ORIGINAL ARTICLE

Optimization of Ibuprofen Nanosponges Herbal Gel for Anti-Inflammatory Action

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Abstract

Introduction: The main purpose of the research was to formulate Ibuprofen nanosponges herbal gel to reduce the inflammation in rheumatoid arthritis patients. **Materials and Methods:** The emulsion solvent diffusion method was used to formulate ibuprofen nanosponges using a 3-factor, 3-level Box-Behnken design. A Box-Behnken design was selected with X1, X2, and X3 (quantity of ethyl cellulose, quantity of PVA, and speed, respectively) as factors and (*ex vivo* drug release, entrapment efficiency, and production yield) as responses which resulted in 15 runs. After running with 15 formulations, they were optimized according to physico-chemical characterization. Further, it was prepared and evaluated. **Results:** After evaluation, the following results were obtained: percent production yield (82.9 ± 0.43%), entrapment efficiency (88.1 ± 0.46%), permeation studies (43.2 ± 0.28% in 6 h), and flux (1.63 ± 0.3 μg/cm^2/h). SEM studies showed the spherical shape with nano-sized pores, particle size (214.3 nm diameter), zeta potential (−37.8 mV), and PDI (0.341). PNS was formulated into gel with 5% tea tree oil and was evaluated. The optimized gel formulation (G3) showed a viscosity of 4.1487 × 10^{5}cps and *ex vivo* release of 24.64 ± 47.72% after 6 h, follows 1st order kinetics and anomalous release mechanism. The optimized gel formulation and marketed diclofenac sodium gel were studied for anti-inflammatory studies for 4 h. By measuring the paw edema in a rat animal model, edema reduction in 4 h shows 0.2 ± 0.1 cm which is equal to control. **Conclusion:** It was found that ibuprofen nanosponges herbal gel reduces the inflammation in rheumatoid arthritis.

Key words: Ibuprofen, nanosponges, tea tree oil, rheumatoid arthritis

INTRODUCTION

There is an advantage with topical drug delivery over other delivery systems, such as bypassing the gastrointestinal tract (GIT), avoiding GI irritation and hepatic first-pass metabolism, and reaching directly to the desired site to reduce adverse reactions (ADRs).[1] Tiny mesh-like structures called nanosponges with a size >1 μm can easily escalate the bioavailability by binding to the drugs that are poorly soluble, leading to better solubility of such drugs.[2]

Rheumatoid arthritis being a chronic inflammatory condition thus results in painful swelling. Joint deformity and bone degradation are brought on by chronic inflammation. CD4 and T-cells are activated by the CD28-CD80 pathway and other pathways, leading to the differentiation of synovial CD4 (clusters of differentiation 4) and T-cells into T helper cells. T-cells also secrete IFN-alpha (Interferon-α) and IL-17(Interleukin-17), which cause bone avulsions. The treatment for rheumatoid arthritis must block the production of COX1(Cyclooxygenase), COX2, and TNF-alpha. NSAIDS (non-steroidal anti-inflammatory drugs), corticosteroids, glucocorticoids, DMARDs, and biological agents are used in rheumatoid arthritis that suppresses COX1 and COX2. Ibuprofen is a class II drug that has low solubility and low bioavailability. Ibuprofen is an anti-inflammatory agent that inhibits the synthesis of COX 1 and COX 2 enzymes.[3,4]

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Received: 16-08-2024
Revised: 17-11-2024
Accepted: 01-12-2024

Inflammation is a process that is concomitant with pain and involves events, such as an increase in vascular permeability, an increase in protein denaturation, and membrane alteration. There are medications for controlling and suppressing inflammation. To achieve increased pharmacological response and low side effects, there is a need to apply natural anti-inflammatory factors within medication therapy.[5,6] Tea tree oil is used to inhibit the synthesis of TNF-α and IL-1, which causes inflammation of joints and leads to rheumatoid arthritis. Water-soluble components can inhibit the lipopolysaccharide-induced production of the inflammatory mediator's tumor necrosis factor-alpha (TNF-α), interleukin-1α (IL-1), and IL-10 by human peripheral blood monocytes by approximately 50% and that of prostaglandin E2 by about 30%. [7,8] However, dosage forms are available along with either tea tree oil or ibuprofen, but there are no formulations found in combination. Hence, efforts were made to formulate nanosponges using natural ingredients such as tea tree oil, as it helps in relieving the pain caused by rheumatoid arthritis. The topical route is preferred for enhanced drug targeting.

MATERIALS AND METHODS

Ibuprofen was acquired from Yarrow chem, Mumbai; ethyl cellulose (EC), polyvinyl alcohol (PVA), and dichloromethane were purchased from Sd Fine-Chem Limited.

INTERACTIONS BY FTIR

The spectra of pure drugs and physical mixtures with excipients were analyzed using FTIR (Shimadzu (Kyoto, Japan) facility (model-8400S)), IR spectrum from 4000$^{-1\ cm}$ to 500$^{-1\ cm}$.

DSC

DSC investigations were performed by DSC-60, Shimadzu Corporation, Japan, to ascertain the compatibility.

PREPARATION OF IBUPROFEN NANOSPONGES[9,10]

Nanosponges are prepared by the emulsion solvent diffusion method. The weighed amount of PVA and distilled water form an external phase. The water phase was added dropwise to the oil phase using a syringe. The emulsion was kept on a high-speed homogenizer to get nanosponges. Using Design Expert 11 (Version 11; Stat-Ease Inc., Minneapolis, MN), a three-factor, and three-level Box-Behnken design (BBD) were used as given in Table 1.

Table 1: Factors and factor levels of the box–Behnken experimental design

Independent factors	Levels		
	Low	Medium	High
	1	1	1
1. X1=Quantity of EC (mg)	200	500	1000
2. X2=Quantity of PVA (mg)	50	100	150
3. X3=Speed (rpm)	1800	2000	2200
Responses (dependent factors)			
1. R1=*ex vivo* drug release (%)	Minimum is better		
2. R2=Entrapment efficiency (%)	Maximum is desirable		
3. R3=Production yield (%)	Maximum is desirable		

EC: Ethyl cellulose, PVA: Polyvinyl alcohol

EVALUATION OF THE FORMULATIONS

All the 15 preparations obtained by BBD were examined for the following:

Percent production yield (%)

The percent production yield is calculated by:

$$Production\ yield = \frac{Pratical\ yield\ of\ nanosponges}{Theoritical\ yield} \times 100$$

Assay

Nanosponges equal to 100 mg equivalent drug were taken, crushed into a powder, and dissolved in methanol. Followed by filtration, and were examined using a UV spectrophotometer.

Angle of repose

The angle of repose was determined using the formula given below, and the funnel method was used for the same.[11]

Tan θ =h/r; Where θ = angle of repose, h = height (in cm), and r = radius (in cm).

Particle size measurement

The particle size was scanned using a microscopic technique. Powder with a drop of glycerin was placed on the slide and it was placed on the stage microscope.[12,13]

Entrapment efficiency

Take 5 mg of ibuprofen in 10 ml of pH 7.4 phosphate buffer saline. Then it is ultra-centrifuged at 15000RPM for ½ h in

2 cycles. It was calculated using the formula.[12,13]

$$Entrapment\ efficiency = Total\ amount - \frac{Unentrapped\ amount}{Total\ amount} \times 100$$

Ex vivo permeation studies[14]

It was conducted in accordance with IAEC approval no:GPRCP/IAEC-1/27/03/2023/PCE/AE-1.Male Wistar rats were sacrificed by cervical dislocation, and hair was removed from the abdomen using an animal hair clipper. Abdominal skin sections were observed for the existence of cuts and wounds. It was cleaned and washed with water, and *ex vivo* release studies were performed.

Permeability parameters[15]

(A) Steady-state flux (µg/cm^2/h)

Steady-state flux (Jss) may be defined as drug permeated per unit cross-section area multiplied by time.

$$Jss = \frac{dQ}{A} dt$$

(B) Lag Time (hrs)

It is calculated by plotting the cumulative amount of drug permeated vs. time. The x-intercept value gives the lag time.

(C) Permeability coefficient (cm/h).

The permeability coefficient (Kp) can be defined as flus per total donor concentration of the formulation.

$$Jss = \frac{dQ}{A} dt$$

(D) Enhancement ratio

Enhancement ratio (ER) may be defined as the flux of the drug with enhancer per flux of the control.

$$ER = Jss\ of\ drug\ with \frac{enhancer}{Jss\ of\ control}$$

Skin deposition studies[14,15]

After performing the permeation test, this was performed in accordance with IAEC approval no: GPRCP/IAEC-1/27/03/2023/PCE/AE-1.The skin was removed, the surface was washed with buffer, and then the skin was cut into pieces. The skin was kept for 24 h in a beaker of 20 ml of methanol, then homogenization was done, and the resultant was then diluted with pH 7.4 phosphate buffer. This was examined using a UV spectrophotometer.

Table 2: Formulation of nanosponges incorporated gel

Ingredients	G1	G2	G3
Ibuprofen (mg)	1000	1000	1000
Tea tree oil (mL)	-	0.5	0.5
Carbopol 974 (mg)	200	200	200
Triethanolamine (mL)	0.1	0.1	0.1
Ethanol (mL)	3	3	3
Distilled water (mL)	100	100	100
Preservatives (methylparaben and propylparaben)	0.1	0.1	0.1

G2: Optimized formulation, G3: Ibuprofen Nanosponges with Equivalent amount of 1000 mg of Ibuprofen

Drug kinetics

To understand the mechanism and kinetics, the *ex vivo* data were related to the kinetic models.

EVALUATION OF THE OPTIMIZED FORMULATION

Further, the optimized formulations were assessed for the SEM, zeta potential, and particle size.

Scanning electron microscopy

This test was accomplished using JIB 4700FIB, JEOL Ltd., Tokyo, Japan. Samples were kept under vacuum and coated with the layer of gold operated at a 15 kV acceleration voltage.

Zeta potential and particle size

Particle size and PDI were checked using Delsa Max PRO Zeta Potential Dynamic Light Scattering Analyzer B2916 at 25°C. The charge of itwas known using a Zeta sizer (Delsa Max PRO Zeta Potential Dynamic Light Scattering Analyzer B2916).

PREPARATION AND EVALUATION OF NANOSPONGES GEL[9]

Preparation of gels

Ibuprofen herbal gel was formulated by dissolving carbopol in the water, then tea tree oil, ethanol, and triethanolamine were added to the same according to Table 2.

EVALUATION OF GEL[16]

Clarity

The clarity of the prepared formulation was determined visually under a black and white background, and it was rated as follows: turbid+, clear ++, and very clear +++.

Determination of pH

The pH was determined using a digital pH meter by dispersing 1 g of formulation in 100 ml of pH 7.4 phosphate buffer saline.

Drug content

100 mg of formulation was taken in 100 ml of buffer, and drug content was calculated after proper filtration and dilutions.

Homogeneity

It was determined by visual inspection for the appearance of gel and the existence of any agglomerates.

Spreadability

This is done by determining the spreading diameter of 1 g of gel between 20 × 20 cm glass plates after 1 min. The spreadability was calculated using the following formula.

$S = m \times l/t$ were, S = spread ability, m = weight tied to the upper glass slide, l = length of the glass slide, t = time taken in seconds.

Determination of viscosity

The viscosity of gels was known by Anton Paar Rheo Compass (2022), and the shear stress of optimized formulations was examined.

Anti-inflammatory and stability studies[17]

Group 1 (control), Group 2 (carrageenan-induced) 0.1 ml of carrageenan, Group 3 (carrageenan + 1.5% diclofenac gel) 0.1 ml of carrageenan, Group 4 (carrageenan + ibuprofen gel), Group 5 (carrageenan + tea tree oil gel), Group 6 (carrageenan + ibuprofen nanospongesgel). 0.1 ml of carrageenan was rubbed to the paw of rats, after which observed and measured edema in accordance with time. Ibuprofen nanosponges with tea tree oil gel were applied to the paw and measured the edema after 2 h.

Procedure[17,18]

Rats were weighed, marked, and divided into groups. Carrageenan solutions were prepared (1%w/v, in normal saline) and used to induce inflammation. After each time interval, the paw edema was measured with vernier calipers at 0, 1, and 2 h after carrageenan administration. The gel formulations were applied to the left hind paw by gently rubbing and compared with the control.

Stability studies[18]

The formulation was assessed for accelerated stability studies as per ICH guidelines for 1 month and evaluated for various tests.

RESULTS AND DISCUSSION

Drug-excipient compatibility study by FTIR

Studies were done to study the interaction between the drug and excipients.

It shows compatibility, as depicted in the Figure 1 below.

In the region of 3000–2800 cm^{-1} (O-H stretching) confirms the alcoholic compound, 1750–1500 cm^{-1} (C=O stretching) shows the Carbonyl group, and 1500–1250 cm^{-1} shows the Alkyne group. This indicates no interaction. There was compatibility between drugs and excipients.

Differential scanning calorimetry

This study of ibuprofen, optimized formulation (PNS), was performed, and the results are observed in Figure 2.

EVALUATION OF FORMULATION BY BOX-BEHNKENDESIGN

15 formulations were studied for the percent production yield (Table 3), assay (90.6 ± 0.05 to 96.479 ± 0.01%), flow properties, Carr's index within 9.09 ± 0.15–20 ± 0.25% range, Hauser's ratio within 1.1 ± 0.19–1.25 ± 0.22 range, and angle of repose within 35.2 ± 0.2°–39.69 ± 0.10° range indicates good-fair flow properties. The particle sizes of 15 formulations were in the range of 45–75 µm. From fifteen preparations, F9 showed a high percentage yield, high drug content, and excellent flow properties.

Entrapment efficiency (E.E.)

E.E. of 15 formulations ranges from 62 ± 0.1% to 95 ± 0.5%. F9 was found to have the highest entrapment efficiency of 95± 0.5%.

Ex Vivo permeation studies

The *ex vivo* permeation studies of all 15 formulations were performed. The pure drug shows (5.04 ± 0.76 % to 22.6 ±

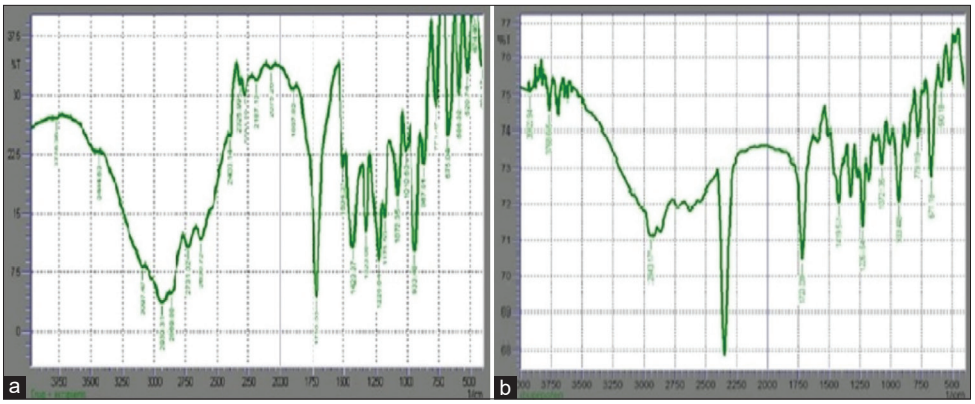


Figure 1: (a and b) FTIR spectra of Ibuprofen Pure Drug, FTIR spectra of Ibuprofen with EC and PVA

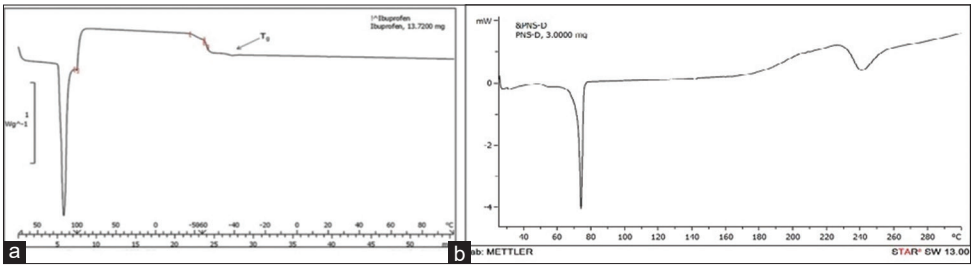


Figure 2: (a and b) DSC of Ibuprofen, DSC of PNS

Table 3: Design layout: Formulation with response

FC	Drug (mg)	EC (mg)	PVA (mg)	Speed (rpm)	E.DR	E.E	P.Y
F1	1000	500	100	2000	29.9±0.7	74.4±0.7	73.3±0.78
F2	1000	1000	100	1800	32.1±0.4	67.2±0.2	80±0.46
F3	1000	200	150	2000	40.1±0.2	85.8±0.56	81±0.53
F4	1000	500	100	2000	29.9±0.1	74.4±0.7	73.3±0.78
F5	1000	200	100	1800	41.5±0.3	89.2±0.36	74±0.87
F6	1000	200	50	2000	33.5±0.3	95±0.5	83±1.03
F7	1000	500	50	2200	35.6±0.1	62±0.1	73.3±0.6
F8	1000	1000	50	2000	39.6±0.4	76±0.9	80±0.2
F9	1000	200	100	2200	23.1±0.2	95±0.5	83±0.43
F10	1000	500	100	2000	29.9±0.2	74.4±0.7	73±0.78
F11	1000	500	150	2200	33.9±0.3	78.8±0.8	76±0.55
F12	1000	1000	100	2200	39.6±0.3	83.2±0.6	67±0.24
F13	1000	500	150	1800	39.5±0.5	80.1±0.5	73.3±0.45
F14	1000	1000	150	2000	39.8±0.4	82.3±0.2	70±0.32
F15	1000	500	50	1800	30.6±0.6	83.4±0.1	73.3±0.45

FC: Formulation code, EC: Ethyl cellulose, PVA: Poly vinyl alcohol, E. DR: *Ex vivo* release, E.E: Entrapment efficiency, P.Y: Practical yield

0.8 %), F1 to F15 shows a maximum range of (7.41 ± 0.4% to 43.1 ± 0.5%) after 6 hrs. F9 formulation, when compared with the pure drug, showed the best results among all.

Skin permeation and deposition studies

These studies were performed for F1-F15 as shown: Flux (0.8831 ± 1.69 to 1.125 ± 2.234 µg/cm²/h), Permeability coefficient (3.06 ±6.45 to 5.57 ± 9.76 cm/hr×10^{-3}), Lag time (0.1 ± 0.2 to 0.53 ±0.7hr), Enhancement ratio (1.286 ± 0.36 to 1.642 ± 0.36), and Skin deposition (0.28 ± 0.23 to 0.98 ±0.69 µg/cm²).

Model-dependent kinetics

From the results of drug release kinetics, the F1-F15formulations follow first-order kinetics and the Fickian drug release mechanism.

OPTIMIZATIONBY BOX-BEHNKEN DESIGN[19,20]

The mathematical relationship between the independent factor and dependent factor is statistically related to the model considering the following parameters: F value greater than 1, $P < 0.05$, the difference between adjusted R^2 and predicted R^2 should not be more than 0.2, and Adequate precision should be greater than 4. Lack of fit should be insignificant. Based on these, the design space can be navigated using the model. These responses are being given in Table 4. Where the quadratic model is significant for *ex vivo* permeation and the % E.E., 2FI model for % yield.

From the 2D counter plot and 3D response surface plot (Figures 3 and 4) it is observed that with increased concentration of EC, there is decreased *ex vivo* permeation, %E.E., % yield. Similarly, with the rise in the quantity of EC, there is a decrease in *ex vivo* permeation, percentage entrapment Efficiency, production yield, and with the increase in the quantity of PVA, there is an increase in *ex vivo* permeation, E.E., and % yield. An increase in the polymer resulted in the thickening of the polymer matrix wall, leading to a prolonged diffusion path and decreased drug release. A mathematical equation of the effect of independent variables on responses is given, where the +ve sign symbolizes the additive effect of that variable on the response and the -ve sign, vice versa.

Quadratic equation of *ex vivo* permeation = +31.90 –1.74 *A+2.54 *C+2.82 *AC+9.29 *A^2

Quadratic equation of entrapment efficiency (%) = +75.39 –5.73*A+ 7.61*A^2

2FI equation of production yield = +75.47−3.00*A −0.1250*C −5.50*AC

Fit statistics measure the predicted and adjusted r^2 value of the responses, and adequate precision shows the signal-to-noise ratio and is used to navigate the design space.

The solutions for the optimized ibuprofen nanosponges formulation were given by design expert software 11, with the criteria for optimization being maximum percent production yield, maximum entrapment efficiency, and least release of the drug. Out of 53 solutions predicted by software, a solution having a desirability of $r^2 = 0.900$ was selected. In the overlay plot (Figure 3), the criteria of highest percentage

Table 4: Fit summary for response R1, R2, and R3

Response model	Quadratic		% yield
	Ex vivo permeation	% E.E	2FI
Sum of square	429.61	478.65	193.12
Degree of freedom	4	2	3
Mean square	107.40	239.33	64.37
F	18.84	5.72	5.34
P	0.0001 significant	0.0180 significant	0.0163 significant
Adjusted R^2	0.8360	0.4026	0.4819
Predicted R^2	0.7271	0.2064	0.3378
Adeq precision	11.2082	4.6101	9.4815
Lack of fit	57.00	502.34	132.61
Pure error	0.0000	0.0000	0.0000

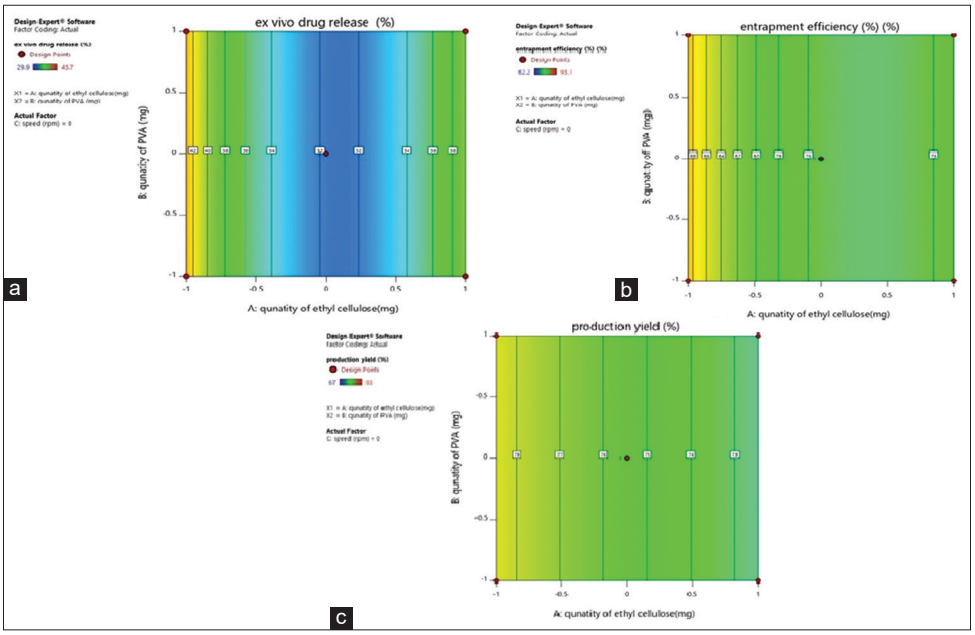


Figure 3: (a-c) Counter plot i.e., the effect of quantity of EC, quantity of PVA on *ex* vivo permeation, percentage entrapment efficiency, and percentage production yield at the centre level of X3

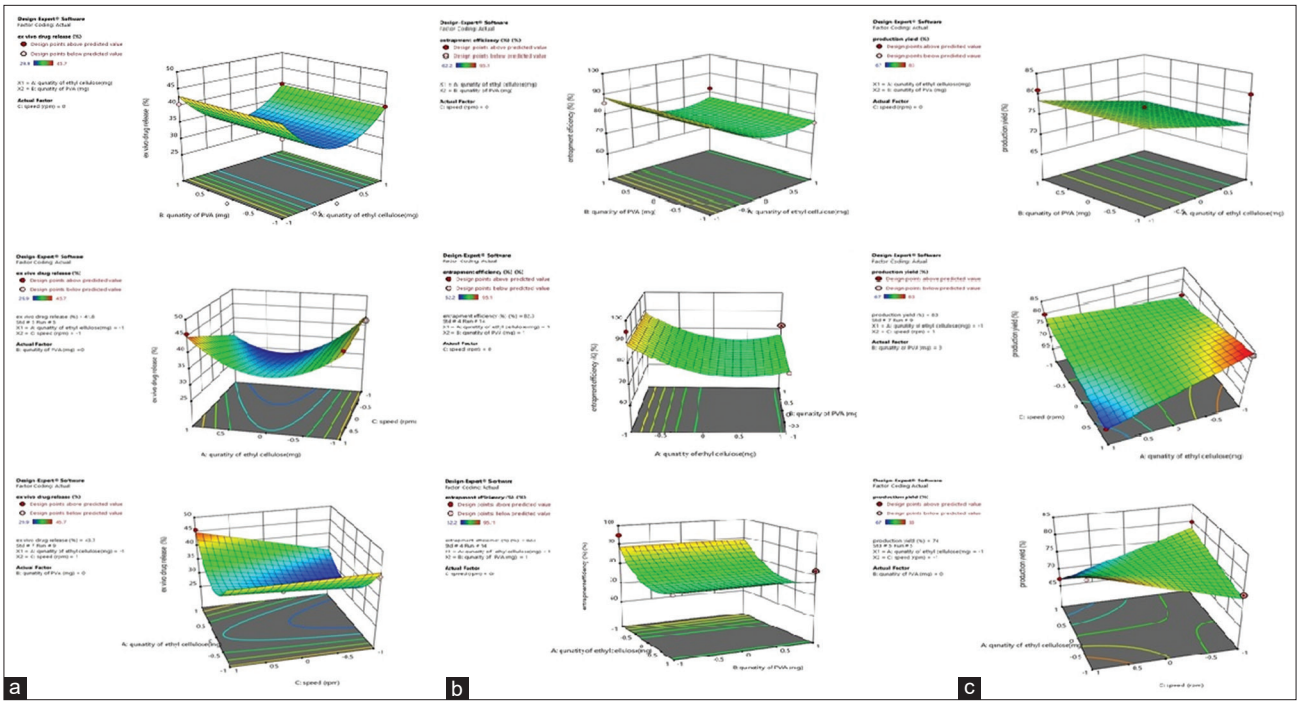


Figure 4: (a-c) Response surface plot showing the influence on the *ex vivo* drug release, percentage entrapment efficiency and percentage production yield

production yield were taken in probability, highest E.E., and *ex vivo* permeation, which are predicted responses and given by software on the overlay plot. Within these, it has predicted the independent variables X1, EC quantity as −1, X2 (PVA) as 0 and speed 1 coded values. Responses were also predicted: 83.8% product yield, 88.7% E.E., and 42.6% permeation. The formulation (PNS) was decoded at 200 mg EC, 100 mg PVA, and speed 2200 rpm, prepared, and evaluated. The observed responses were in correlation with predicted responses with $R^2 = 1$. Among all formulations, the F9 formulation was better, so an optimized formulation close to these was given by the software.

The projected factor concentrations produced in the graph are the same as solutions from numerical optimization, and they are depicted in the overlay plot in yellow as shown in Figure 5.

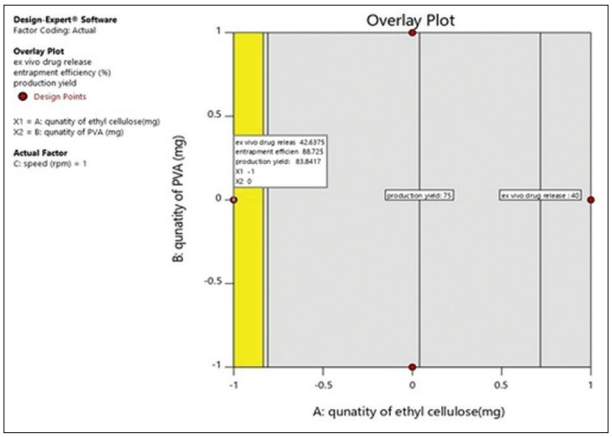


Figure 5: Desirable plot of percentage product yield, entrapment efficiency, *ex vivo* permeation

EVALUATION OF OPTIMIZED IBUPROFEN NANOSPONGES FORMULATION (PNS)

Scanning electron microscopy

The optimized formulation was examined, which shows that particles were non-segmented and homogeneous in nature with spherical-shaped nano-sized holes as shown in Figure 6.

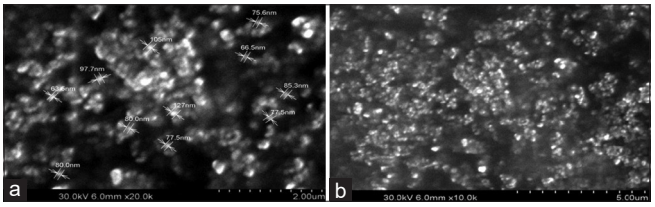


Figure 6: (a and b) SEM image of optimized ibuprofen nanosponges

Particle size and Zeta potential

The particle size of the optimized formulation was found to be 214.3 nm, and PDI was found to be 0.341which indicates an appropriate size range of particles, uniform distribution, and the zeta potential was estimated to be −37.8 mV, which indicates the stability of the formulation as shown in Figure 7.

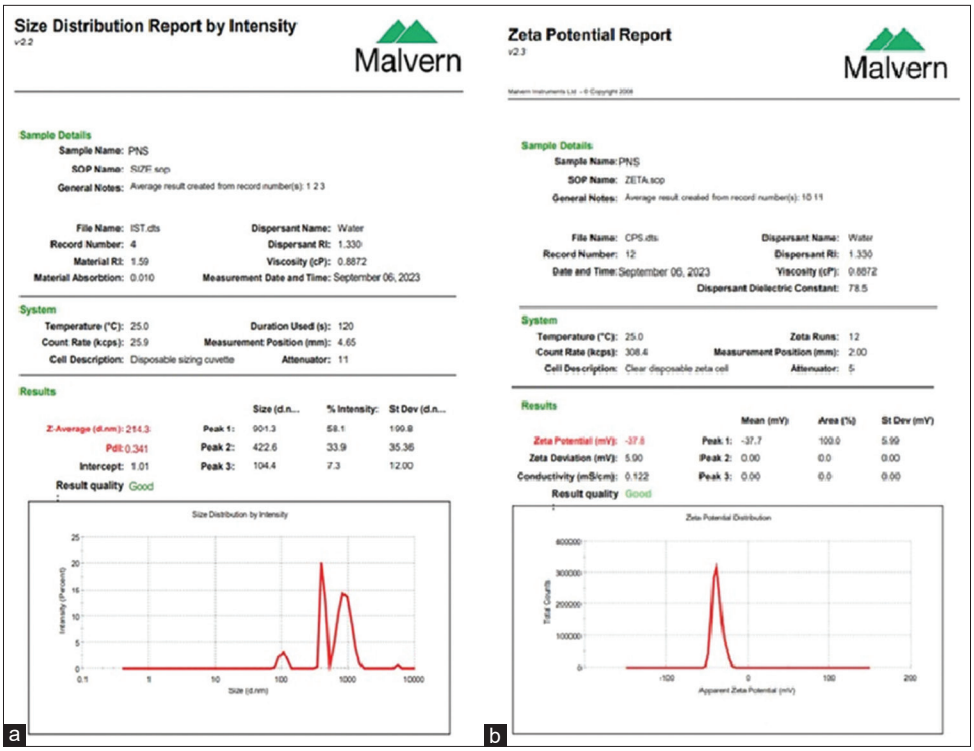


Figure 7: (a and b) Particle size, Poly dispersity Index, zeta potential Ibuprofen nanosponges formulation (PNS)

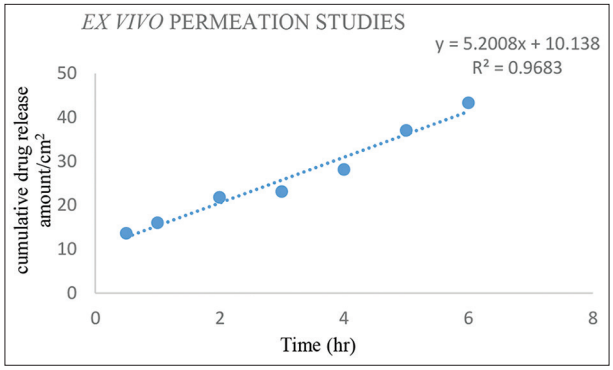


Figure 8: Percentage of drug release of PSN

Ex vivo permeation studies for optimized Ibuprofen nanosponges formulation (PNS)

An *ex vivo* permeation study for the optimized formulation was performed for 6 h, and at the end of 6 hours, a cumulative release of 43.27 ± 76.8% was found as shown in Figure 8. It follows an anomalous drug release mechanism and first-order drug release kinetics.

VALIDATION OF OPTIMIZED FORMULATION OBTAINED FROM DESIGN

The optimized formulation provided by the design is validated based on the predicted vs. obtained responses of yield (%), entrapment efficiency, and *ex vivo* permeation as shown in Figure 9. This shows that acquired values are in relation to predicted values with a R^2 =1.

Gel formulations

The optimized formulation (PNS) was incorporated into the gel base:

Gel formulations were evaluated for clarity, Homogeneity, pH, spreadability, and drug content and were found in limits.

From the results presented in Tables 5,6 and Figure 10, it is confirmed that G3 was optimized as it shows better PH, viscosity, and drug content when compared to others and shows the highest cumulative % permeated, skin deposition, and flux. G3 is followed by first-order kinetics and an anomalous drug release mechanism.

ANTI-INFLAMMATORY STUDIES

Anti-inflammatory studies were done in accordance with IAEC approval no: GPRCP/IAEC-1/27/03/2023/PCE/AE-1.

Tables 7 and 8 shows the anti-inflammatory activity where 6 groups of rats were taken and induced with carrageenan, and after 2 hours the carrageenan-induced rats were treated with gels. It shows the same value as the control after 4 h. The conclusion can be made that it can reduce inflammation in rheumatoid arthritis patients.

Table 5: Evaluations of gel

Serial number	FC	Clarity	Homogeneity	pH	Spread ability (g.cm/s)	Drug content (%)	Viscosity (cps)
1	G1	+++	+++	6.8±0.12	3.58±0.6	92.0±0.27	4.0387×10^5
2	G2	+++	+++	6.6±0.13	3.64±0.9	89.5±0.9	4.114×10^5
3	G3	+++	+++	6.9±0.09	3.3±0.9	92.4±1.19	4.1487×10^5

+++ means excellent, ++ means good, + means poor. FC: Formulation code

Table 6: Skin permeation and deposition studies of gel

Tests	G1	G2	G3
Cumulative percentage permeated	18.77±36.37	20.45±39.63	24.64±47.72
Skin deposition (μg/cm^2)	0.63±0.36	0.68±0.38	0.73±0.46
Flux (μg/cm^2/h)	1.46±0.2	1.33±0.3	1.54±0.2
Permeability coefficient (cm/h×10^{-3})	0.50±0.02	0.56±0.02	0.60±0.02
Lag time (h)	0.2±0.1	0.2±0.1	0.2±0.1
Enhancement ratio	1.53±0.32	1.43±0.22	1.60±0.32

Table 7: Anti- inflammatory studies by rat paw edema model for 4 h

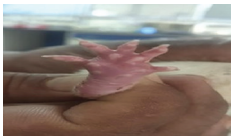
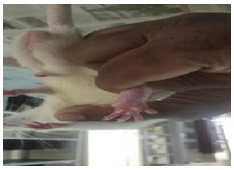
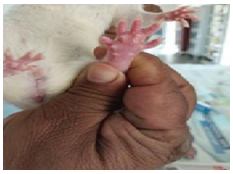
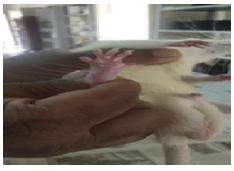
Group	Before treatment	After treatment
Control		
Group 2 (carrageenan-induced)		
Group 3 (carrageenan+marketed gel)		
Group 4 (carrageenan+ibuprofen gel)		
Group 5 (carrageenan+tea tree oil gel)		
Group 6 (carrageenan+nanospongs gel)		

Table 8: Anti- inflammatory studies by rat paw edema model obtained values for 4 h

Time (h)	Group 1 (control) (cm)	Group 2 (carrageen an induced) (cm)	Group 3 (marketed gel) (cm)	Group 4 (ibuprofen gel) (cm)	Group 5 (tea tree oil gel) (cm)	Group 6 nanosponges with tea tree oil gel (cm)
0.5	0.2±0.1	0.3±0.19	0.5±0.3	0.5±0.3	0.5±0.3	0.4±0.2
1	0.2±0.1	0.5±0.3	0.6±0.5	0.6±0.5	0.6±0.5	0.5±0.3
2	0.2±0.1	0.6±0.5	0.5±0.3	0.5±0.3	0.5±0.3	0.4±0.2
3	0.2±0.1	0.5±0.3	0.4±0.21	0.4±0.2	0.6±0.5	0.3±0.1
4	0.2±0.1	0.4±0.2	0.3±0.19	0.3±0.1	0.5±0.3	0.2±0.1

Table 9: Stability studies for optimized Ibuprofen nanosponges herbal gel formulation (optimized Ibuprofen Herbal Gel–G3)

Test	Initial	1st week	2nd week	1 month
Physical appearance	Clear gel	Clear gel	Clear gel	Clear gel
E.E (%)	88.1±0.16	88.0±0.32	87.91±0.24	87.7±0.32
Assay (%)	91.69±0.23	91.15±0.22	91.15±0.22	91.15±0.22
Ex vivo drug release (%)	24.62±0.72	24.54±0.02	24.54±0.17	24.54±0.32
Skin deposition (μg/cm^2)	0.72±0.72	0.80±0.62	0.62±0.22	0.77±0.72
Flux (μg/cm^2/h)	1.53±0.2	1.73±0.2	1.43±0.2	1.53±0.2
Permeability coefficient (cm/h×10^{-3})	0.59±0.02	0.59±0.22	0.59±0.44	0.59±0.2
Lag time (h)	0.2±0.1	0.2±0.12	0.2±0.2	0.2±0.16
Enhancement ratio	1.16±0.39	1.26±0.52	1.18±0.12	1.17±0.32

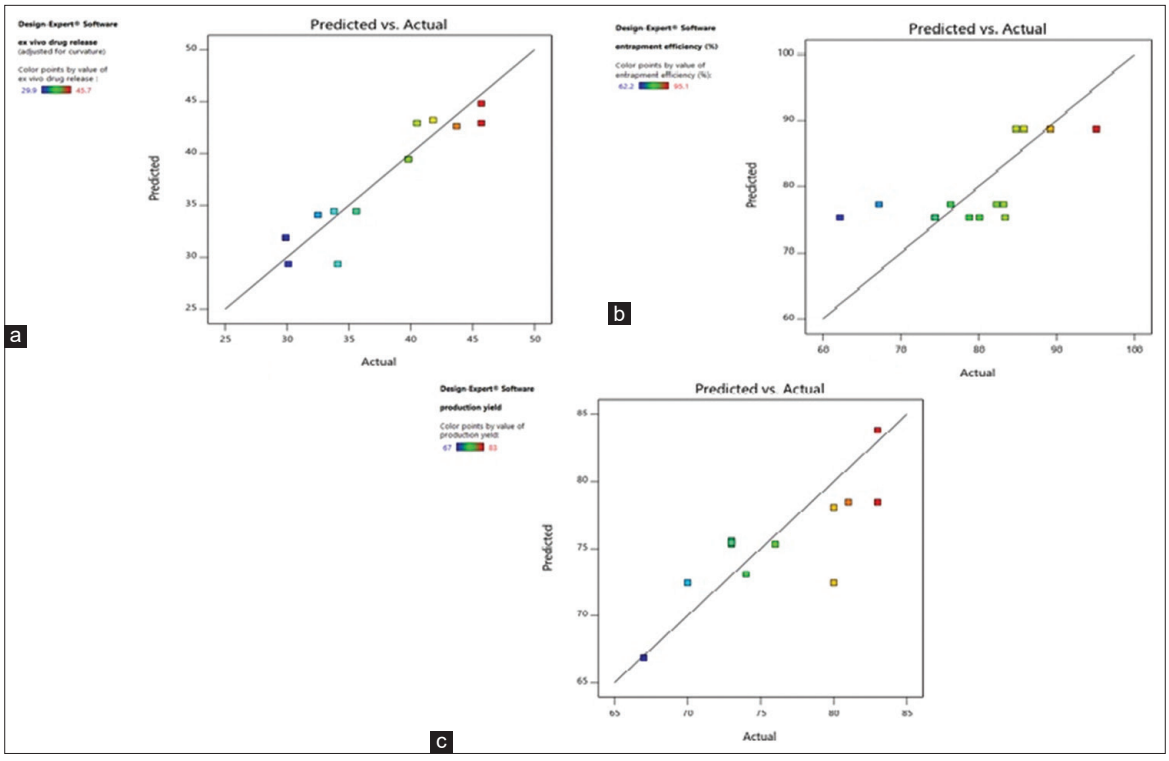


Figure 9: (a-c) Predicted vs. Actual response for R1, R2 and R3

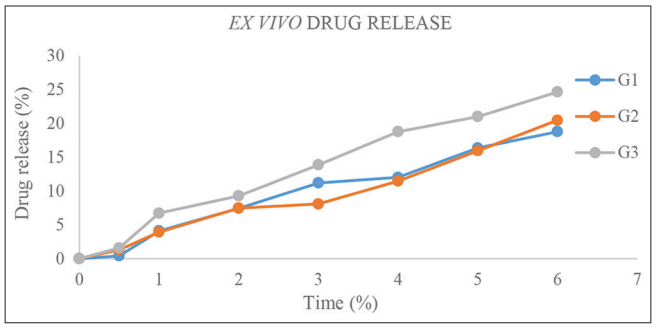


Figure 10: *Ex vivo* drug release of Ibupro fengel formulations (G1–G3)

G3 was examined for any stability changes, and it showed no changes in *ex vivo* drug release studies, drug content, or entrapment efficiency as depicted in Table 9. The formulation was not unstable.

CONCLUSION

The solubility of the drug was enhanced by formulating nanosponges. Tea tree oil as an adjuvant has enhanced the anti-inflammatory action of the formulation. Ibuprofen nanosponges herbal gel (G3) showed a reduction in paw edema in 2 h after application, which is equal to the control. This shows a decrease in inflammation, which is useful for treating rheumatoid arthritis. Further, the pharmacokinetic studies can be studied and commercialized.

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Source of Support: Nil. **Conflicts of Interest:** None declared.