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FORMULATION AND EVALUATION OF FLUFENAMIC NIOSOMAL GEL FOR TREATING RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis (RA) is a symmetrical, chronic inflammatory autoimmune disease that first affects the small joints before spreading to the larger joints, skin, eyes, heart, kidneys, and lungs. Niosomes are non-ionic surfactants with multilamellar vesicles that release the medicine through their bilayer in a regulated manner, allowing for continuous release of the contained substance. Therefore, the purpose of this study is to develop and assess a Flufenamic niosomal gel for the treatment of rheumatoid arthritis. Results showed that the F9 formulation of niosome observed to have average particle size of 155.25 while the % entrapment efficiency was found to be 76.65 %. The Zeta Potential (mV) of F9 formulation was noticed to -39.74. The formulated niosomal gel was observed to have drug content of 99.12±0.15. The pH of gel was within the range of skin pH which is 6.85±0.02. The Extrudability (g) & Spreadibility (g.cm/sec) was found to be 165±6 and 11.35±0.85 respectively. The viscosity was observed to be 3465±15 cps. In-vitro drug release study of prepared gel formulation indicated at 12 hours the % cumulative drug release was 98.85 %. The formulated niosomal gel was observed too be stable for 3 months at 4.0±0.2°C. In conclusion, this study suggests that transdermal formulations of in Flufenamic niosomal gel were successfully developed and could be used as an alternative route of administration.

Keywords: Flufenamic, Niosomes, Transdermal drug delivery, Niosomal gel, Rheumatoid arthritis

INTRODUCTION

The inflammatory autoimmune illness known as rheumatoid arthritis (RA) affects the skin, eyes, heart, kidneys, lungs, and bigger joints first before spreading to other organs like the skin and eyes. Joint bone and cartilage are frequently damaged, and tendons and ligaments become more fragile (lee *et al*., 2017). The morning stiffness of the affected joints for longer than 30 minutes, exhaustion, fever, weight loss, sore, swollen, and warm joints, and rheumatoid nodules under the skin are common symptoms of RA (Singh *et al.*, 2021). The most prevalent kind of arthritis, osteoarthritis (OA), is brought on more by wear and tear than by an immunological disorder. The immune system, the heart, or the lungs are unaffected (Bullock *et al*., 2018). Reducing joint pain and inflammation, enhancing joint function, and preventing joint damage and deformity are the objectives of RA treatment.

This includes elements including disease progression, the affected joints, age, general

health, occupation, adherence, and knowledge of the disease (Staheli, 1998).

Localised drug distribution through the skin, vagina, rectal, and ocular cavities is known as topical drug administration. The stratum corneum, the epidermis' top layer, serves as the skin's primary barrier. The optimum properties of the medications for transdermal distribution include low molecular weight (500 Da), lipophilicity, and efficacy at a low dosage. As a result, by formulating the current medications in a beneficial method, their therapeutic effectiveness is increased. Recently, transdermal drug delivery systems were created with the goal of achieving systemic therapeutic goals through topical application to the intact skin surface. Transdermal medication delivery utilises the skin as a primary target and barrier.The creation of a novel medicine delivery system has received a lot of attention during the last few decades. Chemical and physical methods have been investigated to reduce stratum corneum barrier characteristics in order to enhance permeability. These techniques include iontophoresis, electroporation, tape stripping, and vascular systems like liposomes and niosomes. When synthetic non-ionic surfactants are hydrated, whether or not sterols like cholesterol or other lipids are incorporated, niosomes, which are non-ionic surfactants with multilamellar vesicles, are produced. Niosomes act as drug depots in the body, releasing the drug through its bilayer in a controlled manner to provide prolonged release of the contained drug. The main benefits of these vesicular drug carriers are that niosomes offer good patient compliance in comparison to oily dose forms because they are a water-based vehicle (Sharma *et al*., 2020).

Non-steroidal anti-inflammatory medication flufenamic acid (FFA) has been used as an analgesic for pain associated with rheumatic illnesses (Jiancong *et al.*, 2022). An analgesic, anti-inflammatory, and antipyretic anthranilic acid derivative. It is applied locally and orally to treat musculoskeletal and joint diseases. Analgesic flufenamic acid is used to treat the pain brought on by rheumatoid arthritis. Therefore, the purpose of this study is to develop and assess a flufenamic niosomal gel for the treatment of rheumatoid arthritis.

MATERIALS AND METHODS

Preparation of Flufenamic acid loaded Niosomes

By using the thin film hydration approach, niosomes were created. In a flash evaporator set at 60°C, soya PC and span were dissolved in 500 ml of round bottomed flask with 10 ml of chloroform. To create a dry film, the flask was allowed to spin at 125 rpm for one hour. The film was moistened for 60 minutes with 5ml of pH 7.4 phosphate buffer saline (PBS) containing 10mg of flufenamic acid. Using a bath sonicator, the entire preparation was sonicated for 60 seconds (Kamboj *et al*., 2013).

Preparation of Gel Base

A precise amount of carbopol 934 (0.5-1.5%w/v) was weighed out and added to 80ml of double-distilled water in a beaker. After one hour of continuous 800 rpm stirring, 10ml of propylene glycol was added to the solution. To remove air bubbles, the gel's volume was adjusted to 100 ml, and then it was sonicated

for 10 minutes on a bath sonicator (Aly, 2012).

The gel base's pH was finally adjusted to 6.5. Gel was also made with basic medication by adding 10 mg of medication and properly dispersing it using the same method described above. The same process was utilised to create a noisome-containing gel that substituted a previously made niosomal cake for the plain medication. To achieve the appropriate drug concentration, a niosomes preparation equivalent to 0.1% w/w of the medication was added to the gel base.

Optimization of niosomes

Optimization of lipid: surfactant ratio

The lipid: surfactant ratio in the formulation of niosomes was optimised using multiple ratios, including 10:1, 10:2, 10:2.5, and 10:3, while keeping all other parameters constant. The produced formulation was optimised based on average particle size and% entrapment efficiency.

Optimization of drug concentration

Drug concentration was optimised by using various drug concentrations and creating their formulations while maintaining consistent Soya PC and stirrer time. The formulation was optimised based on entrapment effectiveness and average vesicle size (Abdelaziz *et al*., 2015).

Optimization of sonication time

The formulation was sonicated for various lengths of time, such as 30, 60, and 90 seconds at 40C in three cycles of 10 minutes, with a 5-second break in between each cycle. Average particle size, % Entrapment efficiency, and other factors were taken into consideration during optimising.

Surface charge and vesicle size

Utilising the Dynamic Light Scattering technique (DLS) (SAIF RGPV Bhopal, Malvern Zetamaster, ZEM 5002, Malvern, UK), the vesicles' size, size distribution, and surface charge were determined. Based on the Helmholtz-Smoluchowsky-estimated zeta potential from electrophoretic mobility, the zeta potential of the Niosomes was measured. A zetasizer with a field strength of 20 V/cm was employed on a large bore measurement cell to quantify the zeta potential. Samples were diluted to a conductivity of 50 lS/cm using 0.9% NaCl (El Zaafarany *et al*., 2010).

Entrapment efficiency

The mass of the drug's related formulations divided by the total mass of the drug was used to define the drug's entrapment efficiency. The dialysis method was used to measure entrapment effectiveness. Drug that was captured by niosomes was separated from the free drug using the dialysis technique. The aforementioned formulations were put into dialysis bags, and the unbound medication was dialyzed for 24 hours into 50 cc of buffer pH 7.4. At 282.0 nm, the absorbance of the dialysate was measured in a blank buffer of pH 7.4 along with the absorbance of the equivalent blank. The absorbance difference based on the standard curve might be used to determine the concentration of free medication. By measuring the absorbance at 282 nm for known concentrations of, the standard curve was created (Pando *et al*., 2013).

pH measurements

Using a digital pH metre, the pH of selected optimised formulations was measured. pH metres should be calibrated with buffer solutions of pH 4, pH 7, and pH 9 prior to each pH measurement. Following calibration, the electrode was inserted into the vesicles until it was completely covered. The pH of the chosen formulation was then measured, and the display readings were recorded.

Evaluation of niosomal gel

Determination of pH:

Using a pH metre, the pH of both individual and multi-herbal gel formulations can be found.

Appearance and Homogeneity:

Visual observation can be used to assess the physical characteristics and homogeneity of the created individual and polyherbal gels.

Viscosity:

One can use a Brookfield viscometer (Model RVTDV II) and spindle number 6 to measure the viscosity of single and multiple herbal gels at 100 revolutions per minute.

Spreadability:

By measuring the spreading diameter of 1 g of gel between two horizontal plates (20 cm x 20 cm) after one minute, the spreadability of the gel formulations was ascertained (Jacob *et al*., 2017).

***In -vitro* drug diffusion study:**

Franz Diffusion Cell is used to conduct the in-vitro diffusion investigation. For diffusion, egg membrane is used as a semi-permeable membrane105. The Franz diffusion cell has a receptor chamber with an effective surface area of permeation of 3.14 sq. cm and a potent volume of around 60 mL. In between the donor and the receptor compartment, the egg membrane is mounted. A two-cm2 patch was measured, weighted, and then applied to the donor compartment's side of the membrane. The pH 7.4 phosphate buffer is the receptor medium. To maintain the temperature at 32 0.5°C, a water jacket surrounds the receptor compartment. Heat is produced by employing a magnetic stirrer and a thermostatic hot plate. A Teflon-coated magnetic bead that is stored in the diffusion cell is used to stir the receptor fluid. Samples are obtained and replaced with identical amounts of fresh receptor fluid during each sampling period. The samples taken out are spectrophotometrically examined at the drug's 282nm wavelength.

Drug content:

In a beaker, 1 mg of topical Niosomal gel was accurately measured, and 20 ml of methanol 102 was added. This mixture was properly blended before being filtered via Whatman filter paper. After that, 2.0 mL of the filtered solution was added to a volumetric flask with a 10 mL capacity to make the required volume of 10 mL. UV-Spectroscope with a maximum wavelength of 282 nm was used to examine this solution.

Extrudability:

Standard collapsible aluminium tubes with caps are filled with the gel compositions, and the ends are then crimped shut to seal. The tubes' weights were documented. The tubes were fastened in place between two glass slides. The slides were covered with 0.5 gm, and the cap was then taken off. You can gather and weigh the extruded gel's quantity.

Stability study:

Stability studies were conducted to evaluate the drug and formulation stability. Stability testing of the formulation is a step in the drug development process and culminates with the commercial product. The stability research is conducted to determine the formulation that will work best. The optimised gel formulation is preserved for three months at 40 2 ℃ and 75 5% RH before being examined. The samples are examined for physicochemical analysis, pH, viscosity, and medication content at the end of one month.

Table 1: Optimization of lipid: surfactant concentration

Formulation code	Soya PC: Span 80 (% w/v)	Drug (% w/v)	Average Vesicle size (nm)	% entrapment efficiency
F1	10:0.5	1.0	326.45	72.23
F2	**10:1.0**	**1.0**	**255.65**	**76.65**
F3	10:1.5	1.0	310.25	68.87
F4	10:2.0	1.0	256.65	65.45

Table 2: Optimization of drug concentration

Formulation code	Soya PC: Span 80 (% w/v)	Drug (% w/v)	Average vesicle size (nm)	% Entrapment efficiency
F5	10:1	0.5	268.98	59.98
F6	**10:1**	**1**	255.45	68.85
F7	10:1	1.5	298.65	62.12

Table 3: Optimization of sonication time

Formulation code	Soya PC: Span 80 (% w/v)	Drug (% w/v)	Sonication time (Sec)	Average particle size (nm)	% Entrapment efficiency
F8	10:1	1	30	228.85	71.12
F9	**10:1**	**1**	**60**	155.25	76.65
F10	10:1	1	90	136.74	68.85

Table 4: Characterization of Optimized formulation of Niosomes

Characterization	Average vesicle size (nm) graph add	% Entrapment efficiency	Zeta Potential (mV) graph add
F-9	155.25	76.65	-39.74

Table 5: Characterization of gel based formulation of Niosomes

Parameters						
F. code	**Viscosity (cps)**	**% Drug content**	**% Release after 12 hr**	**Extrudability (g)**	**Spreadibility (g.cm/sec)**	**pH**
FG-9	3465±15	99.12±0.15	98.78±0.21	165±6	11.35±0.85	6.85±0.02

FG* Optimized gel formulation

Table 6: *In-vitro* drug release study of prepared gel formulation

S. No.	Time (hr)	% Cumulative Drug Release	
		Plain gel	Niosomal Gel
1	0.5	36.65	12.32
2	1	65.56	25.65
3	2	79.95	33.32
4	4	98.12	44.45
5	6	-	68.85
6	8	-	89.95
7	12	-	98.85

Table 7: Characterization of optimized formulation of Niosomes formulation

Characteristics	Time (Months)								
	1 Month			2 Months			3 Months		
Temperature	4.0 ±0. 2°C	25-28±2° C	45±2° C	4.0 ±0. 2°C	25-28±2°C	45±2°C	4.0 ±0. 2°C	25-28±2°C	45±2°C
Average vesicle size (nm)	158.23	165.25	173.32	153.32	153.32	169.98	152.25	162.23	178.85
% EE	76.65	73.32	68.85	75.65	73.32	65.58	75.12	74.65	63.32
Physical Appearance	Normal	High turbid	High turbid	Normal	High turbid	High turbid	Normal	High turbid and agglomeration	High turbid

RESULTS AND DISCUSSION

The F9 formulation of niosome observed to have average particle size of 155.25 while the % entrapment efficiency was found to be 76.65 %. The Zeta Potential (mV) of F9 formulation was noticed to -39.74. The formulated niosomal gel was observed to have drug content of 99.12±0.15. The pH of gel was within the range of skin pH which is 6.85±0.02. The Extrudability (g) & Spreadibility (g.cm/sec) was found to be 165±6 and 11.35±0.85 respectively.

The viscosity was observed to be 3465±15 cps. In-vitro drug release study of prepared gel formulation indicated at 12 hours the % cumulative drug release was 98.85 %. The formulated niosomal gel was observed too be stable for 3 months at 4.0 ±0.2°C.

CONCLUSION

Flufenamic loaded niosomes offered an alternative approach to deliver Flufenamic in patients using a stable, low-cost method. In the current study, an attempt was made to develop a niosomal gel for improved systemic availability of Flufenamic via a transdermal route. From all these studies, it can be concluded that a gel formulation containing niosomes loaded with Flufenamic showed prolonged action than formulations containing Flufenamic in non-niosomal form and it can be developed successfully to improve the anti rheumatoid arthritis activity.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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