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Enhanced Delivery of Sulfasalazine via Niosomal Gel: A New Horizon in Rheumatoid Arthritis Therapy

Mrunmayee M. Thombare[1*] and Vaibhav Vaidya[2]

[1]Department of pharmaceutics, Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-411044 (India)
mrunmayeethombare1323@gmail.com
[2]Associate Professor at Dr. D. Y. Patil College of Pharmacy, Akurdi, Pune-411044 (India)
v.vaidya123@gmail.com
[*]Corresponding Author: Email – mrunmayeethombare1323@gmail.com

Abstract

The goal of this research is to create a novel sulfasalazine-loaded niosomes formulation for improved therapeutic efficacy in the management of rheumatoid arthritis. Niosomes vesicles were prepared using the dry ether injection procedure. Enhancing the medicines' bioavailability and subsequently their regulated release from niosomal vesicles received particular focus. Two surfactants, span 60 and tween 80, were combined to improve the biocompatibility and trapping efficiency. To enhance the drug's bioavailability, cholesterol acts as a hard membrane to regulate the pace of release and shield it from deterioration. Because the central composite design provides information about the effects of many variables on the responses, it was used for optimization. Design expert software was utilized to optimize and choose the optimal arrangement of cholesterol and surfactants were employed. Using design expert software, 13 batches of varying compositions were created. The final product was then created by combining the tailored niosomes with a gel foundation. Using a Franz diffusion cell, the produced niosomal gel was assessed for its physicochemical characteristics and drug release investigations. In comparison to traditional dosage forms, the results show that the sulfasalazine loaded niosomal gel had higher drug entrapment efficiency, sustained drug release profile, and enhanced skin penetration. After optimized batches were further examined using SEM and zeta potential, it was discovered that the medication was widely and uniformly distributed throughout the vesicles. Every outcome was appropriate and accurate. Overall, this study shows that niosomal gel has the potential to be a viable carrier system for better sulfasalazine administration, providing new perspectives for the treatment of inflammatory disorders.

Key words : Niosomes, Sulfasalazine, Dry ether injection, Rheumatoid arthritis.

One of the most effective new methods for increasing the bioavailability of weakly water-soluble medications is the formulation of niosomes, which raises the formulations' relative bioavailability. Currently, niosomes are prepared using a variety of techniques, such as the hand shaking method, thin film hydration and the ether injection approach. Niosomes are hydrating mixtures of cholesterol and surfactant that produce non-ionic surfactant vesicles. Normally, niosomes exist on a nano- or micrometric scale[13,19]. Because they are less expensive than current drug delivery methods and have the advantages of being more stable, nontoxic, and economical, niosomes are being investigated as a potential replacement[7]. Niosomes exhibits greater benefits by sustaining pharmacological therapeutic levels over an extended period of time. They may also be able to encapsulate and shield medications from enzymatic and acid breakdown. An inflammatory autoimmune condition, rheumatoid arthritis mostly affects several joints in the body[8,10]. It has been shown that 0.8% of the general population has RA. Women are more likely than men to get RA[5].

Sulfasalazine belongs to the class of pharmaceuticals known as disease-modifying antirheumatic drugs (DMARDs). Sulfasalazine has a half-life of five to seven hours. Sulfasalazine requires frequent administration; hence it is not recommended for oral use. The drug's entrapment in a vesicle has demonstrated enhanced drug delivery at the intended site and decreased side effects, which has improved patient compliance. In addition to having a quicker start of effect than other DMARDs, sulfasalazine may also slow down the radiographic advancement of RA. For RA patients, sulfasalazine is the recommended first line of treatment. For women who are or may become pregnant, it may also be the best DMARD. Numerous cellular effects of sulfasalazine and sulphapyridine seem to have a positive impact on the rheumatoid process. Comparisons with other disease-modifying antirheumatic medications (DMARDs) and placebo-controlled trials have validated the effectiveness of sulfasalazine in RA, which has been reported in numerous noncomparative investigations. Less than 15% of a dose of sulfasalazine taken orally is absorbed as the parent medication. The bioavailability of sulfasalazine is believed to be between 10% and 30%. Therefore, it is advised to put the medication into vesicles to extend its time in systemic circulation and boost its bioavailability[17,20]. Based on the aforementioned results, we deduce that sulfasalazine has anti-arthritic properties. The current study aims to develop a niosomal gel loaded with sulfasalazine in order to boost the bioavailability of medication. In this investigation, several surfactants, including span and tweens, were used to produce niosomal formulations of the medication. The produced formulations were then assessed for formulation stability, drug release, vesicle size, and entrapment efficiency.

Materials :

The model medication was sulfasalazine, which was acquired from Yarrow Chemicals Pvt. Ltd. in Mumbai. Cholesterol, span 20,40,60,80 (LOBA Chemie Laboratory Reagent & Fine Chemicals, Pvt. Ltd.) and Tween 20,40,60,80 (Analab Fine Chemicals, Mumbai) are utilized as surfactants. The gelling agent employed was Carbopol 934 (LOBA Chemie Laboratory Reagent & Fine Chemicals, Pvt. Ltd). The penetration enhancer utilized

was propylene glycol (LOBA Chemie Laboratory Reagent & Fine Chemicals Pvt Ltd). For the purpose of creating vesicles, methanol was determined to be an effective solvent and diethyl ether (LOBA Chemie Laboratory Reagent & Fine Chemicals Pvt. Ltd.) to be a volatile solvent. Analytical grade solvents were all that were used.

1. *Pre-formulation study of drugs :*
   Properties of Sulfasalazine in Physiochemistry
   A) *Organoleptic assessment :*
   The assessment of sensory characteristics, such as taste, look, and odour, was done.

   B) *Solubility (under ambient conditions)*
   The ability of a solute (a solid, liquid, or gaseous chemical entity) to dissolve in a solid, liquid, or gaseous solvent and produce an equal solution of the solute in the solvent is known as its soluble property. The amount of a solute that will dissolve in a specific amount of solvent is known as its solubility. It is a crucial factor to consider when creating a dosage form.
   C) *The Identification Exam :*
   FTIR Spectroscopy: The infrared spectrum is a crucial record that provides adequate details on a compound's structure. This method yields a spectrum with several absorption bands, from which a plethora of knowledge regarding the composition of an organic molecule can be extracted.
   D) *Estimation of λ max of sulfasalazine*
   Sulfasalazine's absorption maxima were found by using a double beam UV spectrophotometer to run the medication solution's spectra[24].

2. *Selection of suitable surfactant :*
   Initially, niosomes were prepared by using various surfactants like span 20,40,60,80 and tween 20,40,60,80. Formulated niosomes were evaluated for parameters like vesicle size and percent entrapment. Surfactants which offer highest entrapment and low vesicle size were selected for further studies.
3. *Sulfasalazine loaded preparation Niosomes*
   a) Niosomes were created via the injection of dry ether. The surfactant and cholesterol were weighed precisely and dissolved in 8 millilitres of diethyl ether.
   b) In a different beaker, the appropriate amount of each medication was dissolved in two millilitres of methanol. There was a blend between the two solutions.
   c) In another beaker, 10 ml of phosphate buffer solution (pH 7.4) was added. The prepared organic phase solution was injected into PBS at a rate of 1 millilitre per minute using a magnetic stirrer with a heated plate at 60 to 65 degrees Celsius.
   d) The solution was allowed to cool after injecting every organic phase into the PBS. After the temperature reached room temperature, niosomes developed.
   e) Use a digital microscope to assess the manufactured niosomes for particle size and determine the percentage of niosomes entrapment efficiency[22].

Optimization of niosomes loaded with sulfasalazine :

Design expert software was used to produce the formulation. We considered many other design strategies before deciding on the randomized central composite design since it is suited for thoroughly examining every potential impact of every variable on response. The concentration of the cholesterol (X2) and surfactant mixture (X1) were chosen as the

two independent variables, while the vesicle size and entrapment efficiency percentage were the dependent variables. Thirteen formulation batches were prepared and assessed in order to identify an optimal batch. Table-1 displays the levels of independent and dependent variables (Factors) for niosomes optimization. The statistical experimental design was created and assessed using the Design-Expert 12 program. For optimization, the Central Composite design was employed. This design makes sense since it enables us to fully comprehend the effects of each variable[11,18]. Every batch that was formulated was prepared and assessed according to a number of criteria. Table-1 displays the levels of independent and dependent parameters for niosomes optimization.

Table-1. Levels of dependent & independent variables

Independent variables	Factors	Levels	
X1	Concentration of surfactant mixture	50	150
X2	Concentration of cholesterol	50	200

Dependent variable	Factors
Y1	Vesicle size
Y2	% entrapment

Evaluation of niosomes :

1. *Vesicle size analysis :*

Using a digital microscope and Pixel Pro software, the size, shape, and lamellar character of the vesicles in the sonicated formulations were examined. A stage micrometre was used to calibrate the microscope. A drop of niosomal dispersion was applied to the slide after appropriate dilution, and the slide was examined under a 10X magnification using a Labomed microscope.

2. *Efficiency of Entrapment :*

To separate the niosomes from the non-entrapped medication, the niosomal formulation was whirled using a cooling centrifuge for 30 minutes at 10000 rpm and 4°C [23]. Following centrifugation, the amount of free drug in the supernatant was measured using a UV spectrophotometer (Shimadzu, UV 1700, Japan) to measure absorbance at 357 nm. The percentage of drug entrapment in niosomes was then computed using the formula,

Percent drug entrapment = (Total drug – drug in supernatant / Total drug) x 100

3. *In vitro drug release studies :*

Using a Franz diffusion cell setup, an in vitro release investigation of niosomes was conducted. The diffusion cell equipment is made out of a glass tube with a 2.5 cm inner diameter that is open at both ends and has a cellophane membrane connected to one end. For twelve to twenty-four hours, cellophane membrane was immersed in buffer. It was carefully positioned between the receptor and donor compartments. 20 millilitres of phosphate buffer 7.4 were added to a dialysis tube containing niosomes equivalent to five milligrams of medication. The temperature was kept at 37ºC while the medium was agitated with a magnetic stirrer. Every now and then, one millilitre of samples was set aside, and the

same amount of medium was refilled after each withdrawal. Following that, the samples were assayed spectrophotometrically at 357 nm using phosphate buffer as blank.

Formulation of niosomal gel :

Selection of suitable penetration enhancer and gelling agent :

Initially, various gelling agents, such as HPMC, CMC, Carbopol 934, and Carbopol 940, as well as various penetration enhancers, such as oleic acid, propylene glycol and PEG 400, were used to make various batches of gels. Further analysis was done on the drug release, Spreadability, and viscosity of the prepared gel batches. The gelling agent and penetration enhancer with the highest drug release was chosen for additional research.

The gelling ingredient was consumed as mentioned above. Niosomes freeze-dried to an equivalent of 50 mg were taken. Calculations were made to determine the appropriate concentrations of penetration enhancer and gelling agent. Niosomes that had been freeze-dried were gradually introduced to the beaker containing the gelling agent[21]. Stirring was done until the gel reached the desired consistency. Various gel compositions were made in accordance with the same process and assessed for additional variables. Using a design expert, several batches of gels were created and optimized following the identification of an appropriate polymer[2]. Formulated niosomal gel is shown in Fig. 5 (c).

Evaluation of niosomal gel :

1. *pH, clarity, and appearance :*

This assessment was carried out to see whether any specific issue was present. A digital pH meter was used to measure the niosomal gel's pH.

2. *Determination of viscosity* :

A digital viscometer was used to determine viscosity. L-4 spindle number was utilized. The created gel was allowed to dip the spindle, and the viscosity was noted[6].

3. *Spreadability :*

Using a Spreadability equipment, the Spreadability of niosomal gel formulations was assessed. A single gram of niosomal gel sample was positioned on the bottom slide, while the upper slide was positioned above the sample.

To calculate Spreadability, use the formula,

$$S = (m \times l)/t.$$

where t is the time, l is the length travelled by the upper slide, m is the weight attached to the upper slide, and S is Spreadability 1.

4. *Drug diffusion :*

For the current work, a cellophane membrane was used in conjunction with the Franz diffusion cell. The pH 7.4 phosphate buffer solution was utilized in the receptor chamber.

In order to ensure that there would be no changes in permeability, the cellophane membrane was pre-incubated with phosphate buffer solution pH 7.4 before the investigation began. The donor compartment was filled with

the niosomal gel formulation. The niosomal gel formulation was approximately 1 millilitre in volume. Regular intervals of 1, 2, 3, 4, 5, 6, 7 and 8 hours were used for sampling. Phosphate buffer solution was used to preserve the sink condition[9]. Phosphate buffer was used to dilute the withdrawal sample before additional measurements were made at 357 nm using a UV spectrophotometer.

Organoleptic properties :

Table-2. Organoleptic properties of the drug

Colour	Yellow fine powder
Odor	Odourless
Taste	Tasteless

Solubility studies

Table-3. solubility studies of the drug

Water	Insoluble
Ethanol	Slightly soluble
Methanol	Soluble
Dimethyl sulfoxide (DMSO)	Soluble

UV Studies using visible spectroscopy

Calibration curve of sulfasalazine By plotting concentration v/s absorbance, the calibration curve of Sulfasalazine was created using DMSO at concentration ranges of 2,4,6,8, and 10 µg/ml. The estimated regression coefficient values for the medication were determined to be 0.9996. A graphical illustration of the calibration curve for sulfasalazine is provided in Figure 1.

FTIR analysis :

Drug's infrared absorption spectra were measured between 4000-200 cm. The main peaks were assessed in order to determine purity. A molecule's distinctive functional groups can be seen in its infrared absorption spectra. Fig. 2 show the IR spectra of sulfasalazine graphically.

DSC study :

Sulfasalazine's DSC thermograph revealed a sharp, distinct endothermic peak at 262.52°C, which corresponded to the drug's melting point temperature. Figure 3(A) indicates that the drug's melting endotherm did not change significantly, indicating that it is pure, and Figure 3(B) indicates that the drug is properly entrapped in the vesicle, shifting the endotherm peak to 100.29°C.

Optimization of Niosomes :

Variables' impact on the size of vesicles

The type of surfactant being employed determines the size of the niosomes vesicles. Since the width of the vesicle is dependent on the length of the surfactant's alkyl chain, it can be explained in terms of surfactant structure. Larger vesicles are typically produced by surfactants with longer alkyl chains[3]. The size of the vesicle grows as the surfactant HLB increases. The vesicle size reduces as the concentration of surfactant increases. The size of the vesicle reduces as the concentration of cholesterol rises. This could be because the presence of cholesterol causes the membrane bilayer to become more rigid, which enhances physical stability and decreases water-soluble substance leakage through the membrane, indicating the homogeneity of the niosomes population as cholesterol content rises[14]. 3D response plot of vesicle size analysis is shown in fig. 4(A).

Effect of Variables on Percent Entrapment:

The percentage of entrapment rises in tandem with the bilayers' increased cholesterol content. Sulfasalazine entrapment in niosomal vesicles rose as the amount of cholesterol in the solution increased. enhanced vesicle size and lipid bilayer width are the causes of the enhanced drug entrapment observed in formulations containing cholesterol[1]. The HLB of the surfactant mixture and entrapment efficiency are inversely correlated. Entrapment Efficiency significantly decreases as surfactant HLB increases[15]. Fig. 4 (B) displays the 3D response plot of entrapment efficiency.

Characteristics of optimized niosomes and niosomal gel

- ***Zeta potential of optimized niosomes***

Zeta potential is one of the key factors that is known to influence stability and plays a significant impact in the stability of niosomes because of the strength of the electrostatic or charge repulsion/attraction between particles[25]. It should be negative or neutral, indicating that the system is stable and that the niosomes particles are charge-free. Zeta Sizer (Malvern Instrument, UK) was used to determine the zeta potential. According to Figs. 5(d) and 5(e), An optimized batch of niosomes formulation was discovered to have a Zeta Potential of -34.17 mV. Niosomes stability is good, as indicated by this negative zeta potential value[12].

- ***Vesicle size of an optimized formulation***

The formulation's vesicle size is examined under a digital microscope. A batch of optimized niosomes was diluted appropriately with distilled water, and the particles were examined at a 10X magnification under a microscope[4,16]. Under a digital microscope, the optimized batch's particle size was determined to be 612.81 nm, as illustrated in Fig. 5(a).

- ***SEM Analysis of an optimized formulation :***

The prepared niosomes have a spherical form with the appropriate particle size, as shown in fig. 5(b), according to the SEM results.

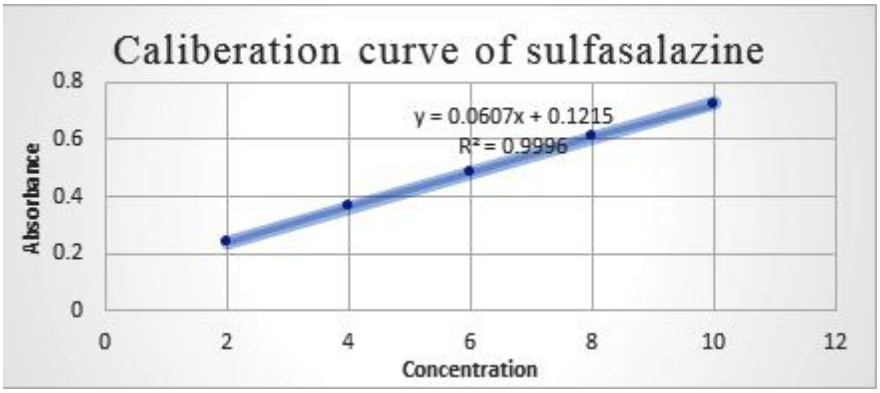

Figure 1. Standard curve of sulfasalazine

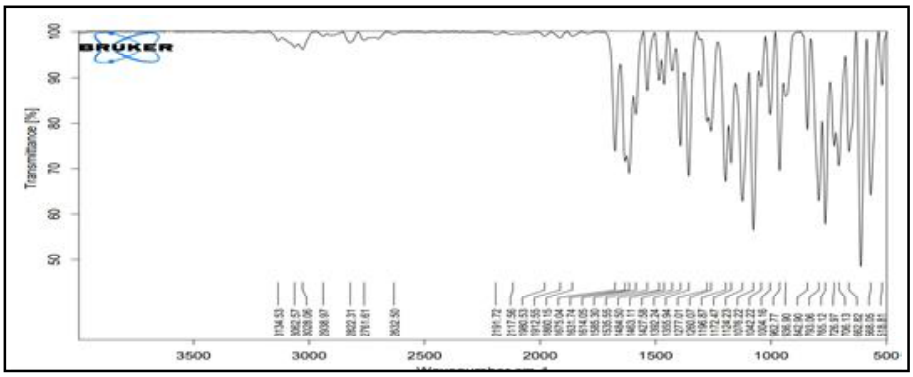

Figure 2. FTIR Spectra of sulfasalazine

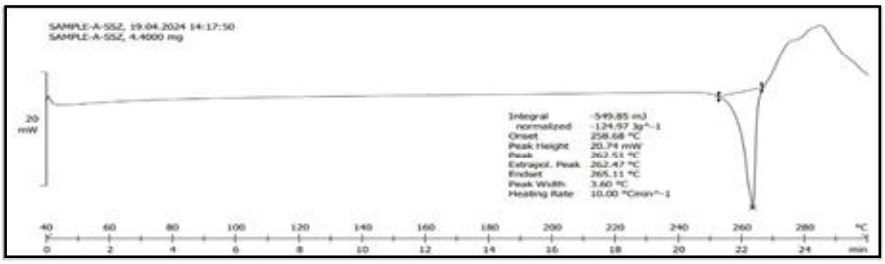

Figure 3 (A). DSC of Sulfasalazine

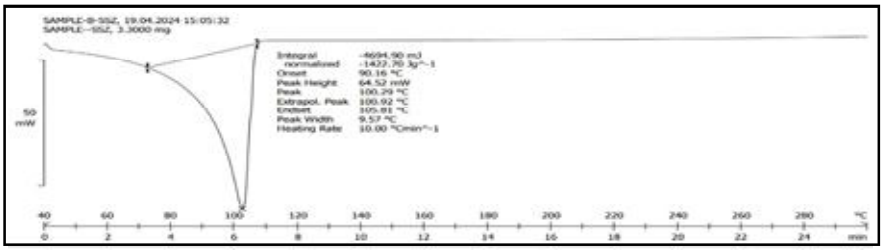

Figure 3 (B). DSC OF Sulfasalazine Niosomes

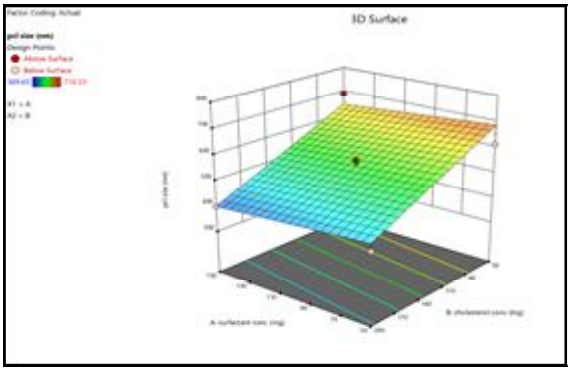

Figure 4(A). 3D Response plot for vesicle size analysis

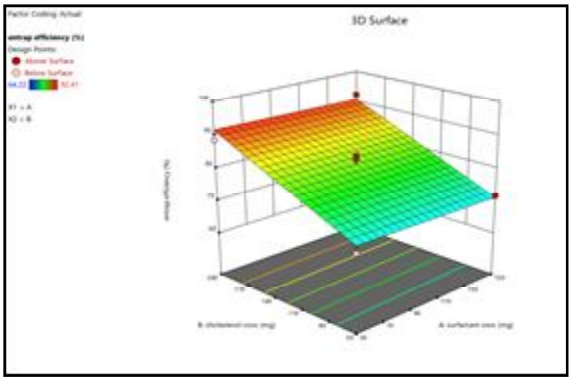

Figure 4(B).3 D Response plot for entrapement effeciency analysis

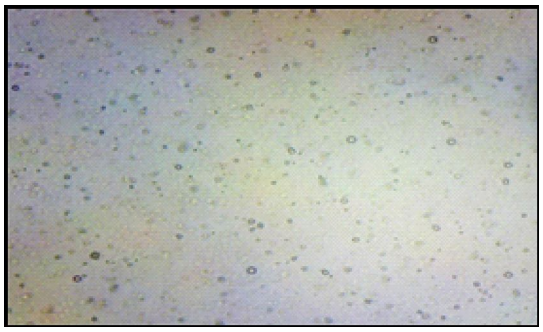

Figure 5 (a). Optimized niosomes

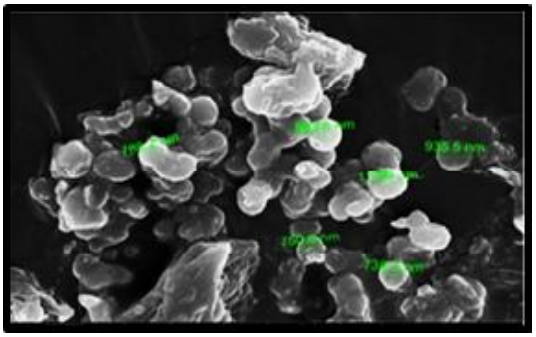

Figure 5 (b). SEM analysis of Optimized batch niosomes

Figure 5 (c). Niosomal gel

Start Date/Time	Sample ID	Zeta Potential (mV)	Mobility (µ/s)/(V/cm)	Conductance (µS)	Sample Count Rate (kcps)	Ref. Count Rate (kcps)	RMS Residual
27-05-2024 16:18:46	SSZ - 1	-33.93	-2.65	95	635	134	3.0732e-02
27-05-2024 16:19:00	SSZ - 2	-34.64	-2.71	95	635	134	4.3556e-02
27-05-2024 16:19:16	SSZ - 3	-33.86	-2.65	95	635	134	2.1945e-02
	Mean:	-34.14	-2.67	95	635	134	3.2078e-02
	Std Err:	0.25	0.02	0	0	0	6.2747e-03
	Std Dev:	0.43	0.03	0	0	0	1.0868e-02

Figure 5 (d). Zeta potential of an optimized niosomal batch

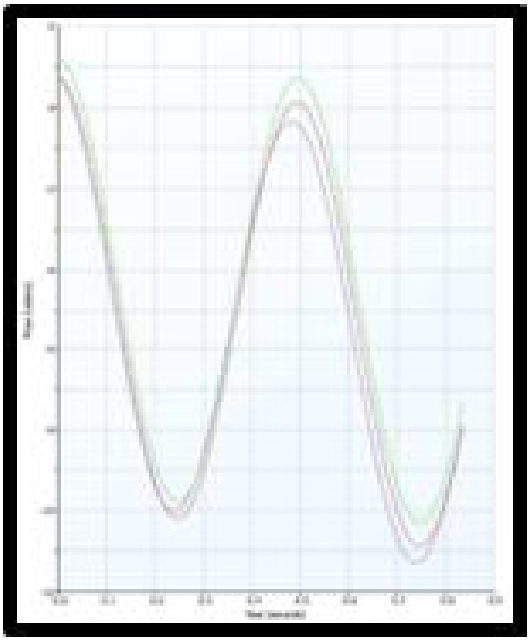

Figure 5 (e). Zeta potential graph
Figure 5 (e) zeta potential curve

Ether injection was used to create niosomes. Software designed for experts is utilized to optimize niosomes. The prepared niosomes were assessed for drug release, vesicle size, and entrapment percentage. Third batch of the thirteen batches that were created were discovered to be an optimized batch of the niosomes. The ideal range for particle size, according to design experts, is between 300 and 800 nm. Furthermore, the real vesicle size was discovered to be 612.81 nm, indicating the vesicle size of an optimized batch is significant. The range of values between 64% to 93% is the optimal range for drug entrapment efficiency. Furthermore, the actual drug's entrapment efficiency value was discovered to be 92.41%, indicating the entrapment efficiency of an optimized batch is significant.

To formulate niosomal gel, optimized niosomes were added to the gel foundation. The prepared gel batch is once more assessed for drug release, Spreadability, and viscosity. It was discovered that the viscosity value was 3046 cps. It was discovered that the Spreadability value was 28.57 gm cm per second. The niosomal gel's drug release was discovered to be 88.12%.

The optimized batches were subjected to zeta potential and SEM analysis, revealing that the drugs were widely and uniformly distributed within the vesicles. Sulfasalazine loaded niosomal formulation was optimized and successfully formulated in the form gel which can be used for topical preparation for the treatment of rheumatoid arthritis.

Conflict of interest

The authors declares that there is no conflict of interest.

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