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Formulation and Evaluation of Niosomal Gel Containing Antiarthritic Drug for Topical Application

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ABSTRACT

Background: The main aim of this research was to address the major challenge of poorly soluble and unpredictable drug absorption following oral administration, which often occurs due to poor water solubility. Niosomes, which are lipid-based vesicles, have been explored as potential solutions to increase the solubility of water-insoluble drugs and helps to enhance the solubility & permeability of the water insoluble drug. **Method:** Niosomal formulations (AF1–AF9) were prepared using the thin- film hydration technique by varying Surfactant and Cholesterol concentrations and optimized by applying QBD. **Result:** The optimized formulation (AF2) exhibited favorable characteristics vesicle size 173 nm, Zeta potential 42.8 mV, entrapment efficiency 92.65%, and *in-vitro* drug diffusion 90.56% and AF2 formulation was also subjected to stability as per ICH guideline, these results confirm the formulation is stable under storage conditions. This optimized niosomal formulation was incorporated into a niosomal gel, with Carbopol 934 as the gelling agent and glycerin as the humectants. The niosomal gel was evaluated for appearance, drug content (NG2-95.13%), surface pH (NG2-6.4), and sustained *in-vitro* drug release (NG2-95.26% over 12 hours). **Conclusion:** In conclusion, the niosomal gel system significantly enhances drug solubility, permeability & *in-vitro* drug release offering a promising, non-invasive therapeutic approach for the effective treatment of arthritis.

INTRODUCTION:

Arthritis is the swelling and aching of one or more joints. There are mainly two common types of arthritis are rheumatoid arthritis and osteoarthritis. Rheumatoid arthritis (RA) is a long-term inflammatory autoimmune disease that affects joints. Osteoarthritis usually comes with age and most often affects the fingers, knees, and hips. Arthritis is clinically manifested by stiffness of the joints, gradual loss of joint function, and long-lasting pain, which together leads to loss of self-support and disturbance of life. Whereas there is no cure for arthritis, symptomatic management can progress the quality of life of patients. This includes pain control using non-steroidal anti-inflammatory drugs (NSAID) analgesics, steroids [1, 2].

Anti-arthritic drugs are obtainable in the market as oral suspension, tablets or capsules. This antiarthritic drug is a very weak acid, with a pka value of 9.68 and it is very lipophilic in nature. It shows variable absorption profiles and delayed onset of action ranging from 3 to 4 hours after oral administration. Some conventional anti rheumatic drugs suffer hepatic first pass metabolism and rapid elimination from plasma. The long-term use induces cardio toxicity after oral administration. Azathioprine is immunosuppressive drug, is effective but causes systemic side effects when administered orally [3].

Niosomal drug delivery systems composed of non-ionic surfactants, improve drug stability, enhance skin permeation, prolong drug release and reduce systemic toxicity[4]. Incorporating niosomes into a topical gel provides localized action, better patient compliance and sustained release [5, 6].

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MATERIALS AND METHODS:

Materials: A sample of the drug Azathioprine was purchased from Yarrow chem. Mumbai and all ingredients i.e. Surfactants, cholesterol, Chloroform DMSO, Methyl parabens, propyl parabens, glycerin chloroform, Carbopol 934, Triethanolamine all ingredients were purchased from Molychem, Mumbai. All the reagents and solvents used were of analytical grade[7].

Preformulation Study: A pre-formulation study was done to optimize the different physicochemical properties of API molecule as well as to design and formulate the niosomal dispersion and niosomal gel. The data collected with Preformulation study gave a possible no chemical and physical interaction of all excipients used for formulation the dosage form. [7]

Physical appearance:
The physical appearance of the drugs was observed visually using a watch glass. The properties checked include: Physical state & Color.

Fourier Transform Infrared Spectroscopy (FTIR) analysis:
The FTIR spectra of Drug and excipients were recorded to check compatibility.
The FTIR spectra were then recorded in the range of 4000–400 cm^{-1} using a Perkin Elmer FTIR.

Preparation and Characterization of niosomes:
Design of experiment
From the literature, the factors that influenced the formation of niosomes were identified as the concentration of surfactant (span 60) and cholesterol. A central composite design was selected to analyze the influence of these factors as shown in Table 1, on responses such as entrapment efficiency and *in-vitro* drug diffusion by using Design Expert Software (version 13)[8].

Table1: Details of Factor

Independent Variables	Levels			Dependent variables	Goal
	-1	0	+1		
Span 60 (gm)	1	1.75	2.5	% Entrapment efficiency	Maximum
Cholesterol(gm)	0.3	2.65	5	% *In-vitro* drug release	Maximum

Table 2: Formulation table as per Design Expert Software

Formulation code	Drug (mg)	Surfactant gm	Cholesterol gm	Chloroform(ml)	Phosphate buffer 7.4 (ml)
1	50	0.68934	2.65	10	15
2	50	1.75	0.68934	10	15
3	50	2.5	5	10	15
4	50	1	5	10	15
5	50	1.75	2.65	10	15
6	50	1	0.3	10	15
7	50	2.81066	2.65	10	15
8	50	1.75	5.9734	10	15
9	50	2.5	0.3	10	15

Method: Niosomes were prepared by the thin film hydration (TFH) method. A specified amount of surfactant and cholesterol were mixed and dissolved in 10 ml of chloroform in a rounded bottom flask. The flask was then continuously vortexed on water bath at 60 ^{0}C until complete evaporation of the organic solvent to obtain a thin lipid film. The lipid film was hydrated by 15 ml phosphate buffer (7.4) containing drug solution at 60 ^{0}C. Finally; the niosomal dispersion was sonicated in 3 cycles of 1 min at 60 ^{0}C to get drug loaded vesicles [9-11].

Evaluation parameters of Niosomes:
Drug content (%): In one ml of niosomal buffer solution, 2 ml Dimethyl sulphoxide was added and further volume was adjusted by distilled water. Addition of DMSO cause breakdown of niosomes and hence drug could freely get dissolve in solvent. Each of this solution was later diluted according to the requirement by distilled water. Absorbance was measured on UV-visible spectrophotometer (Shimadzu 1800) at 280 nm[7, 12, 13]. Drug content was estimated by using the formula:

$$\text{Percent drug content} = \frac{\text{Test abs.}}{\text{Standard abs.}} \frac{\text{Standard Conc.}}{\text{weight of drug}} \times \text{Dilution factor}$$

***In-vitro* diffusion study:** The cellophane membrane diffusion technique was employed to study the *in-vitro* diffusion. A previously activated cellophane membrane was placed in a Diffusion Cell assembly. 2ml of the formulation was placed in the donor compartment. Freshly prepared phosphate buffer (pH 7.4) was used as the receptor medium. The assembly was maintained at 37°C ± 2°C and stirred at 700 RPM. At pre-determined time intervals (0.5, 1, 2, 4, 8, and 12 hours), 0.5 ml aliquots were withdrawn and immediately replaced with the same volume of fresh medium. The aliquots were appropriately diluted with the dissolution medium and analyzed using a UV-Vis Spectrophotometer (Shimadzu 1800) at 280 nm[7].

Entrapment efficiency: The entrapment efficiency

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of the niosomal formulations was determined using the centrifugation method. The prepared niosomal formulation was subjected to centrifugation using a Remi centrifuge at 1400 rpm for 30 minutes. After centrifugation, the supernatant containing the un-entrapped drug was separated, diluted with phosphate buffer (pH 7.4), and analyzed on a UV spectrophotometer. The entrapment efficiency (%EE) was then calculated using the following formula:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total amount of drug added(mg)} - \text{Unentrapped drug(mg)}}{\text{Total amount of drug added(mg)}} \times 100$$

Evaluation parameters for optimized batch:
Particle size
The particle size of the AF2 formulation was determined. The sample was passed through a syringe filter and subjected to analysis with HORIBA SZ-100 (HORIBA Japan)[14].

Zeta potential
The particle size of the AF2 formulation was determined. The sample was transferred to the sample cell with a 1-ml syringe and analyzed with HORIBA SZ-100 (HORIBA Japan) [15]

Scanning electron microscope (SEM) analysis:
SEM images were taken for Span 60 based Azathioprine loaded niosomes (AF2). Scanning was performed using a scanning electron microscope. The working distance of 26 mm was maintained and the acceleration voltage used was 15 kV with the secondary electron image (SEI) as a detector [16, 17].

Stability study: Stability studies were conducted as per ICH Q1A (R2) guideline. The optimized batches of Azathioprine loaded niosomal formulation were stored at refrigerator temperature (2–4°C) for 3 months and Drug content, Entrapment efficiency (EE%) and Drug Release (%) were determined[18, 19].

Preparation of niosomal gel:
Among eleven formulations of niosomes prepared by thin film hydration technique i.e. (AF1-AF9) based on the entrapment efficiency and *in-vitro* drug release, the optimized formulation (AF2) containing 1:2 C/S ratio of span 60 and cholesterol was found to be effective and that formulation was selected as best formulation for the conversion of niosomal gel formulation. Trial formulations were carried out for developing niosomal gel using polymers like Carbopol 934[20, 21].

Four trial formulations of the Niosomal gel were prepared by using gelling agent sufficient quantity of Carbopol 934 was weighed as shown in table 3 and sprinkled onto warm distilled water with continuous stirring. The dispersion was allowed to hydrate for 1-2 hours. Other ingredients like propylene glycol and glycerol were added subsequently to the aqueous dispersion with continuous stirring. Niosomal dispersion (15ml) was added and properly dispersed. The dispersion was neutralized to pH 6 using triethanolamine and the final weight was adjusted with distilled water. The gel was sonicated for 15 minutes and kept overnight to remove air bubbles [8].

Table 3: Formulation table of niosomal gel

Formulation code	Niosomal dispersion (ml)	Carbopol 934(%)	Methyl paraben (g)	Propyl paraben (g)	Propylene glycol(ml)	Glycerin (ml)	Triethanolamine(ml)	Distill water(ml)
NG1	10	0.5	0.18	0.02	10	5	QS	upto100
NG2	10	1.5	0.18	0.02	10	5	QS	upto 100
NG3	10	2	0.18	0.02	10	5	QS	upto 100
NG4	10	2.5	0.18	0.02	10	5	QS	upto 100

Evaluation of Niosomes Loaded Gel
Physical Appearance:
It was determined by visual inspection under black and white background and it was graded as follows: transparent; translucent; and opaque [22, 23].

Homogeneity:
It was determined by visual inspection for the appearance of lump and presence of any aggregate [22, 23].

Spreadability:
A spreadability test was conducted by pressing 0.5 g of gel between two glass slides with the aid of a 20 g weight and leaving it for about 5 min until no more spreading was observed. The diameter of formed circle was measured and used as the value for spreadability [24].

PH:
A 1 g quantity of gel was dispersed uniformly in 100 ml of distilled water using magnetic stirrer. The pH of the 1 %w/v dispersion of the niosomal gel was measured by using digital pH meter [25].

Viscosity: Brookfield viscometer was used to determine the viscosity of the niosomal gel (DV-E viscometer) using spindle no.6 [25].

***In-vitro* diffusion studies:**

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In-vitro diffusion studies of the niosomal gel were carried out using dialysis membrane. The drug release from the niosomal gel was determined from the collected samples. The analysis of the collected samples was done under UV spectrophotometer at 280nm.

Stability studies:
In order to determine stability of gels, the samples were kept in air tight glass vials packed by aluminum foil. As per ICH guidelines: Q1A (R2). The NG2 formulation was stored at 25°C ± 2°C / 60 % ± 5 % RH for 6 months as accelerated study. Samples were also stored at 5°C ± 3°C for 12months as long term study. These samples were evaluated for drug content and physical characteristics [26, 27].

RESULTS:

Preformulation Studies:
Physical appearance: Drug colour, appearance and physical state shown in table no.4

Table 4: Physical appearance

Drug name	Physical appearance	Physical state
Azathioprine	Light yellow, crystalline	Solid

Fourier Transform Infrared Spectroscopy (FTIR) Study: The IR spectrum of the pure Azathioprine sample recorded by FTIR spectrometer as shown in figure No.1. This was compared with standard functional group frequencies of Azathioprine as shown in Table.No.5. In table showed that frequencies of functional group of Azathioprine in were in the reported range which indicates that the obtained sample was of Azathioprine and was pure.

Figure 1: Fourier Transform Infrared Spectroscopy (FTIR) Study (Azathioprine)

Table 5: Observation table of FTIR Frequencies

Sr.No.	Types of observation	Standard frequency cm^{-1}	Observed frequency cm^{-1}
1	C-O stretching	1260-1000	1019
2	C-H Stretching	2950-2850	2807
3	C=o Bending	3550-3200	3260
4	N-H Stretching	3300-3500	3420

Drug & Excipients Compatibility Study: Azathioprine, Cholesterol, Surfactant, Carbopol 934

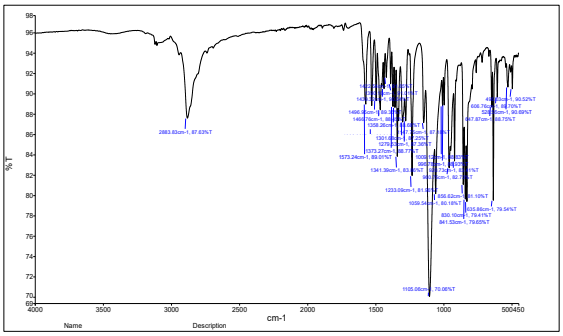

Figure 2: Fourier Transform Infrared Spectroscopy (FTIR) Study drug + Excipients

Table 6: Observation table of FTIR Frequencies

Functional Group	Standard Range (cm^{-1})	Observed Peak (cm^{-1})
C-H Stretching	2850-2960	2934.68, 2891.57, 2853.70
C=O Stretching	1725-1750	1732.69
Aromatic C=C Stretching	1500-1600	1602.12, 1559.56
C-O Stretching	1000-1300	1241.55, 1138.26, 1096.82
Aromatic C-H Bending	675-900	958.84, 825.48

Results of Azathioprine Loaded Niosomes
Appearance: All the developed batches have appeared as milky white dispersion.
Optical microscopy: Vesicle dispersions were analyzed using photo microscopy to assess vesicle formation and morphology. The dimensions and shape of the vesicles in the niosome formulations were examined by optical microscopy with a calibrated eyepiece micrometer, and images were captured at 100× magnification with a digital camera. Optical microscopy was performed to observe niosomal suspension of the formulation under a magnification of 100× (Fig.3)

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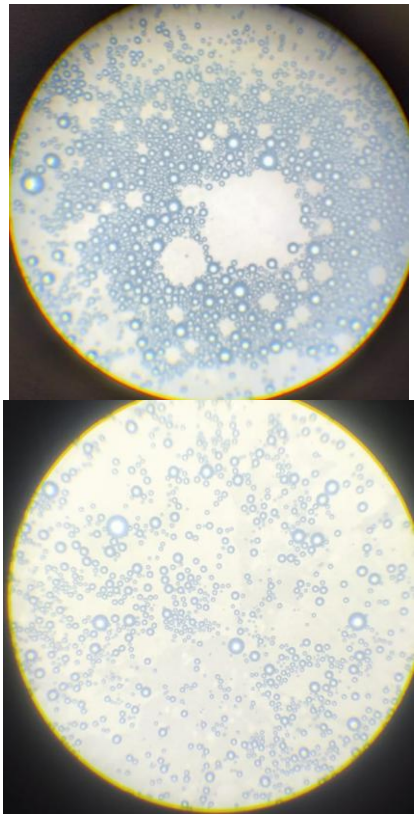

Figure 3: Microscopic Images of optimized batch AF2

Drug content (%):

The drug content was found to 81.89% - 97.87% acceptable for all formulation. I.e. Formulation AF2 shows maximum % of drug content as shown in table 07.

% of Entrapment Efficiency:

The % of Entrapment efficiency was found to be 69.12 to 92.65% acceptable for all formulations i.e. AF2 shows maximum % of entrapment efficiency as shown in table 07.

Table 7: % of Drug content & % of Entrapment efficiency

Formulation Code	Drug content (%)	%Entrapment Efficiency
AF1	86.56	72.45
AF2	97.87	91.25
AF3	92.56	82.16
AF4	93.12	75.62
AF5	82.87	86.74
AF6	80.56	80.12
AF7	82.87	84.36
AF8	82.87	75.85
AF9	82.81	86.45

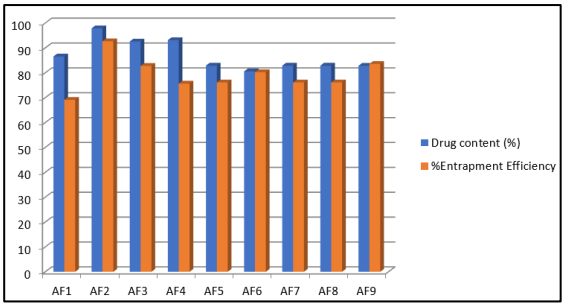

Figure 4: % Drug Content & % entrapment efficiency

% of *In vitro* Drug Release

Table 8: % of *In vitro* Drug Release

Time (hrs)	AF1 (%)	AF2 (%)	AF3 (%)	AF4 (%)	AF5 (%)	AF6 (%)	AF7 (%)	AF8 (%)	AF9 (%)
0	0	0	0	0	0	0	0	0	0
0.5	10.25 ±0.92	13.56 ±0.91	10.23 ±0.63	12.45 ±0.33	11.23±0.53	11.56 ±0.34	10.23±0.53	11.23±0.53	12.23 ±0.09
1	28.79 ±0.88	30.12 ±0.63	25.46 ±0.49	28.12 ±0.73	27.56±1.73	29.56 ±0.29	28.56±1.73	26.56±1.73	27.56 ±1.11
2	39.78 ±0.32	51.56 ±1.63	36.45 ±0.27	45.56 ±0.73	38.25±0.27	44.89 ±0.26	44.25±0.63	40.25±0.23	34.55 ±0.52
4	46.19 ±0.92	68.79 ±0.92	52.56 ±0.63	64.89 ±0.31	55.25±0.11	63.56 ±0.25	62.25±0.11	52.25±0.11	50.11 ±1.82
8	55.63 ±0.72	81.56 ±1.38	68.45 ±0.82	75.89 ±0.83	65.56±0.84	73.36 ±0.77	71.56±0.84	66.56±0.84	63.89 ±0.73
12	83.32 ±1.88	94.85 ±0.82	76.42 ±0.38	80.25 ±0.73	82.63±0.22	85.36 ±0.26	78.24±0.22	72.56±0.22	89.52 ±0.8

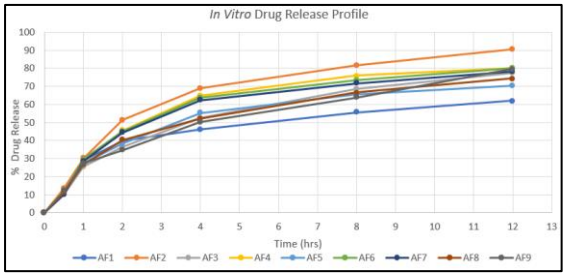

Figure 5: % of *In vitro* Drug release

On the basis of % Entrapment Efficiency and % Invitro drug release AF2 batch was optimized by QBD & therefore AF2 batch further evaluated for Scanning electron microscopy, Polydispersity index, Zeta potential.

Scanning Electron Microscopy (SEM) result of optimized formulation AF2: The surface characteristics of Azathioprine niosomal formulation were studied by scanning electron microscopy. SEM image of prepared niosome formulation shows that the coating of surfactant

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cholesterol mixture on drug particles. Some particles in the images are broken which might be due to handling and processing. Most of the vesicles are spherical and discrete sharp boundaries as shown figure No. 04.

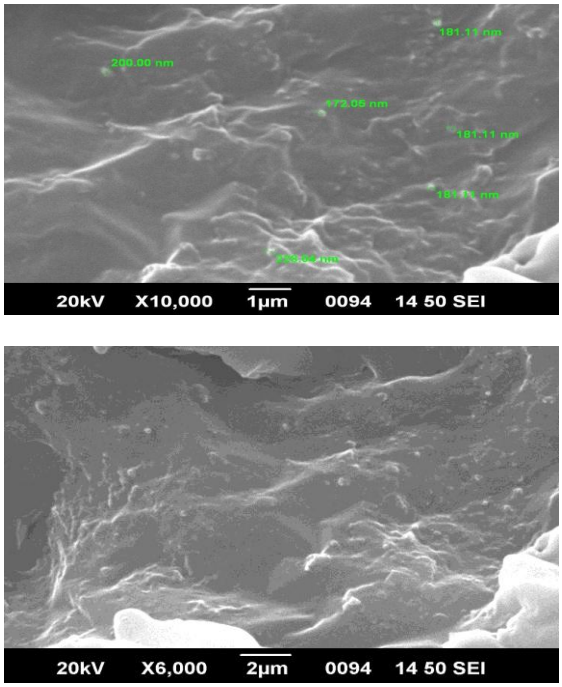

Figure 6: SEM Images of optimized batch AF2

Particle size: The particle size of the niosomes was measured using a particle size analyzer, and the results are shown in Figure. 5**.** The size of the niosomes that were loaded with the drug was 173.4 nm. The polydispersity index was 0.295.

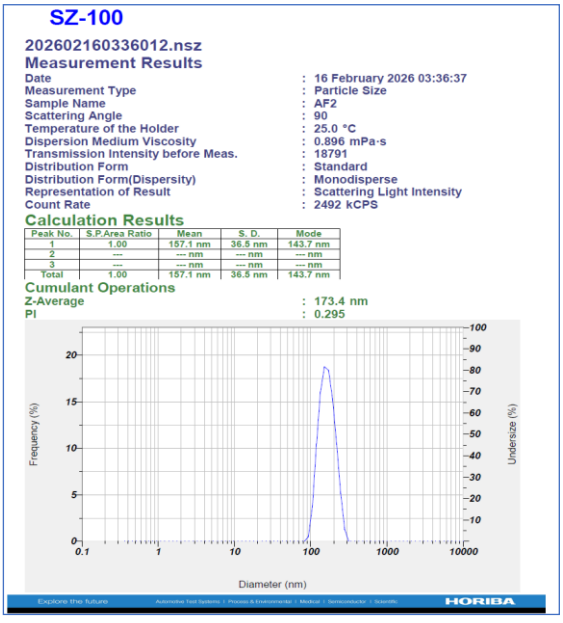

Figure 7: Particle size of optimized batch AF2

Zeta potential: The surface charge of the Azathioprine niosomes was measured with a zeta potentiometer, and the results are shown in Figure.6The zeta potential of the optimal formulation, namely, AF2 batch, which exhibited a high EE, was found to be 42.8mV. The results indicated that the zeta potential is positive hence niosomal **dispersion becomes more stable due to electrostatic repulsion** and Prevents aggregation and fusion that lead to **stable niosomal dispersion.**

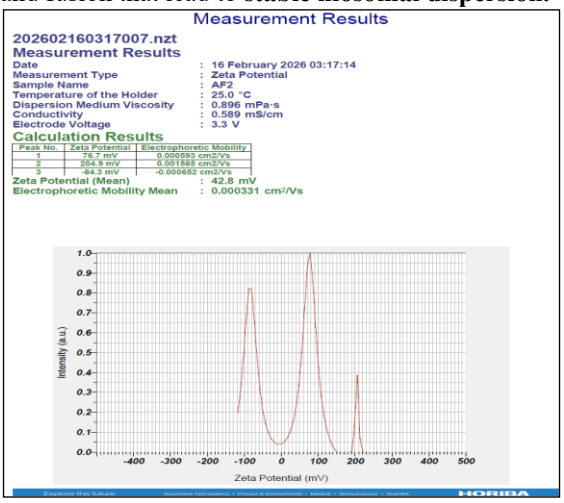

Figure 8: Zeta potential of optimized batch AF2

ANOVA for Quadratic model
Response 1: *In-vitro* drug Diffusion

Table 9: Response 1 *In -vitro drug* release

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	313.80	5	62.76	4.56	0.0361	significant
A-Surfactant	12.31	1	12.31	0.8942	0.3758	
B-Cholesterol	189.68	1	189.68	13.78	0.0075	
AB	6.05	1	6.05	0.4396	0.5286	
A^2	44.23	1	44.23	3.21	0.1162	
B^2	11.28	1	11.28	0.8190	0.3956	
Residual	96.37	7	13.77			
Lack of Fit	51.62	3	17.21	1.54	0.3350	not significant
Pure Error	44.76	4	11.19			
Cor Total	410.18	12				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**
The **Model F-value** of 4.56 implies the model is significant. There is only a 3.61% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 1.54 implies the Lack of Fit is not significant relative to the pure error. There is a 33.50% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack

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of fit is good -- we want the model to fit.

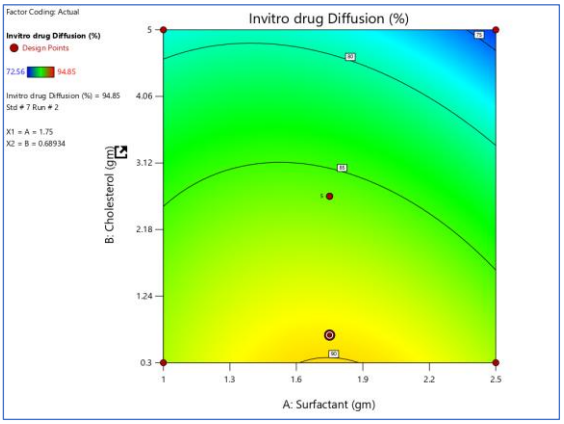

Figure 9: Counter plot of *In vitro* diffusion

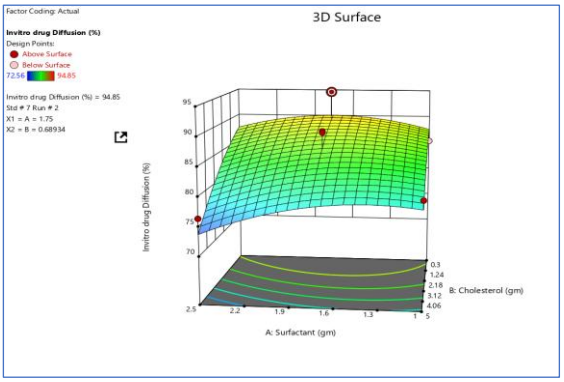

Figure 10: 3D plot of *In vitro* diffusion

ANOVA for Quadratic model
Response 2: Entrapment efficiency

Table 10 -Response 2: Entrapment efficiency

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	391.95	5	78.39	17.62	0.0008	significant
A-Surfactant	129.31	1	129.31	29.06	0.0010	
B-Cholesterol	27.88	1	27.88	6.27	0.0408	
AB	1.25	1	1.25	0.2819	0.6119	
A^2	237.78	1	237.78	53.43	0.0002	
B^2	0.0171	1	0.0171	0.0038	0.9523	
Residual	31.15	7	4.45			
Lack of Fit	16.29	3	5.43	1.46	0.3514	not significant
Pure Error	14.86	4	3.72			
Cor Total	423.10	12				

Factor coding is **Coded**.
Sum of squares is **Type III - Partial**

The **Model F-value** of 17.62 implies the model is significant. There is only a 0.08% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, A^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 1.46 implies the Lack of Fit is not significant relative to the pure error. There is a 35.14% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

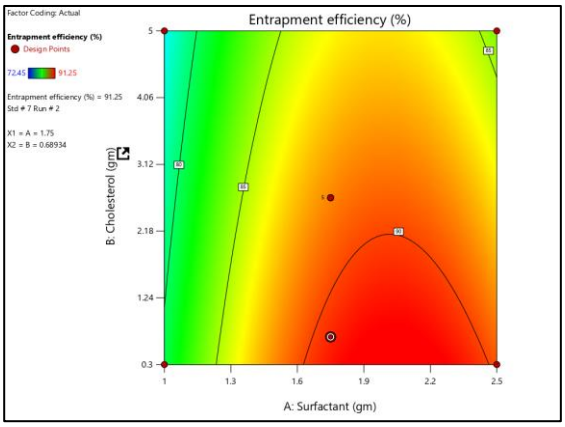

Figure 11: Counter plot of % Entrapment efficiency

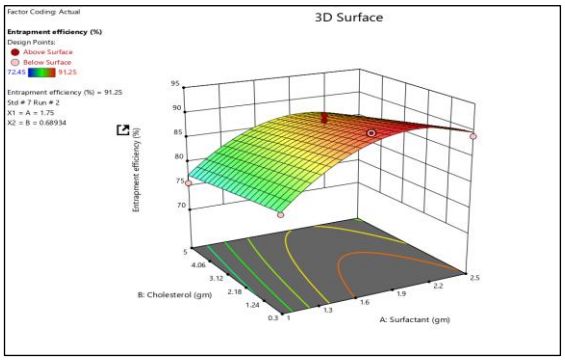

Figure 12: 3D plot of % Entrapment efficiency

Stability study: There was no change found in the % of Drug content, % of entrapment efficiency & % of drug release of niosomal dispersions kept at $5^0C \pm 3^0C$.

Table 11: Stability study of AF2 batchat $5^0C \pm 3^0C$ long term study as per ICH Q1A (R2)

Stability parameter	3 months	6 months	9 month	12 months
Drug Content (%)	97.87	97.86	97.79	97.795
Entrapment efficiency	92.65	92.65	92.58	92.58

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(%)				
Drug release (%)	80.56	80.54	80.52	80.52

Results of Evaluation of niosomal gel:

Physical Appearance: The appearance of niosomal gel was milky White in Colour homogenous smooth with no sign of grittiness.

pH & Viscosity: pH was found to be 6.2-6.4 & viscosity 3562.3 to 4356.8 as shown in table no.12.

Spreadability: In the spreadability test, the gel containing 1.5 % Carbopol 934 was found optimum due to its suitable physiochemical property. The spreadability was found to be 18.26gm.cm/sec. The gel formulation was found smooth and easy to spread on the plate.

Drug content: The niosomal gel was analyzed for drug content spectrophotometrically at 280 nm. All the formulations exhibited fairly uniform drug content. This ensures intended delivery of drug to the site after administration of the gel formulation. The drug content was found to be in acceptable range for all the formulations. The results revealed that drug content of all developed formulations were in the range of 89.56 to 95.13% as shown in table no.12.

Table 12: Evaluation characteristics of niosomal gel

Formulation code	Physical Appearance	pH	Viscosity(cp)	Spreadability gm.cm/sec	Drug content (%)
NG1	Milky white, smooth homogenous	6.2	3562.3	14.50	90.32
NG2	Milky white, smooth homogenous	6.4	4968.4	18.26	95.13
NG3	Milky white, smooth homogenous	6.3	4298.2	13.37	91.20
NG4	Milky white, smooth homogenous	6.2	4356.8	12.26	89.56

***% In vitro*-drug diffusion:** The % of *in vitro* drug release at 12 hr of all the formulations is given (Table13). When compared with all the formulations NG2 showed slower but maximum drug release due to high gelling capacity. For the drug to be released in the medium, it has to pass through the vesicle wall and then through the hydrogel matrix. Initially the un-entrapped drug was released from the gel which can serve as the initial loading dose. There after the niosomes can release drug in a sustained manner making the formulation suitable for once-a-day dosing.

Table 13 *% In vitro* drug diffusion
G2 showed maximum sustained release 95.20 % for a period of 12 hrs.

Time in hrs.	NG1	NG2	NG3	NG4
1	9.6± 0.95	10.5± 0.53	9.5± 0.29	8.5± 0.83
2	16.42± 0.44	18.60± 1.73	17.50± 0.73	16.25± 0.2
3	26.42± 0.18	28.80± 0.63	25.13± 0.66	26.52± 1.72
4	35.78± 0.53	36.78± 0.11	34.60± 1.73	33.47± 0.28
5	43.41± 0.73	48.15± 0.84	42.55± 1.88	43.21± 0.45
6	53.86± 0.30	56.80± 0.22	52.11± 0.63	50.42± 0.56
7	60.80± 0.72	64.85± 0.12	59.23± 1.32	58.23± 0.73
8	62.50± 1.88	70.20± 0.14	61.12± 1.82	60.56± 0.36
9	70.30± 1.38	76.23± 0.37	71.33± 0.81	66.12± 0.32
10	74.22± 0.82	85.80± 0.56	74.23± 0.73	68.58± 0.53
11	76.35± 0.38	90.98± 0.64	69.13± 0.83	70.15± 0.82
12	78.30± 0.36	95.20± 0.32	72.35± 0.76	71.28± 0.82

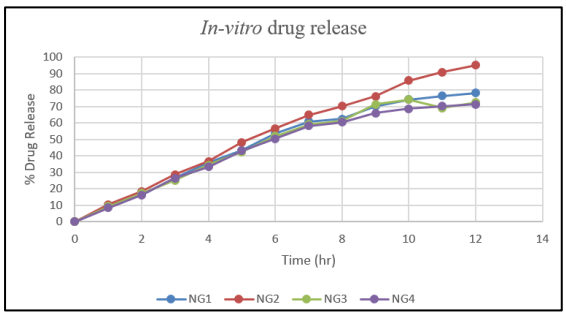

Figure 13: *In-vitro* drug release of niosomal gel

Drug Release Kinetic Studies

Drug release kinetic studies were carried out to investigate the mechanism of Azathioprine release from the optimized niosomal gel formulation (NG2). The cumulative in vitro drug release data obtained over a period of 12 hours were fitted to various mathematical kinetic models, including Zero-order, First-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models. The model showing the highest regression coefficient (R^2) was considered to best describe the release mechanism of the optimized formulation.

The Zero-order kinetic model describes a system in which the drug is released at a constant rate

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irrespective of the concentration remaining in the formulation. The optimized niosomal gel exhibited good linearity with an R^2 value of 0.9635, indicating a nearly constant release of Azathioprine throughout the study period.

The First-order kinetic model assumes that the drug release rate is directly proportional to the amount of drug remaining in the dosage form. In this model, the logarithm of the percentage of drug remaining is plotted against time. The optimized formulation (NG2) exhibited the highest regression coefficient ($R^2 = 0.9894$) with the regression equation:

$$\log(100 - Q_t) = 2.1954 - 0.1063t$$

where Qt represents the cumulative percentage of drug released at time t, 100−Qt represents the percentage of drug remaining in the formulation, and K_1 is the first-order release rate constant. The excellent linearity obtained indicates that the release of Azathioprine from the niosomal gel is concentration dependent.

The Higuchi model was used to evaluate diffusion-controlled drug release from the hydrated gel matrix. The optimized formulation exhibited an R^2 value of 0.9786, suggesting that diffusion of Azathioprine through the hydrated Carbopol matrix and niosomal vesicles contributes significantly to the sustained release profile.
The Korsmeyer–Peppas model was applied to further elucidate the release mechanism. The optimized formulation showed an R^2 value of 0.9728 with a release exponent (n = 0.682), indicating non-Fickian (anomalous) diffusion. This suggests that drug release is governed by a combination of diffusion through the gel matrix and relaxation or swelling of the polymer network.

The Hixson–Crowell model exhibited an R^2 value of 0.9513, indicating that gradual changes in the surface area and diameter of the niosomal vesicles also contribute to drug release, although this mechanism is less dominant than diffusion-controlled release.

Comparison of the regression coefficients demonstrated that the First-order kinetic model exhibited the highest correlation coefficient ($R^2 = 0.9894$), followed by the Higuchi model ($R^2 = 0.9786$), Korsmeyer–Peppas model ($R^2 = 0.9728$), Zero-order model ($R^2 = 0.9635$), and Hixson–Crowell model ($R^2 = 0.9513$). These findings indicate that the optimized Azathioprine-loaded niosomal gel (NG2) predominantly follows first-order release kinetics with a diffusion-controlled sustained release mechanism.

The sustained drug release profile observed over 12 hours, together with the high cumulative drug release (95.20%), confirms the ability of the niosomal vesicular system dispersed within the Carbopol gel matrix to regulate drug diffusion, prolong drug availability at the application site, and improve topical therapeutic efficacy. The sustained release behavior is expected to reduce the frequency of application while enhancing patient compliance in the management of rheumatoid arthritis.

Table 14: Drug Release Kinetic Models of Optimized Niosomal Gel (NG2)

Kinetic Model	Linear Plot	Regression Equation	R^2	Interpretation
Zero-order	% Drug Released vs Time	Qt = 7.78t + 10.54	0.9635	Nearly constant drug release
First-order	Log (% Drug Remaining) vs Time	Log(100−Qt) = 2.1954 − 0.1063t	0.9894	Best fit; concentration-dependent release
Higuchi	% Drug Released vs √Time	Qt = 27.63√t − 13.52	0.9786	Diffusion-controlled release
Korsmeyer–Peppas	Log Qt vs Log t	Log Qt = 0.682 Log t + 1.052	0.9728	Non-Fickian diffusion (n = 0.682)
Hixson–Crowell	Cube root of % Drug Remaining vs Time	(100−Qt) 1/3 = 4.64 − 0.183t	0.9513	Surface area reduction contributes to release

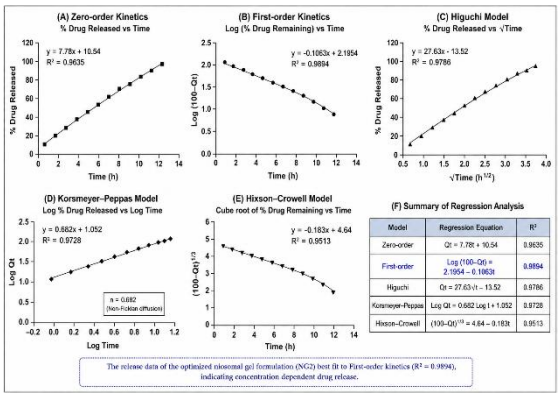

Figure14: Drug Release Kinetic Models of Optimized Niosomal Gel (NG2)

Stability studies: The niosomal gel was found to be off-white in colour there was no change in the appearance, pH and throughout the 12 months of stability studies at $5^0C \pm 3^0C$ & Drug content uniformity showed good uniformity at $5^0C \pm 3^0$ C as compared to room temperature the niosomal gel was more stable than the room temperature.

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Table No.15 Long term Stability result of optimized formulation NG2 at 5°C ± 3°C ICH guidelines: Q1A (R2)

Sr. No	Parameters	After 3 months	After 6 months	After 9 months	After 12 months
1	Physical Appearance	Milky white, smooth homogenous	NC Milky white, smooth homogenous	Milky white, smooth homogenous	Milky white, smooth homogenous
2	pH	6.4	6.4	6.4	6.4
3	Viscosity	4968.4	4968.4	4968.4	4968.3
4	Spreadability	18.26	18.26	18.25	18.24
5	Drug content	96.12	96.10	96.8	95.8
6	*In vitro* drug release	95.20	95.20	95.19	95.19

Table 16: Accelerated Stability study result of optimized formulation NG2 at 25°C ±2°C (Room temp.) ICH guidelines: Q1A (R2)

Sr. No	Parameters	After 2 months	After 4 months	After 6 months
1	Physical Appearance	Milky white, smooth homogenous	Milky white, smooth homogenous	Milky white, smooth homogenous
2	pH	6.4	6.4	6.4
3	Viscosity	4968.4	4968.3	4968.1
4	Spreadability	18.22	18.22	18.15
5	Drug content	96.12	95.10	95.09
6	*In vitro* drug release	95.20	94.13	94.08

DISCUSSION:

Arthritis is clinically manifested by stiffness of the joints, gradual loss of joint function, and long-lasting pain, which together leads to loss of self-support and disturbance of life. Whereas there is no cure for arthritis, symptomatic management can progress the quality of life of patients. This includes pain control using non-steroidal anti-inflammatory drugs (NSAID) analgesics, steroids and drugs are obtainable in the market as oral suspension, tablets or capsules. This antiarthritic drug is a very weak acid, with a PKa value of 9.68 and it is very lipophilic in nature. It shows variable absorption profiles and delayed onset of action ranging from 3 to 4 hours after oral administration [28, 29]. The development of a niosomal suspension for azathioprine aims to increase its efficacy by directly targeting potentially reducing side effects and increasing its solubility and dissolution properties, which are typically low, since azathioprine is a BCS class IV drug[30]. Moreover, niosomal formulations can extend the drug's half-life, improve its circulation in the bloodstream, and provide sustained drug release. The current investigation successfully developed and characterized a novel niosomal gel for the targeted delivery Azathioprine, which is a widely used antiarithatic drug. The niosomal formulation overcomes the limitations associated with conventional oral administration, such as poor bioavailability and systemic side effects [31-33]. The optimized niosomal formulation exhibited desirable characteristics, including high encapsulation efficiency, a suitable particle size range for enhanced cellular uptake, and a positive zeta potential for improved stability. The *in-vitro* drug release profile demonstrated a sustained release pattern, which is advantageous for maintaining therapeutic drug levels for prolonged periods. As reported in previous studies, Preformulation research is essential for evaluating the compatibility between a drug and excipients and for determining compatibility[34].An FTIR study was conducted on Azathioprine, excipients, and the blend.The results suggested that the characteristic peaks of Azathioprine were observed in all the FTIR spectra and that the excipients did not interact with the drug, indicating compatibility between the drug and excipients[21, 35, 36]. Optimization utilizing response surface analysis is considered as an effective approach for reducing both the time and the number of experimental runs required for formulation development and enhancing research output In this study, central composite design was efficiently adopted to explore the influence of various formulation variables on product characteristics and, most importantly, to retrieve an optimized formula. In topical drug delivery, the particle size of nanovesicles is a crucial parameter that control nanocarrier's permeation across skin barrier. It is well recognized that nanovesicles with particle size > 600 nm showed no skin deposition). In contrast, nanovesicles with smaller particle sizes, such as 400 nm, increase cutaneous delivery, whilst nanovesicles with smaller particle sizes, such as 200 nm, might trigger excessive transdermal drug transport.The optimized drug-loaded niosomal formulation AF2 showed an average particle size of 173.4 nm, which is considered suitable for topical delivery. The niosomal dispersion of Azathioprine was prepared with the thin-film hydration method by using different concentrations of surfactant and cholesterol. On the basis of the % of EE and % of *Invitro* drug release, the AF2 formulation was considered the optimal formulation. As reported in previous and current studies, the niosomal dispersion exhibited a characteristic shape and

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dimension for vesicles. The SEM study revealed that the particles appeared to be spherical in shape and were distributed within the size range of 150 to 250 nm, indicating that the formulation was a nano formulation. As per earlier research, the vesicle diameter of niosomes should be less than 1,000 nm. In the present study, the vesicle diameter of the niosomes was found to be 206.1 nm, which is within the acceptable range[7, 16-20].

The PDI (polydispersibility index) is an indicator of a sample's size-based heterogeneity. A sample's size distribution, agglomeration, or aggregation during isolation or analysis can lead to polydispersibility, which should usually be less than 1. The polydispersibility index of AF2 was 0.295, which is a good value for a niosomal suspension, as it indicates that the sample may be stable and free from agglomeration. The zeta potential is a critical parameter of niosomal formulations, as it influences their stability and drug release characteristics. A high absolute value of the zeta potential, typically above 30 mV, indicates strong electrostatic repulsion between niosomes, preventing aggregation and promoting dispersion.Moreover, the zeta potential can affect drug release kinetics by influencing the interaction between the drug and the niosomal membrane. The current research revealed a zeta potential value of 42.8mV, indicating that the formulation was stable [11-13].

EE is a critical parameter of niosomal formulations because it directly influences drug loading capacity and subsequent therapeutic efficacy. High EE ensures that a significant amount of the drug is encapsulated within the niosomes, reducing drug loss during formulation and administration. This leads to a higher drug concentration at the target site, thus enhancing therapeutic outcomes % EE of optimized batch AF2 is 90.65%[5, 6, 8, 9].

Additionally, high EE can improve the stability of a drug, protecting it from degradation and premature release. The drug release profile plays essential role in determining the achievement of transdermal DDSs by directly influencing both skin penetration and therapeutic efficacy. Furthermore, a sustained release profile also minimizes frequent application, improving patient compliance, and it can help bypass first-pass metabolism, improve bioavailability, and reduce systemic side effects. Thus, optimizing the release profile not only prolongs the duration of drug availability but also strategically aligns the release kinetics with the skin permeability dynamics and desired therapeutic outcomes.

An *in vitro* drug release study was conducted over a 12-hrsthe AF2 89.56% demonstrated a greater percentage of drug release in a sustained manner. A study of the stability of the optimal formulation AF2at $5^0C\pm3^0C$was conducted over a12 month period long term stability study. The prepared formulation was stable for a period of 12 months. The stability studies revealed that the niosomal suspensions remained stable under various storage conditions, ensuring long-term viability.

Formulation AF2 was selected as best formulation for the conversion of niosomal gel formulation. Trial formulations were carried out for developing niosomal gel using polymers like Carbopol 934.among the four formulations, the NG2 formulation shows maximum drug content (96.12) & sustained drug release (95.20). The prepared formulation is stable throughout the 12 months of stability studies at $5^0C\pm3^0C$ & Drug content uniformity showed good uniformity at $5^0C\pm3^0$ C as compared to room temperature the niosomal gel was more stable than the room temperature [8].These findings suggest that the developed niosomal gel system has the potential to increase the therapeutic efficacy of Azathioprine by increasing its bioavailability and reducing its systemic side effects. The current study provides a promising approach for developing systems for the targeted delivery of Azathioprine.

CONCLUSION:

The present work was a satisfactory preliminary study in developing the niosomal gel system of Azathioprine. It is a BCS class IV drug, used in the treatment of inflammation, arthritis and was successfully loaded into the niosomal gel system. Central composite design was used to optimize the formulated niosomes using the desirability criteria. The optimized formulation exerted reasonable % EE, and efficiently sustained drug release for up to 12 h. It is concluded that thin film hydration technique is a useful method for the successful incorporation of poorly water-soluble drug & poorly permeable drug. The prepared formulations were evaluated for, vesicle size, entrapment efficiencies, and *in-vitro* diffusion studies. The developed formulation AF2 is an optimized batch of niosomes which is incorporated in gel. Moreover, Azathioprine -loaded niosomal gel exerted suitable pH, viscosity, and spreadability for topical application. Thus, it can be concluded that the niosomes loaded gel can be a promising potential drug delivery system for topical application to efficiently target local pains for prolonged periods and to reduce the frequency of application of gels as compared to conventional gels.

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ETHICS DECLARATION: Not applicable.

DATA AVAILABILITY: All data associated with the manuscript are provided within the manuscript.

AUTHOR'S CONTRIBUTION:
Conceptualization and Methodology: WC; Writing original draft: AS; Critical revision: AS & WC.

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