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RESEARCH PAPER

Development of eucalyptol enriched nano vesicles for better transdermal delivery of curcumin: Preparation, characterisation and *ex vivo* skin analysis

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ABSTRACT

Objective(s): Invasomes are nano-sized vesicular systems made of ethanol, soybean phosphatidylcholine and terpenes that promote skin penetration by disrupting the lipoidal structure of stratum corneum, interacting with the matrix proteins and augmenting the partitioning of the drug moiety in the skin layer. Curcumin is a well-known bright yellow coloured polyphenol which is produced by the plants of curcuma long class belonging to family *Zingiberaceae*. It is being used in the therapeutic field since ancient times for the treatment of several diseases. It is a complex molecule with substantial activity against a number of ailments. Despite having a number of health benefits, curcumin's limited water solubility and poor skin penetration are the major barriers in its transdermal application.

Materials and Methods: In present research work, terpenoid invasomal nano vesicles of curcumin were prepared using mechanical dispersion process with eucalyptol as the permeation enhancer. The prepared invasomal formulations were characterised and optimised in terms of entrapment efficiency, vesicle size, *in vitro* drug release, *ex vivo* permeation and skin retention analysis.

Results: The transmission electron microscope confirmed the presence of spherical-shaped vesicles with a vesicle size of 461.57± 1.38 nm and 80.54 ± 0.38 % entrapment efficiency. *In vitro* release kinetics conformed well with Higuchi kinetic model for release. *Ex vivo* study confirmed that the curcumin permeation across the pig ear skin from the optimised formulation was 2.5 times higher than curcumin solution and had a flux of 179.44±0.26 μg/cm^2/h.

Conclusion: The study suggests that invasomes have a high potential for transdermal administration of curcumin which can increase the topical utility of curcumin in several skin diseases.

Keywords: *Invasomes, Permeation enhancer, Skin, Terpene*

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INTRODUCTION

Curcumin is a well-known bright yellow coloured polyphenol which is produced by the plants of curcuma long class belonging to family *Zingiberaceae*. It is being used in the therapeutic field since ancient times for the treatment of several diseases [1]. It effectively adheres to a range of biomolecules and inhibits the action of inflammatory enzymes, as well as regulates the production of numerous proteins, chemical messengers and adhesion molecules by modifying the functions of various transcription factors. It has significant clinical utility via approaches that target diverse molecular domains, making it a multifunctional chemical moiety with numerous therapeutic benefits such as antifungal, antimutagenic, anticarcinogenic, antibacterial, antiviral and antiulcer [2]. Curcumin is used to manage and cure skin illnesses such as dermatitis, psoriasis, skin irritation, and premature age spots, which is linked to its anti-inflammatory, antioxidant, and bactericidal properties [3-6].

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Despite having several merits and therapeutic significance, curcumin's limited water solubility and relatively low permeability pose serious challenges to its transdermal distribution and effectiveness [1-2].

Invasomes are flexible phospholipid-based nanoscale vesicular systems composed of phosphatidylcholine, ethanol, terpenes or a blend of terpenes with considerably more percutaneous permeation than standard liposomes [7-9]. Terpenes are potent permeation boosters that readily affects the packing of stratum corneum, disrupt its lipid structure, react with intracellular proteins, and significantly augments the stratum corneum drug partitioning [5, 8]. Ethanol and terpenes present in the invasomes extend a synergistic impact which promotes invasomal vesicle penetration through the skin [10-12].

The research focused on the potential of invasomes to promote transdermal administration of curcumin in order to generate a topical formulation with better curcumin transport across the skin for improved therapeutic activity.

METHODS

Materials

Curcumin was obtained from Loba Chemie, India. Soya lecithin and absolute ethanol were bought from Sigma-Aldrich Chemical Co. Ltd., Delhi. Eucalyptol was obtained from Hi media.

Curcumin Invasomes preparation

Curcumin invasomes were produced using mechanical dispersion technique. Soya Phosphatidylcholine was added to ethanol and the mixture was vortexed for 5 min. Eucalyptol and curcumin were added while the mixture was constantly vortexed and sonicated for 5 min. Under constant vortexing, a fine stream of distilled water (up to 10% w/v) was added with a syringe. To obtain the final invasomal preparation, the formulation was vortexed for an additional 5 min [11, 13, 14].

Characterisation of curcumin invasomal vesicles

Entrapment efficiency

Ultracentrifugation method was used for determining the percentage entrapment of the invasomal formulation. Invasomal formulation was centrifuged for 40 min in an ultra-centrifuge at 15000 rpm. The sediment was collected which was further diluted with ethanol. UV spectrophotometry was used for analysing the curcumin content at a wavelength of 426 nm [15, 16].

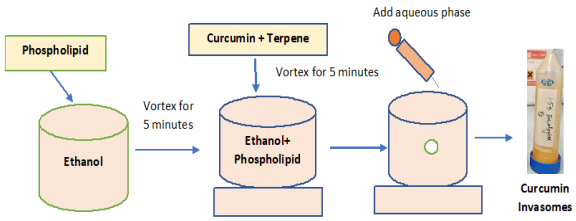

Fig 1. Schematic diagram for the preparation of Invasomes

The percentage entrapment efficiency was then calculated using the equation:

$$\%\ Entrapment\ efficiency = \frac{amount\ of\ entrapped\ drug\ recovered}{total\ amount\ of\ drug} \times 100$$

Particle Size

The size of the vesicles was determined using Zeta Sizer (Nano- ZS, Malvern, U.K.). All experimentation was performed in triplicates [17]

In vitro drug release

In vitro drug release study was conducted using Franz's diffusion cell with receiver cell volume and effective permeation area of 10 ml and 0.196 cm^2 respectively. The donor cell containing the invasomal formulation was clamped over the receptor cell in which phosphate buffer saline (pH 7.4) was filled. The experiment was conducted for 24 hr at a temperature of 37 ± 1°C with constant magnetic stirring at 600 rpm. Samples were estimated for curcumin content using UV spectrophotometer at 426 nm which were withdrawn from the receptor cell at premediated time gaps i.e., 1, 2, 3, 4, 5, 6, 8, 12 and 24 hr. To know the release kinetics of the optimized invasomal formulation, the data was treated according to different release kinetics models [18, 19].

Ex-vivo drug permeation studies

Ex-vivo drug permeation study was conducted

Table 1. Composition of invasomal formulation

FORMULATION	DRUG (w/v)	TERPENE (w/v)	ETHANOL (v/v)	POLYMER (w/v)
F1	1%	0.1%	10%	1%
F2	1%	0.5%	10%	1%
F3	1%	1%	10%	1%
F4	1%	1.5%	10%	1%

using Franz's diffusion cell with receiver cell volume and effective permeation area of 10 ml and 0.196 cm^2 respectively. The amount of drug permeation was determined using the skin of pig ear which was obtained from a local abattoir. The donor compartment was filled with invasomal formulation, which was fixed over the receptor compartment containing phosphate buffer (pH 7.4). Pig ear skin was arranged between the two compartments with the help of a clamp. The experiment was conducted for 24 hr at a temperature of 37 ± 1°C with constant magnetic stirring. Samples were estimated for curcumin content using UV spectrophotometer at 426 nm which were withdrawn from the receptor cell at premediated time gaps i.e., 1, 2, 3, 4, 5, 6, 8, 10 and 24 hr. Using the data obtained from the *ex vivo* study skin parameters like flux (J, µg/cm^2/hr), cumulative permeation of curcumin across the skin per unit area (Q24, µg/cm^2), permeability coefficient (Kp, cm/hr) and enhancement ratio (ER) were determined [19, 20]

Skin retention studies

To ascertain the amount of curcumin that retained in the pig ear skin after the *ex-vivo* permeation study, the pig ear skin was separated and vortexed in methanol (10 ml) for 10 min which was further kept in methanol overnight. Concentration of curcumin in methanol was estimated using UV spectrophotometer at 426 nm [21].

Morphology and vesicular shape

The optimized invasomal formulation was visualised by transmission electron microscope (TEM) (Technai) with an accelerating voltage of 100 kV. The samples were negatively stained using 1% phosphotungstic acid aqueous solution. For staining, the invasomal formulation was dried on a tiny carbon-coated grid. Blotting was used to remove the surplus solution. The material was examined under the microscope after drying [22].

FTIR-ATR studies

For studying the possible interactions taking place between the polymer and the drug FTIR-ATR study was done. The IR spectra of the samples were acquired using an FTIR-ATR spectrophotometer (Bruker) in 4000–400-1 cm range after placing the sample over the sample disc [17].

Stability studies

After storing the optimized curcumin invasomes at 4 ± 1 °C, 30 ± 1 °C and 45 ± 1 °C under controlled humidity of 75% RH for 1, 2 and 4 months, the physicochemical stability was evaluated. Physical appearance, entrapment efficiency and vesicle size were used to assess physicochemical stability. At each storage condition, triplicate samples were used for testing [23].

RESULTS

Vesicle size and entrapment efficiency

The vesicle size of the formulated terpenoid invasomes ranged from 363.46 ± 2.00 to 461.57± 1.38 nm and the polydispersity index was found to be less than 0.35 for all the invasomal formulations. The particle size increased with an increase in terpene content. At 1.5% concentration of eucalyptol the observed vesicle size was 461.57± 1.38 nm which is attributed to the high amount of lipophilic drug loaded in the invasomes with lipophilic terpene which leads to an increased particle size of the invasomes [24]. The PDI values of prepared invasomes affirm good stability and homogeneity of the vesicles.

The percentage drug entrapped in the vesicles directly reflects the drug's ability to incorporate with the lipid content and create vesicles of good stability [25]. The entrapment efficiency

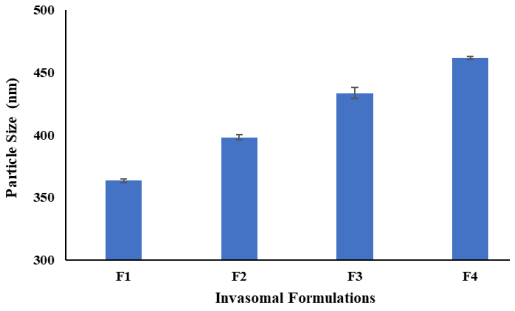

Fig 2. Vesicle size of curcumin invasomes

Table 2. Particle size, PDI and Entrapment Efficiency of Curcumin Invasomes

Formulation Code	Vesicle Size (n=3)	Entrapment Efficiency (n=3)
F1	363.46 ± 2.00	76.35 ± 0.62
F2	398.07± 1.90	77.26 ± 0.43
F3	433.55± 4.21	78.33 ± 0.32
F4	461.57± 1.38	80.54 ± 0.38

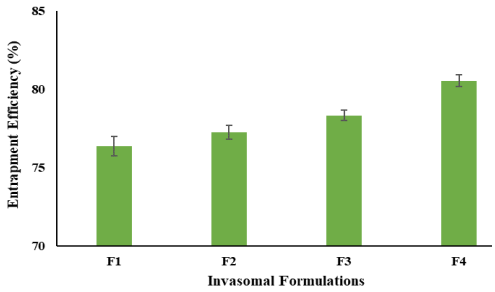

Fig. 3. Entrapment Efficiency (%) of Curcumin Invasomes

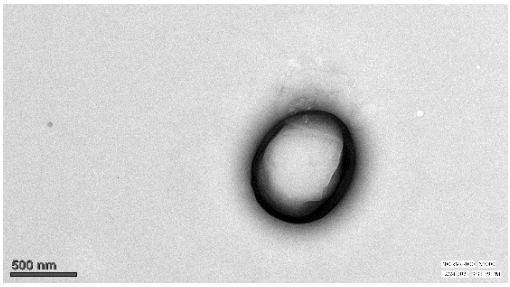

Fig. 4. TEM image of Invasome

of curcumin in different invasomal formulations ranged from 76.35 ± 0·62 to 80.54 ± 0·38 %.

When the concentration of terpene increased in the vesicles its entrapment efficiency also increased which is due to the enhanced solubilization of the lipophilic drug. The lipophilic terpene dissolves in the vesicular bilayer with the phosphatidylcholine during vesicle formation, where phospholipid acyl chains offer a suitable environment for the lipophilic drug and lipophilic terpene [27, 10]. Thus, it is affirmed that as terpene concentration increases, more chains become accessible which increases the solubility of the lipophilic medication in bilayers and therefore entrapment efficiency increases.

Morphology and vesicular shape

Vesicle morphology was analysed by transmission electron microscopy (TEM) which revealed the predominance of unilamellar spherical-shaped vesicles with an even surface (Fig. 3). The particle size determined by TEM agreed with the particle size determined by dynamic light scattering.

In vitro drug release

In vitro drug release studies were conducted on modified Franz diffusion cell and the cumulative amount of curcumin that released from the invasomal formulations was compared to the amount released from curcumin solution. The invasomal formulations F1, F2, F3 and F4 showed a cumulative release of 3985.45 ±2.80, 4169.35 ± 5.14, 4367.19 ± 4.34 and 4568.24 ± 3.4 µg/cm^2 respectively after 24 hr whereas curcumin solution showed a release 1824.34 ± 3.48 µg/cm^2 (Fig. 5). F4 with 1.5 % eucalyptol present in it showed the maximum amount of drug release and it was contemplated that the concentration of terpene had a substantial effect on the drug release profile. Higher amount of curcumin was released with increase in the concentration of terpene.

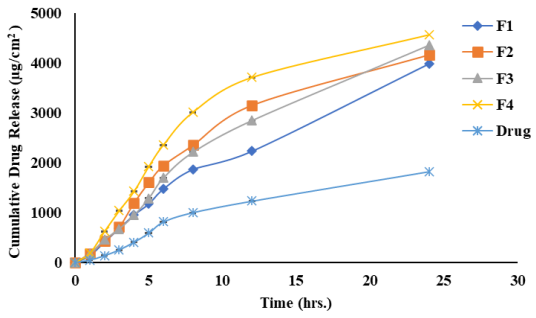

Fig. 5. Cumulative drug release profile (µg/cm^2) from invasomes and curcumin solution

The *in vitro* drug release data was analysed using several release kinetics models such as first order, zero order, Higuchi's equation and Korsmeyer Peppas equation. The best model for describing the drug release kinetics of the optimised invasomes was found to be the one with the highest correlation (R^2) value (F4). According to the greatest correlation (R^2) value, the optimised formulation F4 (R^2=0.953) conformed well to the Higuchi model. The release exponent (n>1) of Korsmeyer–Peppas equation suggest that curcumin release from F4 corresponds to case II transport mechanism where the release of a drug is regulated by erosion and relaxation of the polymer.

Ex vivo drug permeation and skin retention study

Ex-vivo drug permeation study was performed using a modified Franz diffusion cell and cumulative amount of curcumin permeated from the invasomal formulations were compared with curcumin solution.

The invasomal formulations F1, F2, F3 and F4 showed a cumulative permeation 2816.99 ± 1.45, 3564.29 ± 5.1, 3768.25 ±4.29 and 4153.84 ± 4.1 µg/cm^2 respectively after 24 hr whereas curcumin solution showed a permeation of 1633.74 ± 0.88 µg/cm^2 (Fig. 6).

F4 showed the maximum amount of drug

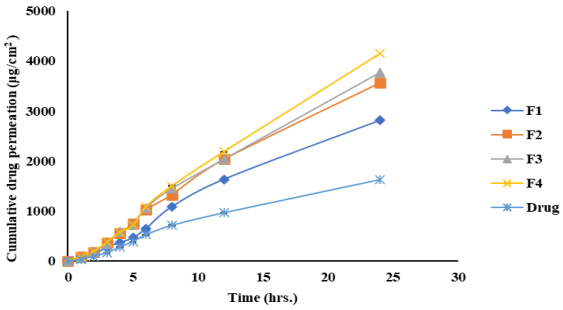

Fig. 6. Cumulative drug permeation (µg/cm²) from invasomes and curcumin solution

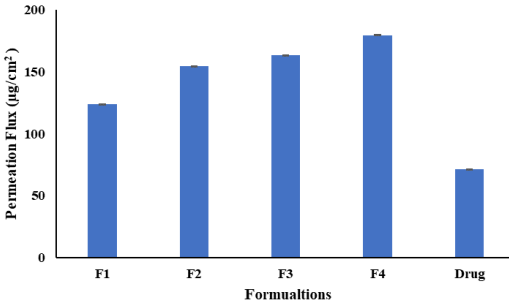

Fig. 7. Permeation flux of curcumin invasomes and simple solution

permeation and it was observed that concentration of terpene had a substantial effect on the drug permeation profile. Higher amount of curcumin permeated the stratum corneum layer of the skin with increase in the concentration of terpene.

The cumulative amount of curcumin that permeated after 24 hr, flux, enhancement ratio, permeability coefficient and skin retention were analysed and Table 3 depicts the results. It was observed that the value of permeation flux ranged from 123.63± 0.02 to 179.44±0.26 µg/cm²/hr for curcumin invasomes whereas curcumin solution had a permeation flux of 71.25±0.19 µg/cm²/hr. Invasomes containing 1.5% eucalyptol showed 2.51 times increase in the permeation flux when compared with curcumin solution. The permeability coefficient of invasomal formulation was found to be 12×10^{-3} to 17.9×10^{-3} cm/hr. Curcumin solution had a permeability coefficient of 7.1×10^{-3} cm/hr whereas the F4 invasomal formulation showed a 2.5 times higher permeability coefficient.

The amount of curcumin that retained in the pig ear skin ranged from 597.58±0.37 to 942.16±0.31 µg/cm². Invasomal formulation F4 demonstrated the highest drug retention 942.16±0.31µg/cm² when compared to the curcumin solution which was 308.43±0.12 µg/cm². The amount of curcumin retained in the skin increased with an increase in concentration of terpene which is attributed to increased size of invasomal vesicle.

Table 3. Release Kinetics

Release Kinetics Model	R^2
Zero order	0.881
First order	0.753
Higuchi model	0.953
Korsmeyer-Peppas model	0.917

FTIR- ATR studies

The FTIR spectra (Fig. 8) of curcumin gave characteristics peaks at 1424.64 cm $^{-1}$ and 1596.98 cm^{-1} which is due to stretching of carbon to carbon within the aromatic ring (C-C vibration). The presence of absorption band at 1500.97 cm^{-1} was observed due to aromatic ring stretching. Clear sharp strong bands were seen at 1626.57

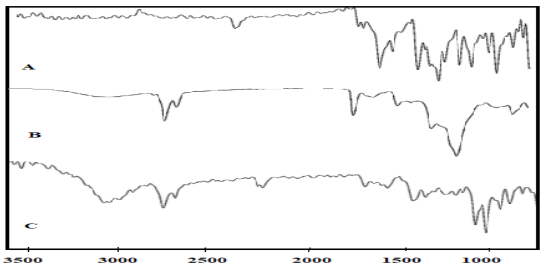

Fig. 8. FTIR spectra of A)Curcumin B)Phospholipid C)Optimized Formulation

Table 4. Skin permeation parameters of curcumin invasomes across the pig ear skin

Formulation Code	Q_{24} Cumulative drug permeated µg/cm²	Permeation flux (J, µg/cm² /h)	Permeability coefficient (Kp, cm/h)	Enhancement Ration (ER)	Amount deposited (µg/cm²)
F1	2816.99±1.45	123.63± 0.02	12×10^{-3}	1.74	597.58±0.37
F2	3564.29 ± 5.1	154.5 ±0.06	15.4×10^{-3}	2.17	758.45±0.18
F3	3768.25±4.29	163.21±0.01	16.3×10^{-3}	2.29	853.63±0.24
F4	4153.84 ± 4.1	179.44±0.26	17.9×10^{-3}	2.52	942.16±0.31
Drug	1633.74±0.88	71.25±0.19	7.1×10^{-3}	1.00	308.43±0.12

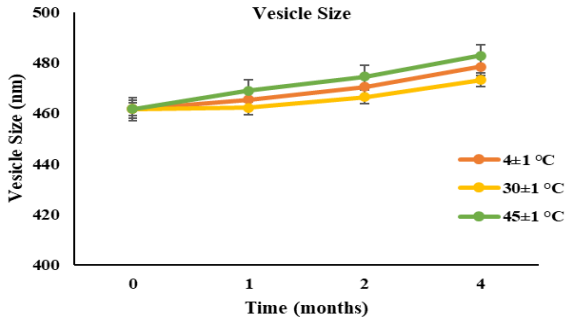

Fig. 9. Effect of storage condition on Vesicle Size

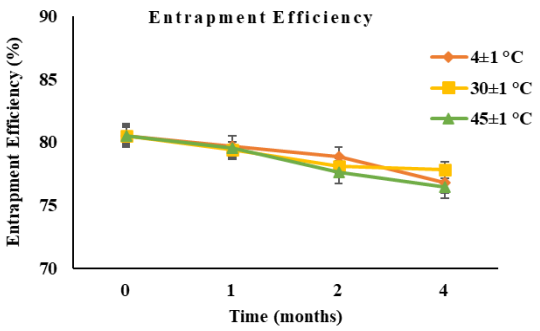

Fig. 10. Effect of storage condition on Entrapment Efficiency

cm^{-1} which is attributed to carbonyl stretching vibration (C=O). Characteristic peak was observed at 1274.92 cm^{-1} due to C-O stretching vibrations and an olefinic CH in plane bending was observed at 1424.64 cm^{-1}.

The spectrum of phosphatidyl choline shows a strong absorption band at 1062.15 cm^{-1} which denotes the presence of P-O-C groups. Peak at 1737.38 cm^{-1} is due to C=O stretching vibration. The aliphatic C-H bands were observed from 2856 cm^{-1} to 2924.33 cm^{-1}. C=C stretching band was observed between 1588.28 cm^{-1} to 1519.22 cm^{-1}.

The IR spectrum of the optimized Curcumin invasome showed the characteristics peaks of both Curcumin and phosphatidyl choline with a minor shift in the absorption band and reduced intensity. A peak at 3275.11 cm^{-1} confirms the presence of mannitol which was used for the lyophilisation of invasomes.

Stability studies

Stability studies were performed at 4 ± 1 °C, 30 ± 1 °C and 45 ± 1 °C under controlled humidity of 75% RH for 1, 2 and 4 months. The optimized invasomal formulation showed good physical appearance at different temperatures during storage. The vesicle size exhibited an increase of 3.63%, 2.50% and 4.57% at 4 ± 1 °C, 30 ± 1 °C and 45 ± 1 °C respectively which is caused by the agglomeration of smaller vesicles together (Fig. 9). The drug entrapment during the 4 months of storage showed a reduction of 4.61%, 3.38% and 5.02% at 4 ± 1 °C, 30 ± 1 °C and 45 ± 1 °C. (Fig. 10). The curcumin invasomal formulation was found to be stable at 30±1°C.

DISCUSSION

The present work explored the potential of invasomes as a possible carrier in delivering curcumin through the transdermal route. Terpenoid invasomal nano vesicles of curcumin were developed using mechanical dispersion process. The prepared invasomal formulations were characterised and optimised in terms of entrapment efficiency, vesicle size, *in vitro* drug release, *ex vivo* permeation and skin retention studies. Ultracentrifugation method was used for determining the percentage entrapment and the maximum drug entrapment was found to be 80.54 ± 0.38%. The vesicle size of the formulated terpenoid invasomes ranged from 363.46 ± 2.00 to 461.57± 1.38 nm and the polydispersity index was found to be less than 0.35 for all the formulations. The optimized invasomal formulation showed spherical-shaped vesicles under transmission electron microscope with a vesicle size of 461.57± 1.38 nm. The PDI values of prepared invasomes affirmed good stability and homogeneity of the vesicles. The FTIR spectra reveals no possible interactions between the drug and excipients. *In vitro* drug release study confirmed that a maximum 4568.24 ± 3.4 µg/cm^2 of curcumin was released from the F4 optimized formulation after 24 hr and the drug release conformed well to the Higuchi model of kinetics. The *Ex vivo* skin permeation study revealed that the curcumin permeation across the pig ear skin from the terpenoid invasome had a flux of 179.44±0.26 µg/cm^2/hr which was 2.5 times higher than curcumin solution. F4 formulation showed the maximum permeability coefficient which was 17.9×10^{-3} cm/hr. The invasomal vesicles were able to attain a higher flux and maximum skin retention when compared to curcumin solution. The higher flux of the invasomes is attributed to the synergistic effect of permeation enhancing effect of eucalyptol and ethanol which acts by disrupting the lipids of stratum corneum, interacts

with the intercellular proteins and enhances the partitioning of curcumin into the skin layer. The optimized invasomal vesicles were stable for 4 months at 30±1°C 75% RH [17]. Results of the study clearly suggest that invasomes actively enhanced the transdermal delivery of curcumin across the stratum corneum [28].

CONCLUSION

Curcumin is a complex molecule with substantial activity against a number of diseases. Despite its numerous advantages and health benefits, curcumin's limited water solubility and low skin penetration offer significant challenges to its transdermal distribution and performance. The developed optimized terpenoid nano invasomal vesicles showed promising results of entrapment, drug release, drug permeation and retention which enhanced the delivery of curcumin across the stratum corneum. The study suggests that invasomes have a high potential for transdermal administration of curcumin which can increase the topical utility of curcumin in several skin diseases.

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CONFLICTS OF INTERESTS

The authors declare that they have no competing interests.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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