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FORMULATION AND EVALUATION OF TRANSDERMAL PATCHES OF 18-B-GLYCYRRHETIC ACID

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ABSTRACT: An attempt was made to formulate and evaluate the 18-β-glycyrrhetic acid (GA) transdermal drug delivery system. Preformulation studies on the drug 18-β-glycyrrhetic acid were done which included description, solubility and compatibility studies. Solvent casting technique was used to prepare the transdermal patches. Four formulations were made with 400mg of 18-β-glycyrrhetic acid and by using polymers Hydoxypropyl methylcellulose (HPMC) and Ethyl cellulose (EC) with different concentration of menthol (2%, 5%) as penetration enhancer. Interaction of drug and polymer was confirmed by FTIR studies. Transdermal patches were evaluated for the weight, thickness, percentage moisture uptake, folding endurance, water vapor transmission rate, in-vitro release and in-vivo release studies. This was done for four formulations F1, F2, F3, and F4. The F1 formulation showed a good release profile due to increase in concentration of penetration enhancer. The accelerated short term stability study indicated that the optimized formulation F1 has very good stability at 4 ^{o} / 40 ^{o} / 60 ^{o} for a period of 3 months.

INTRODUCTION: 18-β-glycyrrhetinic 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 glycyrrhetinic 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 C30H46O4, molar mass 470.68 g mol−1, soluble in ethanol and chloroform [1]. 100µM 18-ß-Glycyrrhetic Acid suppresses LPS-induced TNF-α production and NF-κβ activation in mouse macrophages [2].

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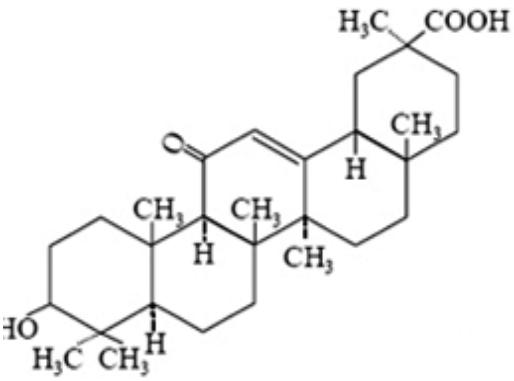
The liposomal gel with GA 0.9% showed a stronger anti-inflammatory activity than triamcinolone acetonide and econazole nitrate cream [3].



GLYCYRRHETIC ACID

18-ß-Glycyrrhetic acid has anti-inflammatory effects in rats and mice. The acute intraperitoneal LD_{50} for 18-ß-Glycyrrhetinic 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]. The pharmacokinetics of 18-β-glycyrrhetic acid was studied in rabbits following intravenous injection at dose 20 mg.kg-1. The half-life was 2.85 h shows poor bioavailability [5].

18-ß-Glycyrrhitic acid (extract of liquorice) as anti-inflammatory agents in an amount from 0.1% to 10% by weight [6]. Transdermal drug delivery system (TDDS) is a well-accepted means of delivering many drugs to the systemic circulation and currently transdermal patch devices are used to treat motion sickness, hypertension, angina, female menopause, severe pain states [7]. Transdermal medication offers many advantages such as it delivers a steady infusion of a drug over an extended period of time. It can increase the therapeutic value of many drugs by avoiding specific problem associated with drug such as GIT, low absorption, decomposition due to first pass effect. The simplified medication regimen leads to improved patient compliance and reduce inter and intrapatient variability [8].

Transdermal patches deliver drugs at a constant rate for 24 hours or longer. Norplant system releases progestin levonorgestel from silicon rubber tubular capsules for several years. The promise of zero order release is to maintain a constant drug concentration in blood for an extended period of time. The zero order release of a drug, however, does not necessarily result in a constant drug concentration in blood. The absorption of the drug by the body usually does not follow the zero order kinetics, except when the drug is directly delivered into the blood stream by an infusion pump [9].

The objective of the present study was to design and evaluate transdermal polymeric matrix films of HPMC and EC containing 18-ß-Glycyrrhitic acid to avoid the hepatic first pass metabolism and improve the therapeutic efficacy of the drug.

MATERIALS AND METHODS:

Materials: Pure 18-ß-glycyrrhetinic acid was purchased from Yucca Enterprises, Mumbai-37. The polymers Ethyl Cellulose (EC) and Hydroxy Propyl Methyl Cellulose (HPMC) were procured from Chemdyes Corporation, Ahmedabad.

All other chemicals and solvents used were of analytical grade.

Methods: It is one of the important prerequisite in development of any drug delivery system. Preformulation studies were performed on the drug, which included solubility and compatibility studies.

Preformulation studies:

1. **Description:** 18-β-glycyrrhetic acid was physically examined for color and odor etc.

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

3. **Drug-polymer interaction study:** The FTIR spectra (Spectrometer Model 2500) was taken and analyzed for any interaction between the drug and the polymers.

4. **Preparation of standard curve for 18-β-glycyrrhetic acid:** The standard stock solutions of 18-β-glycyrrhetic acid was prepared by dissolving 10 mg of glycyrrhetinic acid in Phosphate Buffer (pH-7.4) : Ethanol in 70 : 30 proportion and final volume was adjusted with same solvent in 10mL of volumetric flask to get a solution containing 1000 µg/mL of glycyrrhetinic acid. Aliquots of working stock solutions of glycyrrhetinic acid was prepared with in the same solvent to get concentration in range of 5-35µg/ml of glycyrrhetinic acid. The absorbance of resulting solutions was measured at 254nm [10]. A calibration curve as concentration vs. absorbance was constructed to study the Beer-Lambert's Law and regression equation, as shown in **figure 1 and 2**.

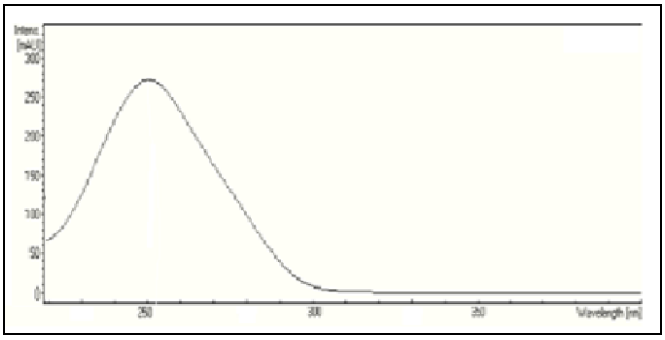


FIG. 1: 18-B-GLYCYRRHETIC ACID WITH λ_{max} 254 nm

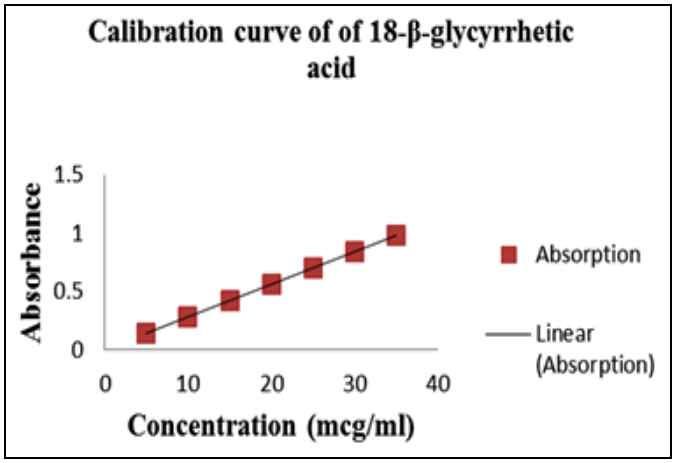


FIG. 2: CALIBRATION CURVE OF 18-β-GLYCYRRHETIC ACID

Preparation of Transdermal patches: The solvent casting technique was used to formulate the 18-β-glycyrrhetic acid transdermal patch. The adhesive patch prepared by dissolving polymer and drug in ethanol: water (10:1). To this menthol (2%, 5% w/w of the polymer) as permeability enhancer, glycerine (30% w/w of the polymer) as plasticizer were added and mixed well. The polymeric dispersion was poured into a petridish (9 cm^2 diameter). To control the rate of evaporation of solvent, the mould was covered with a funnel of suitable size and the casting solvent was allowed to evaporate to obtain the dried films .The films were cut into small patches (4 cm^2) and stored between sheets of wax paper in desiccators for further evaluations.

TABLE 1: FORMULATION OF 18-β-GLYCYRRHETIC ACID TRANSDERMAL PATCHES

Batch code	Drug (mg)	HPMC (mg)	EC (mg)	Menthol (%)	Ethanol: water (ml)	Glycerine (%)
F1	400	500	-	5	30:3	30
F2	400	500	-	2		30
F3	400	-	500	5		30
F4	400	-	500	2		30

Evaluation of Transdermal patches:

1. **Weight of the patch:** Three patches from each batch were taken and weight of each patch was found by using electronic balance. Then average weight of single patch was determined [10].

2. **Thickness of the patch:** The thickness of the patch was assessed by using screw gauge at different points of the patch. From each formulation three randomly selected patches were used. The average value for thickness of a single patch was determined [11].

3. **Percentage moisture content:** The films were weighed individually and kept in a desiccator containing fused calcium chloride at room temperature for 72 hours. The film was again weighed and the percentage moisture content was calculated using the formula [11]:

% moisture content = [initial weight – final weight / final weight] × 100

4. **Percentage moisture uptake:** The weighed films were kept in a desiccator at room temperature for 72 hours and then exposed to 84 % relative humidity using a saturated solution of potassium chloride. Finally, the films were weighed and the percentage moisture uptake was calculated using the formula [11];

% moisture uptake = [Final weight - Initial weight / initial weight] × 100.

5. **Folding Endurance:** The number times the films could be folded at the same place without breaking gave the value of folding endurance. It was expressed a number of times. The patches were folded at same place either to break the patches or to develop visible curves. It was done normally for the prepared [11].

6. **Water Vapor Transmission:** Glass vial of equal diameter were used as transmission cells. These transmission cells were washed thoroughly and dried in an oven. The prepared film was fixed over the edge of the glass vial containing 3 gm of fused calcium chloride as a desiccant by using an adhesive. Then the vial was placed in a desiccator containing saturated solution of potassium chloride. The vial was taken out periodically and weighed for a period of 72 h. The experimental was performed in triplicate and the average values were calculated and given result [11].

$$WVT = WL/S$$

Where, W = Water vapour transmitting in gm, L = Thickness of the patch in cm, S = Exposed surface

7. **Drug content uniformity:** The patch (1 cm2) was cut and added to a beaker containing 100 ml of phosphate buffered saline pH 7.4 (PBS). The medium was stirred (500 rpm) with Teflon coated magnetic bead for 5 hours. The contents were filtered using Whatmann filter paper and the filtrate was analysed by U.V. Spectrophotometer at 254 nm for the drug content against the reference solution consisting of placebo films [11].

8. ***In vitro* drug release studies:** The fabricated film was placed on cellophane membrane (cellulose acetate membrane)and attached to the 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°. 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. The samples were analyzed for drug content using UV spectrophotometer at 254 nm [11].

9. **Anti-inflammatory activity:** Albino rats of Wistar strain of either sex between 130-240 gm breed were selected for the studies. The animals were fastening overnight but allow water *at libidum*. The permission to carry out animal studies was obtained from CPCSEA [1351/c/10/ CPCSEA]. The animals were divided into six group comprising six animals of either sex in each group as,

Group I (Control group)	For Control (co)
Group II (Standard group)	Marketed formulation Brugel (1 g)
Group III	F1 Patch (2*2 cm2)
Group IV	F3 Patch (2*2 cm2)

The anti-inflammatory activity of formulated patches will be evaluated by the carrageenan-induced rat hind paw edema method of Winter et al. One day before the experiment, the left hind thigh of each animal was shaved without damaging the skin. The patch samples were applied to the shaved area in the left hind thigh.

The Group I (control) received orally 0.5ml of normal saline solution. The Group II (standard) received Marketed formulation Brugel. The Group III and IV received F1 and F3 formulation respectively. One hour before patch application 0.1 ml of 1% Carrageenan in isotonic saline was injected subplantarly into left hind paw. The volume of the left hind paw was measured using a displacement of plethysmometer [12, 13].

$$\% \text{ inhibition of edema} = (1- Vt / Vc) * 100$$

Where, Vt = Volume of edema in test, Vc = Volume of edema in control

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, *in-vitro* diffusion studies [14].

RESULTS & 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 IR interactions studies. It shows that there are no interactions found between the drug and polymers.

Physicochemical characterization of Trans-dermal Films: All the Physiochemical Parameters were showed in **Table 2**. The weight variation of formulated films was found to be in the range of 128.17±0.75 to 128.18±0.68 and thickness of formulated films was found to be in the range 0.25±0.13mm to 0.25±0.15mm. A low standard deviation (SD) value in the film thickness measurements ensures uniformity of the films.

The values of folding endurance were found to vary from 262±0.4to 288±0.4which indicates good strength and elasticity The Percentage drug content of formulated films was found to be in the range of 98.24±0.09 to 98.24±0.09per 4 cm^2 strip. However, there was increased moisture content with an increase in hydrophilic polymers.

The film F1 showed maximum WVTR, %MU, and %MC, which may be attributed to the hydrophilic nature of HPMC and EC decreased these values. The results indicated that the hydrophilicity of the polymers is directly proportional to the WVTR, %MU, and %MC. The order of hydrophilicity of the polymers was HPMC >EC. However, the small moisture in the formulations may prevent complete drying and brittleness. Overall, the moisture uptake of the transdermal films was low and thus reduced the bulkiness of the films. % drug content was observed for all the formulations which were 99.01± 0.06 % to 99.17 ± 0.06mg. The results indicated that the film preparation was capable of yielding uniform drug content due to the homogenous dispersion of the drug.

TABLE 2: EVALUATIONS OF TRANSDERMAL PATCHES

Sr. No.	Formulation code	F1	F2	F3	F4
1	Thickness (mm) ± S.D	0.25 ± 0.13	0.25 ± 0.15	0.25 ± 0.13	0.25 ± 0.15
2	Weight variation (mg) ± S.D	128.17 ± 0.75	128.18 ± 0.68	128.18 ± 0.72	128.17 ± 0.75
3	Moisture uptake ± S.D	7.25 ± 0.14	7.24 ± 0.15	6.05 ± 0.16	6.17 ± 0.17
4	Moisture content ± S.D	8.5 ± 0.3	8.4 ± 0.4	6.4 ± 0.2	6.5 ± 0.4
5	Folding Endurance	285± 0.2	288± 0.4	265± 0.3	262± 0.4
6	Water vapour transmission rate (mg/cm/hr)	4.34*10-6	4.02*10-6	1.22*10-6	1.14*10-6
7	% Drug content ± S.D	99.17 ± 0.06	99.07 ± 0.07	99.03 ± 0.04	99.01± 0.06

***In vitro* drug release studies:** The release of a drug from a transdermal drug delivery system occurs by diffusion, which involves transport of a drug from the polymer matrix in to the *in vitro* study medium depending on concentration. The EC was use it retards the release of the drug from the matrix due to the more hydrophobic nature, therefore the prolonged drug release was obtained. The formulation F1 and F2 containing HPMC showed 95.35% & 84.90 % drug release over 10 hrs due to hydrophilic nature of the polymer. Whereas the formulation F3 and F4 containing EC Showed 90.05 % & 81.23 % drug release in 10 hrs due to hydrophobic nature of the polymer, as shown in **figure 3 and Table 3.**

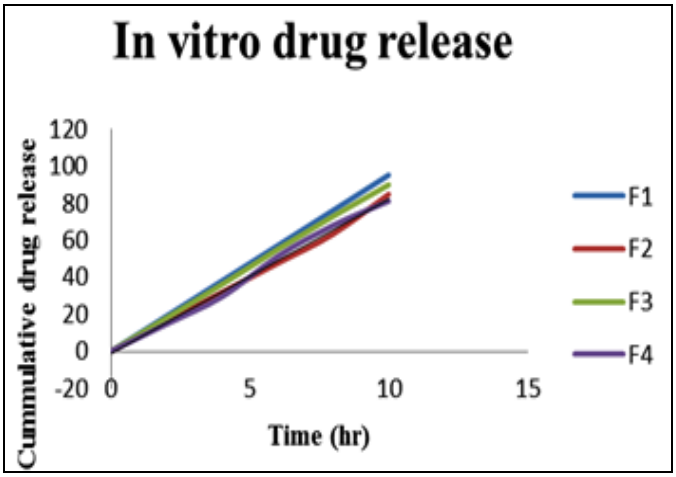


FIG. 3 *IN VITRO* STUDY OF 18-β-GLYCYRRHETIC ACID

TABLE 3: *IN VITRO* RELEASE OF 18-B-GLYCYRRHETIC ACID

Time (hr)	Cumulative % drug release			
	F1	F2	F3	F4
0	0	0	0	0
2	19.05	16.11	18.09	14.78
4	38.09	31.89	36.23	29.56
6	57.11	47.67	55.17	51.34
8	76.23	63.45	73.11	68.12
10	95.35	84.9	90.05	81.23

***In vitro* permeation and the effect of penetration enhancers:** In the presence of penetration enhancers, (5%menthol) film F1 and F3 showed the highest skin permeation of 99.17 ± 0.06% and 99.03 ± 0.04% while F2 and F4 with 2% menthol showed 99.07 ± 0.07% and 99.01 ± 0.06% respectively. It showed the role of permeation enhancer in drug release. %Drug release increase with increase in % menthol, as shown in **Table 3**.

TABLE 3: ANTI-INFLAMMATORY EFFECT IN TERMS OF PERCENT INHIBITION OF EDEMA

Time in min.	Standard	F 1	F 2
Before 30	-	-	-
0	10.14	10	9.82
30	20.28	22.71	21.72
60	30.42	28.90	28.32
120	37.51	38.23	34.79
240	50.21	49.93	48.91

CONCLUSIONS: Based on the results of this study, it can be concluded that a well-controlled release and effective skin permeation of the drug was achieved by the film F1 (HPMC) with 5% menthol as permeability enhancer for extended periods of time. The in vivo study has proved the feasibility of controlled transdermal delivery of 18-β-glycyrrhetic acid in adequate quantity into the circulation. However, to establish the therapeutic efficacy of this formulation, pharmacokinetic studies in humans needs to be conducted.

REFERENCES:

1. en.wikipedia.org/wiki/Glycyrrhetinic acid
2. www.caymanchem.com/pdfs/11845
3. Li S. A novel transdermal fomulation of 18β-glycyrrhetic acid with lysine for improving bioavailability and efficacy. Skin Pharmcol Physiol. 2012;25(5):257-68
4. Glycyrrhetinic Acid: toxicity. Int J Toxicol. 2007; 26 (2):79-112.
5. Xiu-Ping Shen, Pei-Gen Xiao, Chang-Xiao Liu1. Research and application of Radix Glycyrrhizae. Asian Journal of Pharmacodynamics and Pharmacokinetics. 2007; 7(3):181-200.
6. Peter James Ayres, Steven Leigh ster. Pharmaceutical compositions containing urea. EP 0006724 A1. Phares Pharmaceutical Research N.V. Jan 9, 1980.
7. Guy RH. Current status and future prospects of transdermal drug delivery, Pharm Res 1996; 13:1765-1769.
8. Dey BK, *et al*: Development and evaluation of Propanolol hydrochloride Transdermal patches by using hydrophilic polymers. Indian J Pharm. Sci. 2007; 388-393.
9. M.E. Aulton Pharmaceutics- The science of dosage form design, 2nd edition: 656-659.
10. Patil SK, Salunkhe VR, Mohite SK. Development and validation of UV spectrophotometric method for estimation of Glycyrrhetinic acid in hydroalcoholic extract of *Glycyrrhiza glabra*. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2012; 2(4): 617-621.
11. Yadav Shashikumar, *et al*. Formulation and evaluation of transdermal patch for antirheumatic Ayurvedic medicine using different polymer compositions: *in vitro*. Journal of Global Trends in Pharmaceutical Sciences. January–March 2013; 4(1): 999-1006.
12. Vogel H. Gerhard, Drug Discovery and Evaluation Pharmacological Assays, second edition, 759-761.
13. Winter CA, Risley EA and Nuss GW. Carrageenan-induced edema in the hind paw of rat as an assay for anti-inflammatory drugs. Proc. Soc. Biol. Med, 1962; 11: 544-547.
14. S Jayprakash, *et al*. Formulation, *in-vitro* and *in vivo* characterization of Transdermal patches of etoricoxib. International Journal in Review of Life Science 2012; 2(1):17-23.

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