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DEVELOPMENT AND CHARACTERIZATION OF ALLOPURINOL BASED PRONIOSOMAL GEL FOR ANTI-GOUT ACTIVITY

Anurag Sharma[1]*, Dr. Praveen Khirwadkar[2], Dr. Kamlesh Dashora[3]

Institute of Pharmacy, Vikram University Ujjain.

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***Corresponding Author**
Anurag Sharma
Institute of Pharmacy,
Vikram University Ujjain.

ABSTRACT

The objective of this study was to formulate and evaluate a proniosomal gel of allopurinol for effective transdermal delivery. Allopurinol, a xanthine oxidase inhibitor used in the treatment of gout, suffers from poor water solubility, limited oral bioavailability, and gastrointestinal side effects. To overcome these limitations, proniosomal gels were prepared using Span 40, Span 60, cholesterol, and soya lecithin via the Coacervation phase separation method. Preformulation studies including organoleptic evaluation, solubility analysis, partition coefficient determination, melting point, and FTIR spectroscopy confirmed drug-excipient compatibility and physicochemical stability. Nine formulations (F1–F9) were developed and evaluated for drug entrapment efficiency, pH, viscosity, spreadability, particle size, zeta potential, in vitro drug release, and stability. Among these, formulation F9—comprising Span 40 and Span 60 in a 1:1 ratio—exhibited the highest entrapment efficiency (89.93%), ideal pH (7.02), suitable viscosity (8690 cps), and superior spreadability (79.166 g·cm/s). In vitro drug release studies showed sustained release up to 12 hours, with a cumulative drug release of 93.47%. Kinetic modeling indicated that the release followed Korsmeyer-Peppas model and supported by Higuchi model for diffusion-controlled kinetics. Morphological studies using HR-TEM and SEM confirmed spherical, unilamellar nanosized vesicles with uniform distribution. Stability testing under varying storage conditions for 90 days confirmed the physical and chemical stability of the optimized formulation. The study concludes that F9 proniosomal gel offers a promising transdermal drug delivery system for allopurinol, with advantages including sustained release, enhanced stability, improved patient compliance, and reduced systemic side effects.

KEYWORDS: Coacervation phase separation method, proniosomal gel, physicochemical stability, characterisation, Allopurinol, Kinetic modeling, Morphological studies, sustained release.

INTRODUCTION

Advances in nanotechnology have led to the development of innovative drug delivery methods. Using drug carriers to achieve medication targeting, minimizing adverse effects, and maintaining a consistent and effective drug level in the body are the primary goals of NDDS or innovative drug delivery.[1]

The word "dry niosomes" is another name for Proniosomes**.** Drugs encapsulated in the proniosomal vesicular structure have lower toxic effects, improved penetration in the targeted locations, regulated release, and maintained systemic circulation.[2] Proniosomes are surfactant-coated carrier formulations that are dry and free-flowing. They can be rehydrated in a matter of minutes by briefly stirring them in hot aqueous media. Drugs that are hydrophobic or hydrophilic, polar or non-polar, can be captured by proniosomes. Proniosomes reduce aggregation, fusion, and leakage issues that affect proniosomes' physical stability and offer extra convenience in terms of distribution, storage, transportation, and dosage.[3]

Proniosomes decrease the drug's toxicity after extending its time in the systemic circulation. Numerous areas are the focus of Proniosomes, including entrapment efficiency, in vitro drug release, characterisation, preparation, and applications.[4] They are microscopic lamellar structures called proniosomes. Along with cholesterol, they mix a non-ionic surfactant and hydrate in aqueous medium.[5] The surfactant molecule aligns itself so that the hydrophilic ends of the non-ionic surfactant face outward, while the hydrophobic ends face in the opposite direction, forming a bilayer. Additionally, proniosomes are produced by these bilayer liposomes. Surface-active substances that are not ionic comprise the bilayer of proniosomes.[6] Proniosomes can be either unilamellar or multi-lamellar, depending on how they are prepared. They are composed of two layers of surfactants, with the hydrophilic end facing the vesicle's exterior and the hydrophobic end facing each other inside the bilayer. For this reason, both hydrophilic and hydrophobic medicines are stored in the proteasomes. Drugs that are hydrophilic adhere to the vesicle's confined area, whereas those that are hydrophobic are lodged in the bilayer.[7]

Urate crystals that are deposited as a result of either excessive or insufficient excretion of uric acid cause gout. Although not always, the condition is frequently linked to high serum uric acid levels.[8] Interstitial renal disease, tophi, uric acid nephrolithiasis, and both acute and chronic arthritis are examples of clinical symptoms. If uric acid crystals are found in joints, tissues, or bodily fluids, the diagnosis is made. Goals of treatment include stopping the acute attack, preventing future episodes, and avoiding consequences from urate crystal deposition in tissues. Treatment still relies heavily on pharmacologic control. Acute attacks can be stopped by intra-articular injections of corticosteroids, colchicine, or nonsteroidal anti-inflammatory drugs.[9] Allopurinol, probenecid, and sulfinpyrazone can be used to stop recurrent attacks. Obesity, alcohol intake and certain foods and medications can contribute to hyperuricemia. These potentially exacerbating factors should be identified and modified.[10] Approximately one percent of people have gout, a prevalent arthritic condition. As people age, the prevalence rises and is higher in men. Decreased renal excretion of uric acid, elevated blood uric acid levels, disruption of purine metabolism, and the accumulation of monosodium urate (MSU) crystals in the soft tissues and joints are all part of the pathophysiology of gout.[11] Gout usually manifests as episodic acute monoarthritis of the first metatarsophalangeal joint (MTP) with erythema on top. Polyarticular attacks affecting the hand joints and chronic arthritis are two examples of unusual clinical presentations that may occur as the disease worsens.[12]

Allopurinol is a competitive inhibitor of xanthine oxidase. And its major metabolite alloxanthine (oxypurine). Primarily used to manage conditions associated with excessive uric acid production, such as gout and certain types of kidney stones.[13] Allopurinol inhibits xanthine oxidase, the enzyme responsible for converting hypoxanthine to xanthine and xanthine to uric acid. By blocking this pathway, allopurinol reduces the production of uric acid, thereby decreasing its concentration in the blood and urine. It is first drug of choice in the treatment of chronic gout and used in both over producers and excretors of uric acid.[14]

MATERIAL AND METHODS

PRE- FORMULATION STUDY

The preformulation study is a subfield of pharmaceutical science that determines the physicochemical characteristics of a drug's constituent ingredients and other formulation excipients. The objective of preformulation research is to determine the suitable form of the material, assess its physical characteristics, and produce a comprehensive understanding of its

stability. The odds of creating a product that is acceptable, safe, effective, and stable are increased when preformulation parameters are used. Preformulation studies include assay, melting point, lambda max determination, solubility studies, Fourier transform infrared (FTIR) tests, and organoleptic characteristics (colour, odour, and taste).[15]

Organoleptic Properties

The first step in any preformulation attempt should be to describe the drug substance. A consistent colour, flavor, and scent are required for the new medication. Documented using terminology that provides examples the new drug's colour, flavor, and odour must be reported. Making use of illustrative terminology developing a common language to describe them is crucial to avoiding miscommunications. There are several ways in which scientists describe the phenomenon. Similar in quality, a list of appropriate descriptors is used to characterize the most popular colors. Powdered pharmaceuticals have unique scents. A table is provided. They were all the same colour in the first batches. The effectiveness of the novel drug must be substantiated by terminologically descriptive early batch colour records, which are highly beneficial in producing the appropriate type of batch.[16]

Determination of λmax

Preparation of Stock Solution

50mg (Milligram) of working standard Allopurinol was accurately weighed and transferred to 50ml (Milliliter) volumetric flask. Then 20ml of Methanol was added to dissolve the drug by shaking the flask for few seconds. Then the final volume was made upto the mark with methanol to get the concentration of 1000µg/ml (Microgram per Milliliter).

Preparation of Working Standard Solution

From the above standard stock solution, 1ml was pipetted out into a 10ml volumetric flask and the volume was made up to the mark with methanol to get a concentration of 100µg/ml. Then 1 ml of solution taken from 100µg/ml and further dilute with upto 10 ml methanol to make up volume to get 10µg/ml.[17]

Preparation of Calibration Curve

From the working standard solution, 1ml, 2ml, 3ml,4ml, 5ml, 6ml, 7ml,8ml,9ml,10ml were pipetted into 10ml volumetric flasks and volume was made upto the mark with methanol to produce the concentrations ranging from 1-10µg/ml respectively. The analytical wavelength was selected by scanning in the wavelength range of 400-200nm (Nanometer) using methanol

as a blank and the wavelength corresponding to maximum absorbance (λmax) was found to be 250.0nm. Then, the calibration curve was plotted in the concentration range of 1-10µg/ml at 250.0nm by taking concentration on X-axis and absorbance on Y-axis.[18]

Melting Point of Drug

Ensure that the allopurinol sample is dry and free from impurities. Grind the sample gently with a mortar and pestle to create a fine, uniform powder. Seal one end of the capillary tube by heating it in a flame. Fill the open end with a small quantity of the powdered allopurinol (approximately 2-3 mm (Millimeter) height in the tube). Pack the sample by tapping the sealed end gently on a hard surface to settle the powder. Then Place the filled capillary tube in the melting point apparatus or attach it to a thermometer in the setup. For accurate measurement, ensure proper placement of the sample in the heating zone of the apparatus. Heat the sample slowly and evenly. Observe the sample as the temperature rises and record the range where the substance transitions from solid to liquid.[19]

Partition Coefficients

Aqueous Phase: Prepare a known concentration of allopurinol in phosphate buffer (PBS) 7.4 / distilled water. Organic Phase: Use pure n- octanol or chloroform as the organic solvent. Take equal volumes of the aqueous phase and organic phase (e.g., 20 ml each) in a separatory funnel or tightly sealed vial. Add a measured amount of the allopurinol solution into the separatory funnel containing both phases. Shake the separatory funnel or vial vigorously for 10–15 minutes to ensure thorough mixing and equilibration between the phases. Allow the mixture to stand for sufficient time (e.g., 30 minutes) for the two phases to separate completely. Then carefully collect small samples from each phase using pipettes. Ensure no cross-contamination between the phases. Measure the concentration of allopurinol in both the aqueous and organic phases using a UV-visible spectrophotometer or HPLC.[20]

Formula for Partition Coefficient

$$Partition\ Coefficient = Con.of\ Drug\ in\ Aqueous\ Phase / Con.of\ Drug\ in\ Organic\ Phase$$

Drug - Excipient Interaction Study by FTIR

Take a small amount of pure allopurinol and finely grind it to ensure uniformity. Grind each excipient separately to a fine powder. Mix allopurinol with each excipient in a 1:1 ratio (w/w) using a mortar and pestle. Ensure homogeneity of the mixture. For KBr Pellet Method:

Accurately weigh ~2 mg of the sample (pure drug, pure excipient, or physical mixture) and mix it with ~200 mg of KBr. Grind the mixture uniformly, avoiding moisture contamination. Compress the mixture into a thin, transparent pellet using a hydraulic press. For ATR Method (if available): Place a small amount of the sample directly onto the ATR crystal. Press it gently to ensure good contact with the crystal. Place the prepared pellet or sample in the FTIR spectrometer. Record the spectrum over the range of **4000–400 cm^{-1}** with appropriate resolution (e.g., 4 cm^{-1}). Collect spectra for the pure drug, pure excipients, and drug-excipient mixtures. Compare the FTIR spectra of the pure drug and excipients with the spectra of the physical mixtures. Identify characteristic peaks of allopurinol (e.g., N-H stretching, C=O stretching, etc.). Look for any shifts, disappearance, or appearance of new peaks in the mixture spectra, which may indicate possible interactions.[21,22]

Solubility

Prepare buffer solutions of different pH values as per Pharmacopoeial standards. Use pure distilled water and organic solvents as received. Weigh an excess amount of allopurinol (e.g., 50 mg) using an analytical balance. Add it to a test tube containing 5 ml of the chosen solvent. Place the test tubes in a shaking water bath or stir the solution using a magnetic stirrer. Allow the solutions to reach equilibrium (ensure no further dissolution of the drug). Observe for undissolved particles, indicating saturation. Filter the saturated solutions using filter paper to remove undissolved drug particles. Analyze the filtrates to determine the concentration of allopurinol dissolved in each solvent. Use a UV-visible spectrophotometer determine the Solubility of allopurinol (~250 nm).[23]

DEVELOPMENT OF ALLOPURINOL BASED PRONIOSOMAL GEL COACERVATION PHASE SEPARATION METHOD[24]

Weighed quantities of drug, lipid and surfactants are taken in a dry wide-mouthed glass beaker followed by the addition of solvent. The ingredients are mixed well and warmed over water bath at 65 ± 3°C until the surfactant mixture dissolves completely. During the process care must be taken to prevent loss of any solvent due to evaporation. Finally, the aqueous phase is added to the mixture and warmed on water bath. The resultant solution is cooled overnight to obtain proniosomal gel.

Table 1: Composition of the Proniosomal Gel Formulation.

S.no.	Ingredients	Batch code								
		F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
1	Allopurinol (mg)	100	100	100	100	100	100	100	100	100
2	Cholesterol (mg)	100	100	100	100	100	100	100	100	100
3	Soya lecithin (mg)	225	225	225	450	450	450	900	900	900
4	Span40(mg)	112	-	225	-	450	-	900	-	-
5	Span 60(mg)	-	112	-	225	-	450	-	900	-
6	Span40:span60(50:50)(mg)	-	-	-	-	-	-	-	-	900
7	Ethanol (ml)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
8	Pbs7.4 (ml)	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8

CHARACTERIZATION OF PRONIOSOMAL GEL

Surface morphology by optical microscope

The proniosomes gel formulation are mounted on the glass slide and viewed under optical microscope to determine the surface morphology and particle size of formulation.[25]

Determination of entrapment efficiency by centrifugation

The proniosomal gel (0.1g) was taken in a test tube and reconstituted with 10 ml isotonic phosphate buffer of pH 7.4. This aqueous solution was sonicated in a Sonicator bath. The drug containing proniosomes were separated from the dispersion by centrifugation at 3000 rpm for 30 min at 20°C. The supernatant (1ml) was taken and diluted with phosphate buffer (in 10 ml volumetric flask). And again, from stock solution, 1 ml was withdrawn and transferred to a 10 ml volumetric flask and made up to the mark with buffer. The drug concentration in the resulting solution was assayed by UV-visible spectroscopy method.[26]

The percentage of drug encapsulation was calculated by the following formula:

$$EE\% = \left[\frac{Ct - Cr}{Ct}\right] \times 100$$

Where,

EE= Entrapment Efficiency,

Ct = Concentration of total drug,

Cr = Concentration of Un entrapped drug

PH determination

In 25ml of distilled water 1gm of gel was dissolved and the electrode was immersed in to gel formulation for 30 min till constant reading was achieved.[27]

Viscosity determination

The viscosities of different proniosomal gel formulation were determined at 25°C using a Brookfield viscometer. The formulation (more than 5g) was placed in a beaker and was allowed to equilibrate for 5 min before measuring the dial reading using a spindle no S-73 at 20 rpm.[28]

Spreadability determination

Weigh an appropriate amount (e.g., 0.5 g) of the proniosomal gel using a precision scale. Place it at the center of a clean, smooth glass slide. Cover the gel with another glass slide of the same size. Carefully place a standard weight (500 g) on the top slide. Allow the weight to spread the gel for a fixed time (typically 1 minute). After the time has elapsed, carefully remove the top slide without disturbing the gel. Use a ruler to measure the diameter (in cm) of the circular spread area of the gel.[29,30]

Calculate spreadability using the formula

$$s = m.l/t$$

Where:

S = Spreadability (g·cm/s)

m = Weight applied (g)

l = Length of spread (cm)

t = Time taken to spread (s)

In-vitro drug release

The in-vitro drug release study is performed using Franz Diffusion cell. The diffusion area of the cell is 1.75 cm^2 and the receptor compartment had a capacity of approximately 10.5 ml. The membrane is cut to a diameter of 25 mm and saturated for 30 minutes in receptor medium (phosphate buffer pH 7.4) before starting the experiment. The cell is filled with degassed receptor medium and the membrane is placed in the top of the receptor compartment and checked for air bubbles. An aliquot of the sample is measured with the aid of a syringe and then placed in the cavity of a dosage wafer (donor compartment), on top of the membrane. The amount applied is around 50 mg with a spatula, the formulation is spread uniformly filling the donor compartment. A glass disk is carefully placed on the sample to occlude it, and an aligner cap is then used to centralize the assembly, which is held together by a clamp. The receptor medium is maintained at 37 ± 2°C under constant stirring. To

characterize the drug release, 1ml samples were collected after 1, 2, 3, 4, 5, 6 and 7 hrs. Up to 24hr. After sampling, the volume collected is replaced with fresh receptor medium. The amount of drug is assayed by UV analysis after this %CDR and the drug kinetic is calculated by using different models & formula.[31]

Scanning electron microscope (SEM)

Dilute a small amount of the proniosomal gel with a minimal quantity of distilled water or buffer to release the niosomes from the gel matrix. Pipette a drop of the suspension onto a clean glass slide or SEM stub covered with double-sided adhesive carbon tape. Allow the sample to air-dry at room temperature under a dust-free environment to remove moisture. Mount the stub in the sputter coater. Coat the sample with a thin layer of gold or platinum (thickness ~10-20 nm) to make the surface conductive. Follow the manufacturer's instructions for coating time and voltage. Mount the prepared stub onto the SEM stage. Set the operating conditions of the SEM (e.g., accelerating voltage, spot size, and working distance) as per the instrument's manual, typically: Accelerating Voltage: 10-20 kV. Working Distance: ~10 mm. Focus the electron beam onto the sample and adjust magnification to observe the surface morphology of the proniosomal gel. Capture high-resolution images of the surface structure of the gel and the morphology of the vesicles (if visible). Obtain images at various magnifications for detailed analysis.[32,33]

HR-TEM analysis of proniosomal gel formulation

To determine the morphological characteristics, size, and structural integrity of vesicles in a proniosomal gel formulation using High-Resolution Transmission Electron Microscopy (HR-TEM).

Weigh an appropriate amount of proniosomal gel (e.g., 100 mg). Hydrate it with 10 mL of PBS or distilled water under stirring. Then sonicate the hydrated dispersion for 10–15 minutes to reduce vesicle size and ensure uniformity. Filter the sonicated suspension using Whatman filter paper or syringe filter (0.45 µm) to remove large aggregates if necessary. Place a drop (~10 µL) of the diluted proniosomal dispersion onto a carbon-coated copper grid using a micropipette. Allow it to stand for 1–2 minutes for adsorption of vesicles onto the grid surface. Remove excess fluid with filter paper gently by touching the edge of the grid. Immediately add 10 µL of 1% phosphotungstic acid (PTA) or 2% uranyl acetate solution to the grid for negative staining. After 30–60 seconds, blot off the excess stain and air-dry the grid at room temperature. Load the dried grid into the HR-TEM sample holder. Operate the

HR-TEM under suitable accelerating voltage (e.g., 100–200 kV). Capture images at various magnifications to observe vesicle morphology, size, and bilayer structure.[34,35]

Particle size determination

Accurately weigh 100 mg of the proniosomal gel formulation. Reconstitute it in 10 mL of distilled water or PBS (pH 7.4) with gentle shaking then sonicate the suspension for 5–10 minutes to ensure uniform dispersion and vesicle formation. Optionally, filter the dispersion through a 0.45 µm syringe filter to remove aggregates (if instrument requires). Turn on the Dynamic Light Scattering (DLS) instrument and allow it to stabilize. Choose the appropriate mode (intensity-based or number-based distribution).Set the measurement angle (typically 90° or 173°) depending on the instrument model. Maintain the measurement temperature at 25°C or 37°C, as per standardization. Transfer the prepared vesicle dispersion into a clean, dust-free quartz or polystyrene cuvette. Ensure there are no air bubbles in the cuvette Place the cuvette in the instrument. Run the sample for 3 consecutive measurements (each 60–90 seconds). Record the average particle size (Z-average), Polydispersity Index (PDI), and size distribution curve.[36,37]

Zeta potential

Take a small amount of proniosomal gel (e.g., 0.1–0.2 g). Dilute it in 10–20 ml of deionized water or buffer to release the niosomes from the gel matrix. Vortex the mixture for 2–3 minutes or sonicate it for 5 minutes to ensure uniform dispersion of proniosomes. Transfer the prepared sample to a disposable zeta potential cuvette or electrophoretic cell. Ensure that there are no air bubbles in the cuvette. Switch on the DLS instrument or Zetasizer and allow it to stabilize. Set the appropriate parameters, such as: Refractive index and viscosity of the dispersant (e.g., water). Temperature (e.g., 25°C). Measurement angle (typically 173° for backscatter detection). Place the cuvette into the instrument sample holder. Start the zeta potential measurement process. The instrument applies an electric field and measures the electrophoretic mobility of the vesicles, which is used to calculate the zeta potential.[38]

Stability studies of proniosomal gel formulation

The physical, chemical, and rheological stability of the proniosomal gel was evaluated over a period of a 90 days under various storage circumstances. To imitate normal storage circumstances and ascertain the gel samples' shelf-life, they were kept at different temperatures. The storage conditions listed below were used:

Room Temperature (25°C ± 2°C)

Refrigeration (4°C ± 2°C)

Accelerated Conditions (40°C ± 2°C)

At the conclusion of each week and after a 90 days of storage, the gel samples were assessed for changes in appearance (such as phase separation, colour, and texture), pH, viscosity, and spreadability.[39]

RESULT AND DISCUSSION

PRE- FORMULATION STUDY RESULT

Organoleptic Properties

Organoleptic attributes color, aroma and taste were investigated as part of the drugs organoleptic qualities and the investigation result found to be compliance as per the standards. As shown in table.

Table 2: organoleptic properties of allopurinol.

S.no.	Properties	Standard	Inference	Result
01	Colour	White To off White	White	Compliance
02	State	Crystalline Powder	Crystalline Powder	Compliance
03	odour	Odourless	Odourless	Compliance
04	Taste	Bitter	Bitter	Compliance

Determination of λmax

To identify and describe the drug substance, an examination using UV-visible spectroscopy was conducted. To identify the drug sample's absorption maxima, it was dissolved in an appropriate solvent buffer (PBS 7.4 pH) and scanned across a wavelength range of 200–400 nm. And the maximum absorbance of the drug was found at 250 nm. As shown in fig.

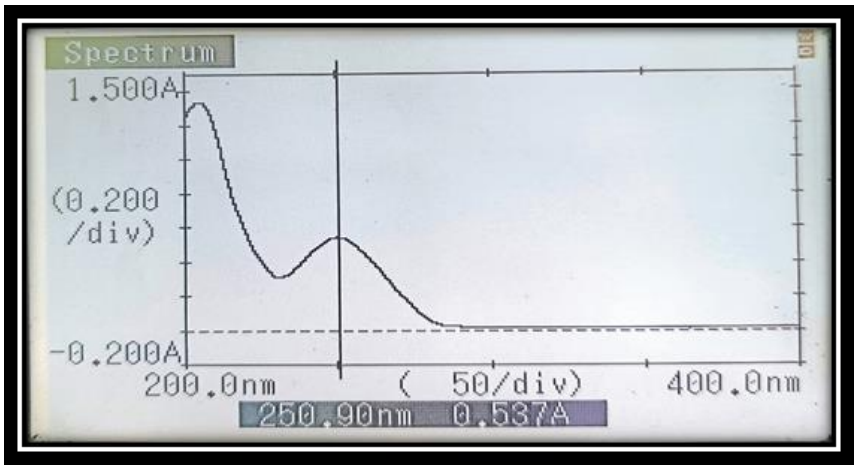


Fig. 1: maximum absorbance of the drug λmax.

Preparation of calibration curve

The drug solution was scanned over a wavelength range of 200–400 nm, and the maximum absorbance was observed at 250 nm. This wavelength was selected for further analysis as it corresponds to the drug's characteristic absorption peak. Stock solution of the drug was prepared by dissolving an accurately weighed amount of the drug in a suitable solvent buffer PBS 7.4pH. A series of standard solutions with concentrations ranging from 1µg/mL to 10µg/mL were prepared by diluting the stock solution. The absorbance of each standard solution was measured by using a UV-Visible spectrophotometer. Calibration curve was plotted by using MS excel graphing the absorbance (y-axis) against the corresponding concentration (x-axis) of the standard solutions. The curve exhibited a linear relationship, as evidenced by the high correlation coefficient (R^2= 0.9953) by using equation (y = mx+c)

Table 3: calibration curve value concentration vs. Absorption of allopurinol.

S.no.	X (concentration in ug/ml)	Y (absorbance)
1	1	0.079
2	2	0.114
3	3	0.145
4	4	0.196
5	5	0.255
6	6	0.311
7	7	0.342
8	8	0.389
9	9	0.428
10	10	0.472

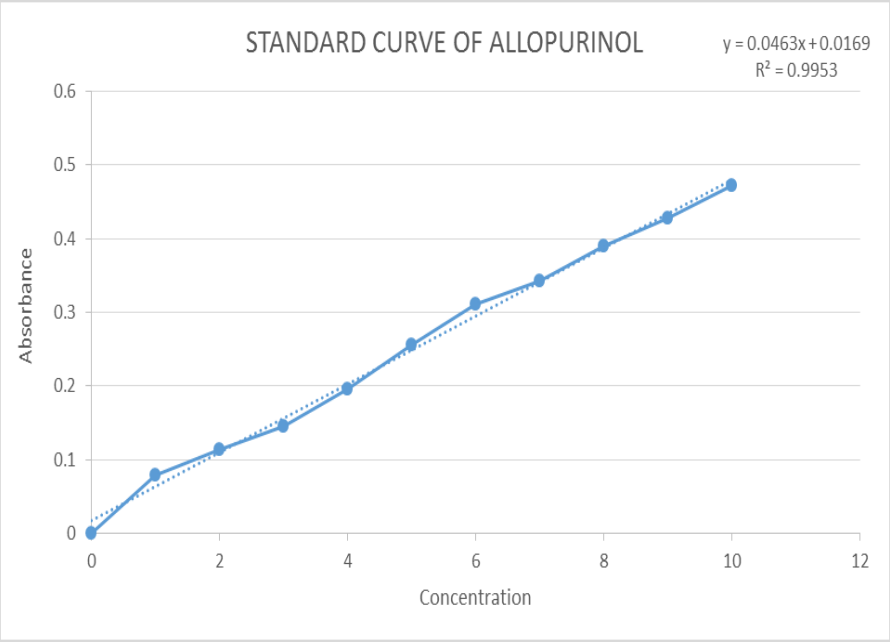


Fig. 2: standard curve of allopurinol.

Melting point of drug

A capillary melting point equipment was used to measure the drug's melting point, which was found to be between the ranges of 350°C to 360°C. The medication appears to be thermally stable at its melting point because no discolorations no breakdown was seen throughout the melting process.

Partition coefficient

For calculating the partition coefficient, the n-octanol/PBS combination was used as the solvent system. 20 mg of the drug were mixed with 20 ml of each of the n-octanol and PBS 7.4 pH. After fully mixing the two phases, they were left to equilibrate at room temperature (25°C) for 30 minutes. Then the drug's concentration in each phase was determined through the use of UV-visible spectrophotometry. The formula was used to compute the partition coefficient (P). And it was discovered that the partition coefficient (log P) was **1.** That indicating both the drug's hydrophilicity and lipophilicity.

Table 04: partition coefficient concentration of allopurinol in different phases.

S.no.	Phase	Concentration (absorbance)
1	Aqueous Phase (P.B.S.- 7.4pH)	4.000
2	Lipid Phase (N- Octanol)	4.000

Solubility

Table 5: solubility.

S.no.	Solvent	Solubility
1	Methanol	4.008mg/ml
2	Ethanol	4.008mg/ml
3	Water	3.231mg/ml
4	P.b.s. 7.4ph	3.941mg/ml
5	P.b.s.- 6.8ph	3.7965mg/ml
6	Chloroform	0.924mg/ml

It was discovered that the drug is freely soluble in methanol & ethanol and phosphate buffers. Slightly soluble in water but practically insoluble in chloroform.

Identification of drug by FTIR

Interpretation of results

The analysis is done by using FTIR instrument shimazu brand and the result shaved that a lot of numbers of peaks were detected, informing the complex structure of molecule.

Key functional group confirmation

N–H Stretch**:** Strong absorption around 3778 cm^{-1} confirms presence of amino-type hydrogen.

C=O Stretch: Observed around 1708–1778 cm^{-1}, indicative of the carbonyl group in allopurinol.

C=N/C=C Stretch**:** Peak near 1597 cm^{-1} supports presence of aromatic or heterocyclic ring.

C–N / N–H bending and stretching**:** Peaks from 1350–1230 cm^{-1} consistent with the purine structure.

Aromatic Ring Vibrations**:** Multiple bands (1487, 896, 810, 713 cm^{-1}) consistent with purine-type heteroaromatic structures.

CONCLUSION

The FTIR spectrum of the analyzed sample shows good concordance with the known functional groups and structural characteristics of Allopurinol.

Characteristic bands for N–H, C=O, C=N, C–N, and aromatic heterocycles are clearly present.

No major extraneous or contaminant peaks dominate the spectrum, though minor signals near 2300–2100 cm^{-1} may be due to ambient CO_2 or trace impurities.

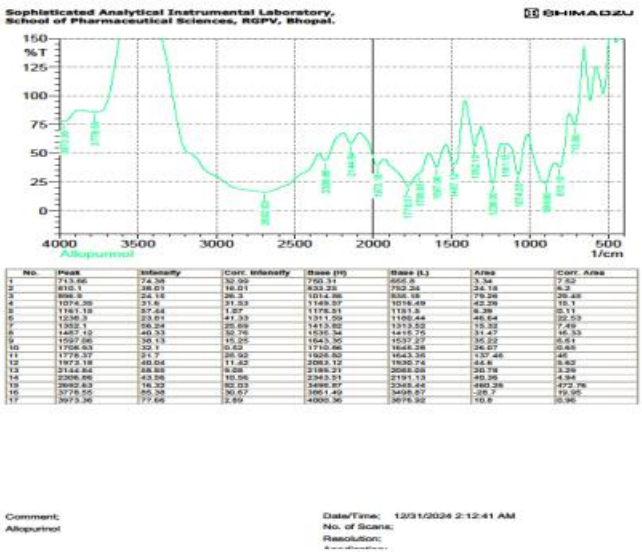
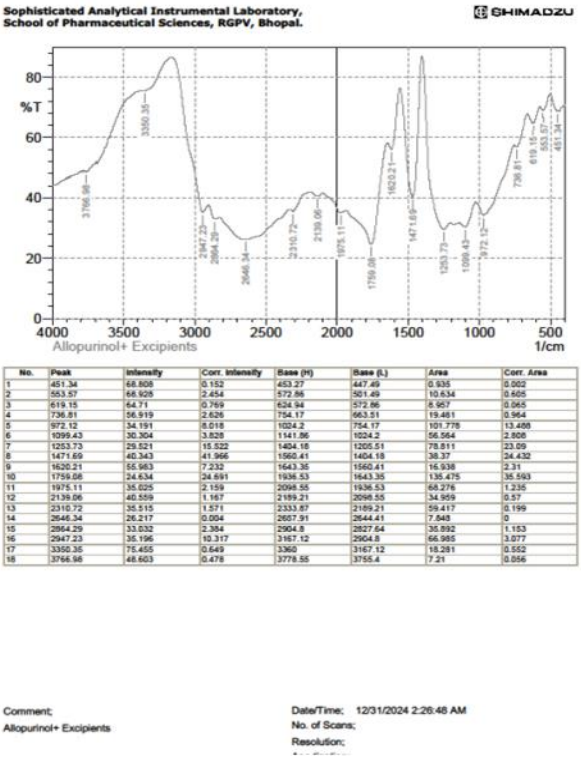


Fig. 3: Identification of drug by FTIR.

Drug excipient interaction study by FTIR



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	451.34	68.808	0.152	453.27	447.49	0.935	0.002
2	553.57	68.928	2.454	572.86	501.49	10.634	0.605
3	619.15	64.71	0.769	624.94	572.86	8.957	0.065
4	736.81	56.919	2.626	754.17	663.51	19.461	0.964
5	972.12	34.191	8.018	1024.2	754.17	101.778	13.466
6	1099.43	30.304	3.828	1141.86	1024.2	56.564	2.808
7	1253.73	29.521	15.522	1404.18	1205.51	78.811	23.09
8	1471.69	40.343	41.966	1560.41	1404.18	38.37	24.432
9	1620.21	55.983	7.232	1643.35	1560.41	16.938	2.31
10	1759.08	24.634	24.691	1936.53	1643.35	135.475	35.593
11	1975.11	35.025	2.159	2098.55	1936.53	68.276	1.235
12	2139.06	40.559	1.167	2199.21	2098.55	34.959	0.57
13	2310.72	35.515	1.571	2333.87	2189.21	59.417	0.199
14	2646.34	26.217	0.004	2657.91	2644.41	7.848	0
15	2864.29	33.032	2.384	2904.8	2827.64	35.892	1.153
16	2947.23	35.196	10.317	3167.12	2904.8	66.985	3.077
17	3350.35	75.455	0.649	3360	3167.12	18.281	0.552
18	3766.98	48.603	0.478	3778.55	3755.4	7.21	0.056

Fig. 4: drug excipient interaction study by FTIR.

Interpretation of results

The analysis is done by using FTIR instrument shimazu brand and the result shaved that a lot of numbers of peaks were detected, informing the complex structure of molecule.

Major functional groups (N–H, C=O, C=N) of allopurinol remain intact in the mixture spectrum.

Some minor shifts observed (especially around 1708 → 1759 cm^{-1}**)** suggesting weak hydrogen bonding or van der Waals interactions with excipients.

No new peaks suggestive of chemical degradation or covalent interaction.

Disappearance of minor bands (713–900 cm^{-1} region) can be due to overlapping or dilution by excipient matrix.

Peaks in the range of 2864–2947 cm^{-1} are typical of aliphatic excipients, not present in pure drug.

CONCLUSION

The FTIR analysis confirms that there is no significant chemical interaction between allopurinol and the selected excipients**.**

Minor physical interactions such as hydrogen bonding may be present, but these are typically non-reactive and pharmaceutically acceptable. The integrity of allopurinol is retained, making the formulation compatible and stable from a physicochemical standpoint.

It show the good argument that his chemical compound is allopurinol & our formulation contain allopurinol drug with cholesterol, soya lecithin, spans are compatible & stable.

EVALUATION PARAMITERS STUDY RESULT

Surface morphology by optical microscope

Microphotograph of formulation is taken from optical microscope for the visual evaluation of formulation.

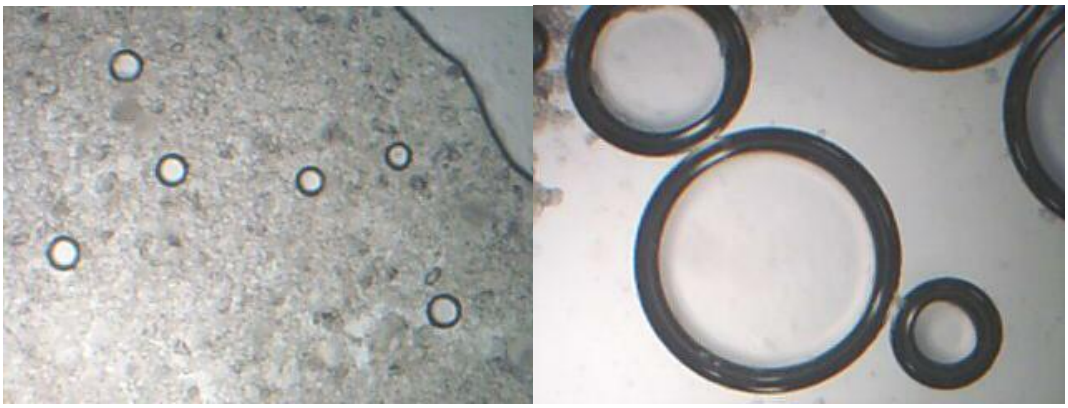


Fig. 5: surface morphology by optical microscope.

Drug encapsulation efficiency of formulations

Proniosomal gel formulation had drug encapsulation efficiency range from 67.30 percentage to 89.93 percentage. Table: shows the drug encapsulation efficiency of formulations.

Table 6: drug encapsulation efficiency of formulations.

S.no	Formulations	EE%
1	F1	67.30%
2	F2	67.25%
3	F3	72.44%
4	F4	72.70%
5	F5	76.44%
6	F6	75.89%

7	F7	80.83%
8	F8	82.30%
9	F9	89.93%

PH measurement of formulations

All formulations had pH close to the skin as shown in table 7, indicating that there was no danger of skin irritation due to their neutral pH.

Table 7: pH measurement of formulations.

S.no	Formulations	pH
1	F1	7.15 pH
2	F2	7.26 pH
3	F3	6.94 pH
4	F4	7.25 pH
5	F5	7.11 pH
6	F6	6.80 pH
7	F7	7.09 pH
8	F8	6.88 pH
9	F9	7.02 pH

Viscosity measurement of formulations

The gel formulations were found to have good viscosity using Brookfield viscometer that shows in table. Gel formulation can be graded in the following order concerning the viscosity of the formulation. F8>F9>F7>F5>F4>F6>F3>F2>F1.

Table 8: viscosity measurement of formulations.

S.no	Formulations	Viscosity (CPS)
1	F1	4650cps
2	F2	4710 cps
3	F3	4890 cps
4	F4	5200 cps
5	F5	6870 cps
6	F6	4992 cps
7	F7	8522 cps
8	F8	9250 cps
9	F9	8690 cps

Spreadability analysis of formulations

The gel formulations were found to have good spreadability. Proniosomal gel formulation had spreadability range from 64.164 g/cm/s to 79.166 g/cm/s. Table shows the spreadability of formulations. Gel formulation can be graded in the following order concerning the spreadability of the formulation. F9>F7>F8>F6>F5>F4>F3>F2>F1.

Table 9: spreadability analysis of formulations.

S.no	Formulations	Spreadability(g/cm/s)
1	F1	64.164 g/cm/s
2	F2	65.926 g/cm/s
3	F3	66.189 g/cm/s
4	F4	68.542 g/cm/s
5	F5	69.190 g/cm/s
6	F6	71.629 g/cm/s
7	F7	72.176 g/cm/s
8	F8	72.414 g/cm/s
9	F9	79.166 g/cm/s

In-vitro release studies of proniosomal transdermal gel of allopurinol

Table 10: in-vitro release studies of proniosomal transdermal gel of allopurinol.

S.no.	Time (Hrs.)	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	0	0	0	0	0	0	0	0	0	0
2	1	9.44	10.13	10.94	9.98	11.2	12.74	12.93	13.65	15.86
3	2	11.96	13.42	14.26	12.84	15.8	17.11	18.18	19.78	21.63
4	3	18.74	20.44	22.49	20.8	25.07	26.04	25.92	26.38	28.17
5	4	24.68	27.18	30.77	27.13	31.67	34.5	35.11	36.04	38.48
6	5	31.04	34.66	36.12	33.41	38.97	41.18	42.46	43.68	48.94
7	6	37.08	41.13	42.76	41.72	45.12	47.98	48.45	48.92	59.44
8	7	45.04	47.19	52.16	50.28	54.26	56.49	56.78	59.24	69.71
9	8	53.07	55.18	60.18	61.98	59.68	64.22	66.71	69.44	77.82
10	12	66.12	69.45	71.89	74.34	77.14	79.11	84.66	89.78	93.47

Table shows the findings of the in-vitro diffusion research over the egg membrane using different gels. In terms of the rates of release of allopurinol from the gel formulations, they can be graded in the following order: F9>F8> F7> F6> F5, > F4, > F3, > F2.> F1. The permeation profile revealed that formulation F9, which contained a span 40 and span 60 in proniosomal gel in a 50:50 ratio, provided the best drug release for up to 12 hours.

OBSERVATIONS

The medication release from all formulations increases gradually over time.

All formulas exhibit the same release pattern, with some achieving greater release percentage sooner than others.

Formulations like F8 and F9 have a higher cumulative release and a faster pace than the others, approaching 89.78% and 93.47% at 12 hours, respectively.

The release rates for other formulation are slower and remain below 85% after 12hours. Since F9 produced the maximum cumulative drug release (93.47%) in 12hours, it is the best formulation according to the data.

The optimal formulation is chosen based on how well it releases the medication over an extended period of time. This balance can be achieved by F9 formulation for shown to superior penetration and hence might be evaluated as a possibility for topical dosage form development.

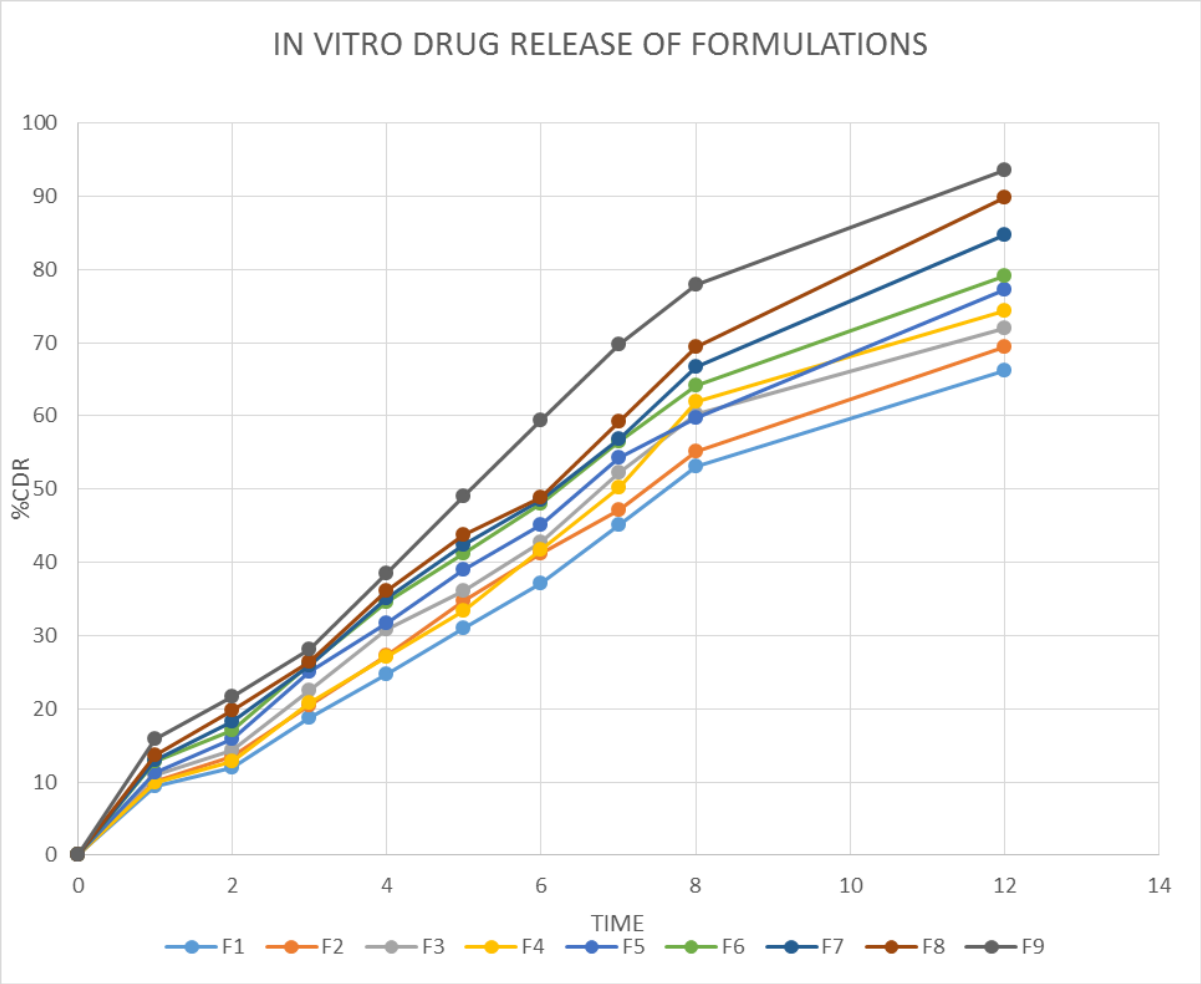


Fig. 6: in-vitro release graph (%CDR vs time) of proniosomal transdermal gel.

Release kinetics of proniosomal gel formulation F9

The in vitro drug release data (% Cumulative Drug Release, CDR) was analyzed using various kinetic models to determine the mechanism of drug release. The models applied included: Zero-order kinetics, First-order kinetics, Higuchi model, Korsmeyer–Peppas model, Hixson–Crowell model.

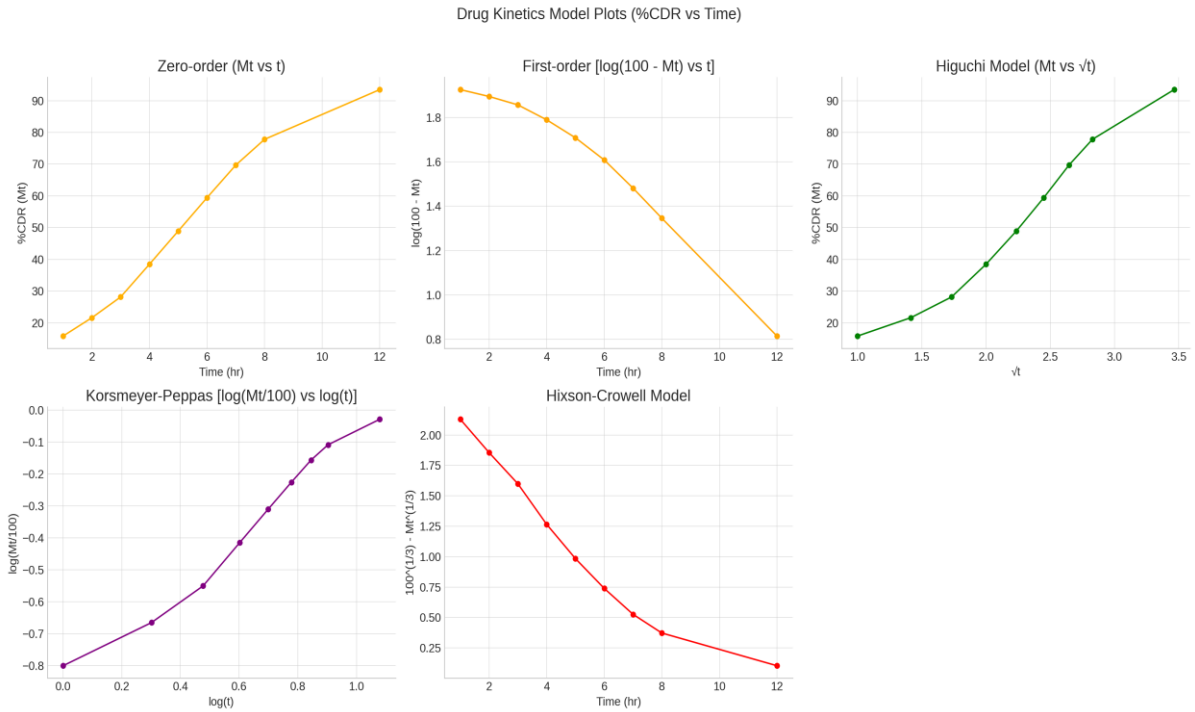


Fig. 7: release kinetics of proniosomal gel formulation F9.

Each model was subjected to linear regression analysis. The R^2 (coefficient of determination) values for each model were calculated to assess goodness of fit:

Table 11: R^2 of release kinetics model.

S.no	Model	R^2
1	Zero-order (Mt(%CDR) vs t)	0.964008
2	First-order [log(100 - Mt) vs t]	0.955856
3	Higuchi (Mt vs √t)	0.970707
4	Korsmeyer-Peppas [log(Mt/100) vs log(t)]	0.974223
5	Hixson-Crowell [(100^1/3 - Mt^1/3) vs t]	0.915927

INTERPRETATION

The Korsmeyer–Peppas model yielded the highest R^2 value (0.974223), indicating the best fit to the experimental data. The Korsmeyer-Peppas model is especially useful for understanding release mechanisms from polymeric systems such as proniosomal gels. This suggests that the drug release follows a diffusion-controlled mechanism, where both diffusion and erosion may play roles. Which is characteristic of matrix-based drug delivery systems. The Higuchi model also showed a good fit (R^2 = 0.970707), supporting the hypothesis of anomalous (non-Fickian) or diffusion-based release kinetics. However, since the Higuchi model and Korsmeyer–Peppas model specifically accounts for diffusion through a porous matrix, and erosion. It is considered the most representative of the actual release mechanism in this case.

HR-TEM analysis

HR-TEM showed that the particles have circular, uniform shapes. The dense, well-distributed pattern observed in Fig. In the electron micrographs of F9 Transmission reveals the structure of hydrated proniosomal vesicles that are well-defined. The proniosomes are unilamellar vesicles, spherical, Nano size, with sharp and well-separated limits.

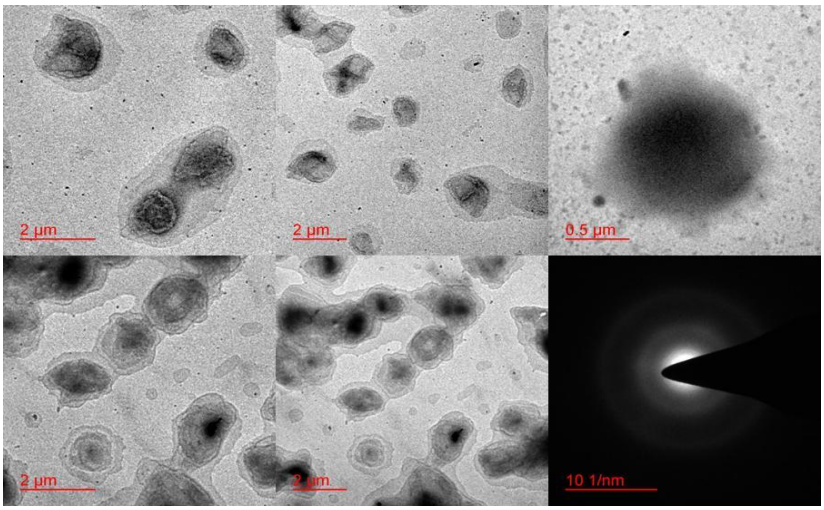


Fig. 8: HR-TEM analysis.

SEM analysis

SEM showed particles shapes in electron micrograph scanning reveals the structure of hydrated proniosomal vesicles that are well- defined.

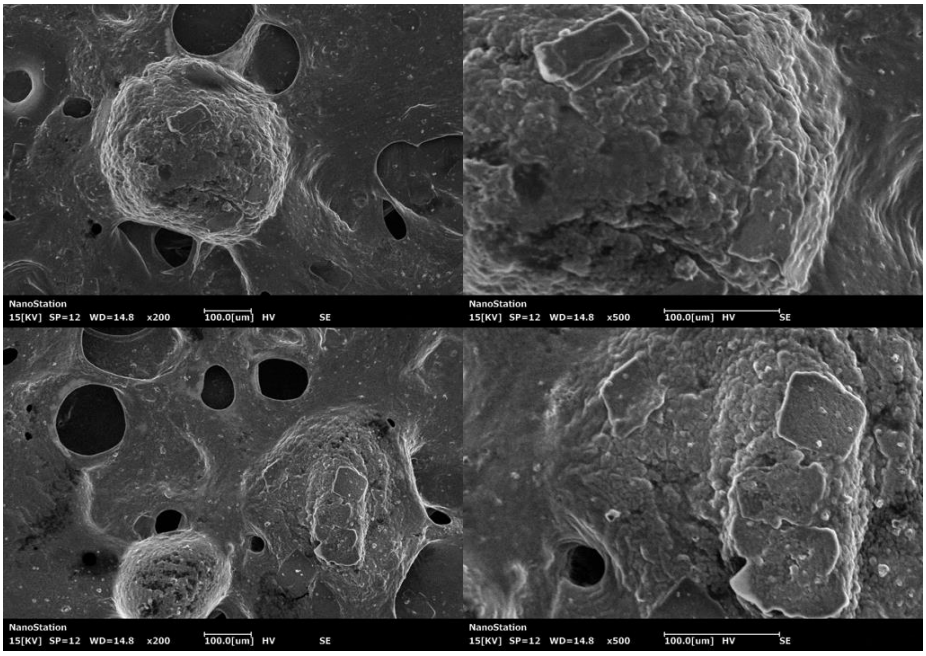
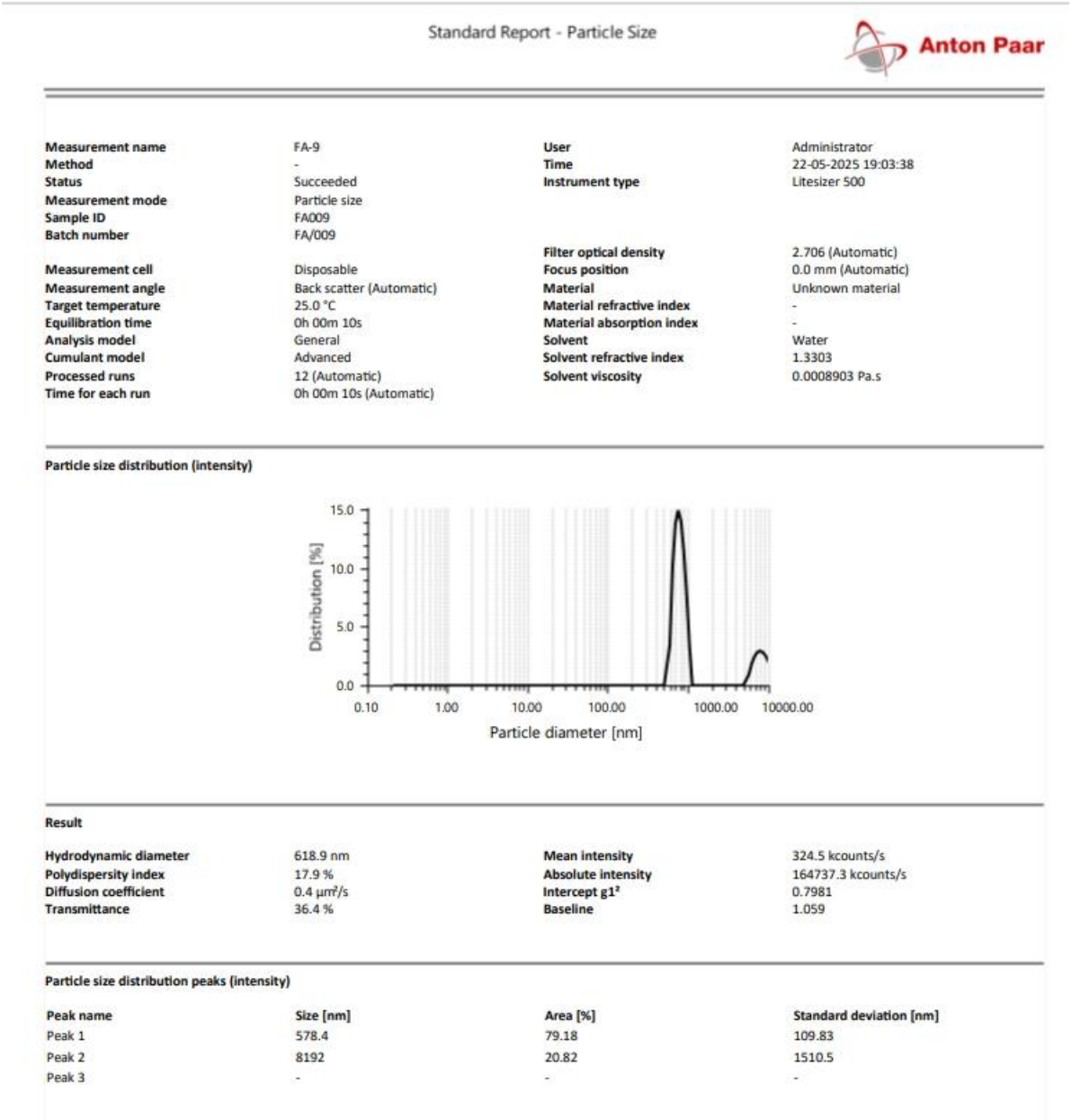


Fig. 9: SEM analysis.

Particle size

Particle size of formulation is analyzed by litesizer 500 and result found to be in nanometer range.



Standard Report - Particle Size

Anton Paar

Measurement name	FA-9	**User**	Administrator
Method	-	**Time**	22-05-2025 19:03:38
Status	Succeeded	**Instrument type**	Litesizer 500
Measurement mode	Particle size		
Sample ID	FA009		
Batch number	FA/009		
		Filter optical density	2.706 (Automatic)
Measurement cell	Disposable	**Focus position**	0.0 mm (Automatic)
Measurement angle	Back scatter (Automatic)	**Material**	Unknown material
Target temperature	25.0 °C	**Material refractive index**	-
Equilibration time	0h 00m 10s	**Material absorption index**	-
Analysis model	General	**Solvent**	Water
Cumulant model	Advanced	**Solvent refractive index**	1.3303
Processed runs	12 (Automatic)	**Solvent viscosity**	0.0008903 Pa.s
Time for each run	0h 00m 10s (Automatic)		

Particle size distribution (intensity)

Result

Hydrodynamic diameter	618.9 nm	**Mean intensity**	324.5 kcounts/s
Polydispersity index	17.9 %	**Absolute intensity**	164737.3 kcounts/s
Diffusion coefficient	0.4 µm²/s	**Intercept g1²**	0.7981
Transmittance	36.4 %	**Baseline**	1.059

Particle size distribution peaks (intensity)

Peak name	Size [nm]	Area [%]	Standard deviation [nm]
Peak 1	578.4	79.18	109.83
Peak 2	8192	20.82	1510.5
Peak 3	-	-	-

Fig. 10: particle size.

Zeta potential

Zeta potential of formulation is analyzed by litesizer 500 and the zeta strength of the prepared structure obtained within a range of 49.5 shows good stability of proniosomal gel.

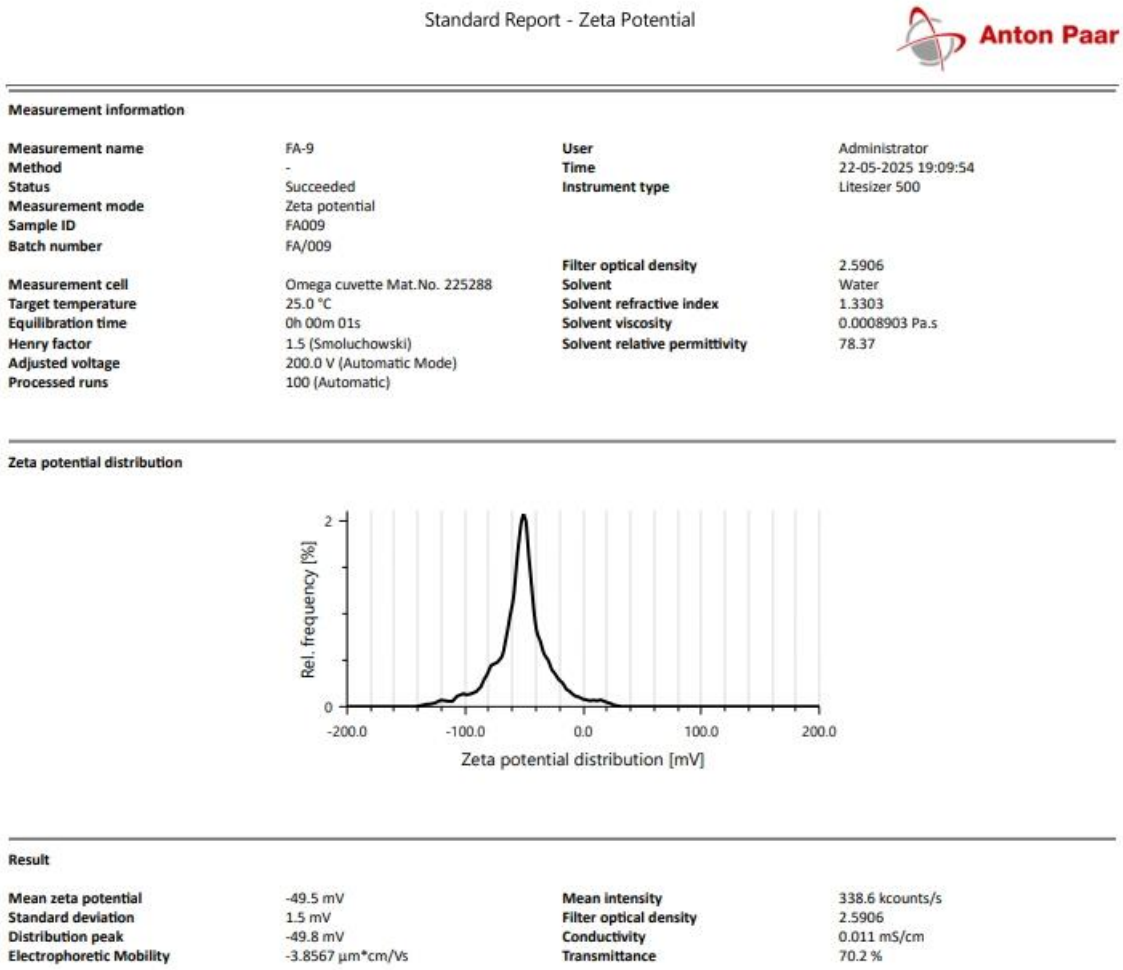


Fig. 11: zeta potential.

Stability studies

There was no change in colour, odour, drug content, rheological properties, pH, or phase separation during 90 days at varied temperature conditions (4°C, 25°C, and 45°C ± 2°C) reported in Table 10, as there was no major change in colour, odour, rheological properties, pH, or phase separation. As a result, it's possible to establish that formulation (F9) was chemically and physically stable.

Table 12: accelerated stability studies of formulation.

S.no.	Parameters	4°C ± 2°C	25°C ± 2°C	45°C ± 2°C
1	pH	7.03 pH	7.02 pH	7.01 pH
2	Viscosity in cps	8712 cps	8696 cps	8698 cps
3	Phase separation	Not found	Not found	Not found
4	Spreadability	Good	Good	Good

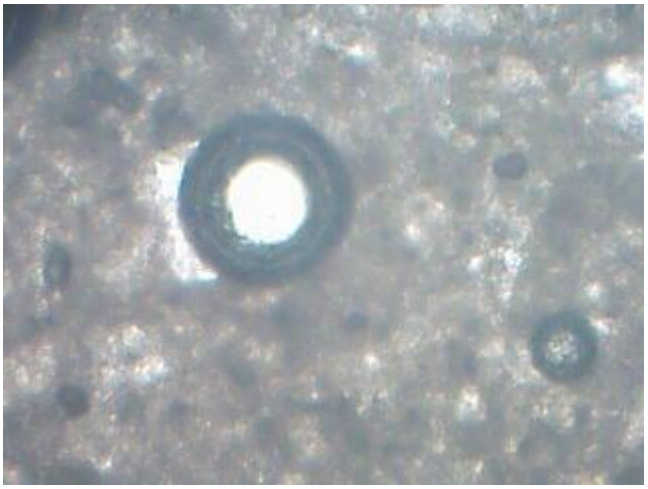


Fig. 12: microphotograph of formulation after 90 days.

DISCUSSION

The Allopurinol transdermal proniosomal gel was formulated, and all formulations were prepared for encapsulation efficiency, stability, dispersion, viscosity, pH & excipient interactions of the drug were examined are the most suitable formulas are containing span 40 and span 60 in equal measure among all species. The pH of the entire composition was about 6.80 to 7.26, indicating that there was no skin irritation. In terms of drug viscosity, the gel's composition can be categorized as follows F8>F9>F7>F5>F4>F6>F3>F2>F1. In comparison to the other gel formulation, the prevalence of F9 formulations, including allopurinol gel was good. The correlation coefficients (r) values suggested that the distribution profile followed Korsmeyer-Peppas model and supported by Higuchi model. In terms of allopurinol release levels, the gel's components can be organized in the following order: F9>F8> F7> F6> F5, > F4, > F3, > F2.> F1. From the permeation profile, it was clear that the F9 formulation containing span 40 & span 60 (50:50) proniosomal gel showed a drug release up to 12 h. The structure of F9 has been found to have better penetration and can be considered a candidate for the development of volume capacity forms. The encapsulation of drug in proniosomal gel formation ranges from 67.30% to 89.93%.

CONCLUSION

Prepared formulation of allopurinol based proniosomal gel using surfactant span 40 and span 60 and cholesterol used as stabilizer & to improve penetration lecithin i.e. from soya is used. Drug excipient compatibility was known through FTIR. Vesicle analysis was carried out through HR-TEM and SEM. By finally carrying out different evaluation parameters, F9 was found as the optimized formulation. The entrapment efficiency of F9 was 89.93% as the

surfactant and lecithin are increased, and the drug release is also increased to a certain extent. The release kinetics was calculated, and the best fit model is the Korsmeyer-Peppas model and supported by Higuchi model mechanism. These carrier systems have broad scope in the future, especially in transdermal drug delivery. This work will provide the benefits of controlled and sustained release activity, first pass metabolism, stability and versatility as a drug transporter, formulation with improved physical and chemical stability, and good bioavailability for a low soluble drug and the drug with short half-life. This Proniosomal gel makes a significant contribution to the development of transdermal research and offers hope for the development of a successful transdermal system that will be of value to society, industries and academy.

ETHICAL APPROVAL

There is no requirement for ethical Approval in this research.

AUTHORS' CONTRIBUTIONS

Carried out the formula have a look at carried out the pre formula have a look at & Drafted the manuscript. TB designed, & supervised it throughout the work.

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CONFLICT OF INTEREST

The authors do not have interests or struggle to share. The authors assert that they have no known conflicting financial interests or personal connections that may have been used to support the arguments made in this paper.

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