

Formulation and Evaluation of Coumarin Loaded Transfersosomal Gel

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ABSTRACT

Coumarin is a naturally occurring phytochemical that exhibits various pharmacological activities, including anti-inflammatory, antioxidant, antimicrobial and wound-healing properties. However, its topical therapeutic effectiveness is limited due to poor aqueous solubility and inadequate skin permeation. Transfersomes are highly deformable lipid vesicles capable of penetrating the stratum corneum and delivering drugs into deeper layers of the skin, thereby enhancing topical drug delivery. The present study aimed to formulate and evaluate a coumarin loaded transfersomal gel for improved topical delivery. Coumarin loaded transfersomes were prepared using the thin-film hydration method with phospholipids and an edge activator. The prepared vesicles were evaluated for vesicle size, polydispersity index, zeta potential and percentage drug content. The optimized transfersomal formulation was incorporated into a Carbopol gel base and further evaluated for appearance, pH, viscosity, spreadability, drug content, in vitro drug release and ex vivo skin permeation. The optimized transfersomal gel exhibited uniform appearance, suitable pH, good spreadability and sustained drug release with enhanced skin permeation compared to the plain coumarin gel. The findings suggest that the coumarin-loaded transfersomal gel is a promising topical drug delivery system capable of improving the therapeutic efficacy of coumarin by enhancing its skin penetration, prolonging drug release and increasing local drug availability. Therefore, transfersomal gel represents a potential carrier for the effective topical treatment of skin disorders.

KEYWORDS: Anticoagulant, Anti-inflammatory, Transfersomal Gel, Coumarin, Thin-Film Hydration Method, Prolongation of Clotting Time, Topical drug delivery system.

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1. INTRODUCTION

Topical drug delivery is widely used for the treatment of skin diseases because it provides direct application of the drug at the site of action, minimizes systemic side effects, improves patient compliance and avoids first-pass metabolism. However the outermost layer of the skin, known as the stratum corneum acts as a major barrier to drug permeation limiting the effectiveness of many therapeutic agents. Therefore, the development of advanced drug delivery systems capable of overcoming this barrier has become an important area of pharmaceutical research¹.

Coumarin (2H-1-benzopyran-2-one) is a naturally occurring benzopyrone compound present in numerous medicinal plants such as *Melilotus officinalis*, *Dipteryx odorata*, *Cinnamomum cassia* and *Murraya paniculata*. Coumarin and its derivatives

possess several biological activities including antioxidant, anti-inflammatory, antimicrobial, antifungal, anticoagulant, anticancer and wound-healing effects. These pharmacological properties make coumarin a promising candidate for topical therapy in the management of skin infections, inflammation and wound healing. However, the clinical application of coumarin is limited due to its poor aqueous solubility, limited skin permeability and low bioavailability².

Nanovesicular drug delivery systems have emerged as effective carriers for improving the delivery of poorly permeable drugs through the skin. Among these systems, transfersomes have attracted considerable attention because of their unique deformability. Transfersomes are ultra flexible lipid vesicles composed mainly of phospholipids and edge activators such as Tween 80 or Span 80. The presence of

edge activators imparts elasticity to the vesicles enabling them to squeeze through skin pores much smaller than their own diameter. Consequently transfersomes enhance drug penetration into deeper skin layers, improve drug retention at the target site and provide sustained drug release while reducing systemic exposure³.

Incorporating transfersomes into a gel base combines the advantages of nanocarrier-mediated drug delivery with the convenience of semisolid dosage forms. Transfersosomal gels are easy to apply, non-greasy, provide prolonged contact with the skin and improve patient acceptability. Carbopol-based gels are commonly employed due to their excellent viscosity, clarity, biocompatibility and stability. The incorporation of coumarin-loaded transfersomes into a gel matrix can further enhance formulation stability and ensure controlled drug release.

The present study was undertaken to formulate and evaluate a coumarin-loaded transfersomal gel for topical application. Transfersomes were prepared using the thin-film hydration technique and optimized based on vesicle characteristics and entrapment efficiency. The optimized formulation was incorporated into a Carbopol gel and evaluated for physicochemical properties, drug content, spreadability, in vitro drug release and ex vivo skin permeation. The developed transfersomal gel is expected to improve the topical delivery and therapeutic effectiveness of coumarin compared with conventional gel formulations, making it a promising approach for the treatment of skin disorders^{3,4}.

2. MATERIAL AND METHOD

2.1 Materials

The coumarin was purchased from Yucca Enterprises, Mumbai and other excipients were obtained from New Neeta Chemicals, Pune.

2.2 Formulation of coumarin loaded Transfersomes

Coumarin-loaded transfersomes were prepared by the thin film hydration method. Accurately weighed quantities of phospholipid, cholesterol, edge activator, and coumarin were dissolved in a chloroform mixture (2:1, v/v) in a round-bottom flask. The organic solvent was removed using a rotary evaporator under reduced pressure at 40–45°C until a thin lipid film was formed on the inner surface of the flask. The deposited lipid film was further dried under vacuum to eliminate residual solvent. The dried film was hydrated with phosphate buffer saline (PBS, pH 6.8) at controlled temperature with gentle rotation to obtain a vesicular dispersion. The prepared transfersomal dispersion was subjected to sonication to reduce vesicle size and improve homogeneity. The optimized transfersomal formulation was stored at refrigerated conditions until further evaluation and incorporation into a suitable gel base. This method was performed based on the thin-film hydration technique reported for preparation of transfersosomal vesicular systems with

suitable modifications according to formulation requirements^{5,6}.

2.3 Preparation of Trasfersosomal Gel of Coumarin

Carbopol 934/940 was accurately weighed and gradually dispersed in distilled water with continuous stirring. The dispersion was allowed to hydrate for 24 hours to obtain a clear polymeric solution. Propylene glycol was added as a humectant and penetration enhancer. The pH of the gel base was adjusted using triethanolamine to form a clear and uniform gel. The prepared transfersomal suspension was slowly incorporated into the gel base under continuous stirring to obtain a homogeneous transfersomal gel. Care was taken to avoid air entrapment during mixing. The final formulation was transferred into suitable containers and stored under refrigerated until further evaluation^{6,7}.

3. CHARACTERIZATION

3.1 Physical Observation

Physical appearance, odor, texture was done for final formulation by physical observation⁵.

3.2 Calibration curve

A standard stock solution of coumarin (100 µg/mL) was prepared. Different aliquots of the stock solution were diluted to obtain concentrations ranging from 2.5–15 µg/mL. The absorbance of each dilution was measured at 274 nm using a UV–Visible spectrophotometer against a solvent blank. A calibration curve was plotted by taking concentration on the X-axis and absorbance on the Y-axis, and the regression equation with correlation coefficient (R^2) was determined to confirm linearity⁷.

3.3 FTIR

A small quantity of coumarin sample was finely triturated with dry potassium bromide (KBr) powder and compressed under pressure to prepare a transparent pellet. The prepared KBr pellet was placed in the sample holder of the FTIR spectrophotometer and the spectrum was recorded over the range of 4000–400 cm^{-1} . The obtained FTIR spectrum was analyzed to identify the characteristic functional groups of coumarin. The FTIR analysis was performed to confirm the purity and structural integrity of the drug before formulation^{8,9}.

3.4 Melting Point

A small quantity of dried coumarin sample was finely powdered and filled into a thin-walled capillary tube sealed at one end. The capillary tube was placed in the melting point apparatus, and the temperature was gradually increased at a controlled rate. The temperature range at which the sample started melting and completely melted was recorded as the melting point of coumarin. The observed melting point of coumarin was found to be approximately 68–72°C, which was in accordance with the reported melting point range, confirming the identity and purity of the compound¹⁰.

3.5 Particle Size and PDI

The particle size and polydispersity index (PDI) of the prepared coumarin-loaded transfersosomal dispersion were determined by the dynamic light scattering (DLS) technique using a particle size analyzer. The transfersosomal formulation was suitably diluted with distilled water to avoid multiple scattering effects and vesicle aggregation. Approximately 1 ml of diluted sample was transferred into a disposable cuvette and analyzed at 25°C. The mean vesicle size was measured based on the Brownian motion of vesicles and expressed in nanometers (nm). The PDI value was recorded to evaluate the homogeneity and size distribution of the transfersomes^{11,12}.

3.6 Zeta Potential Analysis

The prepared coumarin-loaded transfersosomal gel dispersion was appropriately diluted with deionized water to obtain a suitable concentration for analysis. The diluted formulation was transferred into a disposable electrophoretic cell and evaluated using a Horiba SZ-100 zeta potential analyzer at a controlled temperature of 25°C. The zeta potential measurement was performed to determine the surface charge of transfersosomal vesicles and to assess the physical stability of the formulation¹⁰.

3.7 Ph Determination Test

The pH of the prepared coumarin-loaded transfersosomal gel formulation was evaluated to determine its suitability for topical application. Since the skin maintains a slightly acidic environment, the pH of the formulation plays an important role in ensuring skin compatibility, stability, and therapeutic effectiveness. The pH of the developed gel was measured using a calibrated digital pH meter by immersing the electrode into the formulation. The measurement was performed at room temperature^{13,18}.

3.8 Drug Content

1 mL of coumarin-loaded transfersosomal gel was transferred into a 10 mL volumetric flask and diluted with methanol. The dispersion was sonicated for 15 minutes to extract the drug completely. The volume was adjusted with methanol and the solution was filtered. Further, 1 mL of filtrate was diluted with PBS in a 10 mL volumetric flask. The final solution was analyzed using a UV-visible spectrophotometer. The experiment was performed in triplicate for accuracy. Drug content and percentage active ingredient content was computed using below formula¹⁴.

$$\% \text{ Drug Content} = \frac{\text{Amount Of drug content}}{\text{Theoretical amount of drug content}} \times 100$$

3.9 In-vitro Drug Release Study

The in-vitro release of coumarin from transfersosomal gel was evaluated using a Franz diffusion cell containing donor and receptor compartments. A pre-soaked dialysis membrane was placed between the two compartments. The receptor chamber was filled with phosphate buffer (pH 6.8/7.4) and maintained at 37 ± 0.5°C with continuous stirring. A measured amount of coumarin-loaded transfersosomal gel was applied on the

membrane in the donor compartment. Samples were withdrawn at specific time intervals and replaced with an equal volume of fresh buffer to maintain sink conditions. The collected samples were analyzed using a UV-visible spectrophotometer at the λ_{max} of coumarin. The cumulative percentage drug release was calculated and plotted against time to determine the release profile^{15,19}.

3.10 Ex- vivo Skin Permeation Study

The Ex- vivo study was conducted to check the penetration potential of transfersosomal gel containing coumarin. For this study, fresh goat skin was collected from slaughter house. The goat tissue was sliced into the optimal size, prior to the deep washing and removal of hair. The tissue was then sandwiched between the donor and the acceptor compartment of FD cell and 2 ml of drug solution and coumarin containing transfersosomal gel sample was introduced in the donor compartment with phosphate buffer saline (PBS) pH 5 was taken in the acceptor compartment as dissolution media and maintained at 37 °C of temperature and continuously stirred at 300 rpm using magnetic stirrer for 24 h exposure. The test fluids from the acceptor compartment was intermittently withdrawn and replaced by equal volume of PBS pH 5 to maintain the sink conditions. Withdrawn samples were then analyzed by UV spectroscopic method^{14,17,18}.

Skin permeation parameters such as steady- state permeation rate (J_{ss}) and lag time (LT, hrs) were calculated by plotting cumulative amount of drug permeated per unit area Vs time. From the slope and x-intercept of the linear proportion, respectively, the permeability coefficient (k_p) and enhancement ratio (Er) was calculated by following equation:

$$K_p = J_{ss} / C_o$$

3.11 Spreadability

For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 100mg weight for 5 min. weight (50 g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken measure of spread ability^{20,21}.

$$S = M.L / T$$

M- Weight tied to the upper slide; L - Length moved on the glass; T - Time Taken

4. RESULTS AND DISCUSSIONS

4.1 Physical Appearance

The Transfersosomal gel appears as a smooth, white to off-white, homogeneous semisolid gel.

4.2 Calibration curve

Coumarin was quantitatively analyzed using UV-Visible spectrophotometry. It shows maximum absorbance at 274 nm as shown in figure 1a.

In the above figure no. a graph of absorbance (Y-axis) versus concentration (X-axis) was plotted. The resulting calibration

Formulation and Evaluation of Coumarin Loaded Transfersosomal Gel

curve was found to be linear within concentration range of 2.5 to 15 µg/ml.

The linear regression equation obtained from the curve was $y = 0.0558x + 0.0606$. The Correlation Coefficient (R^2) was found to be 0.9802 which indicates good linearity and reliability of the method for quantification of coumarin.

4.3 FTIR Analysis

The FTIR study of the Coumarin and physical mixture was performed to identify the characteristic functional groups of the drug and excipients and to evaluate the compatibility between coumarin and other formulation components used in the transfersosomal gel.

In figure 2 the FTIR spectrum of Coumarin showed characteristic peaks confirming the presence of important

functional groups of coumarin. The broad peaks at 3395.47 cm^{-1} and 3049.43 cm^{-1} indicated O–H and aromatic C–H stretching vibrations. The peak at 2860.30 cm^{-1} represented aliphatic C–H stretching, while the strong peak at 1700.68 cm^{-1} confirmed the presence of the lactone carbonyl (C=O) group, which is the main characteristic peak of coumarin. The peaks at 1601.73 cm^{-1} and 1558.71 cm^{-1} showed aromatic C=C stretching vibrations. Peaks between 1257.57 cm^{-1} and 1041.57 cm^{-1} indicated C–O and C–O–C stretching of the lactone ring. The fingerprint region peaks from 943.28 cm^{-1} to 688.87 cm^{-1} confirmed aromatic ring bending vibrations. Overall, the FTIR study confirmed the identity, purity and structural integrity of coumarin without any significant change in characteristic peaks.

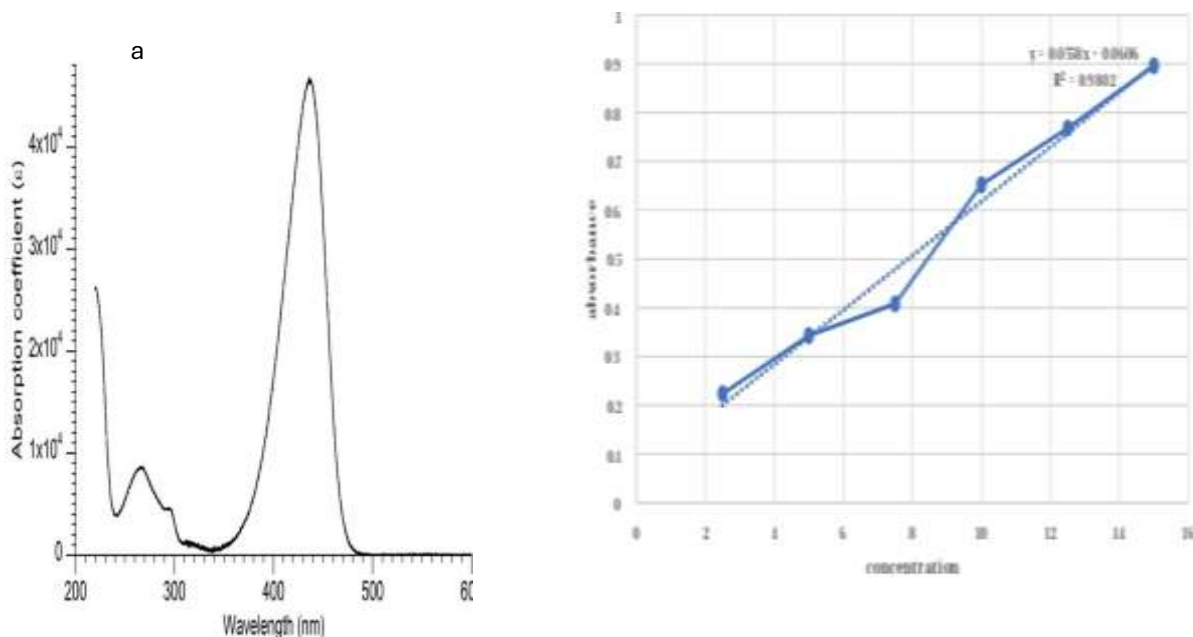


Figure 1: a. UV Spectra of Coumarin

b. Calibration curve of Coumarin.

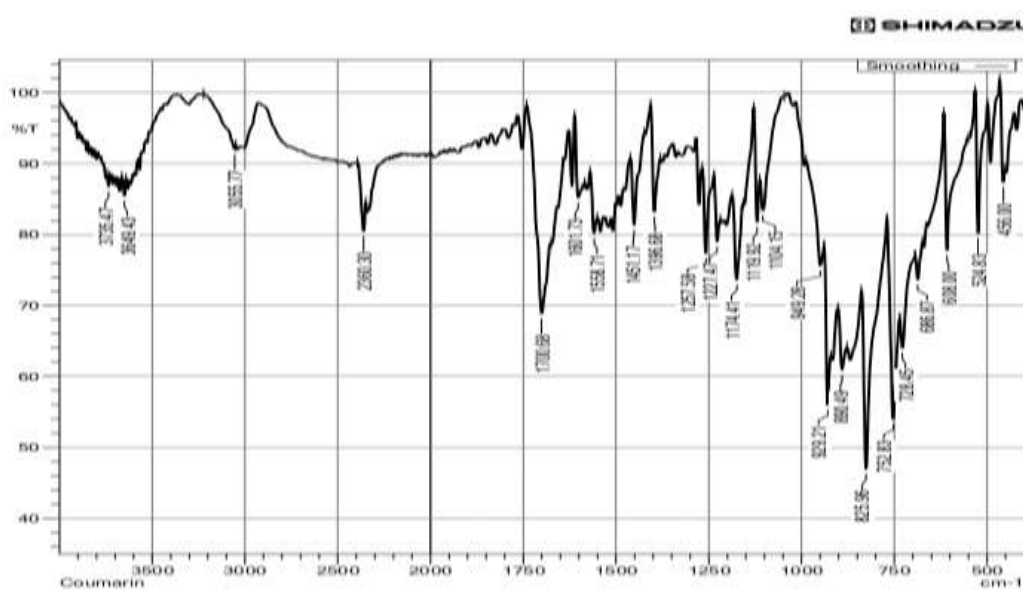


Figure 2: FTIR spectra of Coumarin

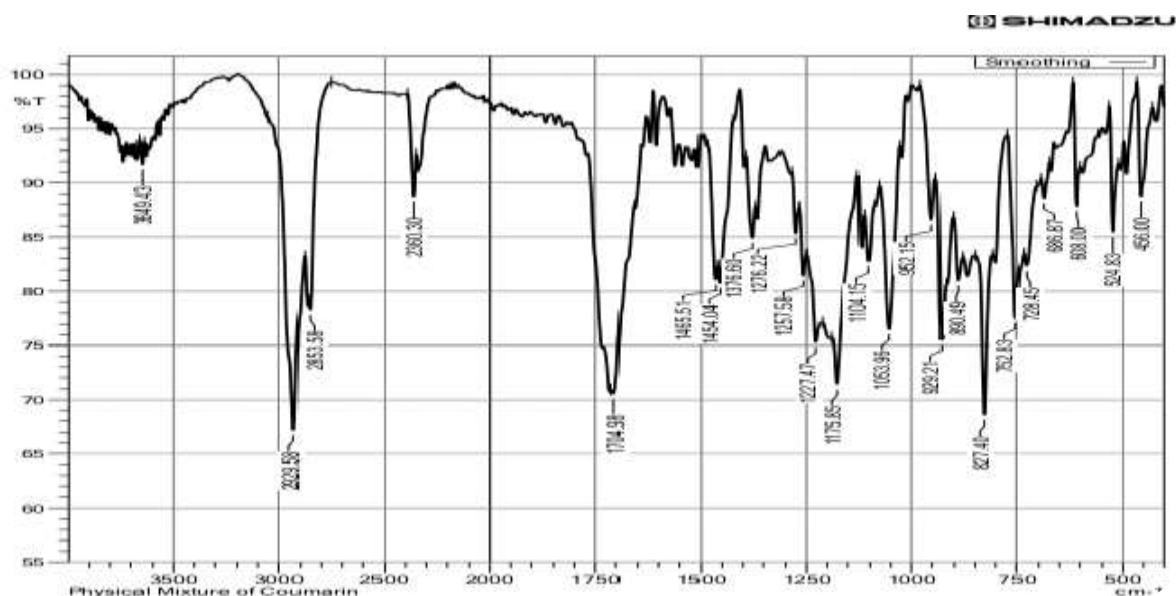


Figure 3. FTIR spectra Physical Mixture of Coumarin

4.4 Melting Point

The observed melting point of coumarin was found to be in the range of 68°C, which is in close agreement with the reported standard melting point of coumarin. The sharp melting range indicates the purity and crystalline nature of the drug sample.

The obtained melting point confirms the identity and purity of coumarin. No significant deviation from the standard value was observed, suggesting that the sample was free from major impurities and suitable for further formulation studies.

4.5 Particle size analysis & PDI

Figure 4 shows the average particle size and polydispersity index (PDI) was measured to assess the size distribution and uniformity of the transfersomes which providing the consistency and homogeneity of the nanoparticles. The diluted suspension of drug batch of coumarin loaded transfersomes subjected for analysis using the Horiba SZ-100 at 25°C. Formulation exhibited particle size of 262.2 nm with PDI of 0.323. The obtained particle size in the nanometer range confirms the successful formation of nanosized transfersosomal vesicles. The PDI value of 0.323 indicates moderate uniformity and acceptable homogeneity of the vesicle distribution. A PDI value below 0.5 generally suggests

a stable and fairly uniform nanoparticle system suitable for topical drug delivery applications^{11,12}.

4.6 Zeta Potential

Zeta potential analysis was performed to evaluate the surface charge and stability of formulation. Transfersosomal vesicles showed a zeta potential value of -38 mV (figure 5). The obtained zeta potential graph showed a sharp and narrow peak, indicating uniform distribution of vesicles with good colloidal stability. The high negative zeta potential value suggests the presence of sufficient electrostatic repulsion between vesicles, which helps to prevent aggregation and fusion of transfersomes during storage. Therefore, the prepared transfersosomal formulation can be considered physically stable, homogeneous and suitable for effective topical drug delivery of coumarin.

4.7 pH

The pH of formulation was found to be 6.8 using a calibrated digital pH meter at room temperature. The obtained pH was within the acceptable skin pH range, indicating good compatibility and stability of the formulation.

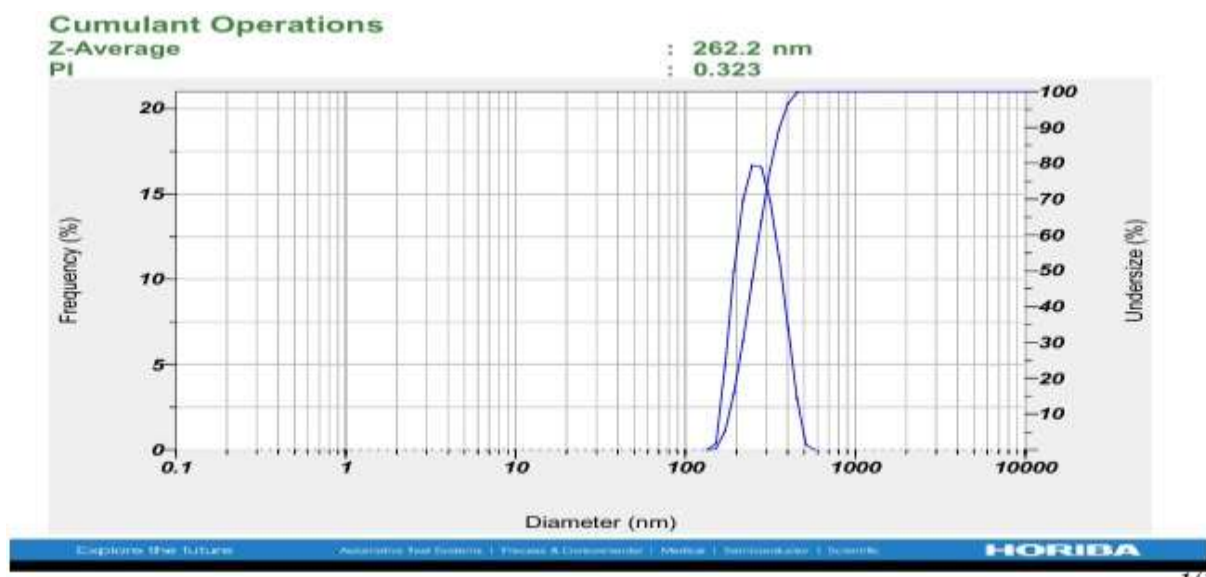


Figure 4. Particle size and PDI Value

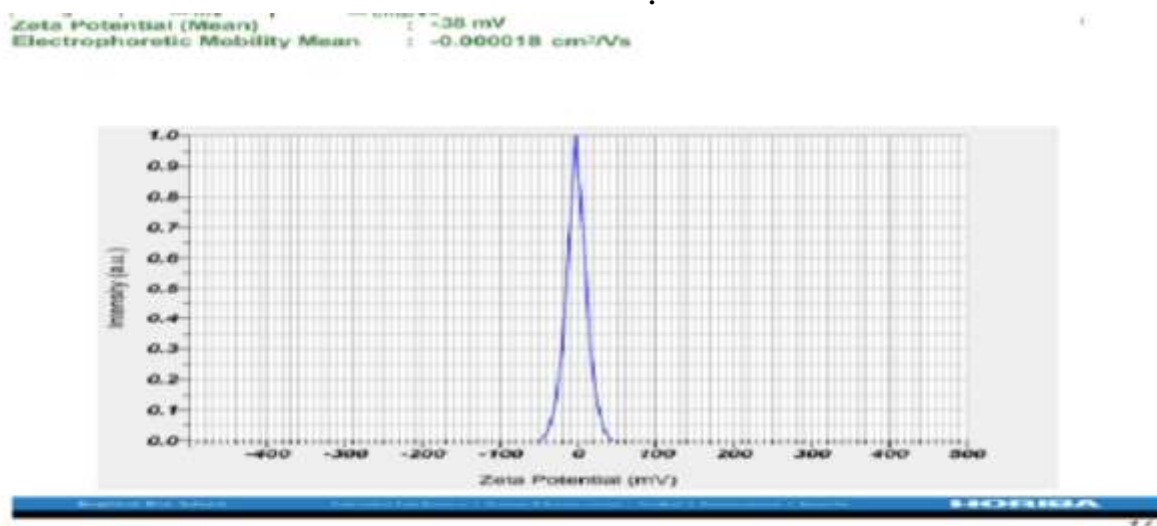


Figure 5: Zeta Potential

4.8 Drug content

The drug content of the formulated coumarin transfersomal gel was determined to evaluate the uniform distribution of drug within the formulation. The prepared formulation showed a drug content of 82.9 ± 0.829 indicating satisfactory incorporation of coumarin into the transfersomal gel system.

4.9 In vitro Drug Release study In figure no.6 in vitro Franz diffusion study showed that the coumarin loaded transfersomal gel released the drug more efficiently than the pure coumarin gel. The transfersomal gel exhibited a rapid initial release approximately 46–51% coumarin within 1 h, which further increased to nearly 68% after 4 h. In contrast, the pure coumarin gel showed a slower and more gradual release profile, with only about 19–22% release during the first 0.5 h and around 43% cumulative release after 4 h. The enhanced drug release from the transfersomal gel may be due to the ultra flexible vesicular structure of transfersomes, which improves drug penetration and diffusion through the

membrane. The transfersomal formulation showed a biphasic release pattern with an initial burst release followed by sustained release, whereas the pure coumarin gel demonstrated a controlled and prolonged release profile. These results indicate that the transfersomal gel can improve the availability and permeation of coumarin at the application site, potentially leading to faster therapeutic action and better topical effectiveness compared to the pure coumarin gel.

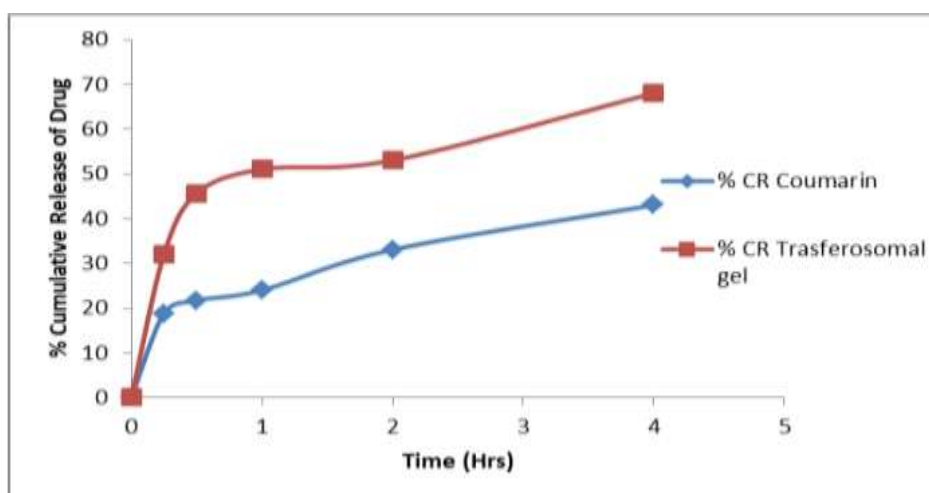


Figure 6. In vitro drug release study

4.10 Ex vivo skin permeation study

The ex vivo permeation study showed (Figure 7) that the transfersosomal gel of coumarin exhibited significantly higher drug permeation through goat skin compared to conventional coumarin gel. The transfersosomal formulation showed rapid and sustained drug release. Whereas the conventional gel exhibited slower permeation. The higher transdermal flux

value of $90.121 \mu\text{g}/\text{cm}^2/\text{h}$ confirmed the enhanced permeation efficiency of the transfersosomal gel. This improved permeation may be due to the ultra deformable nature of transfersomes and the presence of edge activators :Tween 80 which enhance skin penetration and drug diffusion. Overall, the transfersosomal gel demonstrated superior transdermal delivery compared to conventional coumarin gel.

4.11 Spreadability Study

The spreadability of the coumarin-loaded transfersosomal gel was found to be $6.2 \text{ g}\cdot\text{cm}/\text{sec}$, indicating good spreading characteristics and satisfactory consistency of the formulated gel. The obtained value suggests that the gel can be easily applied on the skin surface with minimal shear stress, which is an important parameter for topical and transdermal formulations. Good spreadability enhances patient compliance by ensuring uniform application and better contact with the skin, thereby improving drug distribution and absorption. Overall, the result indicates that the prepared

transfersosomal gel possesses acceptable spreadability suitable for effective topical drug delivery of coumarin.

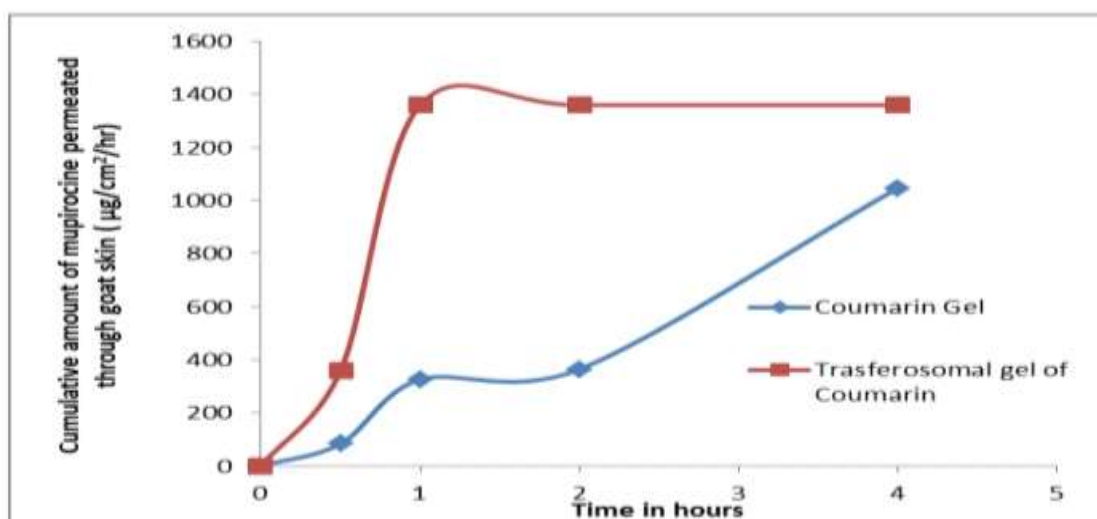


Figure 7. Ex vivo study

4. CONCLUSION

The present study on the formulation and evaluation of coumarin-loaded transfersosomal gel demonstrates that transfersosomal systems are a promising approach for enhancing transdermal drug delivery. The incorporation of Coumarin into ultra-deformable vesicles significantly improves its permeation through the skin, thereby overcoming the limitations of poor solubility and low bioavailability associated with conventional dosage forms.

The formulated transfersosomal gel exhibited satisfactory physicochemical properties such as appropriate pH, good viscosity and spreadability, indicating its suitability for topical application. The vesicles showed desirable characteristics including optimal particle size, optimum zeta potential and uniform distribution within the gel matrix. In addition, the formulation provided controlled and sustained drug release, which is beneficial for maintaining therapeutic drug levels over an extended period. Furthermore, enhanced skin permeation and improved drug retention suggest better therapeutic efficacy with reduced systemic side effects. Overall, the developed coumarin-loaded transfersosomal gel can be considered an effective and patient-friendly drug delivery system. This study supports the advancement of transfersosomal technology as a valuable tool in modern pharmaceutics.

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Formulation and Evaluation of Coumarin Loaded Transfersosomal Gel

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