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Research Article

FORMULATION AND EVALUATION OF TRANSDERMAL PATCH OF INDOMETHACIN CONTAINING PATCHOULI OIL AS NATURAL PENETRATION ENHANCER

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ABSTRACT

Objective: To develop a transdermal patch of Indomethacin using patchouli oil as a natural enhancer to increase transdermal permeation of the drug from the matrix system across rat epidermis.

Materials and Methods: The chemical characterization of patchouli oil was done by gas chromatography-mass spectrometry. Transdermal patches of indomethacin were formulated after studying the drug-excipient compatibility studies by differential scanning calorimetry and Fourier transform infrared spectroscopy (FTIR). The transdermal patches were evaluated for various physiochemical properties. *In-vitro* transdermal permeation was carried using modified Keshary-Chein diffusion cell across rat epidermis. FTIR studies of rat epidermis were done to understand the mechanism of the permeation enhancing effect of the oil from the matrix patch.

Result: The results of physiochemical parameters of the transdermal patch were found satisfactory. The transdermal flux obtained of the different concentration of patchouli oil tend to increase with increasing concentration of the oil and the maximum transdermal flux of 61.92 ± 0.89 μg/cm^2/hr was obtained with formulation F7 (containing 1% w/v of patchouli oil) which is similar to the flux of the formulation F2 containing standard enhancer dimethyl sulphoxide. The skin irritation test did not show any edema and the FTIR data of rat epidermis indicated that patchouli oil enhances transdermal permeation of indomethacin by partial extraction of lipids in the stratum corneum.

Conclusion: Thus, the results showed a potential enhancing effect of patchouli oil on the transdermal permeation of the model drug indomethacin and may be used as natural permeation enhancer in transdermal drug delivery systems.

Keywords: Patchouli oil, Penetration enhancer, Keshary-Chein diffusion cell, Rat epidermis.

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INTRODUCTION

The skin is a large multilayer organ serves as barrier against physical and chemical attack and use as the most desirable site for systemic and topical delivery of drugs because of its easy accessibility [1]. Transdermal drug delivery offers a number of advantages over other conventional methods such as increase patient compliance, sustained release of drugs, and bypassing the gastric first pass metabolism [2].

However, the barrier function of the stratum corneum (outermost layer of the skin) is the main limitation to its use and hence to overcome these barrier properties of the skin penetration enhancers has gain significance in pharmaceutical research [3]. These are the agents helping in increasing the permeation of the skin and are of both natural and synthetic sources which includeazones, pyrrolidones, surfactants, essential oil, etc. [4].

Essential oils are the aromatic compound extracted from natural sources mostly plants, and as skin penetration enhancers they partition into the stratum corneum and cause interaction with components of the tissue to lessen the barrier function of the stratum corneum but do not cause any damage to the skin cell lying underneath [5].

Many types of research have been done to investigated the permeation enhancing effect of essential oils, for example, eucalyptus oil was investigated and it was found to increase permeation of chlorhexidine [6], niaouli oil was investigated and found to be effective as transdermal penetration enhancers for estradiol [7], fennel oil was found to increase the transdermal permeation of trazodone hydrochloride [8].

In this quest of studying the potency of essential oil as permeation enhancers, the present work patchouli oil which is an essential oil was selected to investigate for its penetration enhancing the effect on transdermal patches having indomethacin as a model drug.

Patchouli oil is extracted from *Pogostemon cablin* of the family *Lamiaceae* and is been used widely in traditional medicine practices in China and in India [9]. The model drug selected, indomethacin is a non-steroidal anti-inflammatory drug which is used for the treatment of inflammation, arthritis, and pains but on oral administration in chronic treatment causes various gastrointestinal effects as side effects and hence the development of an effective transdermal system will eliminate its side effects [10].

MATERIALS AND METHODS

Materials

Chemicals

Patchouli oil was collected from North Eastern Development Finance Corporation Limited, Assam, India. Indomethacin, hydroxypropyl methylcellulose (HPMC K100), polyvinylpyrrolidone (PVP K30) was received from Yarrow Chemical Products (Mumbai, India). Polyethylene glycol (PEG 400) was received from Merck Specialties Pvt. Ltd. (Mumbai, India) and dimethyl sulfoxide (DMSO) was received from Avantor Performance Materials India Limited (Haryana, India). All other reagents and chemicals used throughout the study were of analytical grade.

Animals

Adult male Wistar albino rats (250-500 g) and adult male New Zealand albino rabbits (1.5-2 kg) were supplied by Animal House of Girijananda

Chowdhury Institute of Pharmaceutical Science and was inhabited under standard laboratory condition with proper diet. The animals were received after the proposed study was approved by the Institute Animals Ethics Committee, and Committee for Purpose of Control and Supervision of Experiments on Animals, Government of India bearing the number GIPS/IAEC/M.Ph/2017/03.

Methods

Physicochemical characterization of the drug and Patchouli oil

Drug (indomethacin)

Odor and color of the drug were observed by smelling and visual inspection, respectively. The melting point of the drug was determined using melting point apparatus (MAC, Digital melting point apparatus, Macro Scientific Works).

Patchouli oil

The color, odor, and solubility of the essential oil in organic solvents were checked manually and the viscosity of the oil was measured using a Brookfield DV-E viscometer.

Chemical characterization by gas chromatography-mass spectrometry (GC-MS)

The GC-MS analysis of the oil was performed on a Parkin Elmer Clarus 680/600 chromatogram with build in autosampler using a fused DB-5 capillary column (length 30 m × 0.25 mm internal diameter (ID), film thickness 0.25 μm), equipped with an Elite-5 MS capillary column and FID detector. The oven temperature was programmed from 50° to 260°C at a rate of 5°C/min. The injector temperature was set at 250°C and the injection volume was 1.0 μL. The run time was 46 minutes the gas used was helium with a flow rate of 1.1 mL/min [11].

Drug-excipient compatibility study

The compatibility study of the drug with excipients to be used in the formulations was done by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) studies [12-14].

FTIR study

The compatibility of the drug with excipients was studied using a Bruker FTIR spectrophotometer (model 220, Germany) in the range 4000-400 cm^{-1}. The FTIR spectra of drug, excipients, and physical mixture of drug-excipient are compared for presence or absence of incompatibility.

DSC study

For thermal analysis of Indomethacin and Indomethacin-excipient mixtures, a differential scanning calorimeter (Jade DSC, Perkin Elmer 2000, USA) was used. Individual samples of indomethacin, excipients, and physical mixtures of indomethacin and selected excipients were mixed in the ratio 1:1 and scanned in the temperature range of 20-300°C under an atmosphere of dry nitrogen at a heating rate of 10°C/minute. The thermograms hence obtained were checked for any interaction.

Preparation of transdermal patch

Transdermal patches (matrix type) were prepared using solvent evaporation technique. In this method bangles are used, the bottom of which are covered with aluminum foil. The transdermal patches were prepared in accordance with the formula shown in Table 1. HPMC (K100) and PVP (K30) in requisite amount were measured and dissolved in the solvent containing chloroform and ethanol in the ratio 1:1. Indomethacin was added to the homogeneous dispersion followed by addition of PEG which was used as a plasticizer. The uniform dispersion was then cast to the aluminum backing membrane; the evaporation rate was controlled using inverting funnel over the patches. After 24 hrs, the dried films were taken out, wrapped in aluminum foil and kept in a desiccator for further use [15].

Evaluation of transdermal patches

Folding endurance

The evaluation of folding endurance was done by cutting a strip of a specific area which was repeatedly folded at the same place without breaking and which gives the value of folding endurance [16].

Film thickness

The film thickness was evaluated by measuring the film at different places using a screw gauge and taking the average of five readings [17].

Drug content

For estimating drug content, a required area of the patch is cut and is put into 100 ml phosphate buffer (pH 7.4) shaken continuously for 24 hrs. The solution is then subjected to ultrasonication for 15 minutes and after which it is filtrated and the drug content is analyzed by ultraviolet spectrophotometer at lambda max (λ_{max}) of 256 nm [18].

Uniformity of weight

Uniformity of weight is determined by weighing individually 10 randomly selected patches and then the average weight is calculated [19].

Skin irritation study

For performing skin irritation study healthy rabbits (average weight 1.2-1.5 kg) were taken and the samples were applied on the dorsal surface of the rabbit skin, hair of which was removed by shaving and cleaned with rectified spirit. Adhesive tape USP was used as control patch. The formulations (transdermal patch) were removed after 24 hrs and the skin was examined for erythema/edema [20].

Skin preparation and in-vitro permeation study

Preparation of rat skin

The rat skin was prepared by first anesthetizing the male Wistar albino rats with chloroform after which they were sacrificed. The abdominal hair was shaven with the help of electrical clipper, then the epidermal layer was surgically removed and then to remove the adhering fat it was wiped with isopropyl alcohol, it was then soaked in phosphate buffer pH 7.4, wrapped in aluminum foil, and kept in the deep freezer for further studies [21].

Permeation study

The *in-vitro* permeation study was done using a modified Keshary-Chein diffusion cell using rat epidermis. The rat epidermis was mounted

Table 1: Formulation composition of patches containing indomethacin

Formulation code	HPMC K100 (mg)	PVP K30 (mg)	PEG 400 (ml)	Indomethacin (mg)	DMSO (% w/v)	Patchouli oil (% w/v)
F1	130	160	0.3	30	-	-
F2	130	160	0.3	30	0.05	-
F3	130	160	0.3	30	-	0.05
F4	130	160	0.3	30	-	0.25
F5	130	160	0.3	30	-	0.5
F6	130	160	0.3	30	-	0.75
F7	130	160	0.3	30	-	1.0

HPMC K100: Hydroxypropyl methyl cellulose, PVP: Polyvinyl pyrolidone, PEG 400: Polyethylene glycol, DMSO: Dimethyl sulphoxide

between the donor and the receptor compartment of the diffusion cell and the patch was cut into requisite shape and placed over epidermis in the donor compartment. The permeation study was carried for 8 hrs with the receptor compartment containing 34 ml of phosphate buffer pH 7.4 maintained at 37±0.5°C. 2 ml of sample at different intervals was withdraw and analyzed spectrophotometrically at λ_{max} of 256 nm against blank and receptor phase was replaced with equal volume of phosphate buffer at each intervals. Cumulative release curve per cm^2 of the patch was plotted against time and the permeation parameters of indomethacin across rat epidermis treated with patch containing patchouli oil were compared with the placebo patch and appropriate control (DMSO) [22,23].

Permeation enhancement mechanistic study

The permeation enhancement mechanistic study was done by FTIR study of rat epidermis treated with the transdermal patch without the essential oil (patchouli oil) as a permeation enhancer and of the epidermis treated with the patch containing the essential oil (patchouli oil).

Data analysis

For estimating the *in-vitro* release parameters, the flux of indomethacin across rat epidermis was calculated from the slope of cumulative release curve per cm^2 of the patch cross the skin versus time using linear regression analysis. The lag time (T_{lag}) was calculated from the X-intercept from the linear portion of the plot, the cumulative amount of drug permeated Q_{24} (µg/hr) was the amount of drug penetrating through the skin after 24 hrs. The enhancer ratio was also estimated using the equation: $E_{SS}=J_{SS}$ (enhancer treated)/J_{ss} (control). All the data, hence, found was expressed as a mean ± standard deviation (SD) [24-26].

The therapeutic transdermal daily dose for indomethacin was calculated using the equation: $T_d=D_o\times F/100$, where D_o is the oral dose F is the bioavailability after oral administration in percentage [27].

RESULTS AND DISCUSSION

Physiochemical evaluation of drug and oil

Physiochemical properties of drug

After proper observations, indomethacin was found to be colorless, odorless and it was soluble in ethanol, methanol, and acetone and the melting point of the drug sample was found to be 162 ± 0.5°C (mean ± SD, n=3).

Physiochemical properties of oil

The results of physicochemical evaluation of the patchouli oil are presented in Table 2.

GC-MS analysis of patchouli oil

After performing the GC-MS study of the patchouli oil resulted in the identification of 16 components of the major peaks in the gas chromatogram. The results hence found are given in Fig. 1 and Table 3.

Drug excipient compatibility studies

FTIR spectroscopy

All the characteristic peaks of the pure drug indomethacin were retained in drug and polymer physical mixtures, which indicate that the drug and polymer are compatible. The results of the FTIR studies were shown in Table 4 and in the Fig. 2.

DSC studies

From the DSC studies, the results were shown in Fig. 3. The melting point of Indomethacin was found to be at 162.89°C, a mixture of HPMC K100 + indomethacin and PVP K30 + indomethacin at 161.68°C and 153.63°C, respectively, which were in range and thus it was concluded that no type of drug excipient compatibility was found.

Table 2: Physiochemical properties of oil

S.No.	Parameters	Results
1	Color	Slightly brown
2	Odor	Characteristic unpleasant
3	Taste	Characteristic pungent
4	Solubility	Chloroform, acetone
5	Density	0.963±0.07 g/mol*
6	Viscosity (cP)	509 cP (50 rpm/min)* 307 cP (100 rpm/min)*

*All the values are given in mean±SD (n=3). cP: Centipoise, SD: Standard deviation

Table 3: Identified components of patchouli oil

S.No.	Compound	Retention time (minutes)	% Area
1	1,5-cyclodecadiene	26.031	1.618
2	4,7-methanoazulene	26.091	1.524
3	Caryophyllene	26.996	3.831
4	Azulene	27.371	14.274
5	Seychellene	28.926	1.359
6	1H-cycloprop[e]azulene	28.817	2.849
7	1,4-methanocycloocta(D) pyridazine	29.157	0.187
8	7-methannoaazulene	30.682	0.698
9	Cyclohexanone	30.927	0.711
10	Icosapent	31.047	0.836
11	gamma-Elemene	32.078	1.005
12	Methyl 8,11,14-heptadecatrienoate	32.318	0.204
13	Naphthalene	32.938	2.900
14	Patchouli alcohol	33.398	36.708
15	1-propyl 6,9,12-hexadecatrienoate	34.549	0.165
16	2-allyl-4 methylphenol	35.594	0.194

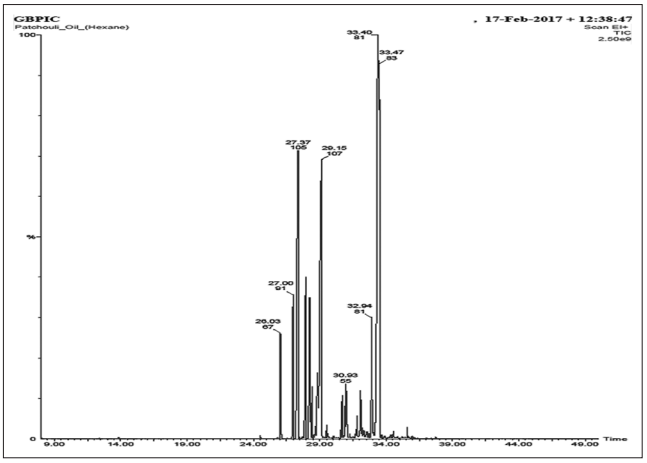

Fig. 1: Gas chromatography-mass spectrometry chromatogram of patchouli oil

Evaluation of transdermal patch

The results of evaluation of various physiochemical parameters of indomethacin transdermal patches were presented in Table 5. The values of weights and thickness were found to be uniform having an almost low value of SD. The drug content was also studied for all the formulations indicating that the method used for the preparation of transdermal patch was suited for giving uniform drug content.

Skin irritation study

On performing skin irritation study test for formulation F7on healthy, a male rabbits for 24 hrs and comparing it to the control (adhesive tape USP);

Table 4: FTIR interpretation of drug and excipients

Functional group	Wave number (cm^{-1})		
	Indomethacin	Indomethacin + HPMC K100	Indomethacin + PVP K30
O-H bending	1219.81	1219.99	1289.41
C=O stretching	1687.98	1688.99	1688.00
C-Cl stretching	749.22	749.25	750.53
C-O stretching	1064.20	1083.84	1065.91
C-C stretching	831.51	831.50	831.50

HPMC K100: Hydroxypropylmethylcellulose, PVP K30: Polyvinylpyrrolidone, FTIR: Fourier transform infrared

Table 5: Drug content and physical parameters of different formulation of patch

Formulation code	Film thickness (mm)*	Folding endurance**	Uniformity of weight (mg)*	% Drug content*
F1	0.130±0.0026	7	530.8±1.65	93.04±0.64
F2	0.132±0.0023	6	537.1±1.71	97.50±1.23
F3	0.129±0.0028	4	525.6±1.86	97.43±0.67
F4	0.121±0.0030	5	534.5±1.92	95.30±1.41
F5	0.128±0.0029	7	536.2±2.14	98.16±0.43
F6	0.132±0.0014	6	529.8±1.69	93.65±0.72
F7	0.120±0.0032	4	539.3±1.76	95.75±0.54

*All the values are given in mean±SD (n=3), **average of 3 observations. SD: Standard deviation

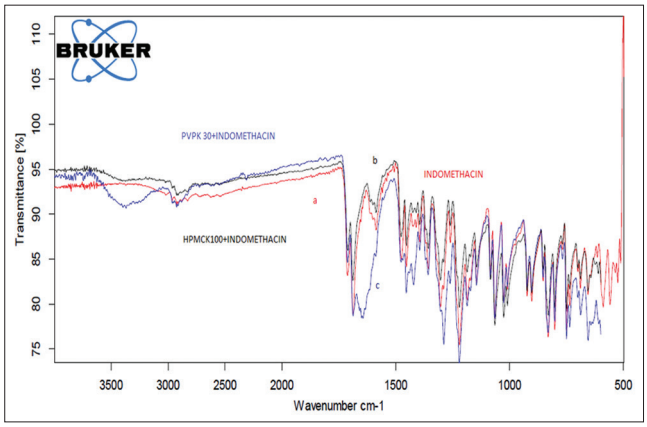

Fig. 2: Fourier transform infrared spectroscopy (FTIR) spectra of drug, excipients, and physical mixture of drug and excipients. (a) FTIR spectra of indomethacin, (b) FTIR spectra of hydroxypropyl methylcellulose K100M + indomethacin, (c) FTIR spectra of polyvinylpyrrolidone K30 + indomethacin

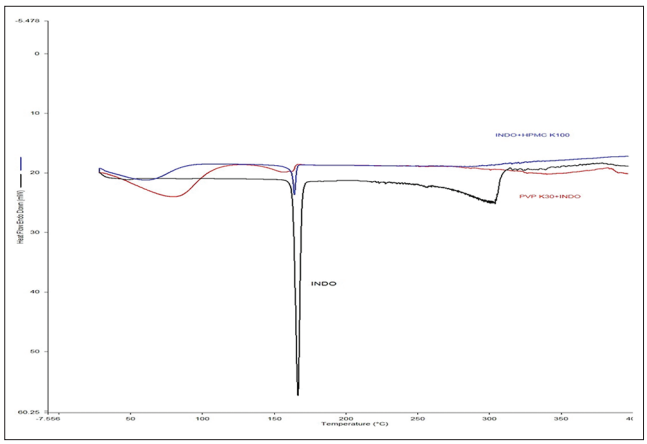

Fig. 3: Differential scanning calorimetry (DSC) thermogram of drug, excipients, and physical mixture of drug and excipients. (a) DSC thermogram of indomethacin, (b) DSC thermogram of hydroxypropyl methylcellulose K100M + indomethacin, (c) DSC thermogram of polyvinylpyrrolidone K30 + indomethacin

no edema or erythema was observed on the site of application of the patch indicating that they are non-irritable to the skin hence can be considered safe for transdermal patches. The observed results are depicted in Fig. 4.

Permeation study

All the seven formulations were subjected to *in-vitro* permeation studies across rat epidermis for establishing the different permeation parameters. The results of permeation studies were shown in Table 6 and Fig. 5. From the data of the study, it was revealed that the transdermal flux of the formulation F1 which contains only the drug is 8.82 ± 0.26 µg/cm^2/hr, which is very less in comparison to the flux value of 62.32 ± 1.09 µg/cm^2/hr of the F2 formulation containing DMSO as the standard permeation enhancer.

The formulations containing the different concentrations of patchouli oil, namely, F3 (containing 0.05% w/v of patchouli oil), F4 (containing 0.25% w/v of patchouli oil), F5 (containing 0.50% w/v of patchouli oil), F6 (containing 0.75% w/v of patchouli oil), and F7 (containing 1% w/v of patchouli oil) shows increase in transdermal flux with the increasing concentration of patchouli oil showing the flux to be 40.99 ± 2.77 µg/cm^2/hr, 46.64 ± 1.54 µg/cm^2/hr, 54.5 ± 2.76 µg/cm^2/hr, 56.88 ± 1.43 µg/cm^2/hr and 61.92 ± 0.89 µg/cm^2/hr, respectively. The cumulative amount (Q_{24}) of indomethacin permeated over 24 hrs was found to increase, ranging from 1830 ± 26.66 to 2660 ± 21.60 µg/cm^2 from the transdermal patches containing 0.05-1% w/v of patchouli oil. This result evidenced that the addition of patchouli oil in the formulation enhanced the transdermal flux of indomethacin which is as comparable as with DMSO used as a standard permeation enhancer.

Permeation enhancement mechanistic study

FTIR study

To further elucidate the effect of patchouli oil-induced alteration on the intercellular lipids in the stratum corneum keratin and protein FT-IR studies were conducted of rat epidermis one as control (epidermis treated with a patch containing no penetration enhancer) and one as at test (epidermis treated with a patch containing patchouli oil as penetration enhancer). The spectra showed a number of peaks due to molecular vibrations of the proteins of the stratum corneum. After scanning the rat epidermis (both control and test) by FTIR, the spectra showed absorption bands at 2921 cm^{-1} (C-H stretching) and 2852 cm^{-1} (C-H stretching) which are in the range from 2700 to 3000 cm^{-1}, which occurs mainly due to the C-H stretching of the alkyl groups present in the lipids and the proteins [28].

Table 6: Permeation parameters of the different formulations of transdermal patches of indomethacin studied across rat skin

Formulation code	Q_{24} (µg/cm²)[a] (mean±SD; n=3)	J (µg/cm²/hr)[b] (mean±SD; n=3)	T_{lag} (hr)[c] (mean±SD; n=3)	ER[d] (mean±SD; n=3)
F1	395.5±6.53	8.82±0.26	3.20±0.76	-
F2	2752±54.00	62.32±1.09	2.40±0.65	7.06±0.10
F3	1830±26.66	40.99±2.77	1.50±0.38	4.64±0.31
F4	2061±42.00	46.64±1.54	2.12±0.48	5.28±0.17
F5	2201±79.33	54.5±2.76	2.10±0.53	6.12±0.35
F6	2535±20.00	56.88±1.43	1.90±0.30	6.44±0.09
F7	2660±21.60	61.92±0.89	1.20±0.46	7.02±0.10

[a]Q_{24} cumulative release of drug in µg/cm² at the end of 24 hrs; [b]J flux of the different formulations; [c]T_{lag} is the lag time of the different formulations; [d]ER is the enhancement ratio of indomethacin. SD: Standard deviation

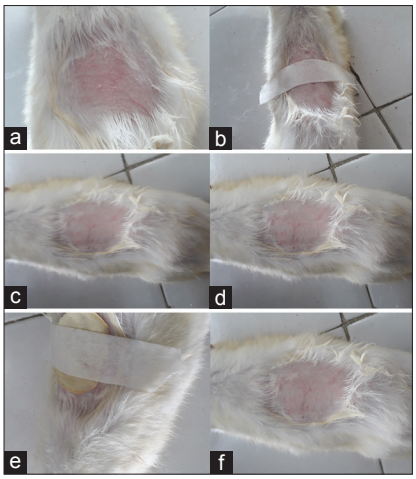

Fig. 4: Photographs of skin irritation study, (a) Before application of transdermal patch, (b) application of adhesive tape USP, (c) After application of adhesive tape USP, (d) before application of transdermal patch, (e) application of transdermal patch, (f) after application of transdermal patch

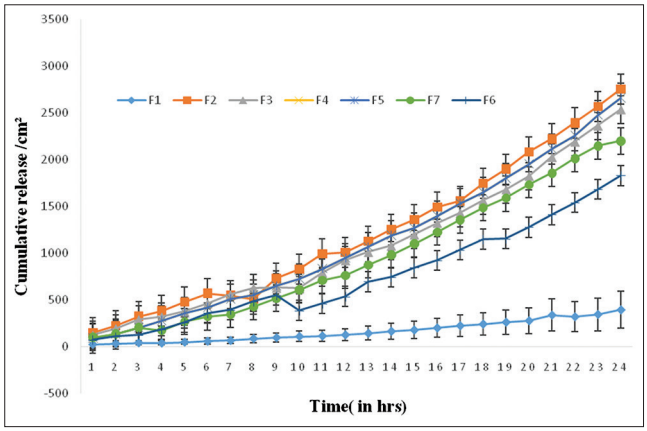

Fig. 5: *In-vitro* skin permeation profiles of various transdermal formulations. All the values are given in mean±standard deviation, n=3

It also showed 2 amide vibrations within the range of 1500~1700 cm^{-1}, namely, amide 1 (~1644 cm^{-1}) and amide 2 (~1539 cm^{-1}), which were one of the important parameters to investigate the alteration in the structure of the lipids and keratin present in stratum corneum induced by penetration enhancers [29]. The frequencies of these amide bonds shifts to lower or higher frequencies due to change in protein conformation [29] and from the spectra (Fig. 6) it was seen that the frequency of the amide band of the test shifted toward the higher frequency in comparison to the control which helps us to conclude that the essential oil under investigation (patchouli oil) enhances the

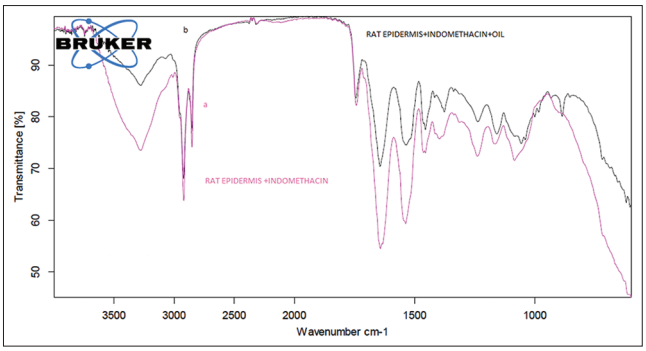

Fig. 6: (a) Fourier transform infrared spectroscopy (FTIR) spectra of untreated ratepidermis, (b) FTIR spectra of rat epidermis treated with patchouli oil as penetration enhancer

permeation by possible disruption of stratum corneum lipids and also by changing protein conformations.

CONCLUSION

In the present study, various formulations of transdermal indomethacin patches were prepared and the effect of patchouli oil was investigated for its permeation property. On account of the results, it was found that all the prepared formulations showed good uniformity with regard to drug content and physical parameters. The skin irritation study performed indicated that the patches showed no skin irritation exhibited by patchouli oil. The *in-vitro* skin permeation studies showed that patchouli oil can remarkably enhance the permeation of indomethacin across rat epidermis with increasing concentration of patchouli oil. The FT-IR studies showed that patchouli oil brings changes in the stratum corneum lipids and protein conformation which possibly results in the alteration of skin permeability. Hence, it can be concluded that patchouli oil can effectively enhance the transdermal permeation of model drug indomethacin and may be used as a natural permeation enhancer for transdermal drug delivery system.

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