Section A-Research paper
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Development and Characterization of Drug loaded Novel Carrier for the Treatment of Arthritis

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ABSTRACT

A urate crystal buildup in joints and connective tissue (tophi) is a hallmark of gout, a metabolic condition that may manifest as acute or chronic episodes of arthritis. When it comes to the painful illness known as gout, high levels of serum uric acid (SUA) aren't necessary. Precipitating and depositing in joints, the kidneys, and the subcutaneous bone occurs when blood concentrations of uric acid are high due to the limited water solubility of uric acid. Hyperuricemia is the sole major cause of gout, but the majority of those who have it do not develop gout or even uric acid crystals. Unlike previous vesicular systems, transferosomes are a completely new kind of vesicular system. In addition to the edge activators, phospholipids, ethanol, and sodium cholate, they are administered to the skin in a non-occlusive manner. This project focuses on the two-factor model. Drug entrapment in transferosome vesicles and skin penetration are both improved by this method. Thereafter, the transferosome formulation was made by choosing the best-reported polymer ratio. At three different levels, we've analysed two independent variables. F1 through F9 have been

formulated in total. Among the F1-F9 batch, Formulation F4 has shown the most promising outcomes. Entrapment of 89.5 percent was achieved with Formulation F4 and in vitro penetration of 55.56 percent was achieved in 3 hours with Formulation F4. Particles with a diameter of 222 nm were observed. When compared to pure febuxostat gel, the transferosome gel demonstrated greater trapping and skin penetration (56.88 percent) (33.00 percent). A combination of febuxostat and quercetin transferosome gel has been shown to be more effective in reducing gout symptoms than a single febuxostat gel. So, the experiment demonstrates the synergistic impact of including bioactives into the equation.
Keywords: Febuxostat, transferosomes, gout

INTRODUCTION

A urate crystal accumulation in joints and connective tissue (tophi) is a characteristic of gout, a metabolic disorder that may present as acute or chronic bouts of arthritis. Even while gout is associated with high blood uric acid levels, it's not essential for this severe disease (SUA). High blood concentrations of uric acid precipitate in joints, renal, and subcutaneous bone [1]. The large majority of people with hyperuricemia do not develop gout or uric acid crystals in their urine. Only 5 percent of patients with hyperuricemia exceeding 9 mg/dl end up with gout, according to the American College of Rheumatology. Furthermore, it is also commonly acknowledged that genetic predispositions play a role in gout. In addition to environmental impacts, renal and intestinal excretion of urate is crucial to the management of serum urate, and genetic variables play a vital role [2]. For example, diuretics are used to treat hypertension, and they boost uric acid levels in the blood, raising the risk of gout. IFN- and NLRP3 inflammasome activation are crucial in the process. Beer or sugar-sweetened drinks, purine-rich foods like liver, shellfish, or anchovies, and obesity elevate gout risk. Crystal in synovium and outside the joint indicate gout. An assault may not produce much uric acid. [2, 3]. Transferosomes are first-generation flexible vesicles that were found to transport the desired concentration of medications via skin under non-occluded settings. They consist of phospholipids and an activator of the edge. Surfactants that destabilize lipid bilayers and increase bilayer deformation are known as edge activators [4, 5]. Because of the liposomes' flexibility, they are less likely to rupture and may follow the water gradient across the epidermis once they are applied [6, 7]. Transferosomes may distort and squeeze through microscopic openings smaller than their own size if they remain intact as a vesicle. Encapsulating drugs in transferosomes may help overcome the barrier function of the skin's outermost layer. Lipophilic, hydrophilic, and amphiphilic pharmaceuticals may all be encapsulated in these bilayer liposomes, making them more effective at penetrating the body than traditional liposomes [8, 9]. Gout causes acute joint discomfort and synovial fluid and lining irritation owing to monosodium urate crystal deposition (MSU). Despite the fact that hyperuricemia is frequently linked to gout, it is not a precondition for this excruciating condition (SUA). Purine metabolism results in uric acid, a water-insoluble compound that precipitates and accumulates in joints, kidneys, and subcutaneous bone when blood concentrations are elevated [10, 11]. The risk of gouty arthritis is roughly the same for men and women at any given concentration, although males are more

likely to develop hyperuricemia. Gout is as common in postmenopausal older women as it is in men [10, 12].

Table 1: Several types of gout [6, 9, 11]

S. No.	Types	Properties
1	**Asymptomatic Gout**	Only uric acid levels in the blood are elevated, but this kind of gout does not cause gouty arthritis or kidney damage.
2	**Acute Gout**	When inflammation spreads quickly and causes pain and swelling in one joint, it's known as septic arthritis. In joints or tissues around joints, uric acid crystals trigger an inflammatory response. However, the knees, and the great toe are all involved. It's fully reversible and cured.
3	**Chronic Gout**	Long-term joint inflammation, which produces discomfort in the joints at rest and/or during movement. It may develop after years of acute gout, and it can cause a wide range of health problems, including peripheral pain, joint stiffness, and renal failure.
4	**Pseudo Gout**	There are crystals of calcium pyrophosphate rather than urate that build up in the joints in this kind of arthritis, which causes inflammation. CPPD (calcium pyrophosphate dihydrate) deposition disease is another name for this disorder.

Prevention

Alcohol, sugar (high fructose corn syrup) [11, 13] and meals high in purine, such as organ meats and shellfish, may reduce gout attack risk. Gout attacks may be prevented by eating dairy products, vitamin C, coffee, and cherries, as well as by losing weight. It is possible that sleep apnoea may cause gout by releasing purines from cells that have been deprived of oxygen. Apnoea treatment may reduce the frequency of episodes [12, 13].

Mechanism of Action

Xanthine oxidase sequentially oxidises hypoxanthine and xanthine to create uric acid in humans. Xanthine oxidase inhibition increases hypoxanthine, xanthine, and uric acid (Figure 1) [13, 14].

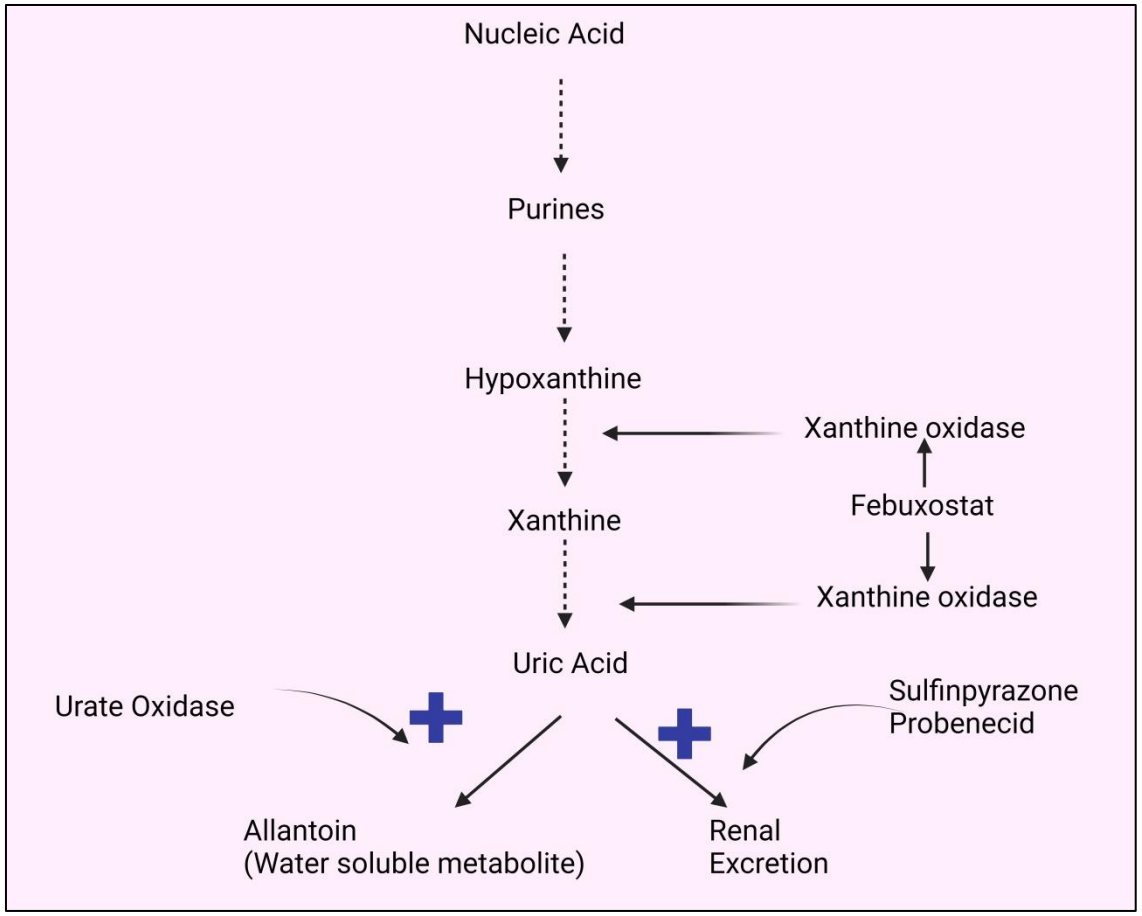


Figure 1: Mechanism of action of febuxostat

This study aimed to develop a transdermal delivery method for allopurinol, a poorly soluble medication. To accomplish this, it was postulated that if we integrate allopurinol into nano drug carriers such as transferosomes and then into transdermal gel formulations, we may obtain improved penetration and release of the medication via transdermal routes. Transferosome gel may be utilised to make superior permeation allopurinol formulations.

MATERIALS AND METHODS

GlR Innovation, Delhi, India, produced Febuxostat. New Delhi's Central Drug House sold Soy Lecithin and Triethanolamine. Mumbai-based Thomas Baker Pvt. Ltd. supplied Tween 80. All compounds utilised were analytical grade. The Institutional Animal Ethics Committee (IAEC) authorized the experimental methods according to CPCSEA criteria (Enrolment No.: IAEC/NIET/2020/01/18).

Preparation of Transferosomes Formulation

Thin film dispersion-hydration prepared the transferosome. Soya lecithin, surfactants were dissolved in 20 ml ethanol and 10 ml chloroform. Using a rotary evaporator, a homogenous thin lipid layer is created. The film is hydrated with pH 6.5 phosphate buffers for 1 hour at 20°C to achieve rough dispersions. Dispersions were sonicated for 9 minutes under 400 w. Size reduction follows extrusion through 0.45 mm Nylon-66 membranes (Figure 2 and 3).

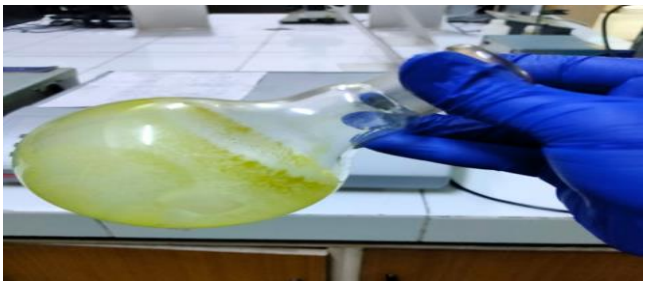


Figure 2: Thin lipid formulation

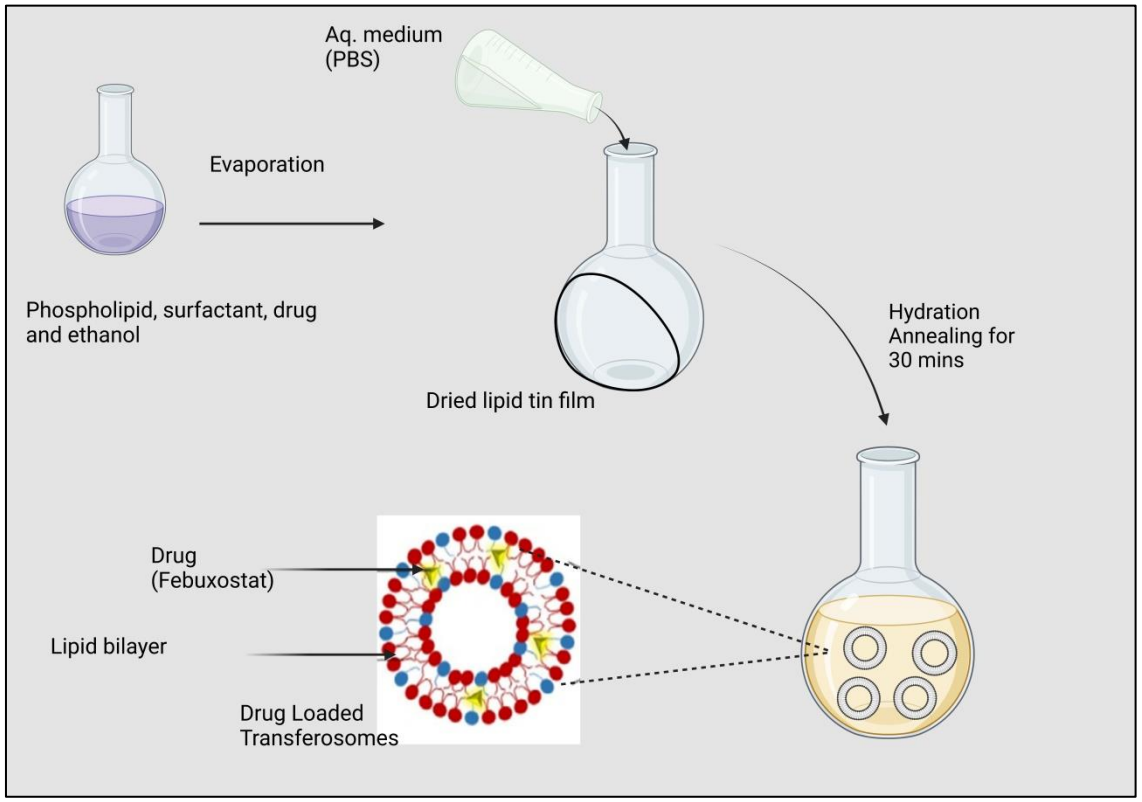


Figure 3: Formation of a transferosomes

Formulation of Transferosomes

Table 2: Ingredients to prepare transferosomes

SI. No	Ingredients	Amounts
1.	Febuxostat	50 mg
2.	Quercetin	10 mg
3.	Soya lecithin	85 mg
4.	Ethanol: Chloroform	2:1
5.	Edge activator	10 mg
6.	Hydration medium	20 ml

Table 3: Optimization of transferosomes development

Formulation Code	Soya Lecithin (mg)	Edge Activator (mg)	Drug (mg)	Solvent	Hydration medium
F1	90	20	50	2:1	20
F2	90	15	50	2:1	20
F3	90	10	50	2:1	20
F4	85	20	50	2:1	20
F5	85	15	50	2:1	20
F6	85	10	50	2:1	20
F7	80	20	50	2:1	20
F8	80	15	50	2:1	20
F9	80	10	50	2:1	20

Characterization of Transferosome Formulation

Percentage Drug Entrapment Study [8, 15]

Transferosomal 170 mg transferosomes are dissolved in 100 ml of pH 6.4 phosphate buffer and sonicated for 4 hours at 30^0C. Sample filtering using 0.2 m membrane filter. After filtering, the sample was compared to a blank using a UV-Vis spectrophotometer at 304 nm.

Percentage Entrapment efficiency (EE) = (WT - WF) /WT×100

Where percent EE is drug entrapment efficiency, WT is total febuxostat in transferosomal suspensions, and WF is free febuxostat in the supernatant.

Particle Size

Particle size and size distribution can be determined by dynamic light scattering (DLS).

FT-IR Spectrophotometric Study of Transferosomal Formulation

Organic functional groups were found in transferosomal solution FTIR. All formulations' FTIR images were taken at 4000-400 cm-1 (Model No. 234, Perkin Elmer).

Scanning Electron Microscopy (SEM)

SEM from LPU Punjab utilising JOEL (Japan) JSM-6490LV discovered transferosome surface morphology.

In-vitro Release Study

Transferosomal formulations were tested using 0.75 cm^2 Franz diffusion cells. The egg membrane separates the donor and receptor compartments. To simulate physiological circumstances, the receptor compartment was agitated by a magnetic bead and kept at 37 ± 1^oC. 1 cc transferosomal suspension was loaded in the donor compartment. Using a UV-Vis spectrophotometer, medication concentrations in aliquots were measured against a blank at 250 nm [16-18].

Stability Study

Stability studies give information on a drug's quality that varies over time owing to heat, moisture, and illumination. Physicochemical characteristics and drug content were used to choose stable formulations. The selected formulations were sealed in vials and stored at 4±2ºC, 25±2ºC and at 40±2ºC and 75±5% RH for 1 month following ICH standards.

RESULTS AND DISCUSSIONS

Physical Appearance: The I.P. describes febuxostat as a white, odourless powder.

Table 4: Physical compatibility studies for febuxostat

Sl. No.	Parameter	Ratio	Initial	Control	40^{O}C, 75 % RH	60^{O}C
1	Febuxostat	Control	White	Nil	Nil	Nil
2	Quercetin	Control	Yellow	Nil	Nil	Nil
3	Soya lecithin	Control	Slightly Yellow	Nil	Nil	Nil
4	Sodium deoxycholate	Control	White	Nil	Nil	Nil
5	Febuxostat + Quercetin	1:1	yellow	Nil	Nil	Nil
6	Febuxostat + Soya lecithin	1:1	Yellow	Nil	Nil	Nil
7	Febuxostat + Sodium deoxy cholate	1:1	Yellow and white	Nil	Nil	Nil
8	Febuxostat + Soya lecithin+ Sodium deoxy cholate	1:1:1	Yellow and white	Nil	Nil	Nil
9	Febuxostat + Soya lecithin + Sodium deoxy-cholate + Quercetin	1:1:1:1	Yellow and white	Nil	Nil	Nil

Melting Point: Melting point of febuxostat was found to be 211ºC.

FTIR Studies: Figure shows FTIR spectra of pure febuxostat, individual excipients, and drug-excipient combo (Figures 4-7).

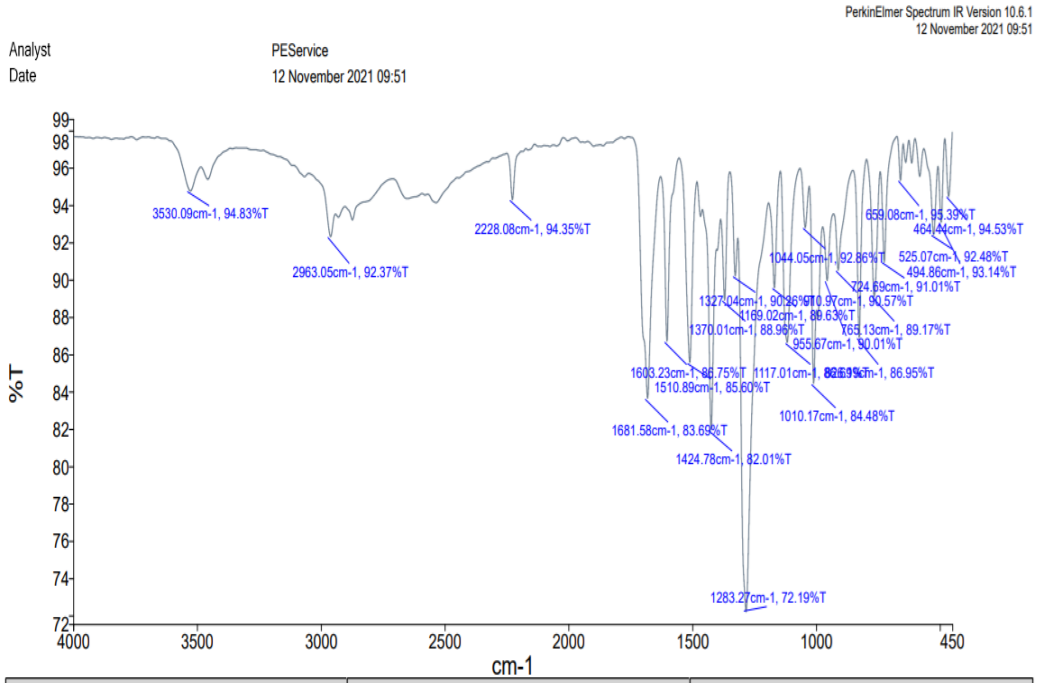


Figure 4: FTIR of febuxostat

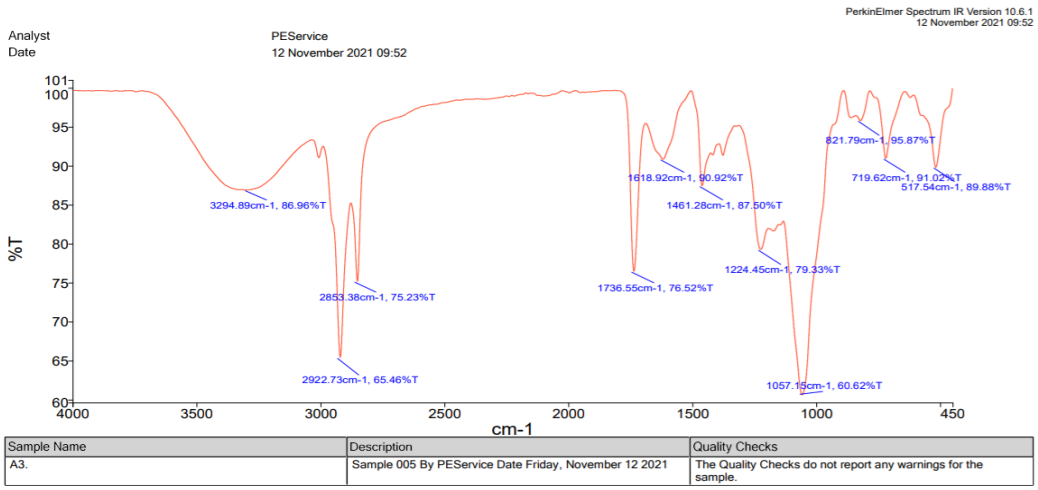


Figure 5: FTIR of Soy Lecithin

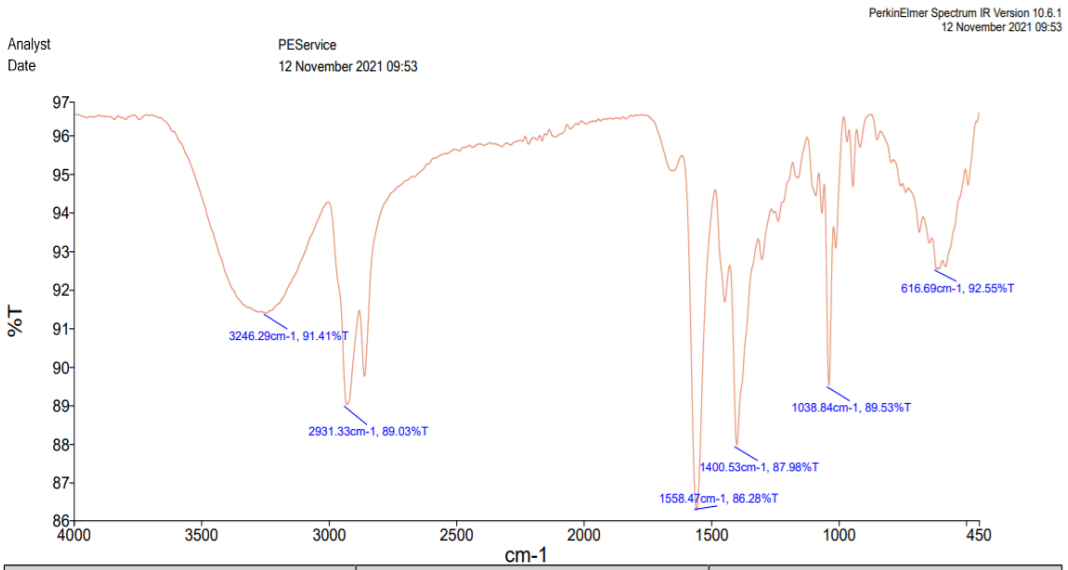


Figure 6: FTIR of EDGE activator

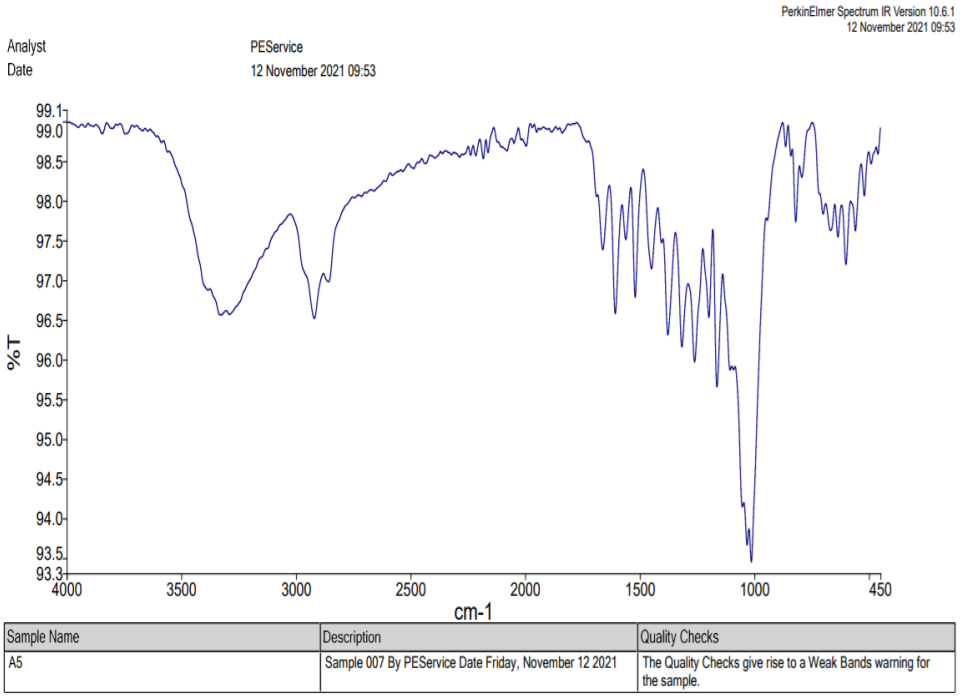


Figure 7: FTIR of F4 formulation

UV Spectrophotometric

Linearity was evaluated by creating standard solutions and graphing absorbance versus analyte concentration [16, 19]. Beer's rule was followed at 2-14 g/ml.

Table 5: Reports a 0.9959 determination coefficient.

Sl. No.	Parameters	Observations
1	Linearity	2-10 µg/ml
2	Intercept	0.42
3	Determination coefficient	0.9959

Table 6. Describes the standard curve data of febuxostat

S. No.	Concentration (µg/ml)	Absorbance at 304 nm
1	2	0.1691
2	4	0.2593
3	6	0.3372
4	8	0.4568
5	10	0.5463

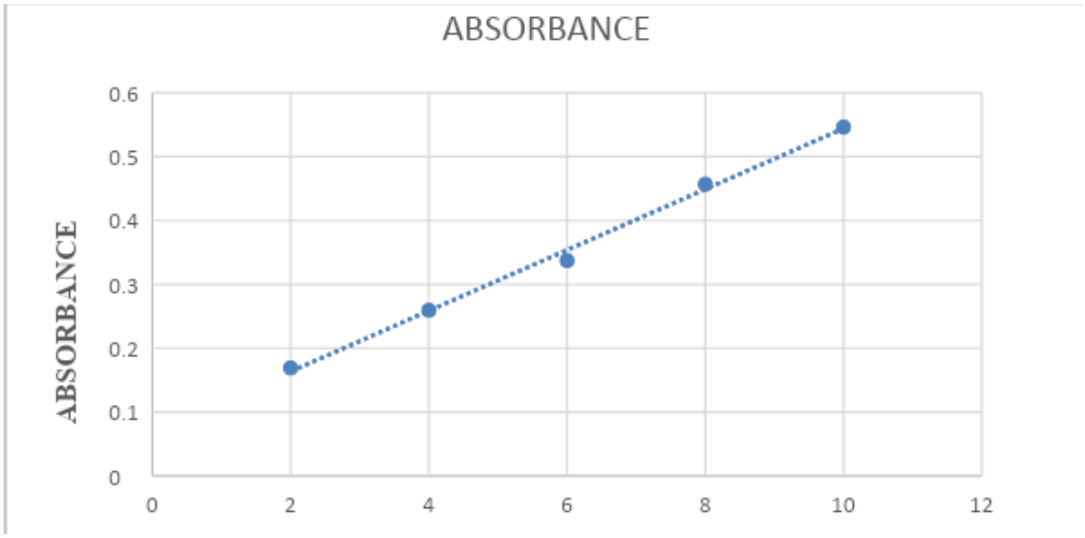


Figure 8: Calibration curve of febuxostat

Percentage Drug Release

Table 7. Drug release profile of optimized formulations

Time (hour)	F1	F2	F3	F4	F5	F6	F7	F8	F9
0	0	0	0	0	0	0	0	0	0
0.5	3.86	5.16	3.17	0.4	7.93	5.38	5.18	4.82	3.92
1	8.43	10.27	5.96	11.6	15.49	11.27	10.7	9.64	7.84

1.5	12.6	15.28	9.73	18.5	27.62	17.29	15.6	15.7	12.76
2	16.83	19.58	12.27	26.4	29.94	21.52	22.9	19.3	15.68
2.5	20.63	24.83	13.95	36.8	34.45	26.9	26	24.1	20.7
3	23.4	29.74	16.74	45.4	41.34	33.18	33.8	29.8	23.52
3.5	27.3	33.04	20.74	53.2	50.72	37.66	36.3	33.7	28.65
4	34.83	39.41	24.68	55.6	50.41	45.92	42.8	40.7	31.36

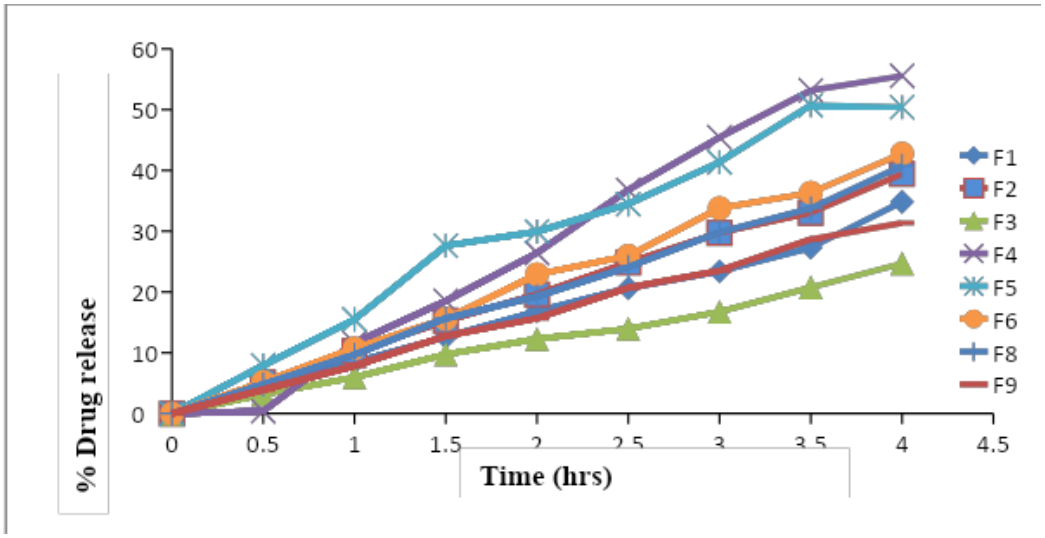


Figure 9: Percentage drug release

Percentage Entrapment Efficiency

Table 8: Percentage of encapsulation efficiency of different drug loaded transferosomes formulation

S. No.	Formulation Code (F1-F9)	EE%
1.	F1	78.9
2	F2	83.4
3.	F3	88.1
4	F4	89.5
5.	F5	87.5
6.	F6	74.4
7.	F7	78.04
8.	F8	74.6
9.	F9	68.02

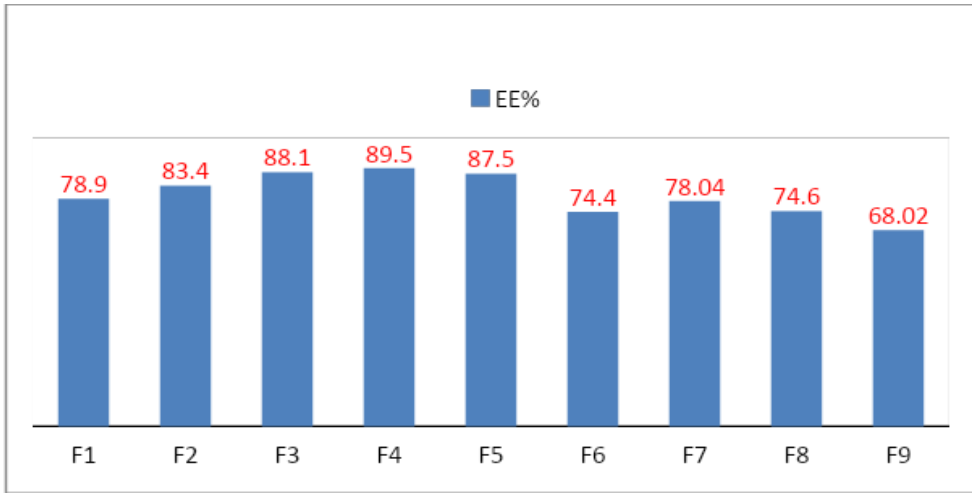


Figure 10: Percentage entrapment efficiency

Mean Particle Size

Table 9: Describes the particle size of different optimized formulation

S. No.	Formulation Code (F1-F9)	Particle size nm
1.	F1	298
2	F2	278
3.	F3	280
4	F4	222
5.	F5	295
6.	F6	310
7.	F7	305
8.	F8	356
9.	F9	317

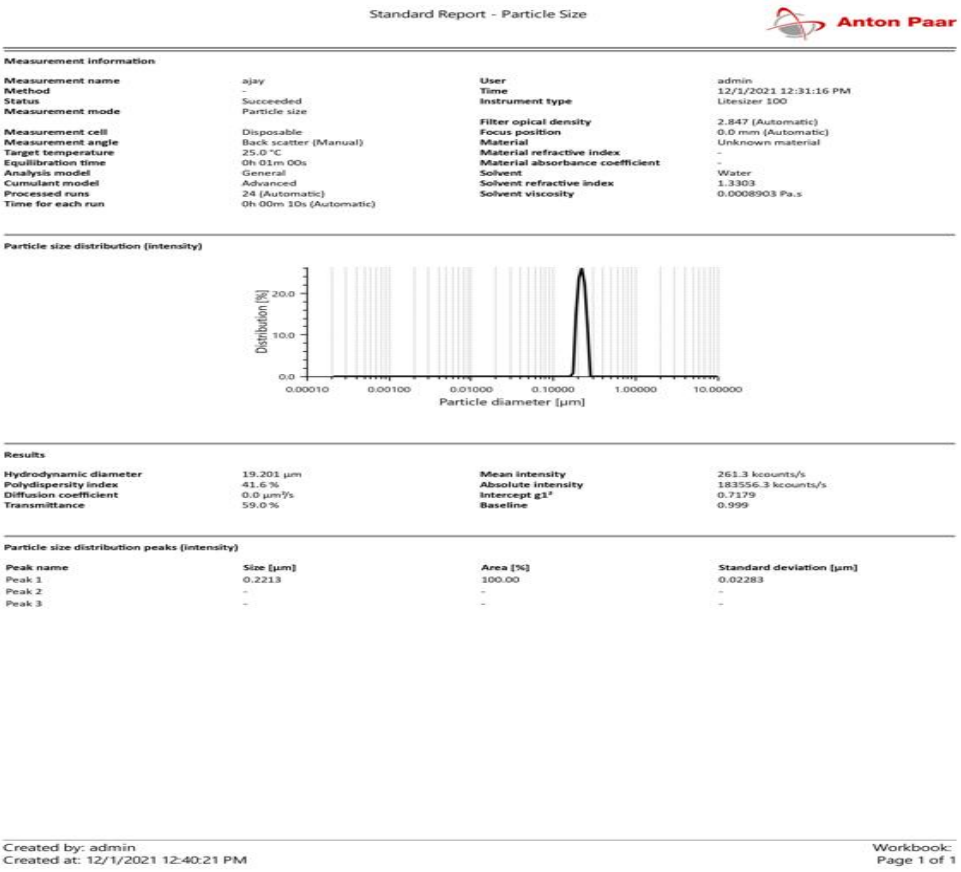
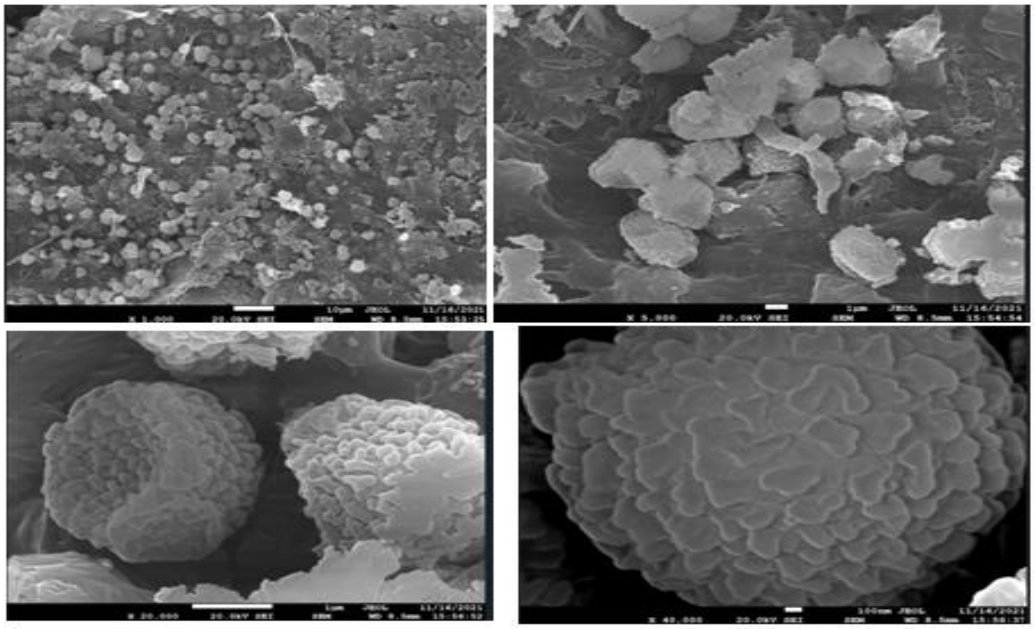


Figure 11: Particle size



SEM of Transferosomes Formulation

Figure 12: SEM of transferosomes

In Vivo Study

Measurements of knee breadth, uric acid in synovial fluids, and X-ray images of joint interspaces were used to evaluate an *in-vivo* study on the transferosomal gel (A1). On day 'First', MSU crystals were infused, and then on day '21', a combination transferosomal gel was applied transdermally. There was a significant improvement in rabbit knee measurements and decreased aggravation at the end of the study (45 days). Treatment levels for both treatment and control groups are listed in a table [20, 21].

Table 10. The knee joints of rabbits were inflamed after being injected with MSU crystals (n=3).

Group	Animal	Number of days		
		1st days	21 days	45 days
Control Group I treated with 0.9% NaCl	C1	1.22	1.22	1.22
	C2	1.20	1.21	1.22
	C3	1.23	1.22	1.23
Test group II treated with transferosomal gel A1	T1	1.26	1.25	1.21
	T2	1.25	1.24	1.22
	T3	1.24	1.25	1.23
Standard Group III treated with standard group febuxostat + quercetin	P1	1.27	1.22	1.18
	P2	1.28	1.23	1.19
	P3	1.26	1.24	1.20

CONCLUSION

Formulation (F4) has 89.5% entrapment effectiveness and 222 nm particles. Combining febuxostat and quercetin into transferosomal gel prolongs its therapeutic efficacy after transdermal delivery. Transferosomal transports in gel showed steady and prospective topical febuxostat dispersion with better trap efficacy and stability. Controlled particle size and optimal permeation flow were the study's most significant results. Transferosomes may breach the skin's barriers. Combination therapy in transferosomal gel was a great alternative for reducing oral medication adverse effects and improving patient compliance. The project's findings show: The improved formulation effectively produced febuxostat transferosome and gel. *Ex-vivo* investigations showed that febuxostat transferosome gel penetrated better than febuxostat gel and transferosome standard formulation.

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