression coefficients and the release rate constants of different kinetic models were tabulated in table 2. The zero-order plots of F4 was found to be fairly linear, as indicated by their high regression values. Therefore it was ascertained that the drug permeation from these formulations could follow either near zero or zero-order kinetics. Hence, to confirm the exact mechanism of drug permeation from these patches, the data obtained were also fitted to Korsmeyer-Peppas model in order to find out the ‘n’ value, which describes the release mechanism. The ‘n’ value for formulation F4 lies between 0.5 to 1 indicating the mechanism of drug release to be diffusion and non-Fickian transport [15,16].
Fig. 10 First order release of F4

Fig. 11 Higuchi model

Fig. 12 Korsmeyer-Peppas model
Table 6: Kinetic modeling of ex vivo drug release

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zero order</th>
<th>First order</th>
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<th>Korsmeyer-Peppas</th>
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<tbody>
<tr>
<td>F4</td>
<td>0.9886</td>
<td>0.8926</td>
<td>0.9867</td>
<td>0.9902</td>
</tr>
</tbody>
</table>

Permeation data analysis
Enhancement ratio was used to evaluate the effect of permeation enhancer on diffusion and permeation of selected drug molecules.

Table 7 Permeation data analysis of F4 formulation

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Flux (J) µg.cm⁻².h⁻¹</th>
<th>Enhancement ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F12 (without enhancer)</td>
<td>56.27</td>
<td>1.9</td>
</tr>
<tr>
<td>F4 (with enhancer)</td>
<td>107.57</td>
<td>1.91</td>
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</table>

Stability study
In order to determine the change in physicochemical parameter and in-vitro release profile on storage, stability study was carried out. The physicochemical parameter of the optimized formulation was not significantly changed on storage. The in-vitro release profile before and after storage is shown in Figure 5. The result indicates that the formulation was stable on the required storage condition.

CONCLUSION
Transdermal patch consisting of the Carbopol 934-GA reservoir with menthol as permeation enhancer and EVA (19% vinyl acetate) as rate-controlling membrane demonstrated sustained and controlled release of the drug across goat skin during ex vivo permeation study. Use of piperine enhanced the bioavailability of the drug. Thus it can be concluded that in vivo bioavailability of GA can be investigated by using animal models.

REFERENCES
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2. www.caymanchem.com/pdfs/11845