Ufasomal Gel for Topical Delivery of Diclofenac Sodium in Arthritis Pain Management

Sonika[1], Kirankumar Rangappa[2], Umesh Dhamodhiran[3], Aiyushi Das[4], Buddekallu Veeran Gouda[5], Akash[6], Rama Bukka[7], Rama Radhakrishna Nargund[8], Shravan Laxmivenkatesh Nargund[9], Shachindra Laxmivenkatesh Nargund[10], Malviya Nidhi[11]

[1,2,3,4,5,6,7,11]*Department of Pharmaceutics, Nargund College of Pharmacy, Bangalore, Bharath*

[8]*Department of Pharmacology, Nargund College of Pharmacy, Bangalore, Bharath*

[9]*The Principal, Dr Gurachar Nargund College of Pharmacy, Koppal, Bharath*

[10]*The Principal, Nargund College of Pharmacy, Bangalore, Bharath*

Abstract*—Rheumatoid arthritis is a chronic inflammatory disorder requiring effective and sustained drug delivery. Diclofenac sodium is widely used as an NSAID but may cause gastrointestinal and systemic adverse effects following oral administration. The present study aimed to develop Diclofenac sodium-loaded ufasomes incorporated into a topical gel to improve topical delivery and provide sustained drug release. Ufasomes were prepared by the thin-film hydration method. Method and Results: Diclofenac sodium-loaded ufasomes were prepared by the thin-film hydration method using oleic acid, surfactant, cholesterol, and diclofenac sodium. The resulting film was hydrated with phosphate buffer (pH 7.4), sonicated, and incorporated into a Carbopol-934, PVP-K90, HPMC K-4M gel. Optimized U21 showed a particle size of 215.2 nm, PDI of 0.246, and 91.48% entrapment efficiency. Gel C exhibited good homogeneity, pH 6.66, viscosity 2890 cps, and spreadability of 12.4 cm². The optimized ufasomal gel demonstrated sustained drug diffusion, reaching 81.20% at 24 h, indicating prolonged topical drug delivery. Conclusion: The study successfully developed a Diclofenac sodium-loaded ufasomal gel using the thin-film hydration method. The optimized formulation demonstrated satisfactory vesicle characteristics, high drug entrapment, acceptable topical properties, and sustained drug diffusion over 24 hours. Short-term stability studies also showed minimal changes in particle size, entrapment efficiency, pH, viscosity, and spreadability during 30 days of storage. The developed ufasomal gel therefore shows potential as a topical delivery system for prolonged Diclofenac sodium administration.

***Index Terms*—Topical drug delivery, Ufasomal gel, Thin-film hydration, Oleic acid, Sustained drug release, Rheumatoid arthritis, Vesicular drug delivery, Transdermal delivery.**

I. INTRODUCTION

Rheumatoid Arthritis (RA) impacts around 0.5% to 1% of adults, totaling about 18 million worldwide, with greater rates seen in women, and 55% of those affected are over 50 years old; the majority of individuals with Rheumatoid Arthritis are located in North America and Europe rather than Asia.[1] Rheumatoid arthritis is a long-lasting autoimmune condition characterized by joint swelling, rigidity, and deformities, frequently limiting mobility. It might impact different organs and is identified by synovitis, systemic inflammation, and particular autoantibodies. Reduced activity heightens the chances of obesity, heart disease, and depression, significantly affecting patients' overall well-being.

Currently, a wide range of drugs for managing rheumatic diseases includes Nonsteroidal Anti-Inflammatory Drugs (NSAIDs), Disease Modifying Anti-Rheumatic Drugs (DMARDs), Biological Therapies, Corticosteroids, and Natural Compounds.[2] Diclofenac sodium is a nonsteroidal anti-inflammatory medication (NSAID) frequently utilized for treating rheumatic disorders. It possesses a brief plasma half-life of 1–3 hours and is commonly administered as 50 mg two times a day or 100 mg in sustained-release once each day. Diclofenac sodium, a Class II BCS drug, exhibits low water solubility but high permeability, featuring a log P

value of 4.5. Its oral bioavailability varies between 50-60%, affected by the formulation type, food consumption, and individual metabolic differences.[3] When taken orally, it leads to gastric ulcers, gastrointestinal bleeding, haemorrhage, and kidney damage. This medication functions by decreasing compounds in the body that lead to pain and swelling. Diclofenac sodium is utilized for alleviating mild to moderate pain, as well as for addressing symptoms and indications of osteoarthritis or rheumatoid arthritis. Voltaren is likewise recommended for the management of ankylosing spondylitis.[4]

Topical application offers an alternate method that reduces systemic side effects by directing the medication straight to the targeted area, ensuring quick relief through enhanced skin absorption. Moreover, they are simple to use, non-oily, and ensure a regulated release of the medication, enabling extended therapeutic outcomes and enhanced patient adherence.[5] In comparison to the oral route, the transdermal drug delivery system offers numerous benefits, including the avoidance of first-pass metabolism and efflux pumps, evasion of the harsh gastrointestinal environment, and decreased frequency of dosing.

Ufasomes are vesicles derived from unsaturated fatty acids, forming bilayer structures skin to other carrier systems, which have surfaced as efficient carriers that boost drug stability by safeguarding against degradation and enhance the solubility and bioavailability of inadequately soluble drugs.[6] Ufasomes, derived from natural fatty acids, are biocompatible and perfect for secure drug delivery. They provide improved skin absorption, stability, and medication retention compared to liposomes. Improving bioavailability and minimizing toxicity, ufasomes are suitable for both hydrophilic and lipophilic medications. They are economical and hold potential for topical gel formulations of Diclofenac sodium.[7] Consequently, creating a drug delivery system that allows for the controlled release of Diclofenac sodium could be extremely advantageous, especially for high dose-dependent therapies in managing chronic conditions like Rheumatoid arthritis.

This study's aim is to create a topical gel that incorporates Diclofenac sodium encapsulated in ufasomal carriers. This approach is expected to increase skin absorption, avoid first-pass metabolism, decrease the frequency of administration, reduce gastrointestinal side effects, and enhance patient compliance.

II. MATERIAL AND METHODOLOGY

Materials

The formulation of Diclofenac sodium loaded ufasomes utilized various chemicals and excipients. Diclofenac sodium provided by Ultra-pure -lab. Oleic acid sourced by S. D. Fine Chemicals Ltd. Mumbai, Span 60 and Tween 80 by Sisco research laboratories Pvt ltd, Maharashtra, India, Cholesterol by Avra synthesis Pvt Ltd. Hyderabad, Methanol by Nice chemicals Pvt Ltd, Chloroform by Nice chemicals Pvt Ltd, Kochi, Kerala. All chemicals and solvents are under analytical grade.

Preformulations studies

Preformulation testing is the first step in the development of dosage form of a drug substance. It can be defined as an investigation of physical and chemical properties of drug substance alone and when compared with excipients.

The overall objective of the pre-formulation testing is to generate information useful to the formulator in developing stable dosage form, which can be mass produced.

Identification of pure drug:

Identification of Diclofenac sodium was carried out by FT-IR Spectrophotometry.

Weighed amount of drug was mixed with IR grade KBr (1:10) and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer over a range of 4000 cm^{-1} to 400cm^{-1} range.[8]

Determination of melting point:

Determination of melting point gives an idea about purity of the drug. Melting point of Diclofenac sodium was determined by capillary tube method. Fine powder of Diclofenac sodium was filled with glass capillary (previously sealed at one end). The capillary was kept in tube containing liquid paraffin and was placed in melting point apparatus. The temperature at which drug starts to melt were noticed.[9]

Analytical method development by UV Visible spectroscopy:

Determination of wavelength maxima (λmax) of Diclofenac Sodium:

Diclofenac sodium (25mg) was weighed and dissolved in 25 ml of methanol. 1ml of this solution was withdrawn and volume was made upto to 10ml with phosphate buffer saline pH 7.4. Appropriate dilutions were made with phosphate buffer pH 7.4 to give concentration of 10µg/ml. The solution was scanned in UV range from 200-400nm, which could be utilized for analysis and spectrum was recorded.[10]

Preparation of Diclofenac sodium calibration curve in Phosphate buffer pH 7.4:

Diclofenac sodium (25mg) was dissolved in 25ml of methanol which gives the concentration of 1000 µg/ml. From the above solution 1ml was taken and made up to 10ml with phosphate buffer pH 7.4 which contains the concentration of 100 µg/ml. Again, from above solution 0.4, 0.8, 1.2, 1.6, 2.0, 2.4 ml were taken and made up to 10ml with phosphate buffer pH 7.4 to get the concentration ranges of 4, 8, 12, 16, 20, 24 µg/ml respectively.

The absorbance of the above said solutions were measured against the phosphate buffer pH 7.4 as blank at 276nm using UV spectrophotometer. The study was carried out in triplicate(n=3). Then the calibration curve was plotted taking concentration on X-axis and mean absorbance on Y-axis.[11]

Preparation of drug loaded ufasomes:

Preparation of Diclofenac sodium loaded ufasomes by thin film hydration method (Table no 1). In this method 100ml round bottom flask was taken, Diclofenac sodium was dissolved in methanol (3ml). In separate beaker tween 80 and cholesterol were together dissolved in 8ml of chloroform and in another beaker the oleic acid was dissolved in methanol (3ml). These organic solvents were mixed, poured into 100ml round bottom flask and the solvent was evaporated until a thin dry film was obtained under reduced pressure using rotary evaporator (Rotation period 5-6hrs). The obtained thin film was hydrated using 20ml of pH 7.4 phosphate buffer. Finally, the prepared suspension was further sonicated to reduce the size.[12]

Table no.1: Formulation chart of ufasomal suspension.

Batch code	Heat at (°C)	Drug (mg)	Oleic acid (mg)	Span 60 (mg)	Cholesterol (mg)	Chloroform (ml)	Methanol (ml)	Thin film
U1	RT	50	10	10	30	4	2	No
U2	40	50	10	20	20	4	2	No
U3	RT	50	10	20	20	4	2	No
U4	RT	50	10	20	30	4	2	No
U5	55	50	10	20	40	4	2	No
U6	40	50	10	30	30	4	2	No
U7	RT	50	10	30	30	4	2	No
U8	45	50	20	30	30	4	2	No
U9	40	50	30	40	10	10	5	No
U10	40	50	30	40	30	10	5	No

Batch code	Heat at (°C)	Drug (mg)	Oleic acid (mg)	Tween 80 (mg)	Cholesterol (mg)	Chloroform (ml)	Methanol (ml)	Thin film
U11	45	50	10	10	10	8	5	Yes
U12	45	50	10	10	20	8	5	Yes
U13	45	50	10	10	30	8	5	Yes
U14	45	50	10	10	30	9	6	Yes
U15	45	50	10	10	40	4	2	Yes
U16	45	50	10	10	40	8	5	Yes
U17	45	50	10	10	50	4	2	Yes

U18	45	50	10	10	50	6	4	No
U19	45	50	10	10	50	10	5	No
U20	45	50	10	20	30	4	2	No
U21	45	50	15	15	30	8	5	Yes
U22	45	50	20	20	10	8	5	No
U23	45	50	20	20	30	8	5	Yes

Evaluation of ufasomal suspension:
All formulations were evaluated for particle size, zeta potential, PDI, entrapment efficiency, and *In vitro* drug release studies.

Particle size: Particle size and PDI were measured using a Horiba SZ-100 at 90° and 25°C after dilution with distilled water. Lower PDI values indicated greater particle uniformity.[13]

Zeta potential: Zeta potential was measured using a Horiba SZ-100 after diluting the ufasomal suspension with distilled water. Measurements were performed in triplicate (n=3), and mean values were recorded to assess colloidal stability.[14]

Entrapment efficiency: Entrapment efficiency was determined indirectly by centrifugation. The supernatant containing unentrapped drug was analyzed by UV–Visible spectrophotometry at 274 nm, and entrapment efficiency was calculated from total and free drug by equation no 1.[15]

$$\%\ EE = [(\text{Total amount of drug} - \text{Amount of drug unentrapped})/\text{Total amount of drug}] * 100$$ Equation no 1

Microscopic examination of ufasomal suspension:
A small drop of the prepared ufasomal suspension was placed on a clean glass slide and gently covered with a coverslip to obtain a uniform thin preparation. The sample was examined under a bright-field optical microscope at suitable magnification (400×). Images were captured using the S-EYE 2.0 image-analysis system coupled to the microscope. The morphology, vesicular shape, and approximate vesicle size were observed and recorded. Measurements were performed after calibration of the image-analysis software using a stage micrometer.[16]

In vitro diffusion studies:
The release of Diclofenac sodium from ufasomal suspension was determined by using high media dialysis membrane bag diffusion technique (Fig no 1A), which is regenerated with seamless cellulose tubing membrane which is soaked overnight in pH 7.4 phosphate buffer. Soaking the membrane in water or buffer overnight allows it to become fully hydrated, achieving its optimal porosity and flexibility. This is essential for proper diffusion and permeation of solutes across the membrane. Diclofenac sodium ufasomal suspension equivalent to 5mg was taken in dialysis bag and the bag was placed in a beaker containing 200 ml of PBS pH 7.4, which acts as receptor compartment. The temperature of receptor medium was maintained at 37 ± 0.5^0C and agitated using magnetic stirrer with the speed of 100 rpm. Aliquots of 5ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The
collected samples were analyzed using UV spectrophotometer at 276 nm.[17]

Formulation of drug loaded ufasomal gel:
Gel was prepared by using Carbopol-934, PVP-K 90, HPMC K-4M in individually or combination and dissolved in 50ml of purified water and allowed to soak for 12hr (overnight). After 12 hours Polyethylene glycol - Penetration enhancer was added. All this are kept on the magnetic stirrer to get good dispersion using a magnetic agitation. To this add drop by drop Triethanolamine to adjust the pH of the formulation. Finally, drug-loaded ufasomes were incorporated into the gel base with continuous stirring.[18](Table no 2)

Table no.2: Formulation chart of drug loaded ufasomal gel.

SI no	Ingredients	Gel A	Gel B	Gel C
1	Carbopol-934	0.25%	0.25%	0.25%
2	PVP-K 90	0.25%	-	0.125%
3	HPMC K-4M	-	0.25%	0.125%

4	Polyethylene glycol	0.0625%	0.0625%	0.0625%
5	Purified water	50ml	50ml	50ml
6	Gel Texture	Low viscous gel	Low viscous gel	Soft Gel with good texture

Evaluation of gel:
The topical gel of ufasome was evaluated for the following characteristics:

Measurement of pH: The pH of gel formulations was determined by a digital pH meter. 1gm of gel is dissolved in 25ml distilled water and stored for two hours. The measurement of the pH of each formulation was done in triplicate and average values were considered.[19]

Viscosity measurement: Viscosity of the gel was determined by using Brookfield viscometer. Accurately weighed 50gm Diclofenac sodium loaded gel was transferred to 50ml glass beaker. Different Spindle numbers were used to check the viscosity and Spindle no 64 was selected and it is immersed into the gel. The viscometer was operated at 10 rpm until the reading gets stabilized and reading was noted in centipoises.[20]

Spreadability: For the determination of spreadability, excess of sample was applied in between two glass slides and was compressed to uniform thickness by placing 125g weight for 5 min, weight (1g) was added to the pan. The time in which the upper glass slide moves over to the lower plate was taken as measure of spreadability.[21]

In vitro diffusion studies:
The *in vitro* drug diffusion of the ufasomal-loaded gel, plain diclofenac sodium gel, and marketed gel was evaluated using a Franz diffusion cell (Fig no 1 B, C, D) fitted with a regenerated dialysis membrane, which was pre-soaked overnight in phosphate buffer (pH 7.4). The membrane was positioned between the donor and receptor compartments, with 135 mL of phosphate buffer (pH 7.4) in the receptor compartment. The respective gel formulations were uniformly applied to the donor side over the membrane. The diffusion assembly was maintained at 37 ± 0.5 °C and stirred at 100 rpm using a magnetic stirrer. At predetermined intervals, samples were withdrawn through the sampling port and replaced with an equal volume of fresh buffer. The samples were analyzed in triplicate (n = 3) at 276 nm using a UV–Visible spectrophotometer. The drug release data were fitted to the zero-order, first-order, Higuchi, and Korsmeyer–Peppas models, and the regression coefficients (R^2) were compared to determine the best-fit model and elucidate the drug-release kinetics and mechanism.[22]

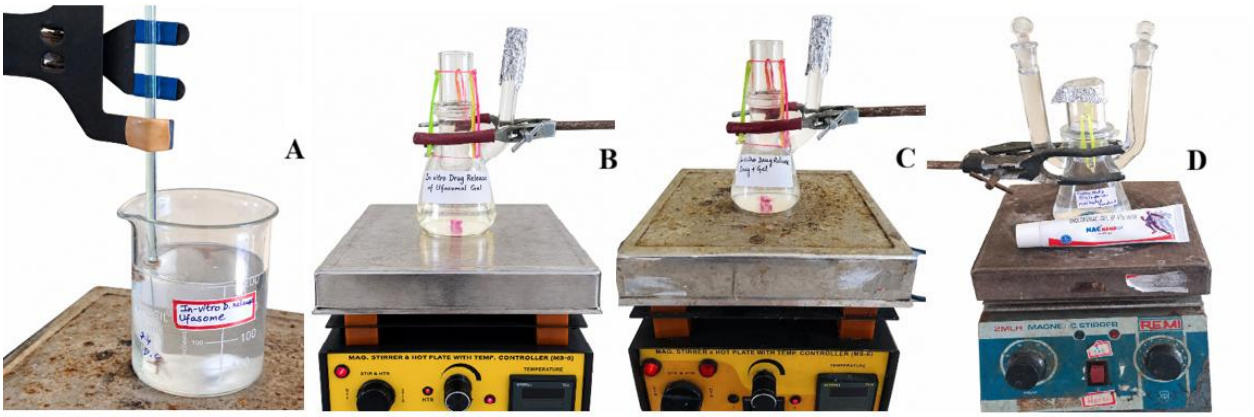


Fig no. 1: *In vitro* drug release studies by diffusion method A. Ufasomal suspension B. Ufasomal-loaded gel, C. Plain diclofenac sodium gel, D. Marketed gel

Drug release kinetic data analysis:
The % drug release from the flurbiprofen loaded ufasomal gel at different time intervals was fitted to zero order kinetics, first-order kinetics model, Higuchi model, and Korsmeyer- Peppas model to characterize the mechanism of drug release.

Zero order Kinetic: It describes the system in which the drug release rate is independent of its concentration.
$Qt = Qo + Kot$
Where,
Qt = Amount of drug dissolved in time t
Qo = Initial amount of drug in the solution, which is often zero
Ko = zero order release constant.
If the zero-order drug release kinetic is obeyed, then a plot of Qt versus t will give a straight line with a slope of Ko and an intercept at zero.

First order Kinetic: It describes the drug release from the systems in which the release rate is concentration dependent.
$\text{Log } Qt - \log Qo + k_1 t / 2.303$
Where,
Q_t = amount of drug released in time t.
Qo = initial amount of drug in the solution
K_1= first order release constant
If the first order drug release kinetic is obeyed, then a plot of log (Qo-Qt) versus twill be straight line with a slope of kt / 2.303 and an intercept at of log Q_O.

Higuchi model: It describes the fraction of drug release from a matrix is proportional to square root of time.
$M_t / M_\infty = kt_{1/2}$
Where,
Mt and M∞ = cumulative amounts of drug release at time t and infinite time,
K = Higuchi dissolution constant reflects formulation characteristics.
If the Higuchi model of drug release (i.e., Fickian diffusion) is obeyed, then a plot of M_t / M_∞ versus $t_{1/2}$ will be a straight line with slope of k.

Korsmeyer - Peppa's model: The power law describes the drug release from the polymeric system in which release deviates from Fickian diffusion, as expressed in following equation.
$M_t / M_\infty = ktn$
$\text{Log } [M_t / M_\infty] = \log k + n \log t$
Where,
M_t and M_∞ = cumulative amounts of drug release at time t and infinite time (i.e., fraction of drug release at time t),
k = constant incorporating structural and geometrical characteristics of CR device,
n = diffusion release exponent indicative of the mechanism of drug release for drug dissolution.
A plot of log $\{M_t / M_\infty\}$ versus log t will be linear with a slope of n and intercept gives the value of log k. Antilog of log k gives the value of k.

Drug-excipients compatibility studies:
To assess the drug-excipients compatibility, the analytical technique Fourier Transform Infrared Spectroscopy (FT- IR) was adopted. Compatibility of the drug with the excipients is determined by subjecting the physical mixture of drug and the excipients of the main formulation to infrared absorption spectral analysis (FT-IR). Any change in chemical composition of the drug after combining it with the excipients was investigated with IR spectral analysis.

Procedure:
Weighed amount of drug (Diclofenac sodium) and other excipients (Tween 80, Oleic acid, Cholesterol) were physically mixed. The mixture is then blended with IR grade KBr (1:10) and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. The pellet was scanned by IR spectrophotometer over a range of 4000-1 cm to 400-1 cm range.

Ex vivo skin permeation study:
Franz diffusion cell with an effective diffusion area of 2.1 cm2 was used for the experiment. Porcine ear skin was brought and the hair was cleaned using the razor and placed in the buffer before using it. The skin was separated by using the scalpel and again placed in buffer. The porcine ear skin was placed between the donor and receptor compartment of Franz diffusion cell with the stratum corneum facing the donor compartment. Ufasomal Suspension was placed on the membrane and release profiles were taken. The receptor chamber was filled with 135ml of diffusion medium (pH 7.4 phosphate buffer). The receptor medium was maintained at 37±2°c and stirred magnetically at 50rpm.
Samples were withdrawn at predetermined time intervals and were analyzed by UV spectrophotometer. The fresh buffer was immediately replenished into the receptor compartment after each sampling. Using the data obtained from the study, % Drug release was determined.[23]

Stability studies:
Stability of the Ufasomal suspension and Diclofenac sodium loaded Ufasomal gel were carried out by storing at room temperatures for one month. The formulation's alterations were tracked by measuring particle size and % Entrapment efficiency for suspension, pH, viscosity, spreadability for gel after 15 days and 30 days of storage.

III. RESULTS AND DISCUSSION

Pre formulation studies
The drug was identified by different methods, including FTIR Spectroscopy, melting point method and analytical method was carried out by UV-visible spectroscopy. All the parameters were found within limits and compiled with the requirements of the official compendia.

Determination of melting point:
The melting point of Diclofenac sodium was found to be 283^0C [283-285^0C]

Identification of pure drug:
The IR spectrum of pure drug was found to be similar to the standard spectrum of Diclofenac sodium. The spectrum of Diclofenac sodium shows the functional groups as per the reference peaks. (Fig no 8 A)
The IR spectrum of the pure drug shows characteristic peaks at 3381, 3030, 1795 and 750 of NH stretching, CH (Stretching Aromatic & Aliphatic), C=O (Stretching Aromatic) and C-Cl stretching groups respectively.

Analytical method development by UV- visible spectroscopy
The λmax was found to be at 276 nm in pH 7.4 phosphate buffer solution and observed absorbance was 0.257 as shown in Fig no 2 A. The calibration curve showed excellent linearity for diclofenac sodium, with regression equation y = 0.0389x and R^2 = 0.9998 as shown in Fig no 2 B.

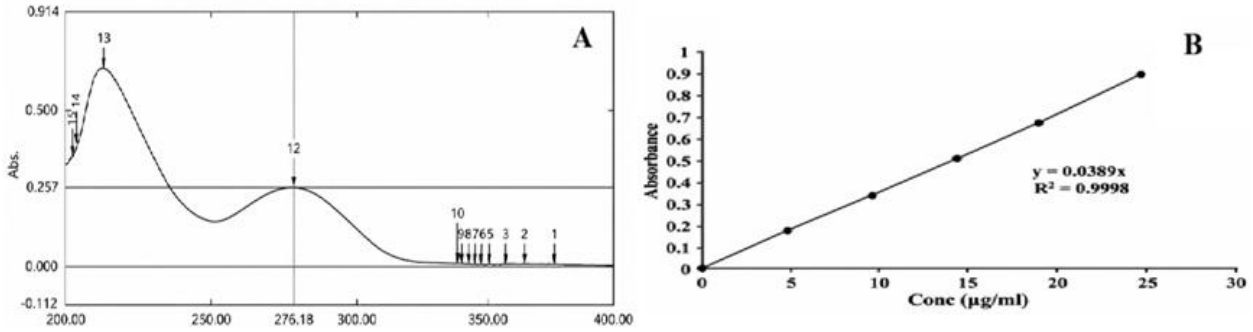


Fig no.2: Analytical method development by UV visible spectroscopy (A. λmax of Diclofenac sodium, B. Calibration curve of Diclofenac sodium)

Formulation studies of drug loaded ufasomal suspension:
Drug loaded ufasomes were successfully prepared by thin film hydration method. The characteristic of thin film was observed as uniform/even, continuous, smooth. (Fig no 4 A, B)

Table no.3: Evaluated parameters for formulation of ufasomal suspension

Batch code	Oleic acid (mg)	Tween 80 (mg)	Cholesterol (mg)	Chloroform (ml)	Methanol (ml)	Particle size (nm)	Zeta potential (mV)	PDI	% EE
U11	10	10	10	8	5	5623	9.5	0.865	65.0
U13	10	10	30	8	5	7263.4	6.3	0.630	57.9
U16	10	10	40	8	5	942	-1.0	0.427	85.4
U21	15	15	30	8	5	215.2	-3.8	0.246	91.48
U23	20	20	30	8	5	2424.6	8.7	0.859	76.81

The evaluation of the ufasomal suspensions showed that the formulation variables significantly influenced particle size, zeta potential, polydispersity index (PDI), and entrapment efficiency (%EE) as shown in Table no 3. Among the tested formulations, U21 demonstrated the most desirable characteristics, with a particle size of 215.2 nm, PDI of 0.246, zeta potential of -3.8 mV, and the highest entrapment efficiency of 91.48%. The smaller particle size observed in U21 may provide a greater surface area and potentially enhance drug skin interaction and penetration.

Formulations U11 and U13 exhibited larger particle sizes of 5623 nm and 7263.4 nm, respectively, indicating comparatively poor vesicle size control. Increasing cholesterol concentration in U16 reduced the particle size to 942 nm and improved %EE to 85.4%, suggesting that cholesterol contributed to better vesicular membrane organization and drug retention. However, further optimization of the lipid and surfactant ratios was required to obtain smaller and more uniform vesicles.

U21, containing 15 mg each of oleic acid and Tween 80 with 30 mg cholesterol, showed the lowest PDI, indicating a relatively narrow particle-size distribution. Its high %EE suggests efficient incorporation of diclofenac sodium into the ufasomal vesicles. Therefore, U21 can be considered the optimized formulation among the evaluated trials and was selected for further characterization and incorporation into the topical gel.[24,25]

Microscopic examination of ufasomal suspension:

Microscopic examination of the prepared ufasomal suspension was carried out using a bright-field optical microscope at 400×magnification. The observed micrograph showed well-defined spherical to oval vesicular structures with relatively uniform distribution and slight variation in vesicle size (Fig no 3).

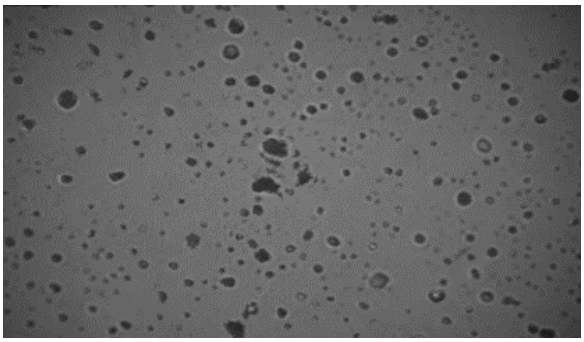


Fig no.3: Microscopic image of ufasomal suspension

The presence of discrete vesicles confirmed the successful formation of ufasomes in the prepared formulation. The vesicles appeared clearly distinguishable from the surrounding dispersion, indicating satisfactory morphological characteristics. The observed spherical or oval shape is characteristic of vesicular systems formed from fatty acid-based components. The relatively uniform distribution of vesicles suggests good dispersion and physical stability of the prepared ufasomal suspension. Microscopic examination is important for the preliminary assessment of vesicle formation, morphology, shape and approximate size. These characteristics are significant because well-formed and uniformly distributed vesicles can provide better drug incorporation and facilitate controlled drug release. Thus, the microscopic findings supported the successful formation and suitability of the prepared ufasomes for topical drug delivery applications.[26]

Optimized formulation of diclofenac sodium loaded ufasomal gel:

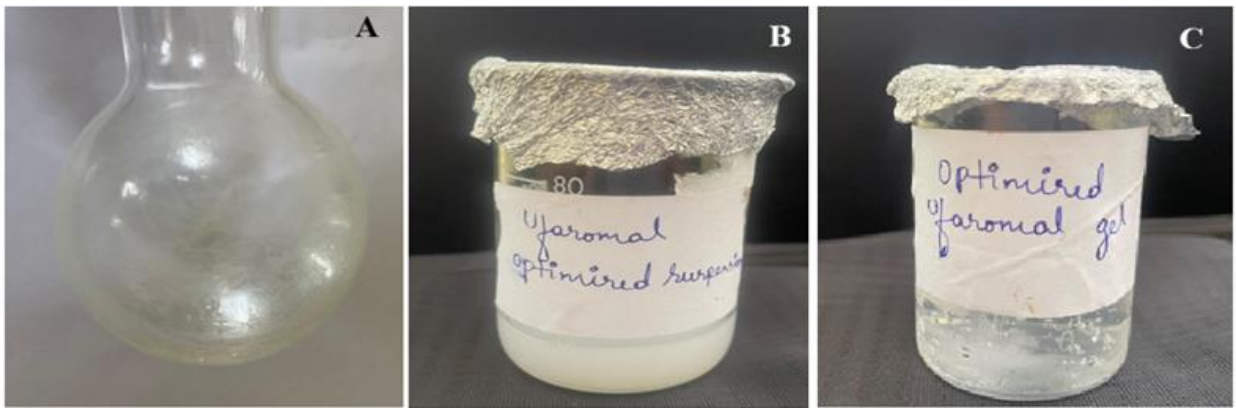


Fig no.4: Optimized formulation A. Thin film, B. Ufasomal suspension, C. Ufasomal gel

Gel C exhibited satisfactory physical properties, indicating good formulation stability and consistency. The off-white appearance suggests an acceptable visual characteristic, while good homogeneity confirms uniform distribution of the formulation components without visible aggregates or phase separation (Table no 4). The pH of 6.66 is close to the physiological skin pH, suggesting good suitability for topical application and minimal risk of irritation. The viscosity of 2890 cps indicates adequate consistency, providing sufficient retention on the skin while allowing easy application. The spreadability of 12.4 cm² demonstrates that the gel can spread effectively over the skin surface, supporting convenient and uniform application.[27]

In vitro drug diffusion studies profile and ex vivo permeability study:

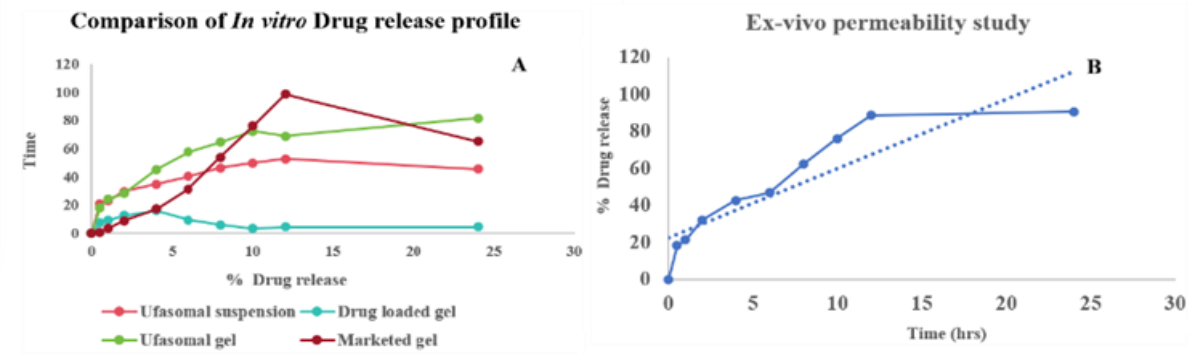


Fig no.5: A. Comparison plot of *In vitro* Drug release of optimized formulation, diclofenac sodium loaded gel, ufasomal gel and marketed gel, B. Ex-vivo permeability study of ufasomal suspension

In vitro drug diffusion studies profile:

The *in vitro* drug diffusion study demonstrated distinct release patterns among the ufasomal suspension, drug-loaded gel, ufasomal gel, and marketed gel (Fig no 5). The drug-loaded gel showed the lowest drug release, reaching only 4.43% at 24 h. This may be attributed to the incorporation of diclofenac sodium within the polymeric gel network, which increases diffusion resistance and restricts drug mobility.

The ufasomal suspension showed a gradual release, increasing from 20.69% at 0.5 h to 52.44% at 12 h, followed by a slight decrease to 45.24% at 24 h. The initial release may result from drug present near the vesicle surface, while the subsequent slower release can be associated with diffusion through the ufasomal lipid bilayer.

The slight decrease at 24 h may be due to experimental variation or sampling-related factors.[28]

The optimized ufasomal gel exhibited sustained drug diffusion, increasing from 17.81% at 0.5 h to 81.20% at 24 h. This prolonged release may be due to the combined barrier effect of the ufasomal vesicles and the gel matrix, allowing controlled movement of diclofenac sodium through the formulation.

The marketed gel showed comparatively rapid release, reaching 98.3% at 12 h and 64.78% at 24 h. Overall, the optimized ufasomal gel demonstrated a more controlled and sustained release profile, indicating its potential for prolonged topical drug delivery and improved residence time compared with conventional gel.[29]

The values for the release rate constant (K_0 and K_1), the correlation coefficients (R^2) were calculated using different equations.

The correlation coefficients (R^2) were used as an indication of the best fit models, for each of the model considered. Based on correlation coefficient (R^2), the best fitted model was determined and formulation followed zero order model.

For the ufasomal suspension(U21), the model that fits the data was zero-order model (R^2- 0.7061), and n value for Korsmeyer-peppas equation was found to be 0.6809.

For the ufasomal gel, the model that fits the data was zero-order model (R^2-0.8216), and n value for Korsmeyer-peppas equation was found to be 0.7290 as shown in fig no 6 A B) and the mechanism of drug release follows non-fickian diffusion.

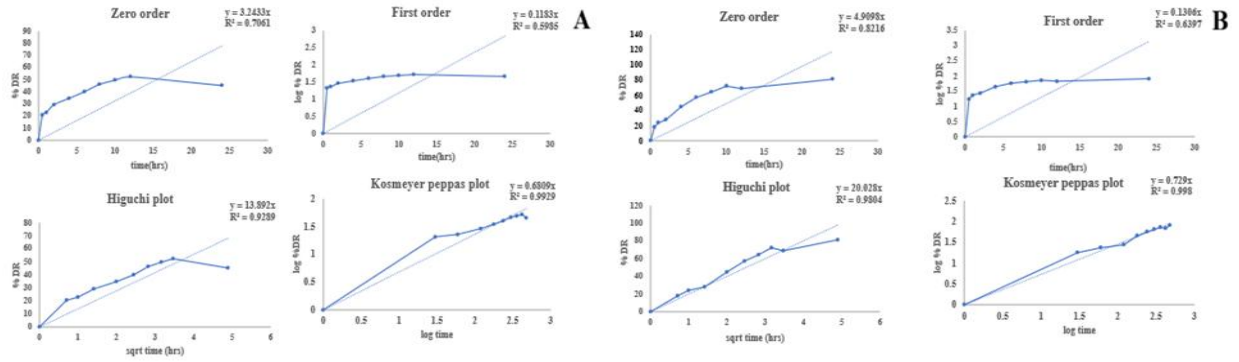


Fig no.6: *In vitro* release kinetic model of A. Ufasomal suspension and B. Ufasomal gel Mathematical modelling of drug release profile from ufasomes

Ex vivo skin permeation study:

The ex vivo skin permeability study demonstrated a progressive increase in diclofenac sodium permeation from the ufasomal suspension over 24 hours. Drug permeation increased from 18.32% at 0.5 hours to 90.58% at 24 hours, indicating sustained drug transport through the skin. This may be attributed to the lipid-based vesicular structure of ufasomes, which can interact with skin lipids and facilitate drug penetration through the stratum corneum. The gradual increase in permeation suggests that ufasomes may act as effective carriers for enhancing topical drug delivery. The high cumulative permeation observed at 24 hours indicates the potential of the formulation to provide prolonged drug availability at the application site. These findings support the suitability of ufasomes as promising carriers for topical diclofenac sodium delivery, although comparison with a control formulation and statistical analysis are required to confirm the enhancement in skin permeability.[30]

Drug-excipients compatibility study:

The FTIR analysis was performed to determine the compatibility of diclofenac sodium with Tween 80, oleic acid, and cholesterol used in the ufasomal formulation. The additional peaks observed in the physical mixtures are mainly due to the characteristic functional groups of the respective excipients. Minor shifts in peak positions may occur because of hydrogen bonding, intermolecular interactions, or changes in the local chemical environment during mixing. However, the major characteristic peaks of diclofenac sodium were retained, with no significant disappearance or development of new dominant peaks. This indicates that no major chemical interaction or degradation of the drug occurred. The results therefore demonstrate good drug–excipient compatibility and support the suitability of Tween 80, oleic acid, and cholesterol for preparation of diclofenac sodium-loaded ufasomes (Fig no 7).[31]

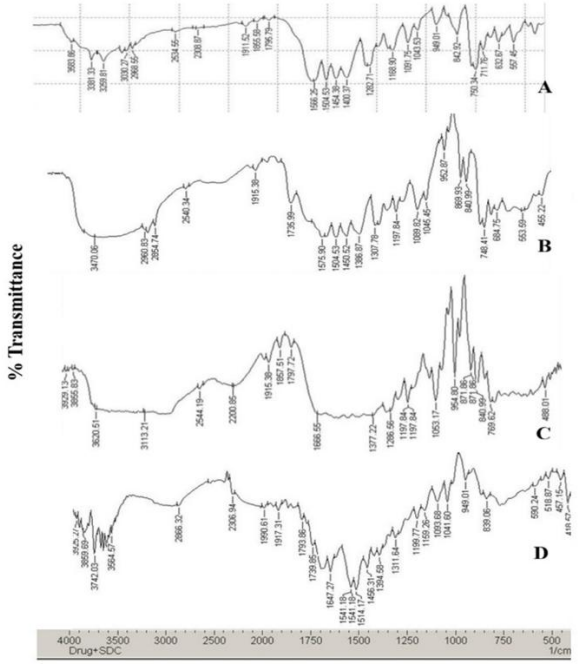


Fig no.7: IR Spectrum A. Pure drug, B. Drug + Oleic acid, C. Drug+ Cholesterol, D. Drug +Tween 80

Table no.4: FTIR peaks of Diclofenac sodium with physical mixture (tween 80, oleic acid, cholesterol)

SI No	Compounds	IR Bands
1	Diclofenac sodium	3583.86, 3381.30, 3030.27, 2969, 1795.79, 750.34
2	Drug + oleic acid	3470.06,2960.83, 2540.34, 1915.38, 1735.38, 1575.90, 1386.87, 1197.84

3	Drug + cholesterol	3929.13, 3620.51, 3113.21, 2544.19, 2200.85, 1915.38, 1666.55, 1377.22
4	Drug + tween 80	3925.27, 3742.03, 3564.57, 2866.32, 2306.94, 1990.61, 1917.31, 1793.86

Stability studies of ufasomal suspension and ufasomal gel:

Table no.5: Results for Stability Studies of ufasomal suspension and ufasomal gel.

Evaluation parameters	Ufasomal suspension (U21)			Evaluation parameters	Ufasomal gel (Gel C)		
	Day 1	Day 15	Day 30		Day 1	Day 15	Day 30
Physical appearance	Off white	Off white	Off white	pH	6.66	6.63	6.61
Average Particle size	215.2nm	207nm	195nm	Viscosity	2890	2886	2882
Entrapment efficiency	91.48%	90.23%	88.53%	Spreadability	12.8	12.76	12.6

Stability studies of U21:

The short-term stability study of ufasomal suspension U21 was carried out for 30 days to determine whether the formulation maintained its particle size and drug-retaining capacity during storage (Table no 5).

The physical appearance remained off-white on Days 1, 15, and 30, indicating the absence of visible colour change, precipitation, phase separation, or aggregation. This consistent appearance suggests that the vesicular system remained physically stable during storage under the study conditions.

The average particle size decreased slightly from 215.2 nm on Day 1 to 207 nm on Day 15 and 195 nm on Day 30. The small reduction may be due to gradual rearrangement and hydration of the vesicular membrane, breakdown of loosely associated vesicles, or redistribution of vesicles within the dispersion. Minor differences can also occur because of sample and measurement variability. Importantly, particle size did not increase, suggesting that vesicle aggregation or fusion was not evident.

Entrapment efficiency decreased gradually from 91.48% on Day 1 to 90.23% on Day 15 and 88.53% on Day 30. This slight decrease may be attributed to limited diffusion or leakage of diclofenac sodium from the vesicular bilayer into the surrounding aqueous phase during storage. Changes in membrane packing or permeability over time may contribute to this drug loss. However, the formulation retained 88.53% entrapment after 30 days, indicating good drug-retention capacity.

Overall, the minimal changes in particle size and entrapment efficiency, together with unchanged physical appearance, demonstrate that U21 exhibited satisfactory short-term stability and maintained its vesicular characteristics and drug-encapsulation capacity throughout the 30-day storage period.[32,33]

Stability studies of Gel C:

The stability results of Gel C demonstrate only minor changes in pH, viscosity, and spreadability over 30 days, indicating that the formulation remained physically stable during the study period (Table no 5)

The pH decreased slightly from 6.66 (Day 1) to 6.63 (Day 15) and 6.61 (Day 30). This minor change may occur due to gradual equilibration between the drug and polymeric components, slight ionization changes of the polymers, or limited interaction between the formulation ingredients during storage. The change of only 0.05 pH units is very small and indicates that there was no significant chemical alteration of the formulation. The final pH remains close to the physiological skin pH, supporting its suitability for topical application.[34]

Viscosity decreased slightly from 2890 to 2886 and 2882. This small reduction can be attributed mainly to minor rearrangement or relaxation of the polymer chains and changes in polymer hydration during storage. Carbopol and HPMC form a hydrated three-dimensional network responsible for gel consistency. Since the decrease was only 8 units, the polymeric network remained substantially intact, indicating good structural stability.

Spreadability decreased from 12.8 to 12.76 and 12.6. Spreadability is influenced by the consistency and viscosity of the gel. The slight reduction is therefore

consistent with the small change in viscosity. Minor structural rearrangement of the hydrated polymer network during storage may also contribute to this variation.

Overall, the changes were minimal and gradual, suggesting that Gel C maintained its physicochemical characteristics without significant instability over the 30-day study period.[35]

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this work.

ETHICAL CLEARANCE

No ethical clearance was required as this research work do not involve any kind of studies on animals.

DATA AVAILABILITY

All data is within this manuscript and no supplementary data is available.

ACKNOWLDEMENT

The authors sincerely acknowledge Nargund College of Pharmacy, Bengaluru, Karnataka, for providing the necessary laboratory facilities, computer facilities, Wi-Fi, and other laboratory accessories required to carry out this research work. The authors are also grateful to Rajiv Gandhi University of Health Sciences (RGUHS), Bengaluru, Karnataka, for providing access to Helinet, access to Drill Bit plag checker, and other essential e-resources. The authors would also like to express their special thanks to the Vision Group of Science and Technology (VGST), Bengaluru, Karnataka, India, for providing the GRD-178 grant, which supported the procurement of the Nanoparticle Analyzer SZ-100 (HORIBA Scientific, Kyoto, Japan) used during this research work.

REFERENCE

[1] R. Handa, U. R. K. Rao, J. F. M. Lewis, G. Rambhad, S. Shiff, and C. J. Ghia, "Literature review of rheumatoid arthritis in India," *International Journal of Rheumatic Diseases*, vol. 19, no. 5, pp. 440–451, 2016.

[2] S. Y. Chuang, C. H. Lin, T. H. Huang, and J. Y. Fang, "Lipid-based nanoparticles as a potential delivery approach in the treatment of rheumatoid arthritis," Nanomaterials, vol. 8, no. 1, Art. no. 42, 2018.

[3] B. Chuasuwan, V. Binjesoh, J. E. Polli, et al., "Biowaiver monographs for immediate release solid oral dosage forms: Diclofenac sodium and diclofenac potassium," Journal of Pharmaceutical Sciences, vol. 98, no. 4, pp. 1206–1219, 2009.

[4] K. P. Garnock-Jones, "Diclofenac potassium powder for oral solution: A review of its use in patients with acute migraine," CNS Drugs, vol. 28, no. 8, pp. 761–768, 2014.

[5] D. Singh Malik, N. Mital, and G. Kaur, "Topical drug delivery systems: A patent review," Expert Opinion on Therapeutic Patents, vol. 26, no. 2, pp. 213–228, 2016, doi: 10.1517/13543776.2016.1131267.

[6] P. M. Luke and T. Joseph, "Ufasomes: Rising technology for delivery of drugs," International Journal of Medical and Pharmaceutical Sciences, vol. 11, no. 11, pp. 1–7, 2021, doi: 10.31782/IJMPS.2021.111101.

[7] S. Jain, V. Jain, and S. C. Mahajan, "Lipid based vesicular drug delivery systems," Advances in Pharmaceutics, vol. 2014, pp. 1–12, 2014, doi: 10.1155/2014/574673.

[8] A. Roy, A. Ghosh, S. Datta, et al., "Effects of plasticizers and surfactants on the film forming properties of hydroxypropyl methylcellulose for the coating of diclofenac sodium tablets," Saudi Pharmaceutical Journal, vol. 17, no. 3, pp. 233–241, 2009.

[9] P. Tudja, M. Z. I. Khan, E. Meštrović, M. Horvat, and P. Golja, "Thermal behaviour of diclofenac sodium: Decomposition and melting characteristics," Chemical and Pharmaceutical Bulletin, vol. 49, no. 10, pp. 1245–1250, 2001.

[10] H. Ali, S. A. Zuberi, and N. Tariq, "Study of ex vivo and in vivo effect of topical ionic solution of diclofenac sodium in male albino rats using iontophoretic technique," Advances in Pharmacological and Pharmaceutical Sciences, vol. 2026, no. 1, Art. no. 7708719, 2026.

[11] S. Talele, P. Nikam, B. Ghosh, C. Deore, A. Jaybhave, and A. Jadhav, "A research article on nanogel as topical promising drug delivery for diclofenac sodium," Indian Journal of Pharmaceutical Education and Research, vol. 51, no. 4S, pp. S580–S587, 2017.

[12] R. R. Arundhasree, R. A., A. R., A. R. Kumar, S. S. Kumar, and S. C. Nair, "Ufasomes: Unsaturated fatty acid based vesicular drug delivery system," International Journal of Applied Pharmaceutics, pp. 76–83, published online Mar. 7, 2021.

[13] A. R. Soares, J. C. D. Andrade, C. D. Lacerda, et al., "Synthesis of polymeric nanoparticles using fungal biosurfactant as stabilizer," Processes, vol. 12, no. 12, Art. no. 2739, 2024.

[14] C. N. Lunardi, A. J. Gomes, F. S. Rocha, J. De Tommaso, and G. S. Patience, "Experimental methods in chemical engineering: Zeta potential," The Canadian Journal of Chemical Engineering, vol. 99, no. 3, pp. 627–639, 2021.

[15] R. Mittal, A. Sharma, and S. Arora, "Ufasomes mediated cutaneous delivery of dexamethasone: Formulation and evaluation of anti-inflammatory activity by carrageenin-induced rat paw edema model," Journal of Pharmaceutics, vol. 2013, pp. 1–12, 2013.

[16] A. Sharma and S. Arora, "Formulation and in vitro evaluation of ufasomes for dermal administration of methotrexate," ISRN Pharmaceutics, vol. 2012, pp. 1–8, 2012, doi: 10.5402/2012/873653.

[17] S. Ghanbarzadeh and S. Arami, "Enhanced transdermal delivery of diclofenac sodium via conventional liposomes, ethosomes, and transfersomes," BioMed Research International, vol. 2013, pp. 1–7, 2013.

[18] A. Sharma and S. Arora, "Formulation and in vitro evaluation of ufasomes for dermal administration of methotrexate," ISRN Pharmaceutics, vol. 2012, pp. 1–8, 2012.

[19] M. S. Latif, A. Nawaz, M. Asmari, J. Uddin, H. Ullah, and S. Ahmad, "Formulation development and in vitro/in vivo characterization of methotrexate-loaded nanoemulsion gel formulations for enhanced topical delivery," Gels, vol. 9, no. 1, Art. no. 3, 2023.

[20] N. Bavarsad, A. Akhgari, S. Seifmanesh, A. Salimi, and A. Rezaie, "Statistical optimization of tretinoin-loaded penetration-enhancer vesicles (PEV) for topical delivery," DARU Journal of Pharmaceutical Sciences, vol. 24, no. 1, Art. no. 7, 2016.

[21] P. Dhillon, M. A. Mirza, M. K. Anwer, A. S. Alshetaili, S. M. Alshahrani, and Z. Iqbal, "Development and optimization of erythromycin-loaded lipid-based gel by Taguchi design: In vitro characterization and antimicrobial evaluation," Brazilian Journal of Pharmaceutical Sciences, vol. 55, Art. no. e17395, 2019.

[22] R. W. Korsmeyer, R. Gurny, E. Doelker, P. Buri, and N. A. Peppas, "Mechanisms of solute release from porous hydrophilic polymers," International Journal of Pharmaceutics, vol. 15, no. 1, pp. 25–35, 1983.

[23] Y. M. Ahmed, R. Orfali, D. S. Hamad, M. E. Rateb, and H. O. Farouk, "Sustainable release of propranolol hydrochloride laden with biconjugated-ufasomes chitosan hydrogel attenuates cisplatin-induced sciatic nerve damage in in vitro/in vivo evaluation," Pharmaceutics, vol. 14, no. 8, Art. no. 1536, 2022.

[24] S. Al-Mahmood and N. R. Ayash, "Ufasomes as topical/transdermal drug delivery system: Structural components, preparation techniques and therapeutic application," Current Drug Delivery, vol. 22, no. 8, pp. 1047–1057, 2025.

[25] R. Mittal, A. Sharma, and S. Arora, "Ufasomes mediated cutaneous delivery of dexamethasone: Formulation and evaluation of anti-inflammatory activity by carrageenin-induced rat paw edema model," Journal of Pharmaceutics, vol. 2013, pp. 1–12, 2013.

[26] J. M. Gebicki and M. Hicks, "Preparation and properties of vesicles enclosed by fatty acid membranes," Chemistry and Physics of Lipids, vol. 16, no. 2, pp. 142–160, 1976.

[27] M. E. Herbig, D. H. Evers, S. Gorissen, and M. Köllmer, "Rational design of topical semi-solid dosage forms—How far are we?" Pharmaceutics, vol. 15, no. 7, Art. no. 1822, 2023.

[28] S. Verma, A. Bhardwaj, M. Vij, P. Bajpai, N. Goutam, and L. Kumar, "Oleic acid vesicles: A new approach for topical delivery of antifungal agent," Artificial Cells, Nanomedicine, and Biotechnology, vol. 42, no. 2, pp. 95–101, 2014.

[29] S. Maanvizhi, V. Iyyappan, and P. G. Bhavishi, "In-vitro release study of diclofenac sodium from topical gel formulations using diffusion cell," Research Journal of Pharmacy and Technology, vol. 13, no. 6, p. 2901, 2020.

[30] S. Al-Mahmood and N. R. Ayash, "Ufasomes as topical/transdermal drug delivery system: Structural components, preparation techniques

and therapeutic application," Current Drug Delivery, vol. 22, no. 8, pp. 1047–1057, 2025.

[31] R. Chadha and S. Bhandari, "Drug–excipient compatibility screening—Role of thermoanalytical and spectroscopic techniques," Journal of Pharmaceutical and Biomedical Analysis, vol. 87, pp. 82–97, 2014.

[32] A. H. Salama and M. H. Aburahma, "Ufasomes nano-vesicles-based lyophilized platforms for intranasal delivery of cinnarizine: Preparation, optimization, ex-vivo histopathological safety assessment and mucosal confocal imaging," Pharmaceutical Development and Technology, pp. 1–10, published online May 21, 2015.

[33] R. Bansal, D. Baloni, and M. K. Mishra, "Self-assembled ufasomes of unsaturated fatty acids: Mechanisms, characterization, and drug delivery potential," Pharmaceutical Development and Technology, vol. 31, no. 7, pp. 1137–1171, 2026.

[34] D. Baviskar, Y. Biranwar, K. Bare, V. Parik, M. Sapate, and D. Jain, "In vitro and in vivo evaluation of diclofenac sodium gel prepared with cellulose ether and Carbopol 934P," Tropical Journal of Pharmaceutical Research, vol. 12, no. 4, pp. 489–494, 2013.

[35] M. T. Islam, N. Rodríguez-Hornedo, S. Ciotti, and C. Ackermann, "Rheological characterization of topical carbomer gels neutralized to different pH," *Pharmaceutical Research*, vol. 21, no. 7, pp. 1192–1199, 2004.