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DEVELOPMENT AND EVALUATION OF GINGER TRANSDERMAL PATCH AND ITS IMPLICATIONS IN MIGRAINE THERAPY

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Keywords:

Ginger, Transferosomes, Transdermal drug delivery, Lipoid P-100

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ABSTRACT: The potential of transferosomal formulation for transdermal delivery of Ginger constituents was investigated. The aim was to reduce the dosage and improve patient acceptance. Transferosomes were formulated by ethanol injection technique using Phospholipids (Lipoid P-100), cholesterol, various edge activators (tween 80, Span 80), and other excipients. Transdermal patches were prepared by solvent casting method using polymers (Ethylcellulose, HPMC), penetration enhancers (Oleic acid, Tween 80) and PEG 400 as a plasticizer. Transferosomes were evaluated for in-vitro release, particle size, zeta potential, entrapment efficiency, and stability. Patches were evaluated for folding endurance, uniformity of weight, drug content, and thickness. Transferosomes with 1:2:0.25 ratio of Phospholipid: ginger extract: Tween 80 showed particles of 318.8nm, the zeta potential of -6.3mV and 74.4% entrapment efficiency and 67% *in-vitro* release. Optimized batch of patches showed folding endurance 7, 0.134 ±0.0012 mm thickness, 97.18 ± 0.78% drug content. Thus, transferosomes with transdermal delivery is a better approach to deliver ginger constituents.

INTRODUCTION: Ginger rhizome (*Zingiber officinale* Roscoe) Zingiberaceae family is commonly consumed in diet throughout the world. It has been reported in the literature that ginger extract and its essential oil processed various pharmacological activities like anti-emetic, analgesic, anti-inflammatory, motion sickness, prevention of coronary artery disease, and arthritis, stomach ulcers, antioxidant, anti-cancer, anti-thrombotic activity. The major constituents in ginger rhizomes are carbohydrates (50-70%), lipids (3-8), terpenes and phenolic compounds.

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Terpene components of ginger include zingiberene, ß- sesquiphellandrene, and α-curcumene, while phenolic compounds include gingerol, paradols, and shogaol. These gingerols (23-25%) and shogoals (18-25%) are found in higher quantities than others [1]. So, it was necessary to reduce the dose and formulate a convenient dosage form to increase patient compliance. The requirement is that the herbal formulation must deliver the active phytoconstituent at a therapeutic level. Nano-technology may be applied to overcome this limitation, ensuring enhanced bioavailability, improved patient compliance, optimized drug delivery, and better stability [2]. The skin is the best biological barrier with a surface area of 1.5–2.0 m². However, the majority of drugs cannot penetrate through the intact skin due to the structural, biochemical, and physiological properties of the organ.

Transferosomes are lipid bundles in the form of aggregates that are ultra-deformable. They are made up of one interior aqueous segment encircled by a lipid bilayer. They are more elastic and stable in the presence of surfactants *i.e*., edge activator in the vesicular membrane [3]. Dermis and Epidermis are the most important layers of skin. The epidermis is the outermost layer of skin which is 100 to 150 µm thick with no blood flow. It has one more layer within it known as the stratum corneum. This layer is most important for transdermal delivery of drugs. The drug which can penetrate the stratum corneum reaches the bloodstream by passive diffusion. This is the only process to transfer both water-soluble and lipid-soluble drugs.

The transdermal drug delivery system is the dosage form in which the drug is administered topically in the form of a patch that delivers the drug at a controlled rate for systemic effects. A patch contains a relatively high dose of the drug and is applied on the skin. The drug enters the bloodstream directly through the skin by passive diffusion. Since there is a high concentration of the drug on the patch, and its concentration is low in the blood. The drug diffuses slowly into the blood, maintaining the steady-state concentration of the drug in the blood flow for a long period of time.

The best mixture for transdermal delivery is about fifty percent of the drug, each being hydrophilic and lipophilic. This is because the intercellular lipid bilayers of the cell membranes are easily accessible to lipid-soluble substances whereas water-soluble drugs are able to pass limiting steps in transdermal drug delivery system [4]. Since ginger contains 6-gingerol and 6- shogaol as major constituents which are lipophilic in nature. So, can be easily formulated into a transdermal drug delivery system.

MATERIALS AND METHODS:

Materials: Lipoid P-100 was obtained as a free gift sample from Lipoid, Germany. Tween 80 and Span 80 were procured from Loba Chem Pvt. Ltd, Mumbai. All other reagents used were of analytical grade.

Extraction of Rhizome: Fresh rhizomes of Ginger were collected from the local market of Vasai, Mumbai, (Maharashtra) during February 2018. The authentication of the fresh ginger plant along with the rhizome was carried out at Saint Xavier's College, Mumbai. The sample matched with the standard herbarium specimen sample number AKC-1, and its identity was confirmed to be *Zingiber officinale*_Rosae. Family- Zingiberaceae. The fresh rhizomes were washed with water and wiped with a clean cloth to remove dirt, dust, and debris. The rhizomes were dried in the sun for 7 days. The dried rhizomes were then grinded to fine powder. 20 g of ginger powder was extracted with 200 ml of methanol by maceration for 2 h and then by ultrasonication (Spectra lab) at room temperature for 2 h. The mixture was filtered. The filtrate obtained was concentrated and evaporated to dryness on an electric water bath, maintaining the temperature at 100 °C.

Determination of Absorption Maxima: 200 µg/ml solution of methanolic ginger extract was dissolved in methanol and phosphate buffer 7.4 separately. The solutions were scanned at the range of 200-800 nm using Thermoscientific evolution 300 UV-Vis spectrophotometer.

Calibration Curve of Methanolic Ginger Extract: 10 mg of methanolic ginger extract was accurately weighed and transferred to 10 ml volumetric flask. The extract was dissolved in phosphate buffer 7.4 and methanol separately. The volume was made up to 10 ml with phosphate buffer 7.4 and methanol respectively to give a concentration of 1000 µg/ml stock solution. From this solution, 0.5 ml, 1 ml, 1.5 ml, 2 ml, 2.5 ml were withdrawn and transferred in different volumetric flask of 10 ml and the volume was made up with Phosphate buffer 7.4 and methanol respectively to achieve a concentration of 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml respectively. The solutions were analyzed at 282 nm using the blank as Phosphate buffer 7.4 and Methanol, respectively.

Interference Study: Interference study was carried out for any interference of drug-polymer, drug-surfactant, drug-stabilizer used in the formulation. The interference study was carried out using FTIR [5].

Preparation of Transferosomes Containing Methanolic Ginger Extract: Transferosomes of ginger extract was prepared by using solvent

injection technique. The drug extract, cholesterol, and the lipid (Soy lecithin) were dissolved in different ratios using various solvents like ethanol, chloroform, methanol, acetone for different batches by trial and error method. This solution was sonicated till it formed a clear solution and added by injection technique at one jet to the aqueous phase. Tween-80, Span 80 were tried as edge activators, and stirring was continued at 1200 rpm for 2 h. The composition of formulations is in **Table 2**. The solution was then homogenized for 5 min. Formed nanoparticles were harvested from the aqueous slurry by lyophilization using 10% w/v D-(+)-Trehalose dihydrate as a cryoprotectant [3].

Characterization of Ginger Transferosomes:

Particle Size and Zeta Potential Measurement: The average diameter and size distribution and zeta analysis of vesicles were determined by Malvern Zetasizer Version 7.12. Serial no. MAL500458. Zeta potential was analyzed to measure the permeation of transferosomes by studying its colloidal property and stability of the vesicle [5].

Entrapment Efficiency: Transferosome entrapped 6-gingerol was estimated by centrifugation method. The prepared transferosome were placed in an Eppendorf tube and centrifuged at 14000 rpm for 30 minutes. 1ml supernatant was withdrawn and diluted with phosphate buffer (pH 7.4). The unentrapped 6-gingerol was determined by UV spectrophotometer at 282 nm. The free 6-gingerol in the supernatant gave the total amount of unentrapped drug. Entrapment efficiency was calculated by an indirect method as follows [5]:

Entrapment efficiency = Weight of total drug - Weight of final drug × 100 / Weight of total drug

***In-vitro* Release Studies:** Ginger extract transferosomes were evaluated for in-vitro drug release using dialysis (Dialysis membrane-50 with a molecular weight cut off 12000 to 14000 Da from Hi-Media Laboratories Pvt. Ltd.) technique. All the batches were evaluated using Phosphate buffer (pH 7.4) as the medium to simulate the pH conditions of skin.

Procedure: 200 ml Phosphate buffer 7.4 was taken in a 250 ml beaker. 5 ml of the transferosomes formulation was filled in a dialysis membrane bag, and other end of bag was tied with thread. The solution was stirred by a magnetic needle at 100 rpm using a magnetic stirrer (Remi). The temperature of the solution was maintained at 37 °C to simulate the body conditions. 2 ml of aliquots were taken at different time intervals 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 22, 24 h and the same amount of fresh medium was replaced immediately. All the aliquots were analyzed at 282 nm using a UV spectrophotometer. The cumulative drug release was then calculated [6].

Stability Studies: The stability studies of the formulation were carried out as per the ICH guidelines. The optimized batch of transferosomes was stored at different temperature conditions i.e., 4° ± 2 °C, 25° ± 2 °C. The optimized batch was then evaluated for physical stability for the period of 3 months. The batch was also evaluated for the amount of drug remains entrapped in the vesicle and the particle size of the transferosomes [7].

Formulation of Transdermal Patch Containing Ginger Transferosomes: Hot water-soluble PVA was used for preparing backing membrane. 2.5% PVA was dissolved in required amount of distilled water by intermittently heating at 60 °C for few seconds. The solution was poured in a petri dish and allowed to dry in hot air oven at temperature not exceeding 60 °C till it formed a membrane. Matrix type transdermal patches compose of different ratios of polymers, surfactant, plasticizer and solvent were prepared by solvent casting technique. Drug matrix was prepared by dissolving requisite amount of drug (ginger extract or ginger transferosomes), HPMC K-15 M and Tween 80 in hydroalcoholic solution (1:1). PEG 400 was added to this solution and stirred. The uniform dispersion obtained was casted on PVA backing membrane and dried at room temperature for 24 h.

1 BATCH P1 CONTAINING:

Ingredients	Quantity
HPMC (k-100 M)	1g gel
Eudragit RS Pro	0.5 g
Oleic acid	1 ml
Propylene glycol	1 ml
Methanol: DCM (1:1)	qs. Up to 5 ml

2 BATCH P2 CONTAINING

Ingredients	Quantity
HPMC 60 S 50	1g gel
PEG 400	1 ml
Hydroalcoholic solution (1:1)	qs. Up to 5 ml

Patch P5:

Procedure: In a beaker, the polymer base containing Ethylcellulose, PEG 6000, and Chloroform were dissolved. In another beaker Chloroform and PEG 400 were dissolved.

This solution was added slowly in polymer base with continuous stirring. The volume was made to 22 ml with Chloroform. 5 ml of the final solution was poured in an evaporating dish covered and allowed to set for 24 h.

3 BATCH P3 CONTAINING

Ingredients	Quantity
i) Polymer base:	
Ethyl cellulose	900 mg
PEG 6000	90 mg
Chloroform	10 ml
ii) PEG 400	0.5 ml
Chloroform	5 ml
Oleic acid	0.5 ml

Patch P4:

Procedure: In a beaker, the polymer base containing HPMC (K-100 M) and Methanol were dissolved. In another beaker Methanol and PEG 400 were dissolved.

This solution was added slowly in polymer base with continuous stirring. The volume was made to 22 ml with Methanol. 5 ml of the final solution was poured in an evaporating dish covered and allowed to set for 24 h.

4 BATCH P4 CONTAINING

Ingredients	Quantity
i) Polymer base:	
HPMC (K-100 M)	90 mg
Methanol	10 ml
ii) PEG 400	0.5 ml
Methanol	5 ml
Tween 80	0.5 ml

Patch P5:

Procedure: In a beaker, the polymer base containing HPMC (K-100 M), PEG 6000, and ethanol were dissolved. In another beaker ethanol and PEG 400 were dissolved.

This solution was added slowly in polymer base with continuous stirring. The volume was made to 22 ml with ethanol. 5 ml of the final solution was poured in an evaporating dish covered and allowed to set for 24 h.

5 BATCH P5 CONTAINING

Ingredients	Quantity
i) Polymer base:	
HPMC (K-100 M)	90 mg
PEG 6000	90 mg
Ethanol	10 ml
ii) PEG 400	1 ml
Methanol	5 ml
Tween 80	0.5 ml

6 BATCH P6 CONTAINING:

Ingredients	Quantity
HPMC K-15 M	700 mg
PEG 400	1 ml
Tween 80	0.5 ml
Hydroalcoholic solution (1:1)	qs. Up to 5 ml

Once the gel base was optimized, ginger extract and ginger transferases respectively were loaded in it, and the solution was poured in petri dish and allowed to settle for 24 h till it gave a clear patch.

Evaluation of Transdermal Patches:

1. Folding Endurance: A patch of area 4 × 4 cm² was cut and was repeatedly folded until it broke. This gave the value of folding endurance. It indicated the resistance of the patch to poor handling.

2. Film Thickness: The film was measured at different places with the help of a screw gauge, and the average of 5 readings gave the film thickness.

3. Drug Content: A patch of area 4×4 cm² was cut and put into 100 ml phosphate buffer (pH 7.4). The solution was shaken continuously for 24 h. It was then ultrasonicated for 15 min and filtered. The drug content was analyzed by ultraviolet spectrophotometer at λ_{max} of 282 nm.

4. Uniformity of Weight: Uniformity of weight was determined by weighing individually 10 patches, and then the average was taken.

5. *In-vitro* Diffusion Studies: Modified Franz diffusion cell with a receiver compartment volume of 33 ml and effective diffusion area of 2.2 cm². The *in-vitro* drug diffusion was performed by using artificial dialysis membrane 50 in Phosphate buffer 7.4. The dialysis membrane was soaked 2 h. Before conducting the diffusion studies. In the receiver compartment of the modified Franz diffusion cell, 33 ml of Phosphate buffer 7.4 was filled. The outer jacket was filled with water.

The dialysis membrane was placed between the transdermal patch and the receiver compartment. The medium was maintained at 37 °C. The medium was stirred by magnetic needle using a magnetic stirrer (Remi) to simulate the body conditions. 2 ml of aliquots were withdrawn at time intervals of 0, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 22, 24 h through the outlet. The medium was immediately replaced by a fresh medium. The aliquots were then analyzed by UV spectrophotometer at 282 nm. The cumulative drug release through the effective diffusion area was then calculated.

RESULTS AND DISCUSSION:

Preformulation Studies:

Determination of Absorption Maxima: 200 µg/ml solution of methanolic ginger extract in methanol and phosphate buffer 7.4 showed λ_{max} at 282 nm.

Calibration Curve of Methanolic Ginger Extract: Standard solution of methanolic ginger extract showed linearity in the concentration range of 50-250 µg/ml at λ_{max} of 282 nm by UV spectrophotometer.

FTIR (Fourier Transform Infrared) Analysis: The FTIR graphs of the Methanolic ginger extract, Methanolic ginger extract and ethanol, Cholesterol, Cholesterol and ethanol, Ginger extract and Cholesterol, Lipoid P-100, Extract and Lipoid P-100, Extract and Tween 80 are shown in Fig 5.5 to 5.11 respectively.

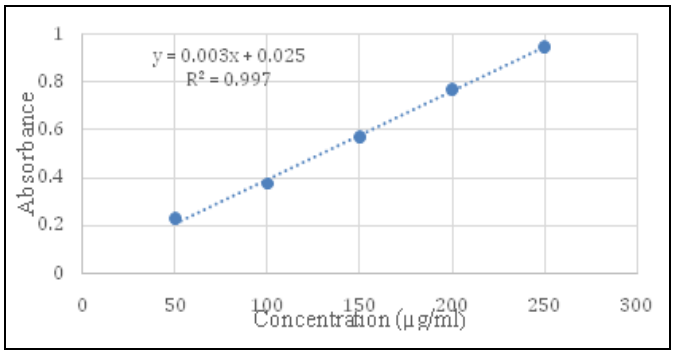

FIG. 1: CALIBRATION CURVE DATA FOR METHANOLIC EXTRACT OF GINGER IN METHANOL

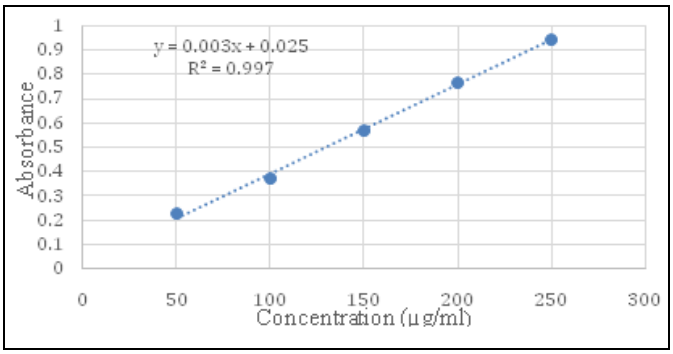

FIG. 2: CALIBRATION CURVE DATA FOR METHANOLIC EXTRACT OF GINGER IN METHANOL

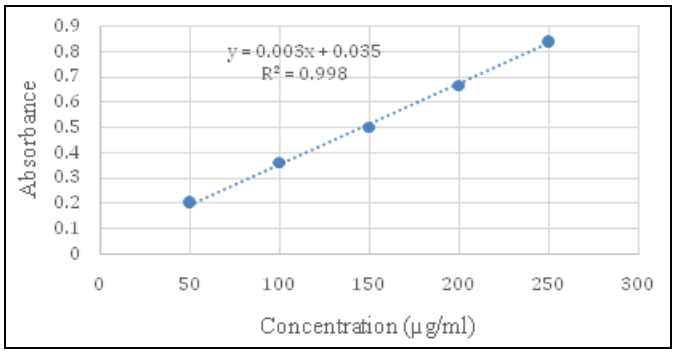

FIG. 3: CALIBRATION CURVE DATA FOR METHANOLIC EXTRACT OF GINGER IN PHOSPHATE BUFFER 7.4

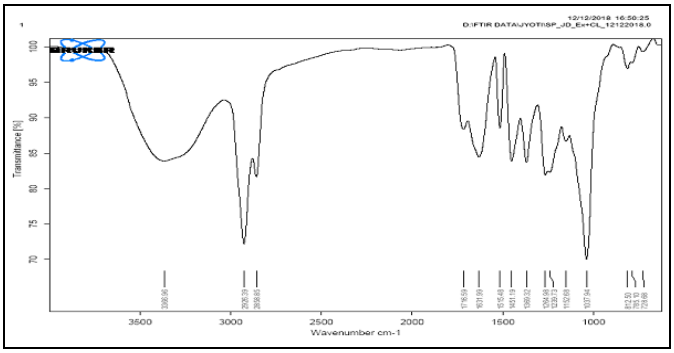

FIG. 4: FTIR GRAPH OF METHANOLIC GINGER EXTRACT

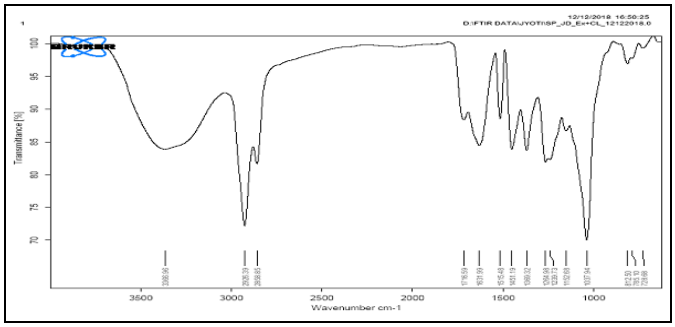

FIG. 5: FTIR GRAPH OF CHOLESTEROL AND METHANOLIC GINGER EXTRACT

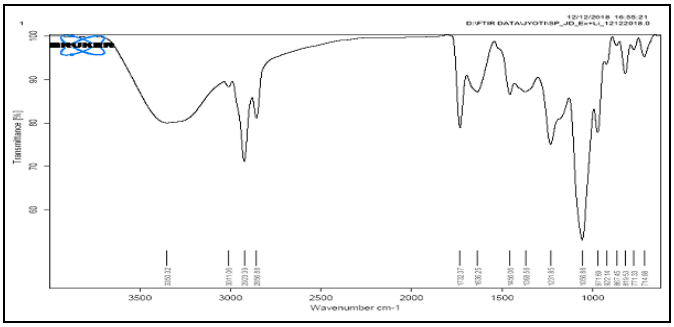

FIG. 6: FTIR GRAPH OF METHANOLIC GINGER EXTRACT AND LIPOID P-100

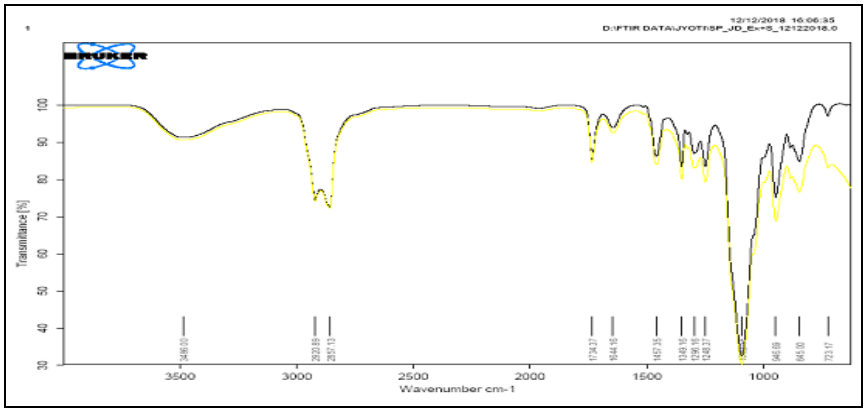

FIG. 7: FTIR GRAPH OF METHANOLIC GINGER EXTRACT AND TWEEN 80

TABLE 1: FTIR INTERPRETATION OF METHANOLIC GINGER EXTRACT AND EXCIPIENTS

Functional Groups	Wavenumber (cm^{-1}) Methanolic Ginger extract	Methanolic Ginger Extract + Ethanol	Methanolic Ginger Extract + Cholesterol	Methanolic Ginger Extract + Lipoid P-100	Methanolic Ginger Extract + Tween 80
O-H bending	3391.80	3336.54	3326.96	3350.32	3466.00
CHO	1712.18	1715.18	1716.59	1732.37	1734.37
C=O stretching	1662.72	1663.17	1631.99	1636.25	1644.16
O-CH3	1074.84	1044.46	1044.81	1056.88	1023.44
C-H stretching for alkanes	2925.18	2925.54	2926.20	2923.39	2920.89
C=C stretch	1614.99	1515.96	1515.48	-	-
C-C multiple bond stretch for aromatics	1515.04	1515.96	-	-	-

TABLE 2: FORMULATION DEVELOPMENT OF TRANSFEROSOMES OF GINGER

Formulation no.	Ginger Extract	Lipid (P-100)	Cholesterol	Solvent	Edge Activator	Phosphate Buffer 7.4
A1	20 mg	20 mg	2 mg	6 ml Ethanol	0.5 ml Tween 80	14 ml
A2	20 mg	2o mg	3 mg	6 ml Ethanol	0.5 ml Span 80	14 ml
A3	2o mg	40 mg	3 mg	6 ml Ethanol	0.5 ml Tween 80	14 ml
A4	20 mg	60 mg	3 mg	6 ml Ethanol	0.5 ml Tween 80	14 ml
A5	40 mg	20 mg	3 mg	6 ml Ethanol	0.5 ml Tween 80	14 ml
A6	20 mg	20 mg	2 mg	5 ml Ethanol	0.5 ml Tween 80	15 ml
A7	20 mg	40 mg	3 mg	5 ml Ethanol	0.5 ml Tween 80	15 ml

Characterization of Transferosomes: Particle size and zeta potential measurement. The average particle size of ginger extract transferosomes with Lipoid P-100, cholesterol, and Tween 80 ratio of 1:2:0.15:0.025 formulation (A7) was found to be 318.8 nm, and the polydispersity index value was 0.123, and Zeta potential value was found to be -6.3 mv indicating good stability of the formulation. The results were graphically represented in **Fig. 8** and **9.**

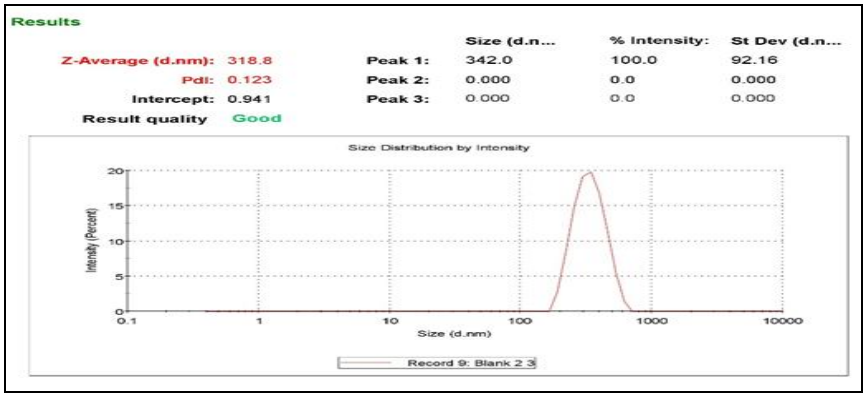

FIG. 8: PARTICLE SIZE MEASUREMENT OF OPTIMIZED A7 BATCH OF GINGER TRANSFEROSOMES

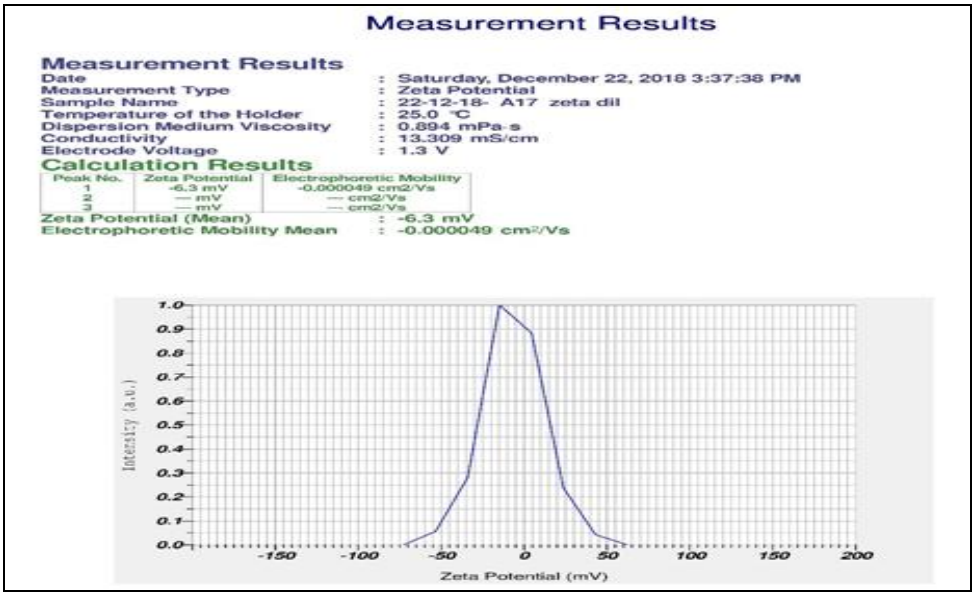

FIG. 9: ZETA POTENTIAL MEASUREMENT OF OPTIMIZED A7 BATCH OF GINGER TRANSFEROSOMES

Entrapment Efficiency: The entrapment efficiency of all the batches was determined by the cold centrifugation method.

***In-vitro* Drug Release Studies:** The cumulative release of ginger extract was maximum *i.e.* 53.75% at 1 h., whereas the ginger transferosomes showed a maximum cumulative release of 67% at 22 h.

Stability Studies of A7 Batch of Formulation of Ginger Transferosomes: A7 batch showed good storage stability at 4 ± 2 °C where there were no significant changes in the entrapment efficiency.

TABLE 3: ENTRAPMENT EFFICIENCY OF DIFFE-RENT FORMULATIONS OF GINGER TRANSFEROSOME

Formulation Code	% Entrapment Efficiency
A1	12.75
A2	34.6
A3	29.58
A4	18.01
A5	34.570
A6	54.73
A7	74.4

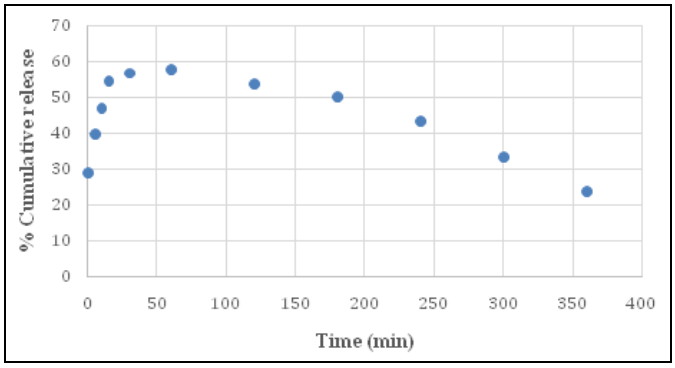

FIG. 10: GRAPH OF *IN-VITRO* RELEASE STUDY OF GINGER EXTRACT

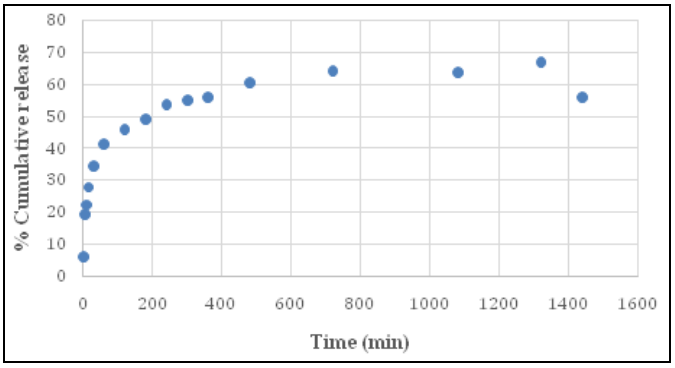

FIG. 11: GRAPH OF *IN-VITRO* DRUG RELEASE STUDY OF A7 BATCH

TABLE 4: STABILITY STUDIES OF A7 BATCH OF FORMULATION OF GINGER TRANSFEROSOME

S. no.	Temperature	Time	Particle size (nm)	Entrapment Efficiency
1	4 ± 2°C	Initial	318.8	74.4
		1 month	321.4	71.2
		2 months	326.9	68.3
		3 months	334.5	53.1
2	25 ± 2°C	Initial	318.8	74.4
		1 month	328.0	63.8
		2 months	337.2	52.5
		3 months	380.4	42.0

Formulation of Transdermal Patches:

Preparation of Optimized Batch of Transdermal Patch Containing Ginger Transferosomes: 700 mg of HPMC K-15 M was soaked in a hydroalcoholic solution (1:1) till it forms a smooth gel. In a beaker, 1 ml of PEG solution, 0.5 ml Tween 80 and 20 mg of lyophilized ginger transferosomes were dissolved using ultrasonication.

This solution was poured slowly in the polymer base. The gel was then kept for ultrasonication till all the air bubbles were removed. This final solution was poured in a petri dish, covered with aluminum foil, and allowed to dry for 24 h at room temperature.

TABLE 5: LIST OF INGREDIENTS AND THEIR ROLE IN PREPARING TRANSDERMAL PATCH

Ingredients	Role
HPMC K-15 M	Polymer
PEG 400	Plasticizer
Tween 80	Penetration enhancer
Hydroalcoholic solution (1:1)	Solvent system

Evaluation of Transdermal Patches:

Folding Endurance: The folding endurance of P6 batch was found to be 7 which ensured that the prepared transdermal patch had the capability to withstand mechanical pressure and has a good flexibility.

Film Thickness: The thickness of the film of P6 batch was found to be 0.134 ± 0.0012 mm.

Drug Content: The drug content of the optimized batch was found to be 97.18 ± 0.78%.

Uniformity of Weight: The drug content of P6 patches of the optimized batch was in the range of 19.54 ± 0.92 mg.

***In-vitro* Diffusion Studies:** Transdermal patch of ginger extract showed maximum *in-vitro* diffusion of 63.27 % at 9 h. whereas the patch containing transferosomes of ginger showed maximum *in-vitro* diffusion of 75.82 % at 22 h.

TABLE 6: DRUG CONTENT AND OTHER PHYSICAL PARAMETERS OF DIFFERENT FORMULATION OF TRANSDERMAL PATCH

Formulation Code	Folding Endurance	Film Thickness	Drug Content	Uniformity of Weight
P1	No patch formation	No patch formation	No patch formation	No patch formation
P2	No patch formation	No patch formation	No patch formation	No patch formation
P3	No patch formation	No patch formation	No patch formation	No patch formation
P4	No patch formation	No patch formation	No patch formation	No patch formation
P5	4	0.128 ±0.0031 mm	92.83 ± 1.56 %	18.64 ± 2.18 mg
P6	7	0.134 ±0.0012 mm	97.18 ± 0.78%	19.54 ±0.92 mg

*All the values are given in mean± standard deviation (n=3) *

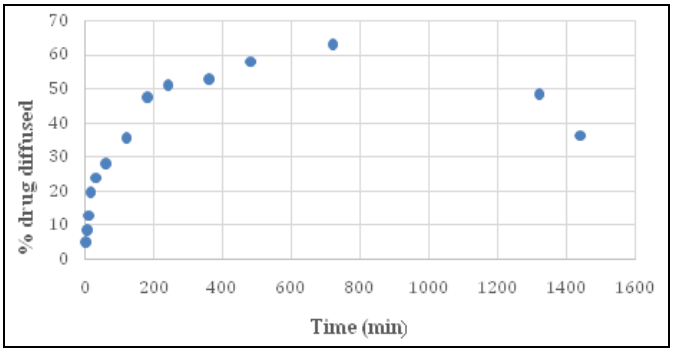

FIG. 12: GRAPH OF *IN-VITRO* DIFFUSION OF TRANSDERMAL PATCH CONTAINING GINGER EXTRACT

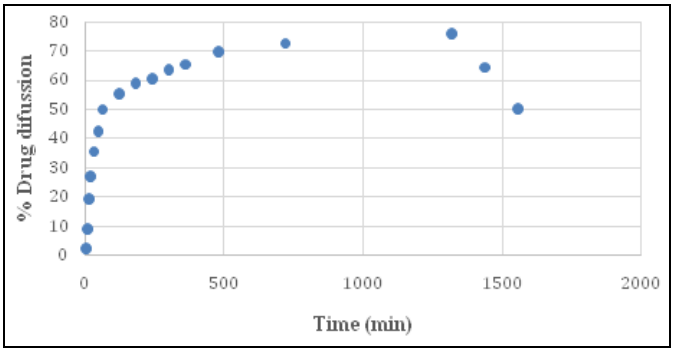

FIG. 13: GRAPH OF *IN-VITRO* DIFFUSION OF TRANSDERMAL PATCH CONTAINING GINGER TRANSFEROSOMES (P6)

CONCLUSION: Transferosomes of ginger were formulated by solvent injection technique. The most stable formulation of transferosomes was 1:2:0.15:0.025. These were formulated into transdermal patch. The formulation showed sustained release when compared to simple ginger extract. Hence, it may be concluded that the transdermal patches with ginger transferosomes are a better approach as compared to simple ginger extract. The frequency of dose is reduced, and the bioavailability of ginger constituents is increased.

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