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Formulation, evaluation, and in vitro drug diffusion of niosomal gel of selected drug

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Abstract---Aim: To minimise toxicity and alter pharmacokinetic and bioavailability, niosomes are becoming more significant in medication delivery. Rheumatoid arthritis, juvenile rheumatoid arthritis, and degenerative joint disease may benefit from this new non-steroidal anti-inflammatory, antipyretic, and analgesic drug. Materials and Methods: Non-ionic surfactants (Span 40, 60, and Tween 60) and cholesterol were used in various ratios to create tolmetin sodium niosomes in a thin film hydration process (CHO). Evaluations of the formulations were conducted in terms of size, shape, encapsulation efficiency, and in vitro drug release. Results: In vitro drug release ranged from 94.87±0.45 to 93.19±0.45 percent in 24 hours for niosomes that looked spherical in entrapment efficiency. Incorporating Tolmetin sodium niosomes with Span 60 and CHO in the ratio of 1:2.1 into Carbopol gel was shown to be promising. Tolmetin drug noisome has a drug content and pH of 99.19±1.20 and 7.0±0.06, respectively. Conclusion: An improved bioavailability gel formulation with Tolmetin sodium in niosomal form demonstrated longer effect than gel formulations containing Tolmetin in non-niosomal form.

Keywords---Tolmetin, *in-vitro* drug release studies, niosomes, Cholesterol.

Introduction

Non-steroidal anti-inflammatory drug (NSAID) tolmetin sodium (TS) is often used to treat rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and periarticular illness [1]. Therapeutic advantages may be attributed to the fact that prostaglandin production is stifled by the drug. Rheumatoid arthritis, juvenile

rheumatoid arthritis, and degenerative joint disease may benefit from this new non-steroidal anti-inflammatory, antipyretic, and analgesic medicine. Because it inhibits cyclooxygenase activity, the tissue production of prostaglandins such PGE and PGE2 is diminished [2]. Since it has a half-life of 30-60 minutes after oral administration and requires regular dosage, its utility is limited. A combination of acetaminophen and several nonsteroidal anti-inflammatory drugs (NSAIDs) is regarded safe by TS as an alternate treatment [3, 4]. Tolmetin, like many other NSAIDs, causes damage to the mucosa of the stomach and small intestines when taken orally [5, 6]. Oral administration of Toxicity Syndrome (TS) is commonly done in split doses of 2, 3, or 4 depending on the patient's response and disease severity. No attempts have yet been made to administer TS topically by vesicular administration. There is a need to create gel formulations and test a topical administration route for Tolmetin sodium to produce sustained and long-lasting effects and increase therapeutic effectiveness in the current investigation.

Materials and Methods

Materials

Tolmetin sodium was given to Hyderabad's Sura lab as a gift. SD Fine Chemicals Ltd. in Mumbai supplied the Span 40, Span 60, and Tween 60. Loba Chemie Pvt. Ltd., Mumbai, etc., was the source of cholesterol. Analytical-grade materials were used for everything else.

Method of preparation of niosomes

To create niosomes, several ratios of surfactant and cholesterol were utilised, but the drug concentration remained constant. Thin-film hydration was used to make the niosome formulations. Non-ionic surfactants (span 20, span 40, and span 60) dissolved in 5ml of solvent mixture were weighed (Chloroform: Methanol 2:1 ratio). Then, it was transferred to a 100ml round bottom flask with a graduated cylinder. To get an ultra-thin layer, the researchers used a rotating flash evaporator rotating at 100rpm and 55°C to create a thin film. A dry film formed on the flask walls after the organic solvent has evaporated. For a satisfactory dispersion of the combination, 10 ml of phosphate-buffered saline pH 7.4 was added to a round bottom flask coated with a thin layer of surfactant and cholesterol, and the mixture was vortexed continuously for 45 minutes at 55°C. For 24 hours, the niosomal suspension was kept at 2-8°C [7].

Characterization of Drug Loaded Niosomal Dispersions
Organoleptic Properties

The colour, odour, and look of the dispersion were all evaluated [8].

pH Measurement

The pH of the dispersion of niosomes was measured using a digital pH meter (Hicon, Grover enterprises, New Delhi, India) [8].

Drug Content

The U.V. technique was used to test drug-loaded niosomal dispersions. Buffer solution was made by dissolving 2ml of niosomal dispersion in 50ml of Phosphate buffer solution with an acidic pH of 7.4. To shatter the niosomes, the sample was agitated at 100 rpm after the addition of 1 percent isopropanol. A UV spectrophotometer (UV – 1700 Spectrophotometer, Shimadzu Corporation, Japan) was used to measure the amount of drug in each sample at its corresponding absorption maxima [9].

Transmission Electron Microscopy (TEM)

Using an electron microscope (TEM; Jeol JEM 1230, Tokyo, Japan) set to scan at 70 kV, a 50-fold dilution of the drug (tolmetin sodium) showed its shape and the best possible formulation of noisome. Three to five minutes were given for the carbon-coated copper grid to settle after each diluted drug drop was placed on it. At room temperature, the additional fluid dried for 10 minutes before it was studied under an electron microscope at 70 kV using filter paper [10].

Encapsulation Efficiency

The unentrapped drug was removed from the niosomal dispersion using centrifugation (R-4C, Remi centrifuge, Vasai, Maharashtra, India). At 20,000 rpm and 4 °C, niosomes were centrifuged for 60 minutes. UV spectroscopy was used to measure the unentrapped drug's absorption peaks. The niosome entrapment of the medication was validated using the equation below [11]. Entrapment efficiency (%) = [(Ct — Cf)/Ct] 100, Where, Ct total Drug concentration and Cf free Drug concentration

In-vitro Drug Diffusion

Design and development of drug delivery systems may be greatly enhanced by the use of in-vitro diffusion studies. It was observed that the release mechanism of API from niosomal dispersion and the experimental findings were linked by dialysis; this resulted in a quantitative and qualitative drug release pattern [12]. For the duration of the experiment, the sacks were immersed in a saline solution. Niosomes carrying 5ml of drug-loaded fluid were used to fill dialysis bags. The drug-loaded niosome dialysis bag was kept heated in a 37 °C beaker. At intervals of 0.5, 1, 2, 3, 4, 5, 6, 12 & 24 hours, one mL samples were changed with buffer media using a UV spectrophotometer (UV – 1700 Spectro-photometer, Shimadzu Corporation, Japan).

Preparation of Niosomal Carbopol Gel

In order to further develop the gel, the niosomes with the best entrapment effectiveness were chosen from the formed batches. In addition, a gel containing pure tolmetin sodium was prepared to compare the results of the evaluations [13]. An important step in making gel bases is to disperse 1% carbopol 934 in a combination of water and propylene glycol, neutralise the dispersion and add enough triethanolamine to make it more viscous [13]. Ten minutes at 3 degrees

Celsius and 8000 revolutions per minute were required for centrifugation of the measured amounts of various niosomal formulations (TSF1-TSF6). It was done by employing an electric homogenizer to separate the semisolid mass of niosomes from the supernatant. It was also developed to compare various parameters the gel containing just tolmetin sodium (TSF gel) [13].

Characterization of Drug Loaded Niosomal Gel
Clarity

A milky white backdrop was used to evaluate the clarity of several formulations and the grades were as follows: murky, clear, extremely clear (glassy) [14].

pH Measurements

With the use of a digital pH metre, we took three separate readings to ensure accuracy. First, the pH metre was calibrated and the glass electrode was used to acquire gel formulation readings [14].

Spreadability of Gel

It is essential for gel dosage forms to have a strong spreadability value. Gels that spread easily over the skin are called "spredability" in this context. The spreadability of gel determines its therapeutic efficacy. The spreadability value was calculated with the use of an equipment. The spreadability gadget is based on a wooden block with two glass panes. Glass plates were placed on top of each other and a gel sample was placed between them. A weight of around 300 g was put on the top plate in order to remove the air and form a homogeneous gel. Finally, a 100-gram weight was attached to a hook and pulled by a thread across the top plate. The spreadability grade was based on the time it takes to pull the top plate by 10 centimetres, and the shorter the duration, the better [14].

Content Uniformity

The lysis procedure was used to determine the drug content of the niosomal preparations. The niosomes were lysed with 50% n-propanol. It was taken in a conventional 100 ml flask, and 1ml of the niosomal formulation was added to it. The appropriate volume of 50% n-propanol was then added and thoroughly agitated until all of the vesicles had been lysed. Distilled water was used to get the volume to 100ml. Diluted to 100 ml with the same distilled water, 10 ml of the aforesaid solution was used. With a UV-1700 spectrophotometer, the absorbance was now measured at 289 nm (Shimadzu Corporation) [14].

Viscosity of Gel

Topical treatments' efficacy is heavily influenced by their viscosity. Brookfield Engineering Laboratories Inc. used a viscometer to measure the gel's viscosity (Brookfield, DV-II, MA, USA). Spindle number 18 was used to test viscosity at 0.3 rpm at a temperature of 25 °C [14].

In-vitro Drug Diffusion Study

It was found that niosomal gel diffusion investigations were done in a Franz diffusion cell (EMFDA, Orchid Scientific & Innovative India Pvt. Ltd, Maharashtra). All formulations were tested on a cellulose nitrate membrane for their in vitro release profile. A cellulose nitrate membrane was used to conduct diffusion experiments using a 15 ml phosphate buffer (pH 7.4) and a 0.5 g gel sample (TSG5) at 371 °C. The quantity of medicine in 2 mL samples collected every 0, 1, 2, 3, 4, 5, 6, 12 or 24 hours and replaced with fresh medium was measured using a UV spectrophotometer [15].

Stability Studies

Experiments on the stability of the improved formulation of tolmetin sodium niosomal gel were performed (TSFG 5). Stability experiments were conducted to assess the shelf life of a newly created formulation. A product's quality may be affected by a variety of environmental factors, including temperature, humidity, and more. In the course of a drug's shelf life, it is subjected to a variety of storage conditions, including those encountered during shipping and handling. As a result of this, we have to research the stability of pharmaceuticals and their formulation under different storage circumstances in various nations. A variety of formulation criteria, such as physicochemical characteristics, are tested at various points in time for the product. Researchers test a drug's stability in the identical closure container in which it will be packaged.

Results and Discussion

Formulation of selected drug niosomes

Thin-film hydrolysis of niosome formulations employing various surfactants (Span 20, Span 40, Span 60) and Cholesterol with varying ratios as shown in table 1 resulted in a steady, uniform dispersion of niosomal particles. The formation of niosomal vesicle was confirmed by Transmission Electron Microscopy (TEM).

Table 1
Formulations of Tolmetin sodium niosomes

Form. code	Drug tolmetin sodium	Surfactant Grade (mg)	Cholesterol (mg)	Ratio (Drug: Surfactant: Cholesterol)	Solvent (Chloroform: Methanol
FTS 1	100	Span 60	100	1:1:1	6:2
FTS 2	100	Span 60	100	1:2:1	6:2
FTS 3	100	Span 60	200	1:.75:2	6:2
FTS 4	100	Span 40	100	1:1:1	6:2
FTS 5	100	Span 40	100	1:2:1	6:2
FTS 6	100	Span 40	200	1:.75:2	6:2
FTS 7	100	Span 20	100	1:1:1	6:2
FTS 8	100	Span 20	100	1:2:1	6:2
FTS 9	100	Span 20	200	1:.75:2	6:2

Characterization of Tolmetin Sodium loaded Niosome Dispersion
Organoleptic Properties

The tolmetin sodium niosomal dispersion was off-white in appearance, odourless, and liquid. No sedimentation could be seen in it (Table 2).

Table 2
Evaluation of Tolmetin Sodium Niosomal Batches

Batch no.	Appearance	Odour
FTS 1	Milky white	Odourless
FTS 2	Milky white	Odourless
FTS 3	Milky white	Odourless
FTS 4	Milky white	Odourless
FTS 5	Milky white	Odourless
FTS 6	Milky white	Odourless

pH and Drug Content Determination

The resulting solution was adequately diluted with methanol as well as with the absorbance at 289 nm was deliberated. The FTS 5 formulation shows the maximum percentage of drug content, i.e. 99.19%, and pH 7.0, therefore, is selected as a final formulation. The result shown in table 3 and figure 1 and 2.

Table 3
Drug Content and pH

S. No.	Formulation Code	Drug Content	pH
1	FTS 1	98.65±1.52	5.5±0.04
2	FTS 2	98.56±1.05	5.8±0.01
3	FTS 3	99.12±1.09	5.3±0.03
4	FTS 4	98.89±2.03	5.9±0.02
5	FTS 5	99.19±1.20	7.0±0.06
6	FTS 6	98.54±1.26	6.2±0.09

n=3, all values are expressed as mean±SD

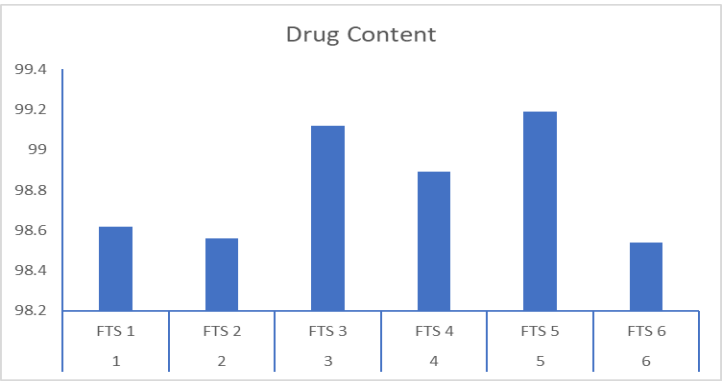

Figure 1. % Drug Content of Niosome

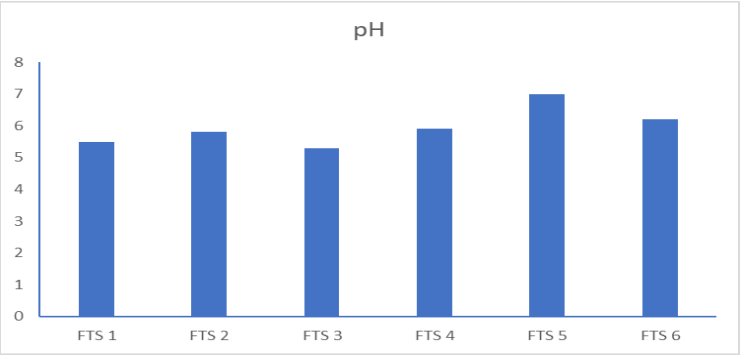

Figure 2. pH of Niosome

Transmission Electron Microscopy (TEM)

As shown in the TEM photomicrograph, the blank noisome had a less cluster formation of noisome which are not much spherical in shape and also size is not uniform and also the particles shows dark background. whereas after drug encapsulation in the niosome the best formulation of noisome (FTS-5) shows uniform spherical morphology with smooth plane characteristics. The vesicles are created due to the self- assembly of non-ionic surfactants after their interface with the aqueous medium which leads to the formation of a concentric double layer shape that has a morphology related to liposomes however without phospholipids which gives improved constancy. The TEM report is shown in figure 3 and 4.

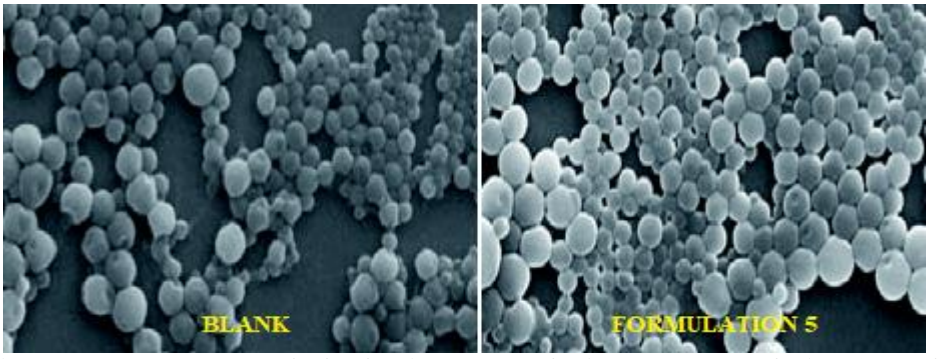

Figure 3. TEM Report of Blank Niosome and Niosome Formulation (FTS 5)

Entrapment Efficiency

Efficacy ranged from 79.12 to 98.89% (w/w). The % encapsulation efficiency of tolmetin sodium in prepared inclusion complexes was found to be 98.65±1.62%, 90.18±0.24%, 98.89±1.35%, 79.12±0.34%, 93.25±1.32% and 97.28±1.32%. It was found that for the the trapping efficiency of FTS 5 was the greatest 98.89% (w/w). The result are shown in Table 4 and figure 4.

Table 4
Entrapment Efficiency of Tolmetin Sodium Niosomal Batches

S. No.	Formulation Code	% Entrapment efficiency
1	FTS 1	98.65±1.62%
2	FTS 2	90.18±0.24%
3	FTS 3	93.25±1.32%
4	FTS 4	79.12±0.34%
5	FTS 5	98.89±1.35%
6	FTS 6	97.28±1.32%

n=3, all values are expressed as mean±SD

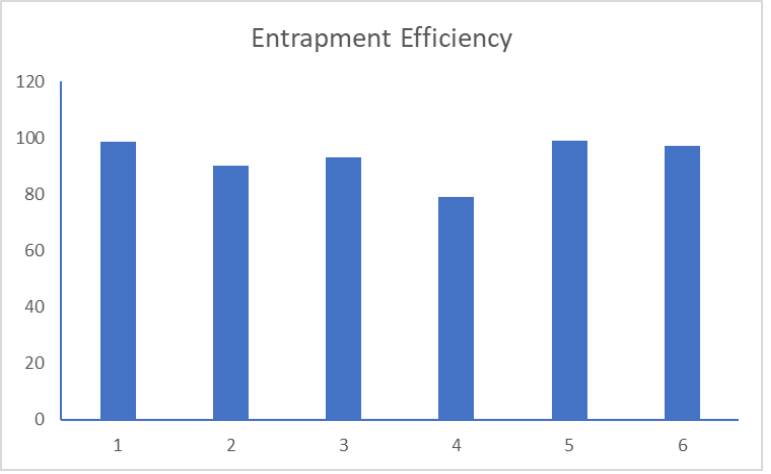

Figure 4. Entrapment Efficiency

***In-vitro* Diffusion Profile**

The *in-vitro* release of all developed niosome formulations were carried out by diffusion method. *In-vitro* release profiles were treated, and results are illustrated in table 5 and figure 5. The drug's release pattern from all formulations. After 24 hrs about 84.45%, 86.87%, 94.13%, 91.91%, 95.06% and 88.11%. of tolmetin sodium was release from formulation FTS1, FTS2, FTS3, FTS4, FTS5 and FTS6 respectively. In the release of *in-vitro* drugs performed for the optimized formulation of FTS5 highest. Niosomal dispersion showed sustained released than conventional dispersion. The result are shown in Table 6 and figure 6.

Table 5
Cumulative % Drug Release of Niosomes of Tolmetin Sodium

Time (h)	FTS 1	FTS 2	FTS 3	FTS 4	FTS 5	FTS 6
0.5	1.07	1.25	1.96	1.54	2.98	1.31
1	3.65	4.65	8.94	8.19	2.95	6.12
2	8.23	9.75	18.04	16.06	20.04	10.05
3	15.54	18.66	29.55	26.55	25.95	20.03
4	25.99	28.91	37.9	35.95	39.05	30.16

5	36.77	37.9	48.36	46.97	49.25	38.12
6	44.21	48.9	56.03	53.56	59.04	51.86
12	53.32	54.99	61.35	59.16	64.54	55.93
24	84.45	86.87	94.13	91.91	95.06	88.11

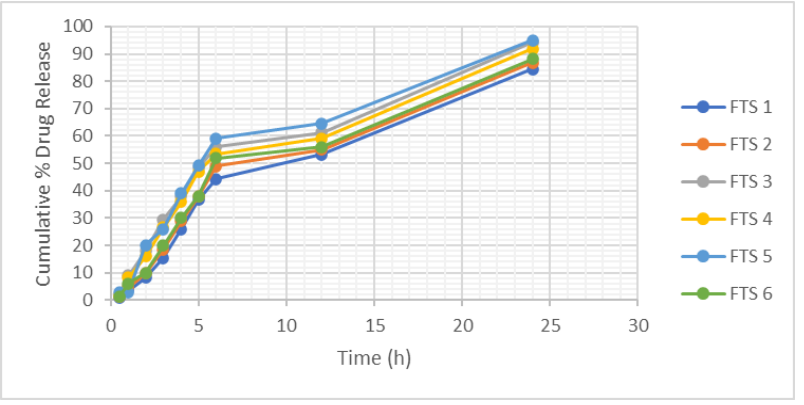

Figure 5. Cumulative % Drug Release of FTS 5 Formulation

Table 6
Comparative Cumulative % Drug Release Tolmetin Sodium Niosome

S. No.	Batch No.	Mean % Drug release of Niosome dispersion	Mean % Drug release conventional dispersion
1	FTS 1	90.23±0.65	88.61±0.15
2	FTS 2	89.24±0.60	88.95±0.45
3	FTS 3	94.35±0.17	91.15±0.05
4	FTS 4	93.89±0.15	92.39±0.44
5	FTS 5	94.87±0.45	93.19±0.45
6	FTS 6	90.25±0.15	89.5±0.015

n=3, all values are expressed as mean±SD

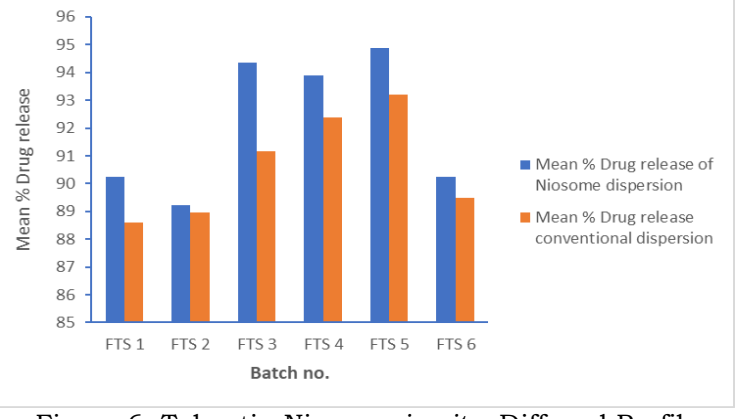

Figure 6. Tolmetin Niosome *in-vitro* Diffused Profile

Formulation of tolmetin sodium loaded niosomal gel
Preparation of Niosomal Gel of Tolmetin Sodium

The gels were prepared using Carbapol 934, Propylene Glycol, Triethanolamine and Water and in different ratios as shown in table 7.

Table 7
Formulation of Niosomal Gels

Form. code	Niosomal Suspension (ml)	Carbopol 934 (%)	Propylene Glycol (ml)	Triethanolamine (%v/v)	Water (ml)
FTS 1	10	0.5	5	0.5	30
FTS 2	10	1.0	5	0.5	30
FTS 3	10	1.5	5	0.5	30
FTS 4	10	2.0	5	0.5	30
FTS 5	10	2.5	5	0.5	30
FTS 6	10	3.0	5	0.5	30

Characterisation of the prepared tolmetin sodium niosomal gel

The formulations pH was in the range of 6.3 to 7.2, considered acceptable to avoid the risk of skin irritation after application to the skin. The pH of the optimized formulation (FTS5) was found to be 6.85. The TSFG5 formulation shows maximum % Drug content *i.e.,* 94.23% and thus selected as a final formulation. Niosomal gels agent exhibited spreadability values ranging from 12.64-15.89 g.cm/s. The average viscosity of formulations lies in the range from 1937.49 to 3850.63 cps. The result are shown in Table 8 and figure 7-10.

Table 8
Drug Content and pH of Tolmetin Sodium Niosomal Gel

Batch No.	Drug Content	pH	Spreadability	Viscosity (cps)
TSFG 1	98.63±1.28	5.4±0.02	11.62	2360.85
TSFG 2	98.49±1.52	5.5±0.05	14.17	2012.48
TSFG 3	99.21±1.41	6.1±0.04	14.35	3204.09
TSFG 4	98.45±1.04	5.7±0.01	13.10	2496.09
TSFG 5	99. 91±1.63	7.2±0.09	15.72	3750.51
TSFG 6	98.60±1.03	6.3±0.03	13.85	2942.98

n=3, all values are expressed as mean±SD

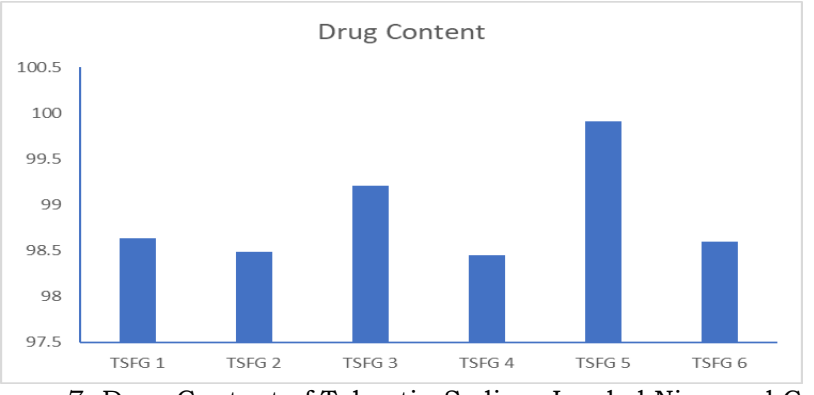

Figure 7. Drug Content of Tolmetin Sodium Loaded Niosomal Gel

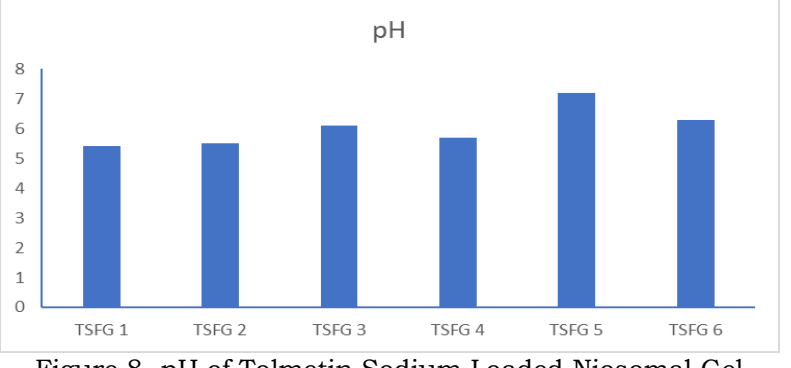

Figure 8. pH of Tolmetin Sodium Loaded Niosomal Gel

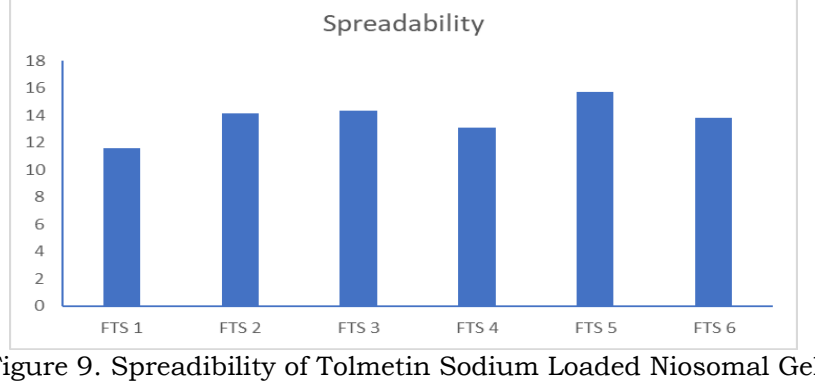

Figure 9. Spreadibility of Tolmetin Sodium Loaded Niosomal Gel

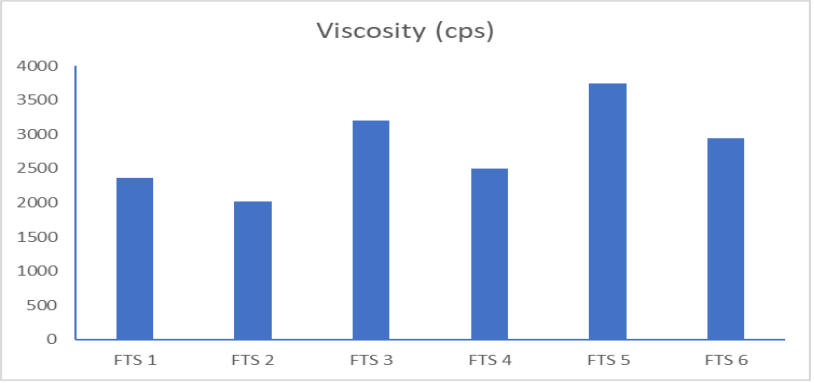

Figure 10. Viscosity of Tolmetin Sodium Loaded Niosomal Gel

In-vitro Release Studies of Tolmetin Sodium Loaded Niosomal Gel

In-vitro studies have been conducted on the release of drugs through the cellulose nitrate membrane. The results of *in-vitro* release after incorporation of the niosome into gels. The cumulative drug release rate over 8 hours was higher for the TSFG5 formulation using carbopol 934. The results are shown in table 9.

Table 9
In-vitro Drug Release Study

Time (h)	Niosome formulation FTS5	Niosomal Gel TSFG5	Marketed Formulation
0.5	21.23± 2.8	22.1± 1.8	20.1± 1.8
1	26.13 ± 6.8	28.23± 1.8	25.23± 1.8
2	35.01 ± 6.1	37.24± 2.9	32.24± 2.9
3	43.11 ± 6.2	45.14± 1.9	40.14± 1.9
4	49.32 ± 1.3	51.23± 3.2	47.23± 3.2
5	59.76± 6.9	61.24± 2.8	58.24± 2.8
6	71.63 ± 5.5	73.13± 1.5	70.13± 1.5
12	77.21± 2.8	78.23± 1.6	76.23± 1.6
24	86.23 ± 1.2	91.23± 1.8	81.23± 1.8

n=3, all values are expressed as mean±SD

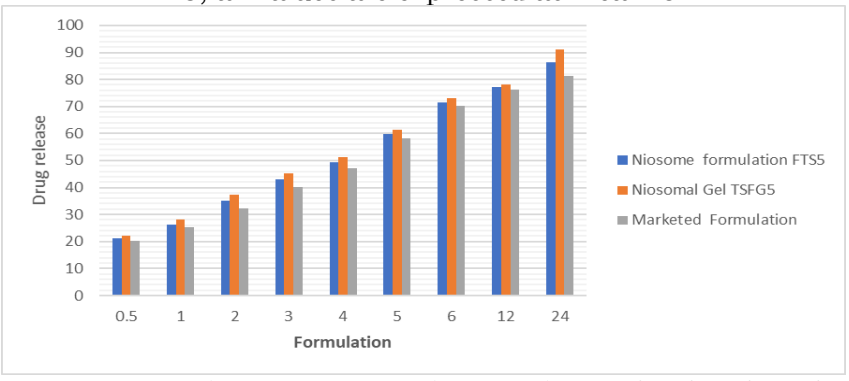

Figure 11. Comparison Between, Noisome, Niosomal Gel and Marketed Formulation

In the release of *in-vitro* drugs performed for the optimized formulation of Nisomal FTS5, the optimized formulation of niosomal gel TSFG5 and the marketed formulation cream. The niosomal gel formulation presented a greater drug release than the niosome formulation and the marketed formulation. Drug release profiles of niosomal tolmetin sodium gels are shown in table 9. Drug release from the niosomal gel of tolmetin sodium was found to range from 21.23% to 86.23% for all formulations. Niosomal gels differ from normal microspheres due to their extremely porous surface. This function provides the property of releasing the drug throughout the pores at a faster rate.

Stability Studies

Stability experiments was being conducted on the tolmetin sodium niosomal gel formulation (TSFG5). The study was carried out to evaluate the effect of storage conditions on essential attributes of gels such as appearance, entrapment, vesicle size, viscosity and drug content after specified time intervals. Niosomal formulation was selected on the basis of entrapment efficiency and *in-vitro* release studies. Stability studies were assessed by keeping FTS5 niosomal suspension and niosomal gel in sealed glass vials period of 30 days. The present study involves investigation of the stability of the formulated niosomal gels under influence of 25°C + 2°C/60 % RH ± 5% RH and 4°C + 2°C. It was observed from the results slight reduction in residual drug content at room temperature. The developed niosomal formulation (TSFG5) was found to be stable at the end of the study on storage condition.

Conclusion

The tolmetin sodium loaded niosomal gels were successfully formulated by thin film hydration method using cholesterol, span 20, span, 40 and span 60 with different ratio. Systemic absorption of tolmetin sodium formulation (FTS5) is low compare than tolmetin sodium niosomal gel (TSFG5). In the current study, an effort was made to originate niosomal gels of tolmetin sodium for effective drug release on the skin by in-vitro release study. In the current study, a topical tolmetin sodium gel was formulated for proficient administration of drug through the skin. With several side effects, Oral utilization of tolmetin sodium is not highly suggested, so this formulation is made for healthier patient compliance and to decrease the dose of the drug as well as to avoid side effects.

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