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Formulation and Characterization of Transdermal Patch Containing Eucalyptus, Curcumin and Ginger Oils with its In-vitro Permeability Study Using Goat Skin and GC-MS Analysis

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Abstract

The present research has been carried out to formulate the transdermal patch containing medicinal oils like eucalyptus, curcumin, and ginger with its characterization and in-vitro permeability checking by GC-MS analysis. Three formulations (F1, F2, and F3) of transdermal patches containing different concentrations of oils were prepared. The patches were evaluated for thickness, weight uniformity, percentage moisture content, and percentage moisture uptake. The average thickness was found to be 0.434, 0.433, and 0.438 mm for F1, F2, and F3 respectively. 0.1876, 0.1861, and 0.1866 gm weight uniformity were observed for F1, F2, and F3 respectively. The moisture content for F1 was 3.96 %, for F2 was 2.48 % and for F3 was 3.67 %. The moisture uptake for F1 was 5.68 %, for F2 was 4.32 % and for F3 was 4.82 %. The F2 formulation produced optimum results, so it is better than F1 and F3. The in-vitro permeation of oils from the transdermal patch was checked by Franz diffusion cell using goat skin as a semi-permeable membrane. The transferred sample was collected and investigated by chemical identification tests at time intervals of 1, 2, 3, 4, and 5 hours for the presence of active chemical constituents. The GC-MS analysis showed the presence of active chemical moieties which transferred through the goatskin including D-limonene, (+)-4-carene, Fenchone, linalool, endo-borne, citronellal, 2,6-octadienal,3,7-dimethyl-,(Z)-,longifolene, caryophyllene, carotol, diethyl phthalate, etc. The above research proves our approach to formulation and delivery of polyherbal oils in a single transdermal patch which may open the door for delivery of formulation containing herbal oils through the skin.

Keywords: Transdermal patch**,** In-vitro permeability, GC-MS, Franz diffusion cell, D-Limonene, Caryophyllene.

Introduction

The transdermal drug delivery system (TDDS) has an essential component of emerging technologies for the release of medications (1). A TDDS applies a reasonably prescribed amount of the medicine to the inside of a patch; that is applied on the local site of the skin for an incredibly longer effect. The medication reaches the systemic circulation via the skin by diffusion mechanism (2). From the patch a balanced concentration of drug diffuses into the bloodstream for an extended period, ensuring a constant concentration of drug in the systemic circulation. This not only improves the drug effectiveness and safety, but it also improves patient fulfilment and overall gives therapeutic benefit (3). TDDS has some benefits such as

being less invasive or non-invasive, avoiding first-pass metabolism, being easy to apply, not requiring expert personnel, and having the potential to reduce the rate of administration (4).

Several transdermal therapeutic systems have been developed for topical administration to regulate therapeutic drug delivery. There are several topical ayurvedic drugs formulated and reported as an herbal patches (5-8). There are different types of transdermal patches reported like Drug-in adhesive with a single layer type of patch, Drug-in adhesive with multiple layers, Matrix type of patch, etc (9). Eucalyptus oil is obtained from Eucalyptus spp. and has a variety of biological activities like anti-microbial, anti-fungal, cardinal, insecticidal, herbicidal, anti-inflammatory, and analgesic, etc (10-11). The chemical moieties responsible for all the above biological activities present in eucalyptus oil are 1, 8-cineole, piperitone, citronellal, citronellol, linalool, *p*-cymene, a -phellandrene, a-pinene, a -terpineol, limonene, alloocimene, g -terpinene, geranyl acetate, spathulenol, a -thujene, etc (10).

Curcumin essential oil is obtained from *Curcuma longa* and reported many biological activities such as antihyperlipidemic (12), antidiabetic and hypoglycaemic (14), antiobesity (15), antioxidant (14-16), neuroprotective (17-19), antiplatelet and antithrombosis (20-21), cytotoxic (22-23), anti-inflammatory (19, 24), antiarthritic and joint-protective (25), hepatoprotective and antihepatotoxic (26), antiatherosclerotic (27), hypothermic (28), anxiolytic (28), anticonvulsant (28), pasmolytic (29), antimutagenic (30), sedative and anesthetic (28, 31), antivenom (32), antibacterial (33), antifungal (34, 35), insecticidal (36, 37), mosquitocidal (38), phytotoxic (39), antitumor (40), hypoglycemic (41), larvicidal (42) etc. The above reported biological activities are because of presence of phytochemical constituents such as a-turmerone, b-turmerone, a-curcumene, zingiberene, curcumenol, xanthorrhizol, curcumol, germacrone, curdione, curzerenone, curzerene, 8,9-dehydro-9-formyl-cycloisolongifolene, b-caryophyllene, (E)-b-farnesene, b-elemenone, b-elemene, b-pinene, camphor, 1,8-cineole, piperitenone, b-myrcene (43) etc.

Ginger oil is obtained from dried rhizomes of the *Zingiber officinale* having a variety of reported biological activities like antioxidant (44), anti-tumour (45-46), anti-inflammatory and analgesic activity (47), anti-microbial activity (48), hepato-protective activity (49-50), etc. All above reported biological activities are because of the active phytoconstituents present and reported in ginger oil and which are paradol, shogoal, zingerone, zerumbone, 1-dehydro-(10) gingerdione, gingerol, gingerenone A, etc (51).

By considering all the above information from the literature, we are reporting here an attempt to formulation and characterization of a transdermal patch containing eucalyptus, curcumin, and ginger oils for treating inflammation and pain. The in-vitro permeability study by using goat skin and GC-MS analysis was carried out for the formulated transdermal patch.

Materials and Methods

Collection of volatile oils (Eucalyptus, Curcumin and Ginger), chemicals, polymers etc.

The oils were procured from a local ayurvedic drug supplier from Kolhapur (Bawadekar ayurvedic shop). The oil samples were then checked for their purity by organoleptic tests (physical state, color, odor, etc), density, and refractive index. The collected oil samples were stored in an airtight amber-colored container at room temperature. HPMC K4M, EC 7 cps, PVP K30, and propylene glycol (PG) were procured from Sigma-Aldrich. Synthetic PVA was procured from Loba Chemie Pvt. Ltd., Mumbai, India. PEG-4000 was obtained as a gift sample from BASF Mumbai, India. All other materials and chemicals used were of either pharmaceutical or analytical grade.

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Formulation of herbal transdermal patches

The herbal transdermal patches were prepared by different methods.

Method 1

Transdermal patches containing volatile oils were prepared using 4 gm of polymer PVA as a backing membrane and as a dispersion polymer. The patches were formulated by first preparing PVA as a backing membrane and then dispersing HPMC polymer in different concentrations in water and ethanol. The backing membrane was prepared and allowed to dry for 24 hours at room temperature. 2 ml of curcumin oil and HPMC were mixed with the appropriate solvent for 1 hour, then 1gm of PEG and plasticizer PEG 4000 were added and the stirring was continued for another hour. Then using a pipette, 5 ml of a dispersion-containing mixture was poured over the previously prepared backing layer of PVA. By placing an inverted funnel over the glass Petri plate, the solvent was allowed to evaporate at room temp. The film was collected and analyzed 24 hrs after drying at room temperature.

Method 2

PVP (1000 mg) and EC (1000 mg) was used as the skeletal type of polymeric material. PG as a penetration enhancer and PEG-4000 was used as a plasticizer. The polymer PVA and EC were weighted in required ratios and mixed into a 50 ml solution containing methanol and distilled water (1:1) ratio. Stirred the mixture over a hot water bath until dissolved, after the mixture was cooled down to 25 ^{0}C the volatile was added. After that, PG (0.5 ml), was added, and the mixture was then poured into a glass Petri dish and dried at room temperature for 24 hrs. The Petri dish was left and placed at room temperature for one day. The patch was obtained intact by slowly lifting from the Petri dish and collected, and stored until further use.

Method 3

PVA (1000 mg) and PVP (1000 mg) and drug were weighted in requisite ratios and mixed in 10 ml distilled water. Stirred the mixture over a hot water bath until it dissolved. After that mixture was cooled down to 25 ^{0}C, added glycerol (0.5ml), PG (0.5 ml), and pressure-sensitive adhesive such as Di-n-butyl phthalate (2 ml). PEG-4000 was used as a plasticizer. The solution was mixed by using a mechanical stirrer at 600 to 800 rpm for 20 min. under occluded condition, and then the mixture was cast on the Petri dish and kept at room temperature for 24 hrs. In table 1 the different ingredients with their quantity used in formulation F1, F2, and F3 are given.

Table 1: Showing different polymers and oils used for preparation of transdermal patches

F1 (Method 1)		F2 (Method 2)		F3 (Method 3)	
Name of Ingredient	Quantity	Name of Ingredient	Quantity	Name of Ingredient	Quantity
PVA	1000 mg	EC	1000 mg	PVP	1000 mg
HPMC	1000 mg	PVP	1000 mg	PVA	1000 mg
PG	0.5 ml	PEG-4000	1000 mg	PEG-4000	1500 mg
Glycerol	0.5 ml	PG	0.5 ml	Glycerol	0.5 ml
Dist. Water : Ethanol (1:1)	50 ml	Dist. water: methanol (1:1)	50 ml	PG	0.5 ml
Curcumin oil	1 ml	Glycerol	0.5 ml	Di-n-butyl phthalate	2 ml
Eucalyptus oil	1 ml	Curcumin oil	1 ml	Dist. water: methanol (1:1)	50 ml
Ginger oil	1 ml	Eucalyptus oil	1 ml	Curcumin oil	1 ml
		Ginger oil	1 ml	Eucalyptus oil	1 ml
				Ginger oil	1 ml

F1 = formulation 1, F2= formulation 2 and F3 = formulation 3

Formulation and characterization of transdermal patch containing eucalyptus, curcumin and ginger oils with its in-vitro permeability study using goat skin and GC-MS analysis

Evaluation of patches (53, 56, 57)

The patch thickness

The thickness of prepared drug-loaded patches was measured at different points by using a vernier calliper and determined the average thickness (56-57).

Weight uniformity

Specified areas of prepared patches were cut into different parts and weighed on a digital balance, and the average weight was calculated (56).

Percentage moisture content

The prepared films were weighed individually and kept in desiccators with fused calcium chloride at room temp for 24 hrs. After 24 hrs the film was reweighed and determined percentage moisture content (53, 56).

Percentage moisture uptake

The weighted films were kept in desiccators at room temp for 24 hours in a saturated potassium chloride solution. After 24 hrs the films were reweighed and determined the percentage of moisture uptake (53).

In-vitro oil constituents release study (Permeation study) by Franz diffusion cell method

Preparation of goat skin

The goatskin was used as semi-permeable membrane, collected from local slotter house nearby college campus. Freshly excised skin was placed for 1 min in distilled water maintained at 55°C to remove fats and subcutaneous tissues. The skin was washed with fresh distilled water and dipped in a phosphate buffer at pH 7.4 for 5 min. The excised skin was carefully checked visually for integrity. Any damaged skin was rejected. The prepared skin was wrapped in aluminium foil, stored at -20°C, and used within 2 weeks of storage (58-59).

Permeation Experiment

The in-vitro oil constituents release (permeation) study was carried out by the Franz diffusion cell method. Franz diffusion cells consist of two compartments i.e. donor and acceptor with an outer jacket. The outer jacket was placed in a water bath at 37 °C to provide a temperature of 32°C±1°C in the receptor compartment. The goatskin was used as a semi-permeable membrane. The skin was allowed to maintain a temperature of 32 °C by placing the skin in a phosphate buffer of pH 7.4 for 1 hour. The receptor compartment was filled with a phosphate buffer pH 7.4.

The goatskin was mounted between the donor and the receptor compartments in such a way that the stratum corneum was facing toward the donor compartment. A circular piece of the patch was cut from a larger patch with the help of a cutter. A circular patch was placed on the skin with the releasing side facing toward the mounted skin. The receptor fluid was continuously stirred at a speed of 120 rpm with magnetic bars on a magnetic stirrer (REMI equipment Pvt ltd., India) to provide sink conditions. At predetermined intervals of 1, 2, 3, 4, and 6 hrs, 1 mL of receptor fluid was withdrawn from the sampling port with the help of a long needle syringe.

The receptor fluid was replaced with fresh phosphate buffer. Care was taken during sampling to avoid any bubble formation in the receptor compartment, because trapped air may reduce the permeation area. In case of air entrapment, the Franz diffusion cell was tilted to remove the trapped air through the sampling port. The donor compartment was covered with aluminium foil (59).

GC-MS study

The GC-MS study was conducted in the common facilities center (CFC), Shivaji University, Kolhapur. The analysis was carried out on a Shimadzu instrument (model TQ8050). The sample collected at 6 hrs time interval

of formulation F3 was injected into the gas chromatography. The method setting and programming details are given in table 2.

Table 2: Showing Gas Chromatographic programming and method details (60).

Column oven Temperature programme	50 hold for 2 minutes at 10 ^{0}C, 200 hold for 5 minutes, 250 hold for 5 minutes, 275 holds for 5 minutes.
Injection temperature	25 ^{0}C
Injection mode	Splitters
Carrier gas	Helium (He)
Column flow	1 ml/min.
Sample volume	1µl

The separated compounds in the form of different peaks at different retention times (Rt) were transferred into the MS. The MS was carried out by setting of programming parameters given in table 3.

Table 3: MS programming parameters [60]

In source temperature	24 ^{0}C
Interface temp	25 ^{0}C
Solvent cut time	5.8 min.
m/z range	45 to 650
Detector voltage	0.76

The ionization, filtration and detection were carried out and the fragments of different ions were recorded.

Result and Discussion

Physicochemical characterization of procured oils

Organoleptic tests

The organoleptic tests for eucalyptus, curcumin, and ginger oils resemble the official monographs.

Density

The relative density is a basic physical property of an oil/substance that can be used to characterise it. The density is the ratio of mass of a substance to volume of substance at given temperature. The relative densities for all three oils i.e. eucalyptus, ginger, and curcumin were 0.866g/ml, 0.970g/ml, and 0.871/ml respectively. The standard values for the same are 0.865g/ml, 0.960 mg/ml and 0.871 g/ml respectively at room temperature.

Refractive Index

The Refractive indeces of volatile oils were determined by using Abbe's refractometer. The refractive index is one of the physicochemical properties of the substance. The refractive index (n) of a substance with reference to air is the ratio of the sine of the angle of incidence to the sine of the angle of refraction of a beam of light passing from air into the substance. It is mostly applied for identify a particular substance, confirm its purity, or measure its concentration. Generally it is used to measure the concentration of a solute in an aqueous solution. It can be used also in determination of drug concentration in pharmaceutical industry (52). The refractive indeces of Eucalyptus, Ginger, and Curcumin oil were 1.461, 1.400, and 1.450 respectively, and the standard refractive index is 1.450, 1.485, and 1.500 respectively.

Formulation and evaluation of herbal transdermal patches

The patches were prepared by using combinations of polymers such as hydroxyl propyl methyl cellulose K4M (HPMC), ethyl cellulose 7 cps (EC), synthetic polyvinyl alcohol (PVA), polyvinyl pyrrolidone K30 (PVP), and polyethylene glycol 4000 (PEG-4000). The formulated patches get softened and extend because of plasticizers. The formulation was subjected to a physical examination; films appeared slightly translucent suggesting that the transdermal patch containing oils was successfully prepared. Further, the patches were evaluated for thickness, weight uniformity, moisture content, and moisture uptake, and the results are tabulated in Table 1.

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Table 1: Showing result of thickness, weight uniformity, moisture content, moisture uptake of prepared transdermal patches

Name of formulation	Average thickness (mm)	Weight uniformity (gm)	Moisture content (%)	Moisture uptake (%)
F1	0.434	0.1876	3.96	5.68
F2	0.433	0.1861	2.48	4.32
F3	0.438	0.1866	3.67	4.82

Each patch's thickness was measured with a vernier calliper at different points on the patch, the average thickness for F1, F2 and F3 was 0.434, 0.433 and 0.438 mm respectively. All the patches were found to be having uniform thickness. The average weight for F1, F2 and F3 was 0.1876, 0.1861 and 0.1866 gm respectively. It was found that the weights were uniform for all prepared formulations and the weight variation was within acceptable range. The moisture content for F1, F2 and F3 was 3.96, 2.48 and 3.67% respectively. The moisture uptake for F1, F2 and F3 was 5.68, 4.32 and 4.82 respectively. The moisture content and moisture uptake studies provide information regarding stability of the formulations. For maximum stability the moisture content and moisture uptake of transdermal patch should be minimum (53-55). In this case formulation F2 showed optimum results so it is better than F1 and F3. The results obtained were promising and the prepared transdermal patches were stable.

Permeation study

The permeation studies of the transdermal patches helps to predict the in vivo absorption of the drug. The permeation studies were performed by Franz diffusion cell method using goat skin as a semi-permeable membrane. The drug samples were collected at an interval of 1,2,3,4 and 6 hrs. The GC-MS study was carried out for the presence of different chemical moieties in the receptor compartment.

GC-MS analysis

The GC-MS analysis of batch F3, for 6 hrs receptor sample was carried out. The GC spectra are shown in figure 1 showing different peaks at different retention times (Rt). The detailed chromatographic separation data with retention time (Rt), initial time (It), final time (Ft), peak area, % area, and name of chemical moiety representing it is given in table no. 2. The major components were D-limonene (14.95), +)-4-carene (2.20), fenchone (1.71), linalool (1.67), acetaldehyde (9.27), camphor (6.09), endo-borneol (1.39), L-.alpha.-terpineol (4.80), citronellal (3.64), 2,6-octadienal,3,7-dimethyl-, (Z)- (1.59), isobornyl acetate (4.44), geranyl acetate (8.55), 2H-2,4a-Methanonaphthalene,1,3,4,5,6,7-hex (1.91), longifolene (2.87), caryophyllene (3.94).

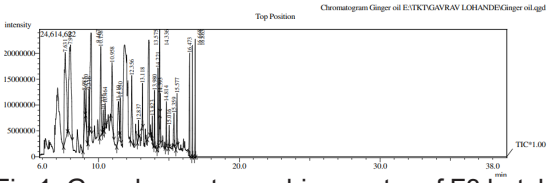

Fig 1: Gas chromatographic spectra of F3 batch (6 hrs time interval samples)

Table 2: Showing time (Rt), initial time (It), final time (Ft), peak area, % area, and name of chemical moiety separated by GC.

Peak no.	Rt	It	Ft	Area	% Area	Name of chemical moiety separated
1	7.999	7.835	8.135	196633623	14.95	D-Limonene
2	8.985	8.890	9.050	28894459	2.20	(+)-4-Carene
3	9.110	9.080	9.200	22457757	1.71	Fenchone
4	9.316	9.255	9.355	21973067	1.67	Linalool

5	9.467	9.355	9.515	121845201	9.27	Acetaldehyde
6	10.158	10.070	10.260	80075561	6.09	Camphor
7	10.336	10.280	10.385	12830938	0.98	(1R,2R,5S)-5-Methyl-2-(prop- 1-en-2-yl) cyclo
8	10.464	10.410	10.530	18235358	1.39	endo-Borneol
9	10.958	10.875	11.085	63118662	4.80	L-.alpha.-Terpineol
10	11.410	11.295	11.505	47805732	3.64	Citronellal
11	11.550	11.510	11.600	20919610	1.59	2,6-Octadienal,3,7-dimethyl-,(Z)-
12	12.356	12.270	12.480	58390518	4.44	Isobornyl acetate
13	12.837	12.760	12.870	8747719	0.67	(2R,2'S,5S,5'S)-2,5'-Dimethyl-5-(prop-1-en-2-
14	13.118	13.065	13.155	17261624	1.31	6-Octen-1-ol,3,7-dimethyl-,propanoate
15	13.575	13.470	13.650	112483497	8.55	Geranyl acetate
16	13.823	13.785	13.860	8893979	0.68	Cyclohexane
17	13.980	13.935	14.025	25137325	1.91	2H-2,4a-Methanonaphthalene, 1,3,4,5,6,7-hex
18	14.221	14.160	14.270	37786059	2.87	Longifolene
19	14.336	14.270	14.270	51780013	3.94	Caryophyllene
20	14.393	14.360	14.430	8294650	0.63	Bicyclo[3.1.1]heptane,6-methyl-2-methylene-
21	14.814	14.755	14.855	18496067	1.41	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-
22	15.016	14.955	15.055	9930168	0.76	Carotol
23	15.359	15.280	15.415	19665565	1.50	2-Octen-1-ol,7-ethoxy-3,7-dimethyl-, (E)-
24	15.577	15.490	15.625	32777390	1.50	2-Octen-1-ol,7-ethoxy-3,7-dimethyl-, (E)-
25	16.473	16.380	16.505	47432263	3.61	Diethyl Phthalate
26	16.668	16.625	16.705	26235615	1.99	Diethyl Phthalate
27	16.863	16.820	16.895	26199705	1.99	Diethyl Phthalate

The GC-MS study confirmed the presence of various chemical moieties in transdermal patch which passes the semi permeable membrane (goat skin) and appears with different retention time as illustrated in figure 1. The mass spectrometer analyzes the compounds eluted at different retention times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios The MS fragmentation pattern of different separated compound in gas chromatography is given in supplementary material which is attached separately.

Conclusion

In conclusion, taken together the results showed that all the three formulations i.e. F1, F2 and F3 were successfully formulated incorporating the combination of oils like eucalyptus, ginger and curcumin oil. All the formulations produced optimum results but formulation F2 showed minimum % of moisture content (2.48) and moisture uptake (4.32), so it is better than F1 and F3. This may be because all the three formulations were prepared by three different methods. The in-vitro permeation study revealed that the active chemical moieties were transferred into the hydro-alcoholic solution through semi-permeable membrane (goat skin) and which were detected and identified by GC-MS study. The above results are promising for further preparation of transdermal patches containing herbal drugs and oils in a combination for the treatment of various diseases like inflammation, pain, gout, rheumatoid arthritis and many more diseases where herbal drugs

and oils are applied externally.

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