QUALITY BY DESIGN-BASED FORMULATION DEVELOPMENT AND CHARACTERIZATION OF SULFASALAZINE-LOADED UFASOMAL TOPICAL GEL FOR RHEUMATOID ARTHRITIS

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Abstract:

Rheumatoid arthritis (RA) is a persistent, developing autoimmune condition that significantly impairs joint function and quality of life. The current treatment regimens often involve systemic administration of drugs, which can lead to undesirable side effects and limited localized efficacy. Ufasomes, unilamellar fatty acid vesicles, present a promising drug delivery system due to their biocompatibility, ability to encapsulate hydrophilic and hydrophobic drugs, and potential for improved skin penetration. This study focuses on designing and evaluating a sulfasalazine-loaded ufasomal topical gel, aimed at enhancing localized drug delivery and therapeutic efficacy for RA. Sulfasalazine-loaded ufasomes were prepared by ether injection method using oleic acid and cholesterol. The formulation was optimized using a Design of Experiments (DoE) approach to achieve the desired encapsulation efficiency, drug release, and stability. Different concentrations of lipid and surfactant were used in the formulation using 2^2 central composite designs. The formulation was assessed for percentage entrapment efficiency and in vitro drug release. The percentage entrapment efficiency was found to be in the range of 55 to 94 and the percentage of drug release was between 55% to 85%. The optimized Sulfasalazine ufasomes were loaded into a topical gel and evaluated for their physical and in vitro drug release characteristics. The results indicated that the sulfasalazine-loaded Ufasomal gel exhibited a drug content of 87% and 70% of drug release at 24 hours. The drug release profile of the optimized sulfasalazine Ufasomal gel followed first-order kinetics with regression of 0.9674 and best fit with the Higuchi equation with R^2 value 0.9942 showing sustained release through diffusion mechanism from the matrix. The stability studies showed that the formulation maintained its physiochemical properties. In conclusion, the sulfasalazine-loaded Ufasomal topical gel offers a targeted, non-invasive treatment option for RA, minimizing systemic side effects while maximizing local therapeutic action.

Keywords: Ufasomal gel, Rheumatoid arthritis, Sulfasalazine ufasomes, Central composite design, Topical delivery.

1. Introduction

The skin, the largest organ in the human integumentary system, covers the entire body, regulating body temperature, producing vitamin D, and protecting against infections and toxins. Its main function is to act as a protective barrier, preventing pathogens and foreign substances from entering the body while shielding against UV light and retaining moisture. The skin is composed of five layers in the epidermis.[1] Rheumatoid arthritis is an autoimmune inflammatory condition that affects the synovial joints, causing pain, swelling, and eventual deformity. It is more prevalent in women due to hormonal and genetic factors. RA affects up to 0.75% of the Indian population and has a global incidence of 0.35% to 1.27%. Approximately 3.6% of women and 1.7% of men may develop RA in their lifetime. Sulfasalazine has been used since the 1940s for rheumatic polyarthritis and has been established as a disease-modifying antirheumatic drug (DMARD).[2] It is broken down in the large intestine into Sulfapyridine and mesalazine, although it is unclear which compound is responsible for its effectiveness in treating rheumatoid arthritis (RA). Studies have shown that sulfasalazine is comparable to methotrexate in efficacy but may be less tolerated. Sulfasalazine is considered safe for pregnant women, making it a preferred treatment option for individuals with RA who are pregnant or planning to become pregnant. The drug may offer a quicker onset of action compared to other DMARDs and can help slow the progression of RA. Combining sulfasalazine with other DMARDs, such as methotrexate, has shown to be more effective than monotherapy. [3] Ufasomes, a type of vesicle made from unsaturated fatty acids, have been developed as a drug delivery system to enhance medication absorption through the skin. Fatty acid vesicles like ufasomes offer a potential solution to overcome skin irritation caused by fatty acids while improving drug delivery efficiency. Ufasomes have been shown to increase drug retention in skin cells and may provide a novel approach to targeted drug delivery. [4]

In our present study, an attempt was made to prepare unsaturated fatty acid vesicular drug delivery systems or ufasomes of sulfasalazine using oleic acid for aiming rheumatoid arthritis which affects the deeper epidermal layer of the skin. This formulation is capable of providing local effects with higher penetrability and reduced dose and toxicity. The ether injection method was used to prepare ufasomes of sulfasalazine. The different characterization parameters were also addressed in this research.

2. Materials and methods

Materials:

Sulfasalazine was Purchased from Yarrow Chemicals, Mumbai. Cholesterol, span 20, Methanol, DMSO, Polyvinyl Alcohol, Diethyl ether Carbopol 940 was purchased from S.D. Fine Chemicals, Tamil Nadu. All other materials used were of pharmacopeial grade.

Methods:

Design of Experiments (Doe) Using Central Composite Design :

Central Composite Design (CCD) is a statistical approach used in the Design of Experiments (DoE) to effectively optimize processes or systems. It includes choosing factors and their levels, deciding on the number of experimental trials, and performing experiments based on a set design matrix. Through the application of regression analysis or ANOVA on the data,CCD aids in identifying important factors and their interactions, facilitating the creation of mathematical models to illustrate the relationship between factors and outcomes. These models aid in optimizing to identify the ideal operating conditions. CCD's methodical strategy offers important insights for enhancing processes and making decisions, rendering it a useful resource across multiple sectors, such as manufacturing, engineering, and research.

Formulation By Design of Expert:

Formulation variables and their levels in central composite Design:

Factors/ Independent variables	**Levels**			**Responses/ Dependent variables**
	-1	**0**	**+1**	
Oleic acid	100	0	200	**Entrapment efficiency**
Cholesterol	50	0	150	**Drug release**

Table 1: Experiment plan for central composite design in terms of actual and coded values

The ether injection method made ufasomes by slowly introducing oleic acid and cholesterol dissolved in diethyl ether mixed with 2 ml methanol previously containing a weighed quantity of the drug. The resulting solution was slowly injected using a micro syringe into 10 ml deionized water at a rate of 1 ml/min on a magnetic stirrer, and the temperature was maintained at 60-65°C. Then the lipid solution was injected slowly into the aqueous phase. Differences in temperature between the phases caused rapid vaporization of ether andresulted in the formation of Ufasomal vesicles [4].

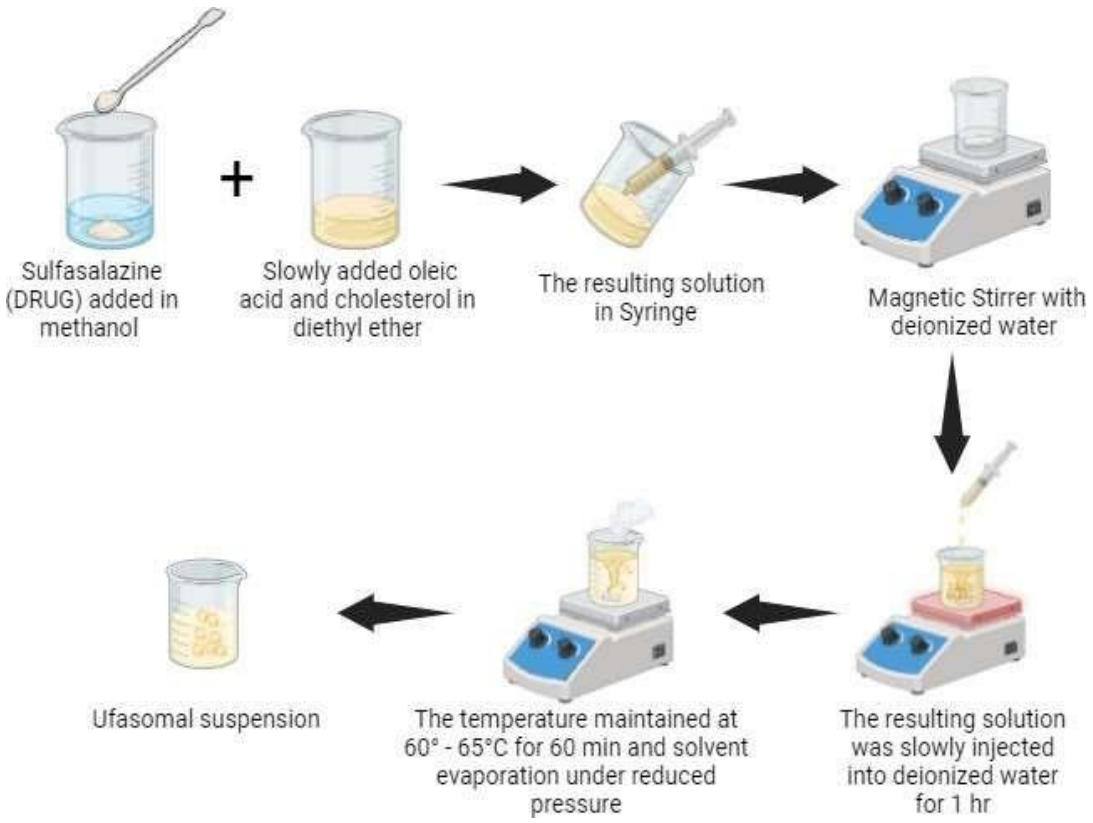


Figure 1: Formulation of ufasomes

Formulation code	Factor 1 A: Oleic Acid (Mg)	Factor 2 B: Cholesterol(Mg)	Response 1 EE (%)	Response 2 Drug release (%)
F1	150	100	90	65
F2	79.2893	100	76	68
F3	150	100	89	70
F4	150	170.711	77	58
F5	150	29.2893	58	75
F6	100	50	57	80
F7	100	150	92	62
F8	150	100	93	60
F9	200	150	69	55

F10	**150**	**100**	**94**	**85**
F11	220.711	100	66	66
F12	**200**	**50**	**55**	**70**
F13	150	100	90	68

Table 2: Optimization of Formula

3. Characterization of Ufasomes

Entrapment efficiency:

The ultra-centrifugation method was employed to measure % of drug entrapped. Centrifugation was performed on the ufasomal formulations having 1 mg equivalent weight of sulfasalazine at 4°C for 1hr at 6000 rpm. To analyze the absorbance under an ultraviolet-visible spectrophotometer at 360 nm, a supernatant solution containing free drug was pipetted and diluted to 10 ml with pH 7.4. The following equation is used to determine the % entrapment efficiency [(5)].

Percentage Drug release:

The drug release from the ufasomes was done using Franz diffusion cells and drug release kinetics were also predicted. There are two compartments in the Franz diffusion cell: one for the donor and one for the receptor. A polycarbonate membrane with pore sizes of 50 nanometers separates these two compartments. The donor compartment contained 1 ml of ufasomal dispersion, whereas the receptor compartment contained phosphate-buffered saline (PBS), pH 7.4, which was kept at 37°C and stirred at a constant rate using a magnetic stirrer. Aliquots of samples are withdrawn and replaced with equal quantities of fresh PBS at predetermined intervals (pH 7.4) [(6)].

Surface morphology:

The surface morphology of the optimized sulfasalazine-loaded ufasome was studied with the aid of Field Emission Scanning Microscopy (FE-SEM). For FE- SEM analysis, a drop of diluted ufasomes dispersion was placed on the carbon-coated copper grid to form a thin film on the grid. The grid was air-dried and samples were viewed under FESEM [(7)].

DSC:

Thermal analysis of the formulation is an important evaluation technique to find any possible interaction between the drug and other used additives. The thermal behavior of thedrug along with oleic acid and cholesterol was studied using the DSC instrument. Calibration of the heat flow scale was done before adding the samples for analysis. A small sample of 15 mg was weighed in aluminum pans followed by crimping. The thermogram was recorded

at 20 ml/min nitrogen gas flow rate and a heating rate of 5^{O}Cper min over a temperature range of 20^{O}C to 350^{O}C [8].

Preparation of Sulfasalazine Ufasomal Gel:

The polymer Carbopol 940 1%w/w was initially soaked in water for the gel for 2 hrs. and dispersed by agitation at 600rpm by using a magnetic stirrer to get smooth dispersion. Triethanolamine was added to neutralize the pH [9].

COMPONENT	QUANTITY
Sulfasalazine loaded ufasomes	1 ml
Carbopol 940	1% w/w (1 gram)
Distilled Water	100 ml
Agitation Speed	600 rpm
Stirring Time	2 hours

Table 3: Components of Preparation of Sulfasalazine ufasomal Gel

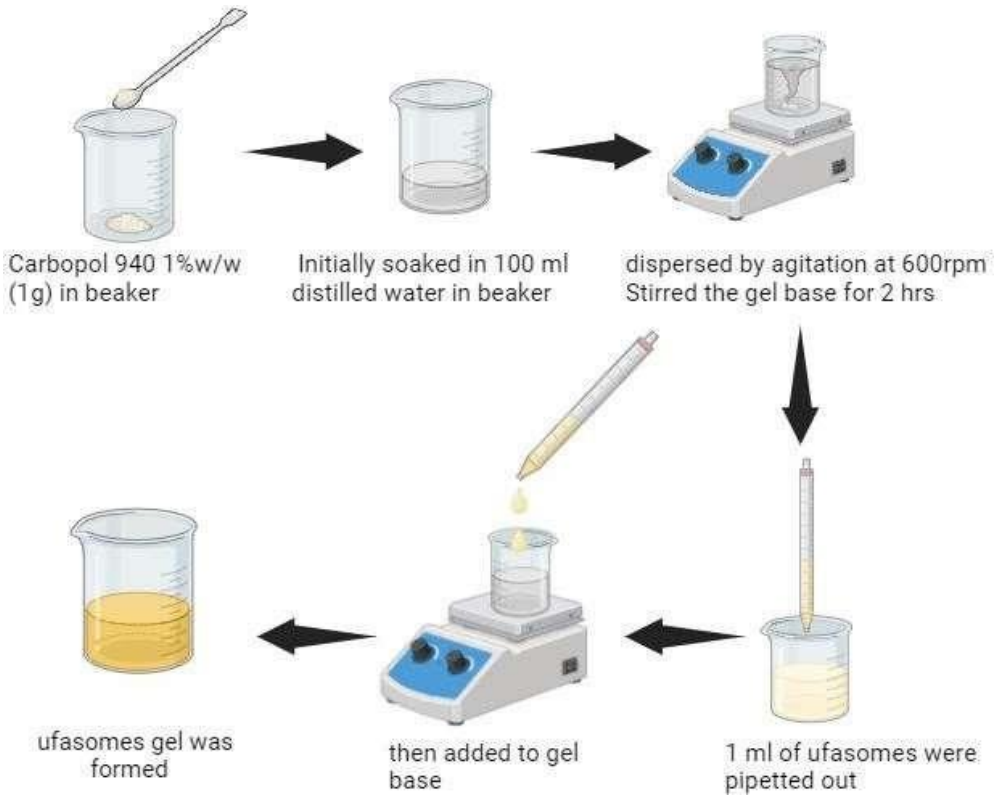


Figure 2: Sulfasalazine Ufasomal Gel

4. Evaluation Studies of Prepared Ufasomal Gel

Physical Examination:

Gels should have a pleasant appearance concerning color, consistency, etc. The prepared ufasomes loaded gel were inspected visually for their color, homogeneity, and consistency.

Drug content estimation:

Accurately weighed equivalent to 100 mg of topical ufasomal gel was taken in a beaker andadded 20 ml of phosphate buffer. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 ml of filtered solution was taken in a 10 ml capacity volumetric flask and the volume was made up to 10 ml with 7.4 phosphate buffer. This solution was analyzed using a UV-Spectroscope at λmax 360 nm for Sulfasalazine respectively and %drug content can be calculated using the following formula [(10)].

Spreadability studies:

Spreadability is a term expressed to denote the extent of the area to which the gel readily spreads on application to the skin. The therapeutic efficacy of a semisolid formulation also depends on its spreading value.1 g of the formulation was placed within a circle of 2 cm diameter pre-marked on a ground glass slide. The gel formulation was sandwiched between this slide and the second slide having the same dimension. The increase in the diameter due to gel spreading was noted.

Determination of pH:

The pH of Sulfasalazine Ufasomal loaded topical gel was checked directly by dipping it into the gel and allowed to equilibrate, then the pH was measured by a calibrated pH meter maintainedat 25°C. The sample was tested in triplicate.

Viscosity and rheological studies:

The viscosity of Sulfasalazine ufasomal gel was measured by a Brookfield viscometer using spindle number 62 rotated at a speed of 12 rpm at room temperature[(11)].

In-vitro Diffusion studies:

The *in-vitro* diffusion experiments were conducted in a Franz diffusion cell. One end of the cell is immersed in the 7.4 pH phosphate buffer solution via the diffusion layer, allowing the drug to diffuse from the dosage form into the diffusion medium. The configuration is kept at 37±0.5 ^{o}C while stirring magnetically. At consistent intervals of 1 to 8 hours, a 4 ml sample was taken out, and 4 ml of 7.4 phosphate buffer was added back into the beaker to uphold the sink conditions. The collected samples are measured spectrophotometrically with a UV

spectrophotometer at a maximum wavelength of 360 nm to determine the drug's cumulative release [12].

Drug release kinetics model fitting of the release data:

The results obtained from the diffusion studies of the dosage form are fitted into several kinetic models like zero order, first order, Higuchi and Korsmeyer-Peppas model[13,14].

Stability Studies:

The stability studies of gel formulation were determined at 5±2°C in a glass container for 90 days. The gel formulations were checked for the change in physical appearance and drug content was analyzed by applying a spectrophotometrically at 360 nm and phosphate buffer was used as blank.

5. RESULTS AND DISCUSSION

MODEL STATISTICAL SUMMARY

Based on the sequential sum of squares (Type-I) and the summary of the fit, the quadraticmodel was selected for all responses. For selecting the model, the F-value, P- *P-value*, and R^2 values were assessed. Furthermore, the quadratic model exhibits the highest polynomial order, with a p-value (indicating degree of statistical significance) of 0.0001.

FIT SUMMARY

Response 1: Entrapment efficiency

Response 2: Drug release

Response	Source	Sequential p-value	Lack of fit p-value	Adjusted R^2	Predicted R^2	Remarks
EE%	Linear	0.1254	0.0007	0.2078	-0.0821	
	2FI	0.4592	0.0006	0.1746	-0.4599	
	Quadratic	< 0.0001	0.0660	0.9381	0.7823	**Suggested**
	Cubic	0.0214	0.5498	0.9814	0.9413	**Aliased**
%Drug	Linear	0.0247	0.9879	0.4276	0.3841	**Suggested**
	2FI	0.8298	0.9745	0.3674	0.3031	

release	Quadratic	0.8022	0.9357	0.2363	0.0803	
	Cubic	0.7889	1.0000	0.0275	0.3669	**Aliased**

Table 4: Fit Summary

For %Drug Release, the Linear model is suggested, with a significant sequential p-value (0.0247), non-significant lack of fit (p = 0.9879), and moderate adjusted (0.4276) and predicted R^2 (0.3841) values, indicating a reasonably good fit and predictive ability. The Quadratic model, while suggested, has lower adjusted (0.2363) and predicted R^2 (0.0803) values, implying weaker performance compared to the Linear model. The 2FI and Cubic models are not favored, as they show poor predictive capabilities and are either aliased or have weak fit statistics. Overall, the Quadratic model is best suited for EE%, while the Linear model is the mostappropriate for % Drug Release.

Std. Dev.	3.72	**R^2**	0.9639
Mean	77.38	**Adjusted R^2**	0.9381
C.V. %	4.80	**Predicted R^2**	0.7823
		Adequate Precision	14.8915

Table 5: Fit Statistics of % EE

The **Predicted R^2** of 0.7823 is in reasonable agreement with the **Adjusted R^2** of 0.9381; i.e. thedifference is less than 0.2.

Adequate Precision measures the *signal-to-noise* ratio. A ratio greater than 4 is desirable.Your ratio of 14.892 indicates an adequate signal. This model can be used to navigate the design space.

Std. Dev.	6.45	**R^2**	0.5230
Mean	67.85	**Adjusted R^2**	0.4276
C.V. %	9.50	**Predicted R^2**	0.3841
		Adequate Precision	6.5103

Table 6: Fit Statistics of % Drug release

The **Predicted R^2** of 0.3841 is in reasonable agreement with the **Adjusted R^2** of 0.4276; i.e. thedifference is less than 0.2.

Adequate Precision measures the *signal-to-noise* ratio. A ratio greater than 4 is desirable.Your ratio of 6.510 indicates an adequate signal. This model can be used to navigate the design space.

ANOVA for quadratic model

Response 1(EE%)

Table 7: Response of EE%

Source	Sum of squares	df	Mean Square	F-Value	P-value	Remarks
Model	2584.32	5	516.86	37.39	< 0.0001	Significant
A- oleic acid	191.51	1	191.51	13.86	0.0074	
B-Cholesterol	719.53	1	719.53	52.06	0.0002	
AB	110.25	1	110.25	7.98	0.0256	
A^2	745.20	1	745.20	53.91	0.0002	
B^2	1018.50	1	1018.50	73.69	< 0.0001	
Residual	96.75	7	13.82			
Lack of Fit	77.95	3	25.98	5.53	0.0660	Not significant
Pure Error	18.80	4	4.70			
Cor Total	2681.08	12				

Factor coding is **coded.**

The sum of squares is **Type III – Partial.**

The **Model F-value** of **37.39** implies the *mode is* significant. There Is only a **0.01%** 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, AB, A^2, and B^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 **5.53** implies there is a **6.60%** chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad -- we want the model to fit. This relatively low probability **(<10%)** is troubling.

Response Analysis through Polynomial Equations

Effect of Variables on Entrapment Efficiency

The Effect of oleic acid concentration and cholesterol on the % EE was assessed by studying the surface plots. An increase in the concentration of cholesterol showed a positive effect on %EE whereas oleic acid showed a negative impact. Comparing the contour plot it was evident that 150:100 should good % EE.

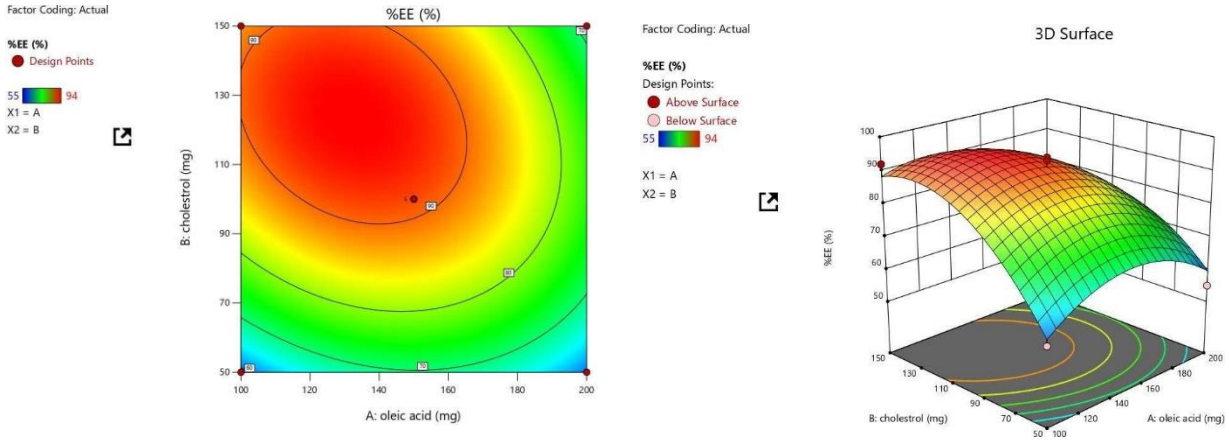


Figure 3. 2D contour plots (A) and 3D response surface plots (B) for evaluating the influence of entrapment efficiency

Response 1: Entrapment efficiency

EE: + 91.20 – 4.89*A +9.48*B – 5.25*AB -10.35*A^2 -12.10* B^2

Entrapment Efficiency (EE) is positively influenced by B (coefficient +9.48) and negatively impacted by A (-4.89), indicating that increasing B enhances EE while A reduces it. The negative interaction term (-5.25) suggests that the combined increase of A and B diminishes EE. Quadratic effects (A2, B2A^2, B^2A2, B2) are strongly negative (-10.35 and -12.10), highlighting diminishing returns or reduced EE at higher levels of these factors. Overall, maximizing EE requires optimizing B while keeping A within a controlled range to minimize adverse effects from interactions and quadratic terms.

Effect of Variables on % Drug Release

Increases concentration of oleic acid show a positive response in drug release which indicates that oleic acid improves the permeability characteristics of the ufasomes and enhancesthe drug release. Lower cholesterol concentration shows an increased effect the drug releasetends to increase and decrease in the concentration of cholesterol. Cholesterol concentration influences the encapsulation efficiency and stabilizes the bilayer but reduces the membrane fluidity, potentially limiting drug diffusion.

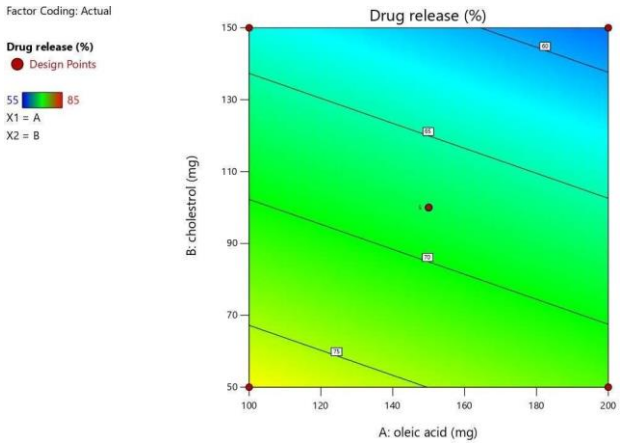


Figure 4: 2D contour plots

Response 2: Drug release(%): + 67.85 – 2.48*A – 7.13*B

The Drug Release (%) is negatively influenced by factor, (-2.48) indicating that increases in either factor reduce drug release. The absence of interaction or quadratic terms suggests a predominantly linear relationship between the factors and drug release. To maximize Drug Release(%), it is essential at lower levels within the experimental range, with a particular focus on minimizing to its stronger negative effect.

Vesicular Size

Using various microscope imaging techniques, the generated vesicular formulations were characterized for morphological properties such as vesicle shape, lamellarity, surface morphology, and aggregation. Before sonication, the creation of vesicular structures and the shape of the produced vesicles were examined using an optical microscope. The prepared ufasome suspension was first viewed under the microscope. A drop of the diluted vesicular suspension was kept on a clear microscope slide, spread uniformly by putting it on the cover slip and viewed under a 10 X magnification optical microscope.

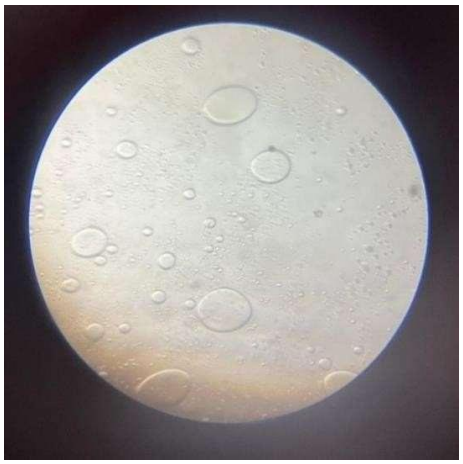


Figure 5: Microscopic image of ufasomes

Ufasomes are usually spherical or oval-shaped vesicles. Their size might vary depending on preparation conditions. Uniformity in size and shape indicates successful preparation. Aggregation or irregular shapes could point to issues in formulation or stability.

Drug entrapment efficiency (EE %)

It was found that the prepared sulfasalazine–ufasomes exhibited a good percentage of entrapment (EE %), with values ranging from (89%) for UF-3.

Formulation	EE (%)
UF-3	89

Table 8: Results for EE%

% Drug release:

It was found that the prepared sulfasalazine–ufasomes exhibited a good percentage of % Drug release with values ranging from (70%) for UF-3.

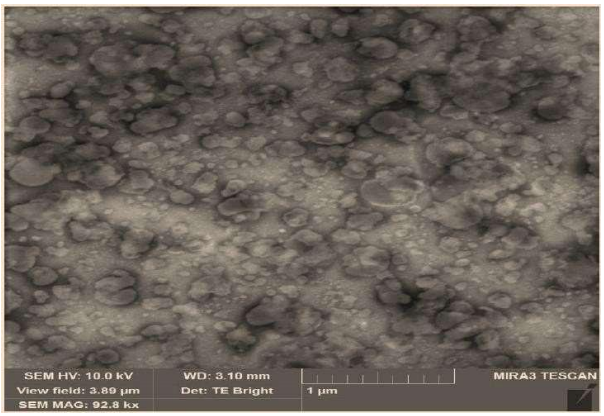
Formulation	Drug release (%)
UF-3	70

Table 9: Results for % Drug release

Scanning electron microscopy

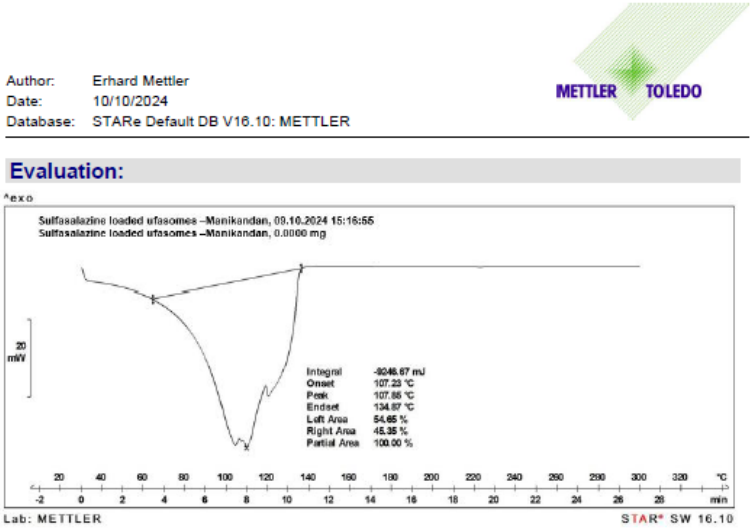
The morphology of the ufasomes vesicles was evaluated using an optical microscope and scanning electron microscopy to validate the vesicular features. Spherical vesicles of uniform size distribution were identified as sulfasalazine ufasomes created from oleic acid, span 20, and cholesterol.

Fig 6: SEM Images of UF-3 Formulation



DSC Studies:

The DSC analysis of sulfasalazine-loaded ufasomes shows an onset temperature of 107.23 °C, with a peak temperature recorded at 107.85 °C. The integral value of the analysis is -9246.67mJ. The partial area distribution shows 54.65% on the left and 45.35% on the right.



Curve: Sulfasalazine loaded ufasomes-Manikandan, 09.10.2024 15:16:55
Sample: Sulfasalazine loaded ufasomes-Manikandan, 0.0000 mg
Module: DSC 3 /500/1754, 02.04.2019 12:54:50
Sample Holder: Aluminum Standard 40ul
Material: Aluminium
Method: 30-300
dt 1.00 s
[1] 30.0. 300.0 °C, 10.00 K/min
Synchronization enabled
User: METTLER
Results: Integral -9246.67 mJ
Onset 107.23 °C
Peak 107.85 °C
Endset 134.87 °C
Left Area 54.65 %
Right Area 45.35 %
Partial Area 100.00 %

Figure 7: DSC of Optimized Ufasomes

6. EVALUATION STUDIES OF PREPARED UFASOMAL-LOADED GEL

Physical Examination

All the prepared ufasomal gel formulations containing 1% Carbopol 940 were visually evaluated for homogeneity. The prepared formulations were clear and transparent**.**

Spreadability studies

Spreadability is a significant factor to take into account when creating gels. The Spreadability of the created UF- 3 formulation was determined to be 48.71%, indicating that this formulation is suitable for topical use.

Formulation	Spreadability %
UF-3	48.71 ± 0.470

Table 10: Spreadability of UF- 3

Determination of pH

Since an increase in pH can irritate the skin, pH is crucial for creating topical formulations. Theformulations' pH values were determined to be 5.20 ± 0.17. This demonstrates that the pH readings were within the skin's range. As a result, the ufasomal gel formulation's pH was found to be appropriate for skin application [15]

Formulation	Ph
UF- 3	5.2 ± 0.17

Table 11: pH of UF 3

Viscosity

The viscosity of the gel is influenced by the specific type of polymer grade and its concentration in the formulation. Significantly, the gel's viscosity rises as the polymeric ratio increases. In the formulation of ufasomes, 1% of Carbopol 940 was employed as a consistent measure across all the preparations. The viscosity of NS was determined to be 1005 cP[16]

Formulation	Viscosity
UF 3	1005

Table 12: Viscosity of UF 3

In vitro drug release studies:

The Ufasomal gel formulation UF-3 underwent in-vitro drug release testing utilizing a Franz diffusion cell. The movement of trapped drug molecules into the vesicular system is controlledby the transfer of drugs from vesicles into the surrounding aqueous environment and then diffusion through the eggshell membrane into the receptor medium. This research was conducted over 6 hours. The total quantity of drug released was determined forthe formulation. The release patterns of sulfasalazine from the formulated ufasomal gel.

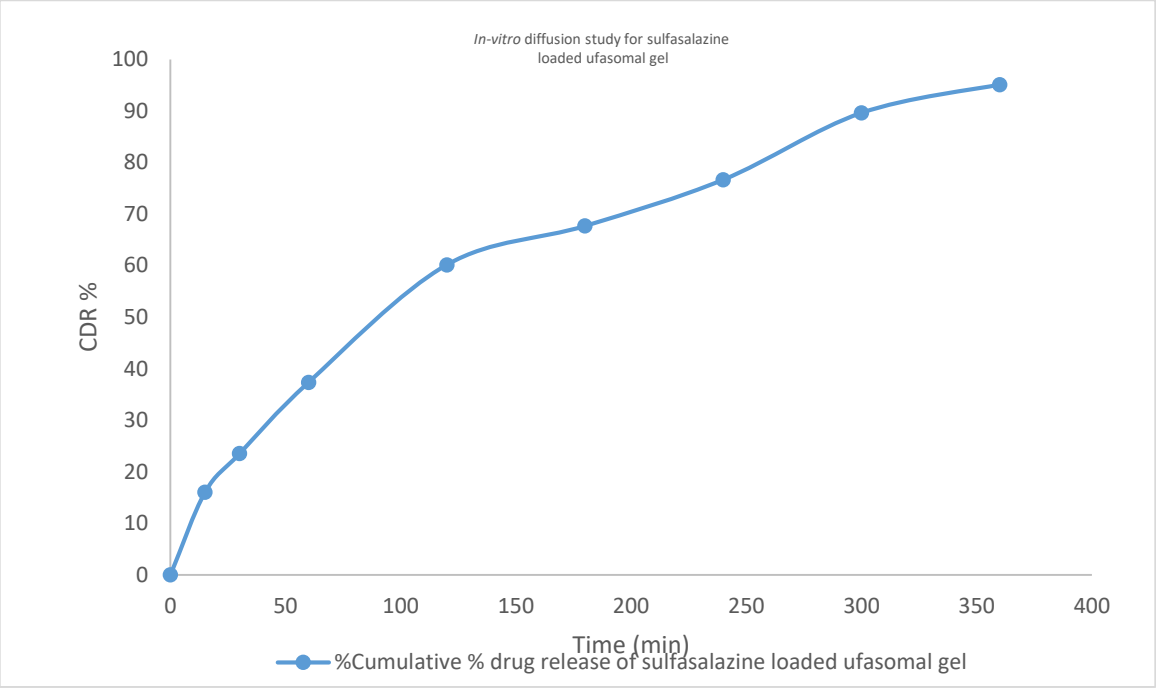


Figure 8: In vitro drug release studies.

Kinetics of In *Vitro* Drug Release

To predict and correlate the *in-vitro* drug release from sulfasalazine ufasomal gel, it is necessary to fit into a suitable mathematical model. Investigation for the drug release from the ufasomal gel was done by substituting the drug release profile into various kinetic equations (Zero order, First order, Higuchi's equation, Hixon Crowell and Korsmeyer-Peppas). The different release kinetics patterns and release mechanisms of the sulfasalazine ufasomal gel for the optimized formulation as well as the best-fit release pattern for the sulfasalazine ufasomes formulations. [17]

Formulation	**Kinetic models**			
F3	**Zero order**	**First order**	**Higuchi model**	**Korsmeyer-peppas model**
R^2 values	0.9269	0.9674	0.9942	0.9711

Table 13: Drug release data of UF 3 – Kinetic models

The drug release studies revealed first-order kinetics with correlation coefficient (R^2) for the formulation value of **(R^2 = 0.9674)** and kinetics equations are presented and it also showed

best fit with the Higuchi model **(R^2 = 0.9942).** This demonstrated that the drug release from the ufasomes *follows a diffusion* mechanism in a sustained pattern from the matrix formed.

Stability Studies

The stability study of the optimized formulation (UF-3) was carried out for 90 days at 5±2°C in a glass container. After prolonged storage, the ufasomal gel of formulation UF-3 was evaluated for various parameters like physical appearance, pH, drug content, and percentage of drug release.

Days	**Physical appearance**	**pH (*±SD) n=3**	**Drug content (*±SD) n=3**	***In vitro* drug release (%) (*±SD) n=3**
0	Clear and colorless	5.22 ± 0.01	87.32 ± 0.09	95.10 ± 1.69
90	Clear and colorless	5.20 ± 0.15	87.24 ± 0.02	89.62 ± 0.09

Table 14: Stability studies

SD = Standard deviation, **n**=3.

Formulation UF-3 after 90 days of storage indicates that there are no significant changes in the formulation compared to the initial state. The research indicates there is no significant difference between *pre-and* post-storage, with all results falling within the satisfactory range. Consequently, the formulation stays stable for an adequate duration following 90 days of storage.

CONCLUSION

In Conclusion, the sulfasalazine-loaded ufasomal gel offers a potential and innovative approach for topical drug delivery in rheumatoid arthritis. This formulation offers the advantage of sustained drug release, which may contribute to prolonged therapeutic efficacy and reduced dosing frequency. Furthermore, the ufasomal gel enhances the stability of sulfasalazine, mitigating degradation and ensuring consistent potency throughout its use. Improved patient adherence is also anticipated due to the topical application route, which bypasses gastrointestinal side effects associated with oral administration. Collectively, these attributes support the sulfasalazine-loaded ufasomal gel as a promising alternative in rheumatoid arthritis.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

SEM: Scanning Electron Microscope; **ML:** Milli Liter;
°**C:** Degree Centigrade; **µg:** Microgram;
Rpm: Rotations per minute; **µm:** Micrometer; **nm:** Nanometer.

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SUMMARY

The objective of the present study is to prepare and evaluate Sulfasalazine-loaded ufasomal topical gels for treating rheumatoid arthritis. Ufasomes were prepared using the ether injection method with varied concentrations of oleic acid, cholesterol, and Span 20. Preformulation studies confirmed the drug met pharmacopeial standards, and FTIR revealed no compatibility issues. Surface morphology analyzed via optical and scanning electron microscopy showed spherical vesicles. An optimized formulation was selected using a central composite design with entrapment efficiency (EE%) and drug release as responses. The optimized ufasomal gel, prepared with Carbopol 940, was evaluated for pH, spreadability, viscosity, drug content, and *in-vitro* diffusion. Results showed sustained drug release following the Higuchi model, indicating matrix-based diffusion. Short-term stability studies revealed no significant changes in pH, drug content, or cumulative drug release. This formulation reduces application frequency and enhances patient compliance. Overall, sulfasalazine-loaded ufasomal gel is a promising transdermal delivery system for rheumatoid arthritis.