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Research Article

FORMULATION OF NIOSOMAL GEL OF ACECLOFENAC AND IT'S *in-vitro* CHARACTERIZATION

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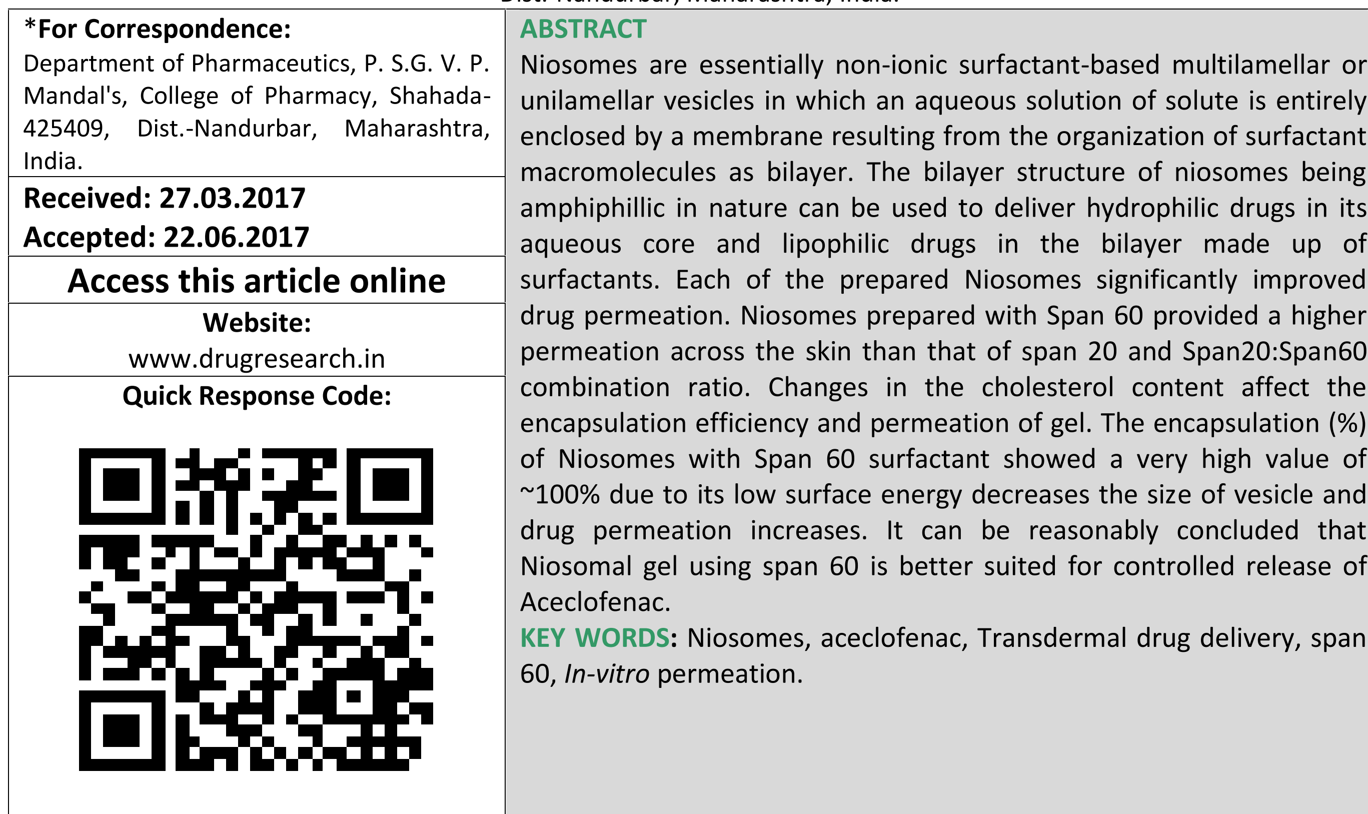

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ABSTRACT

Niosomes are essentially non-ionic surfactant-based multilamellar or unilamellar vesicles in which an aqueous solution of solute is entirely enclosed by a membrane resulting from the organization of surfactant macromolecules as bilayer. The bilayer structure of niosomes being amphiphillic in nature can be used to deliver hydrophilic drugs in its aqueous core and lipophilic drugs in the bilayer made up of surfactants. Each of the prepared Niosomes significantly improved drug permeation. Niosomes prepared with Span 60 provided a higher permeation across the skin than that of span 20 and Span20:Span60 combination ratio. Changes in the cholesterol content affect the encapsulation efficiency and permeation of gel. The encapsulation (%) of Niosomes with Span 60 surfactant showed a very high value of ~100% due to its low surface energy decreases the size of vesicle and drug permeation increases. It can be reasonably concluded that Niosomal gel using span 60 is better suited for controlled release of Aceclofenac.

KEY WORDS: Niosomes, aceclofenac, Transdermal drug delivery, span 60, *In-vitro* permeation.

INTRODUCTION

At present, the most common form of delivery of drugs is the oral route. While this has the notable advantage of easy administration, it also has significant drawbacks namely poor bioavailability due to hepatic metabolism (first pass) and the tendency to produce rapid blood level spikes (both high and low), leading to a need for high and/or frequent dosing, which can be both cost prohibitive and inconvenient. To overcome these difficulties there is a need for the development of new drug delivery system; which will improve the therapeutic efficacy and safety of drugs. The topical administration of drugs for the local treatment of skin diseases has been used for a long time, but the use of transdermal delivery for the systemic action is relatively new and increasingly used. The rapid development of transdermal delivery formulations in the last few years is due to certain unique advantages of transdermal administration. Now a day's vesicles as a carrier system have become the vehicle of choice in drug delivery and lipid vesicles are found to be of value in immunology, membrane biology and diagnostic technique and most recently in genetic engineering. Vesicular system has achieved new heights during the last few years as an essential component of drug development. [4]

Niosome:

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. The vesicle is composed of a bilayer of non-ionic surface-active agents and hence the name niosome. The niosome are very small, and microscopic in size. Their size lies in the nanometric scale. Although structurally similar to

liposomes, they offer several advantages over them. Niosome have recently been shown to greatly increase transdermal drug delivery and also can be used in targeted drug delivery. [1]

Material and Method

Aceclofenac was obtained as gift sample from Ajanta Pharma (Bharuch, India), Span20, Span60 were Obtain from Research lab. (Mumbai, India) and Cholesterol, chloroform and carbopol were from Himedia laboratories (Mumbai, India).

Niosomal Gel of Aceclofenac [4]

- **Preparation of Drug Loaded Niosome**

Aceclofenac loaded niosomes were formulated by using thin film hydration technique and the different nonionic surfactants (span 20 and span 60) grades in different drug:surfactant:Cholesterol ratios as 1:1:1, 1:2:1, 1:1:2. Accurately weighted quantities of surfactant and Cholesterol were dissolved in 5 ml chloroform using a 100 ml round bottom flask. The lipid solution was evaporated by rotary shaker. The flask was rotated at 135 rpm until a smooth and dry lipid film was obtained. The film was hydrated with 5 ml phosphate buffer saline (PBS) of pH 7.4 containing drug for 3 hours with gentle shaking. The niosomal suspension was further stabilized by keeping at 2-8ºC for 24 hours.

- **Preparation of Niosomal gel**

The preparation of niosomal gel using carbopol 934, the gel base was prepared by dispersing 0.2% w/w carbopol 934 in a mixture of water and glycerol (7:3), the dispersion is then neutralized and made viscous by addition of sufficient amount of triethanolamine. The measured amount of selected niosomal formulations were centrifuged by using centrifuge apparatus for 30 min at 2500 rpm. The semisolid mass of niosomes was separated from the supernatant and mixed in the 0.2% carbopol gel base by using electric homogenizer.

Table 1: Formulation and Composition of Niosomal gel

Formulation Code	Surfactant Used	Drug: Surfactant : Cholesterol Ratio	Solvent	Carbopol 934 (%)
F1	Span 20	1:1:1	Chloroform	0.2%
F2		1:2:1	Chloroform	0.2%
F3		1:1:2	Chloroform	0.2%
F4	Span 60	1:1:1	Chloroform	0.2%
F5		1:2:1	Chloroform	0.2%
F6		1:1:2	Chloroform	0.2%
F7	Span 20: Span 60	1:1:1	Chloroform	0.2%
F8		1:2:1	Chloroform	0.2%
F9		1:1:2	Chloroform	0.2%

Characterization of Niosome:

1. Physical appearance:

The prepared niosome was viewed by naked eye to characterize color and physical state. Niosome was also viewed by optical microscope at 40 X magnification, to observe crystal characteristics by spreading a thin layer niosome on a slide and placing the cover slip on it. [5]

2. Entrapment Efficiency:

The entrapment efficiency was determined after separating the unentrapped drug. niosomal gel (100mg) was hydrated with 10 ml of phosphate buffer saline (pH 7.4) solution. The aqueous niosomal dispersion should be sonicated in a bath sonicator for 10 min. The drug containing niosomal dispersion is separated from entrapped drug by centrifuging at 15000 rpm for 30 minutes. The clear supernatant was filter off carefully to separate the unentrapped drug. The recover supernatant is assayed by its analytical technique. The percentage of drug encapsulation is calculated by the following Equation [6].

$$\textbf{EE (\%)} = [(Ct-Cf)/Ct] \times 100$$

Where,

Ct is the concentration of total drug.

Cf is the concentration of free drug.

3. Vesicle size and shape characteristics:

- **Visualization of vesicles by optical microscopy:** Hydration of niosome was performed with phosphate buffer saline (pH 7.4) with slight agitation to produce niosome. A drop of niosome suspension was placed on a slide and after placing cover slip observed under microscope. Photomicrographs were taken at 100X magnification.
- **Visualization of vesicles by transmission electron microscope (TEM):** To further evaluate the surface characteristics of vesicle transmission electron microscopy (TEM) were performed. The niosome suspension was negatively stained with a 1% aqueous solution of phosphotungustic acid (PTA). Niosome suspension was dried on a microscopic carbon coated grid for staining. The excess solution was removed by blotting. After drying the specimen was viewed under the microscope.
- **Visualization of vesicles by scanning electron microscope (SEM):** The shape, surface characteristics, and size of the niosomes were observed by scanning electron microscopy (SEM). Weighed quantity of the Niosome in a glass tube was diluted with 10 ml of phosphate buffer saline (pH 7.4). The lyophilized niosomes were mounted on an aluminum stub using double-sided adhesive carbon tape. Then the vesicles were sputter-coated with gold palladium (Au/Pd) using a vacuum evaporator (Edwards) and examined using a scanning electron microscope JSM-5510 (Jeol Ltd, Tokyo, Japan) equipped with a digital camera, at 20 kV accelerating voltage[6].

4. pH of gel:

The pH of formulated niosomal gel was determined using pH meter. The electrode was immersed in gel and readings were recorded on pH meter. [7]

5. Spreadability:

Spreadability of formulations was determined by an apparatus suggested by Multimer et al. which was fabricated in laboratory and used for study. The apparatus consist of a wooden block, with a fixed glass slide and movable glass slide with one end tied to weight pan rolled on the pulley, which was in horizontal level with fixed slide. Apparatus is shown in figure 2.

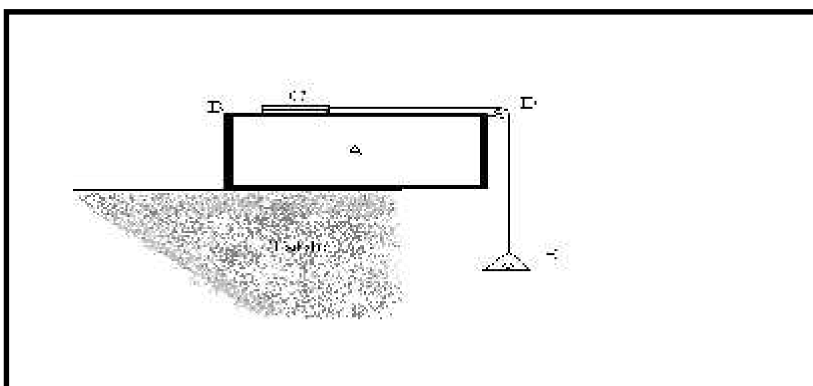

Figure 2: Spreadability Test Apparatus. A: Wooden Box B: Lower Glass Slide C: Upper Glass Slide D: Pulley E: Weighing Pan

Procedure: An excess of gel sample 2.5 g was placed between two glass slides and a 1000g weight was placed on slides for 5 minutes to compress the sample to a uniform thickness. Weight (60g) was added to the pan. The tie (seconds) required to separate the two slides was taken as a measure of spreadability. It was calculated using the formula:

$$S = M.L / T$$

Where,

S - Spreadability in gm.cm / sec

M – Weight tied to upper slide

L- Length of glass slide

T- Time in second

Length of glass slide was 7.5 cm and weight tied to upper slide was (60g) throughout the experiment. [7]

6. Degree of Deformability:

In the case of niosomes, the permeability study is one of the important and unique parameter for characterization. The deformability study is done against the pure water as standard. Niosomes preparation is passed through a large number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 50 nm and 400 nm, depending on the starting niosomes suspension). Particle size and size distributions are noted after each pass by dynamic light scattering (DLS) measurements. The degree of deformability can be determined using the following formula,

$$E = J \times (rv/rp)^2$$

Where,

J= the amount of the suspension extruded during 5min

rv = the size of the vesicle

rp = pore size of the barrier. [8]

***7. In-vitro* drug release:**

Permeation of niosome was studied using a Franz glass diffusion cell. The effective permeation area of the diffusion cell and receptor cell volume was 1 cm^2 and 10 ml, respectively. The receptor compartment contained aliquot of 1%:99% (v/v) ethanol: PBS (pH 7.4) and maintained at 37^0C ± 1°C by magnetic stirrer. Egg membrane was mounted between the donor and receptor compartment. NG (200mg) containing 1 mg of drug was applied to the surface of the egg membrane. Samples were withdrawn through the sampling port of the diffusion cell at predetermined. Intervals and analyzed by UV-Visible Spectrophotometer. An equal volume of fresh PBS, pH 7.4 was replaced into the receptor compartment after each sampling [9, 10].

8. Stability study:

Stability studies were carried out by storing the prepared niosome gel at various temperature conditions like refrigeration temperature (2°-8°C), room temperature (25°± 2°C) and elevated temperature (45° ± 2°C) from a period of one month to three months. Drug content and variation in the average vesicle diameter were periodically monitored [11, 12, 13].

RESULTS

The formulated niosomal gel were evaluated for drug encapsulation efficiency, pH of formulation, Viscosity, Spreadability, Vesicle size and shape, Drug uniformity content, Extrudability, Degree of deformability, SEM, Zeta potential, *In- Vitro* drug permeation as well as Stability study of gel.

Physical Evaluation

The prepared Niosome formulations were viewed by naked eye to characterize color and physical state of gel. The Niosomal gels were translucent to white in color with smooth in appearance depending upon the amount of polymer present in the formulation. All the formulations were odorless.

Encapsulation efficiency

Niosomal gel formulations (F1 to F9) were prepared as per the procedure mentioned earlier and compositions reported in table 1. Then all the formulations were tested for entrapment efficiency for Aceclofenac. The

results of encapsulation efficiency determination in table 2 indicated that formulation containing Span-60 (F4-F6) had high encapsulation efficiency than formulations containing surfactants in different order (F1-F3 and F7-F9).

pH of formulation

The results for pH determination are given in following Table 2.It was found that the pH of all the formulations were in the range of 6.54 to 7.31 that suits the skin pH This indicating skin compatibility and which is the primary requirement for a transdermal formulation.

Vesicle size and shape

Vesicle size and shape can be determined by optical microscope under 45x optical lens. From the figure 3 Span 60 (F5) have lower size among all the tested formulations of surfactant (F1-F9) due the relationship observed between niosome size and Span 60.

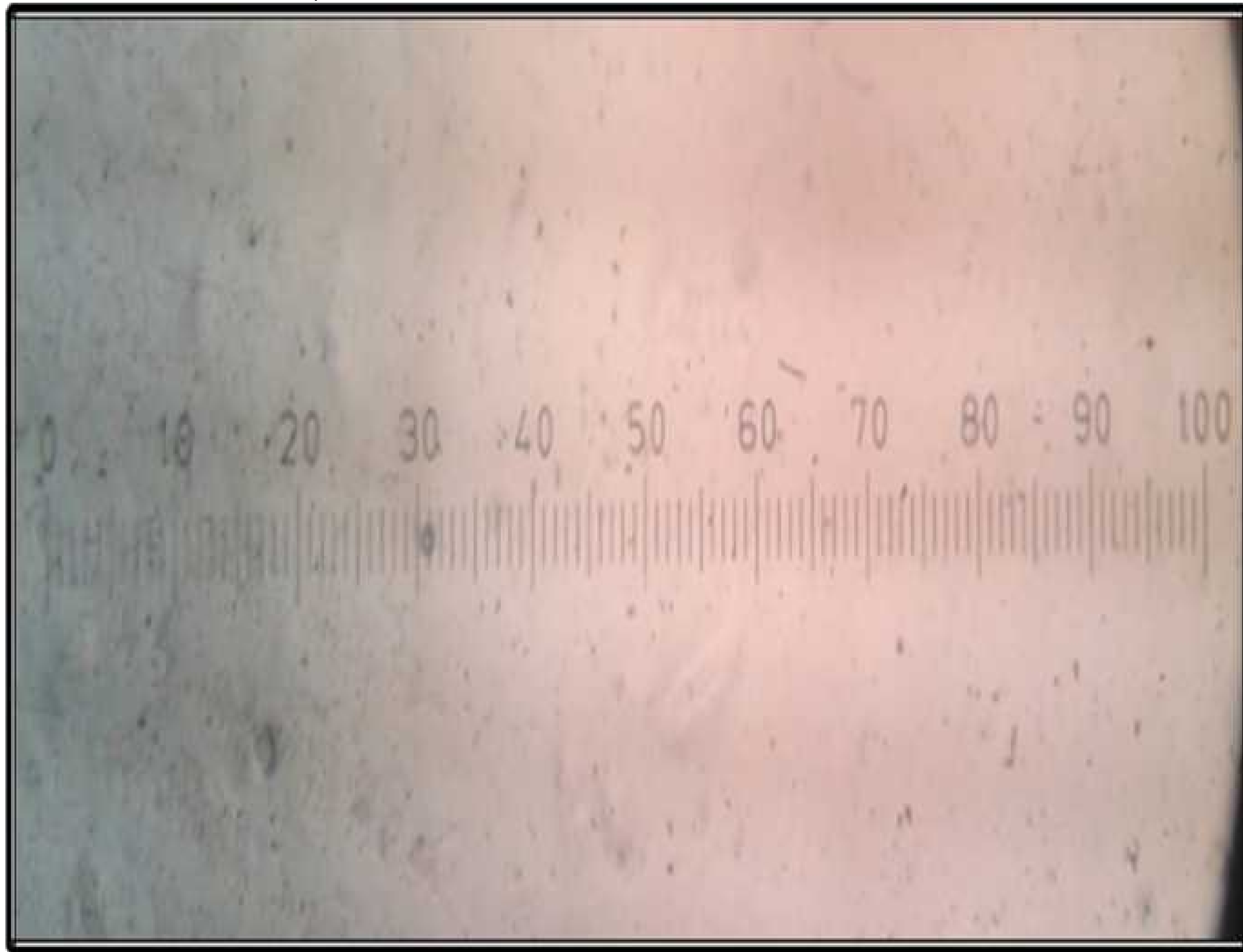

Figure 3: Vesicle size of formulation F5

Viscosity Determination

Viscosity is important parameter for characterization of gel as it affects the release of drug. Viscosity measurement revealed that all formulation shows optimum consistency as shown in table 2.

Spreadability:

Spreadability of all formulation is recorded in table 2 and it shows good spreadability of all formulation (F1-F9).

Drug uniformity content:

The drug content of the Niosomal gel were found to within range by UV spectrophotometer to in range between to 88.66 – 97.86 of the theoretical value for formulation (F1- F9) as shown in table 3.

Table No.2: Observations for Encapsulation Efficiency, Ph, Vesicle Size, Viscosity.
Represents mean ± S.D. (n = 3**)**

Sr. no.	Formulation code	Encapsulation efficiency (%)	pH	Vesicle size (µm)	Viscosity (cps)
1	F1	76.93±1.86	6.7 ± 0.15	5.42 ± 1.66	12480 ± 0.28
2	F2	81.13±2.15	6.74 ± 0.177	4.97 ± 1.22	12489.3± 2.18
3	F3	76.26±2.32	6.54 ± 0.09	6.5 ± 1.56	10608 ± 179
4	F4	85.27±1.92	7.10 ± 0.035	1.56 ± 0.89	12788± 0.51
5	F5	89.45±3.87	7.16 ± 0.35	1.32±0.59	13499±0.21
6	F6	83.02±2.13	7.31 ± 0.04	2.69 ± 0.43	10509 ± 1.89
7	F7	82.26±1.88	6.72 ± 0.13	2.32 ± 2.15	11900.4 ±1.071
8	F8	84.27±0.67	6.98 ± 0.580	3.03 ± 0.14	12890 ±0.75
9	F9	80.14±2.11	7.3 ± 0.27	2.74 ± 1.86	10228 ± 1.07

Extrudability

The extrudability test results are summarized in table 3 and all the formulations show good extrudability Formulations F1,F2, F3, F6, F9 showed good extrudability and Excellent extrudability was observed for formulations F4, F5, F7 and F8.

Degree of Deformability

Deformability index of various Niosome formulations were found to vary significantly with surfactant and cholesterol concentration. It was observed that deformability index first increased significantly with increase in cholesterol concentration .However, deformability index was found to decrease on further increase in cholesterol concentration (table 3.

***In-vitro* drug permeation**

The Niosomal gel formulations were characterized for their drug permeation through the Egg membrane. The niosomal formulations were prepared to study the permeation of drug from the various formulations with fixed concentration of Drug and varying concentration of surfactant and cholesterol. The drug permeation was maximum for F5 formulation i.e. Span 60 among the all tested formulations (F1-F9) as shown in table 4. From the release profile it is clear that after 12 hr. the gel shows maximum drug release of F5 formulationis 96.58 % means it shows controlled release formulation.

Table No 3: Observations for Drug Content, Extrudability, Degree Of Deformability.

Sr. no.	Formulation code	Drug content (%)	Extrudability	Degree of Deformability
1	F1	90.53±1.21	++	16.09 ±0.25
2	F2	91.19± 0.86	++	22.26±0.64
3	F3	88.66 ±1.23	++	11.27 ± 0.64
4	F4	95.48±1.17	+++	28.47 ± 0.47
5	F5	97.86±0.72	+++	34.21 ±0.19
6	F6	94.99± 3.49	++	30.57 ± 0.54

7	F7	92.01± 0.77	+++	23.26 ± 0.45
8	F8	95.38±1.54	+++	27.56 ± 0.89
9	F9	93.54 ± 1.30	++	25.05 ± 0.62

Represents mean ± S.D. (n = 3**)**

Table 4: ***In-vitro*** **drug permeation of Niosomal gel formulation F1-F9 and marketed Preparation**

Time (hr)	**Percent Drug Permeation**				
	F1	**F2**	**F3**	**F4**	**F5**
1	7.98 ± 0.93	15.37 ± 0.54	10.112 ± 0.77	12.32± 0.93	18.88 ±1.23
2	14.17 ± 1.22	21.23± 0.76	15.24± 0.67	16.23 ± 0.7	21.73 ±0.53
3	20.92 ± 0.88	29.87 ± 0.56	17.228 ± 0.98	25.32 ± 0.43	2.56 ±0.83
4	26.15 ± 0.35	35.33 ±0.34	25.187 ± 0.11	35.34 ± 0.31	37.19 ±1.17
5	28.36 ± 0.12	39.97 ± 0.9	19.392 ±0.34	44.75 ± 0.4	43.99 ±1.48
6	34.78 ± 0.24	47.87±0.42	30.404 ± 0.12	49.65 ± 0.2	45.76±0.42
7	37.03 ± 0.82	52.68± 0.68	49.007± 0.65	55.90 ± 0.42	52.53 ±0.87
8	41.44 ± 0.95	58.82 ± 0.42	55.7 ± 0.87	65.987 ± 0.72	55.7 ±0.94
9	44.83 ± 0.77	62.456± 0.84	70.72 ± 0.81	70.785± 0.74	60.06 ±0.75
10	66.13 ± 1.24	79.24 ± 0.35	77.86 ± 0.32	80.897 ± 0.88	83.39 ±0.33
11	82.34 ± 0.38	84.85 ± 0.87	90.925 ± 0.75	87.97 ± 0.89	92.35 ±0.96
12	89.82 ± 0.81	92.53 ± 0.5	91.81±0.11	96.291 ± 0.7	96.58±0.74

Time (hr)	**Percent Drug Permeation**				
	F6	**F7**	**F8**	**F9**	**MKD**
1	11.75± 0.32	8.98 ± 0.32	11.28 ± 0.76	7.32± 0.19	6.79± 1.34
2	13.12± 0.2	11.30 ± 0.24	15.40 ± 0.68	14.52 ±0.12	10.89±0.43
3	19.34± 0.58	16.34 ± 1.39	22.04 ± 0.32	20.28± 0.45	16.61±0.35
4	23.902 ± 0.52	21.58 ±1.86	25.78 ± 1.32	27.9± 0.56	20.86±0.67
5	31.64 ± 0.65	26.96 ± 1.01	30.76 ± 0.8	40.11± 0.16	32.23±1.42
6	37.97 ± 0.30	37.23 ± 1.09	34.46 ± 0.97	47.005± 0.32	36.74±0.69
7	48.97 ± 0.43	40.17 ± 0.91	41.80 ± 1.26	57.47± 0.56	42.12±0.83

8	55.90 ± 0.47	44.54 ± 0.54	45.87 ± 0.78	65.71± 0.43	46.91±0.35
9	59.12± 0.21	51.92 ± 0.88	49.10 ± 0.4	73.864±0.22	50.92±0.59
10	67.90± 0.43	68.69 ± 0.76	59.68 ± 0.36	81.796± 0.32	71.44±0.97
11	81.77± 0.12	87.36 ± 0.16	71.77 ± 0.27	91.382± 0.44	86.18±0.21
12	92.987± 0.31	92.47 ± 0.33	95.56 ± 0.49	92.03±0.87	92.66±0.9

Represents mean ± S.D. (n = 3)

Shape of vesicles by SEM

Optimized formulation (F5) loaded vesicles of Aceclofenac were analyzed by SEM for studying particle shape surface structure. From figure 5. optimized formulation gives circular vesicle shape.

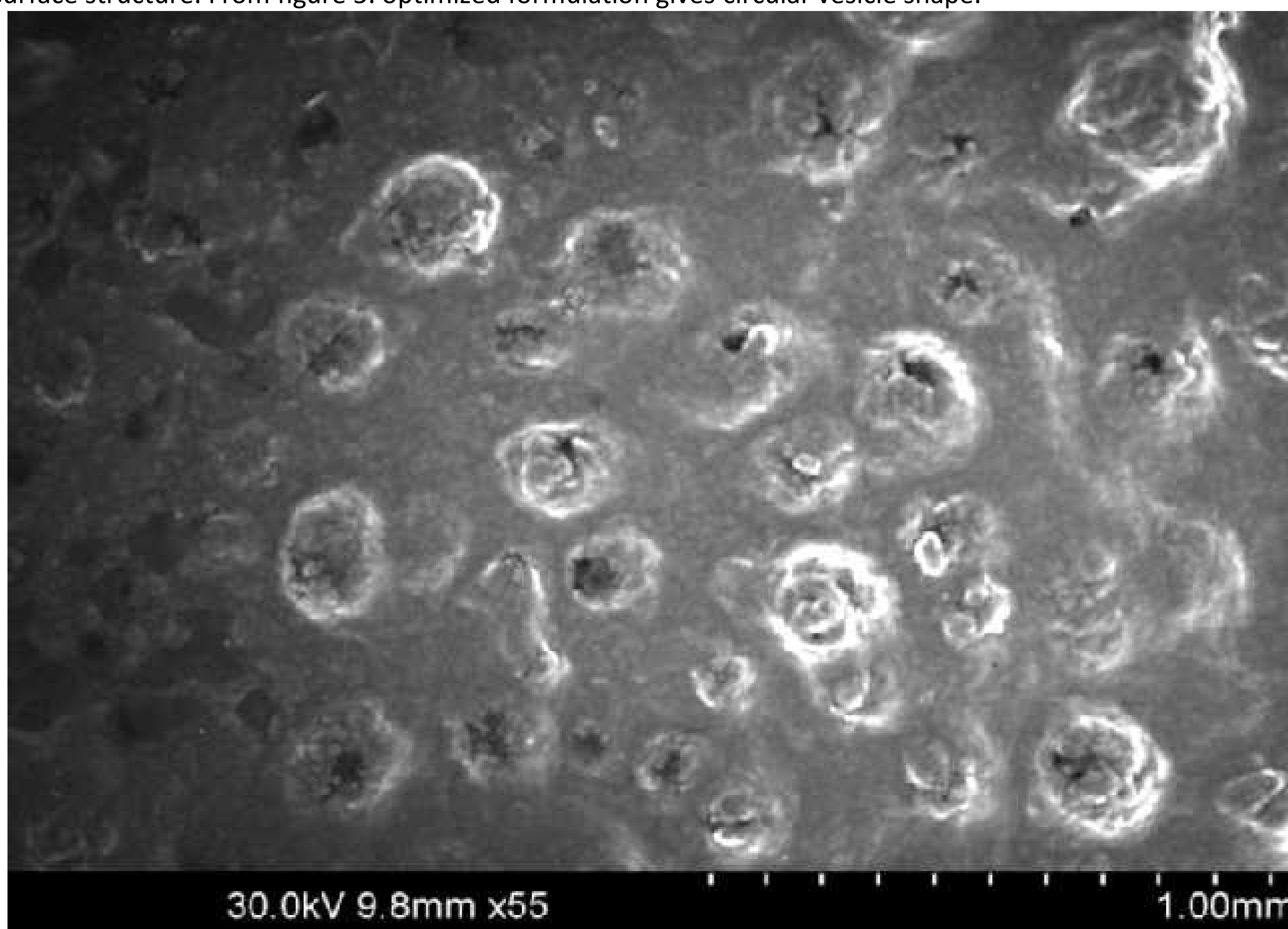

Figure 5: SEM image of vesicle shape

Zeta Potential

Zeta potential of the prepared aceclofenac loaded Niosomes was measured using Zeta Sizer. Niosomes prepared by span 60 (F5) 1: 2:1 ratio showed higher stability and zeta potential of optimized formulation was found to be -17.8 Mv is shown in fig.6

Zeta Potential Report
v2.2

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Sample Details

Sample Name: H zeta 1
SOP Name: mansettings.nano
General Notes:

File Name: Dr.mahajan sir new.dts
Record Number: 28
Date and Time: 6march2017 12:14:03
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

System

Temperature (°C): 25.0
Count Rate (kcps): 4.1
Cell Description: Clear disposable zeta cell
Zeta Runs: 12
Measurement Position (mm): 2.00
Attenuator: 6

Results

			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-17.8	Peak 1:	-18.1	99.0	12.1
Zeta Deviation (mV):	14.1	Peak 2:	37.0	0.8	3.72
Conductivity (mS/cm):	0.210	Peak 3:	-64.9	0.1	1.85

Result quality : See result quality report

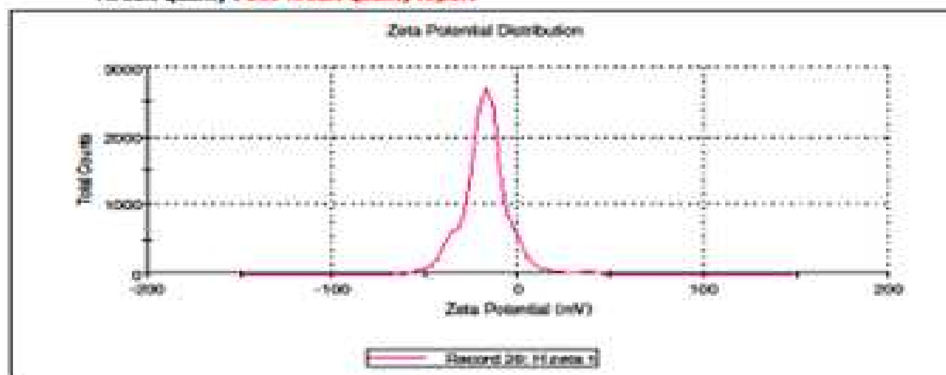

Figure 6: Zeta potential of optimized formulation (F5)

DISCUSSION

The aceclofenac loaded niosomes were successfully prepared by the thin film hydration technique using cholesterol and different grades of span as nonionic surfactant. The presence of cholesterol and nonionic surfactants made the niosomes more stable. All the formulations (F1-F9) were evaluated for the encapsulation efficiency, Drug content, vesicle size and shape. The results were found in the acceptable range. Niosome formulated with span 60 have shown the best entrapment efficiency compared with niosomes prepared with other grades like span 20. The vesicle size and drug permeation was greater for Span 60 (F5) due to its low surface energy decreases the size of vesicle and drug permeation increases. Incorporation of cholesterol was to known to influence vesicle stability, permeability and entrapment efficiency. The Drug permeation retards by increasing the concentration of cholesterol (F3, F6 & F9) due to increase in hydrophobicity. The result of In-vitro permeation study also revealed that the optimized gel formulation (F5) having showed the good permeation as compared to marketed gel.

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