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Formation and Characterization of Naproxen Niosomes Prepare from Pro-Niosomes

Arjun Sony[1]*, Neeraj Sharma[1]

1.Bhabha Pharmacy Research Institute, Bhopal, MP

ABSTRACT

Naproxen is a non-steroidal anti-inflammatory drug (NSAID) commonly used for the reduction of mild to moderate pain, fever, inflammation and stiffness caused by conditions such as osteoarthritis, rheumatoid arthritis, psoriatic arthritis, gout, ankylosing spondylitis, injury, menstrual cramps, tenditis, bursitis and the treatment of primary dysmenorrheal. The reason behind incorporation of Naproxen in Niosomes as this non-ionic surfactants vesicles offer several advantages over other drug carriers like liposome with respect to its biocompatibility, simple and controllable preparation, their capacity to carry large amount of drug, commercially availability, capable of entrapping hydrophilic and hydrophobic drug, cheaper in cost, any special condition not require for the use of the surfactant. The niosome system which achieves the site specific delivery of drug with controlled release kinetics of drug in predictable manner. Before this preparation, prior preparations had been made known as Pro-niosomes with Cholesterol, Surfactant, and Ethanol which later on converting to Niosomes.

Keywords: - Cholesterol, Ethanol, Tween-80, Naproxen

*Corresponding Author Email: arj_arj@rediffmail.com

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INTRODUCTION

Niosomes are vesicular delivery systems which can be formed by aqueous dispersion of non-ionic surfactant films. They are known as analogues of liposomes, and have been used in cosmetic formulations and experimentally as drug carriers. Apart from conventional spherical vesicles, various structures of Niosomes can be formed by varying the vesicle membrane compositions of certain mixed surfactant systems. For example, mixtures of hexadecyl diglycerol ether (C16G2): cholesterol: polyoxyethylene 24 cholesteryl ether were previously shown to form spherical, tubular, polyhedral and disk-like vesicles, depending on the molar ratio.[1]

Non- ionic surfactant vesicles results from self assembling of hydrated surfactant monomer. Non- ionic surfactant of wide verity of structural types is use full alternatives to phospholipids, in fabrication of vascular system. There is chemical difference between Niosomes and liposome, Niosomes posse's physical properties, similar to liposomes which are formed from phospholipids but as the name indicated Niosomes, non- ionic surfactant vesicles prepared by incorporation of non- ionic surfactant. Niosomes also prepared by various ionic amphiphiles such as dicetylphophate stearylamine etc, to achieve a stable vesicular suspension. Non- ionic surfactant forms a verity of aggregates from micelles to large vesicles, which can be used as vehicles for drug delivery.Niosomes are essentially non-ionic surfactant vesicles in which the aqueous solution of solute is enclosed by a bilayer of surfactant macro-molecules.[2,3,4]

Niosomes are microscopic lamellar structures, which are formed on the admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Structurally, Niosomes are similar to liposomes, in that they are also made up of a -bilayer. However, the bilayer in the case of Niosomes is made up of non-ionic surface active agents rather than phospholipids as seen in the case of liposomes. Most surface active agents when immersed in water yield micellar structures; however some surfactants can yield bilayer vesicles which are niosomes. Niosomes may be unilamellar or multilamellar depending on the method used to prepare them.[5]

The niosome is made of a surfactant bilayer with its hydrophilic ends exposed on the outside and inside of the vesicle, while the hydrophobic chains face each other within the bilayer. Hence, the vesicle holds hydrophilic drugs within the space enclosed in the vesicle, while hydrophobic drugs are embedded within the bilayer itself.[6] A typical niosome vesicle would consist of a vesicle forming ampiphile i.e. a non-ionic surfactant such as Span-60, which is usually stabilized by the addition of cholesterol and a small amount of anionic surfactant such as diacetyl

phosphate, which also helps in stabilizing the vesicle.[7]

MATERIALS AND METHODS

Cholesterol, Ethanol, Tween-80, Tween-20, Naproxen

The above ingredients such as Tween-80 & 20 are procured from Merck Pvt. Ltd, Mumbai, while Cholesterol & Drug are from Hi-media & Divis Laboratories, Mumbai & Ethanol from Jiangsu Huaxi International Trade Co. L, China

Formation of Niosomes from Proniosomes

Another method of producing niosomes is to coat a water-soluble carrier such as sorbitol with surfactant. The result of the coating process is a dry formulation. In which each water-soluble particle is covered with a thin film of dry surfactant. This preparation is termed "Proniosomes". The Niosomes are recognized by the addition of aqueous phase at T > Tm and brief agitation.

T = Temperature.

Tm = mean phase transition temperature.

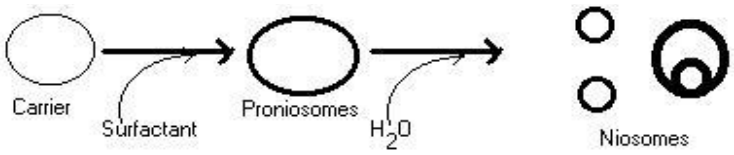


Figure 1 Formations of Niosomes from Pro-Niosomes

Blazek-Walsh A.I. *et al.* have reported the formulation of Niosomes from maltodextrin based proniosomes. This provides rapid reconstitution of Niosomes with minimal residual carrier. Slurry of maltodextrin and surfactant was dried to form a free flowing powder, which could be rehydrated by addition of warm water.[8]

Section of Surfactants

Surfactants are the backbone of the basic composition of niosome. In the present study the nonionic surfactant of sorbitane esters class were selected. From this class tween 80and tween 20 were chosen for the preparation of niosome. These surfactants were used in combination with cholesterol.

Preparation of Proniosome

Proniosomes were prepared by a method modified from Perrett et al. (1991). 50mg of naproxen with surfactant, and cholesterol were mixed with 6 ml absolute ethanol in a wide mouth glass tube. Then the open end of the glass tube was covered with a lid and warmed in a water bath at 60-65°C for 5 min. then 10ml ethanol was added and warmed in water bath for 3 minute. 100µml hot water was added and still warmed on the water bath for about 2 min till the clear solution

was observed. The mixture was allowed to cool down at room temperature till the dispersion was converted to proniosomal gel.

Conversion of Proniosome to Niosome

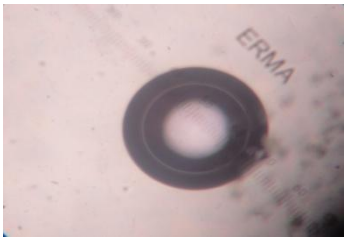
1ml of proniosome gel was taken and add 5ml water in to it shake and allow to stand for 5 min, and then observed microscopically. [9]

Formulation of different batches of Niosomes

Table 1 Different batches of Niosome

Code	Cholesterol	Drug	Tween-80	Tween-20
F1	50	50	100	100
F2	50	50	50	150
F3	50	50	150	50
F4	50	50	75	125
F5	50	50	125	75

Microphotograph of niosome



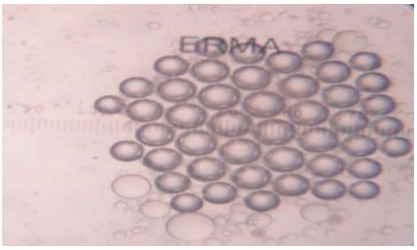


Figure 2 Niosome at 10x magnification **Figure 3 Group's niosome at 10x magnification**

Out of many method of preparation of niosome "formation of niosome from proniosome" was selected. Cholesterol and Tween 80 and Tween 20 were used in niosome formation. Different concentration of surfactant was used for preparation of niosome. Temperature was maintained between 60-70°C. Below 40°C Temperature the niosome was not formed and above temperature 80°C the formulation changes in color before formation of proniosomal gel.

EVALUATION OF NIOSOMES

Entrapment Efficiency

The entrapment of niosome prepared by "formation of niosome from pronisome" method was determined by freeze thawing/centrifugation method. 1 ml Niosomal dispersion was prepared from the proniosomal gel were frozen for 24 hr at -20°C in Eppendorf tubes. The sample were removed from the freezer let to thaw at room temperature then centrifuge at 13000 rpm for 40 mint at 4° C, then 0.2 ml supernatant was analyzed for free naproxen at 330.1 nm after diluted up to 3 ml. The amount of entrapped drug was determined by following formula by subtracting free drug concentration from total drug concentration.[10]

$$EE\ (\%) = C_e / C_t \times 100$$

Where,

EE (%) - % entrapment efficiency

$\mathbf{C_e}$ - concentration of entrapped drug

$\mathbf{C_t}$ - concentration of total drug

Particle Size

Particle size of the niosome was determined by binocular microscope. About 50 particles individually were selected random and their size was measured using calibrated ocular micrometer scale average was taken and size distribution range and mean diameter were calculated. Microphotographs were taken by using digital camera.[9, 11]

Optimization of Process Variable

The preparation procedure was accordingly optimized and validated on the basis of the following process variables:

- Effect of temperature
- Effect of warming time
- Effect of surfactant

Excipients and Active Compatibility Studies by FT-IR Spectroscopy

Compatibility of the drug with excipients was determined by FT-IR spectral analysis, this study was carried out to detect any changes on chemical constitution of the drug after combined it with the excipients.

***In-Vitro* Drug Release Study**

The dissolution cell consisted of a hollow glass cylinder (length 14.6 cm and internal diameter 2.5 cm) made up of Borosil glass. One end of the cylinder was covered with got intestine membrane. The dissolution cell was placed in a 50 ml Borosil beaker that served as the receptor cell. The contents of the dissolution cell were agitated with the help of a glass stirrer. The receptor cell contained a magnetic bead and was rotated at a constant speed. The temperature in the dissolution and receptor cells was maintained at 37±2°C, with the help of a thermostat. Two milliliters of each formulation was subjected to release studies. Phosphate buffer (50 ml) pH 7.4 was placed in the receptor cell. 2 ml sample of each formulation was transferred to the dissolution cell. Two milliliter samples were withdrawn from the receptor cell at specified time intervals of 1,2,3,4,5,6,7,8,9,10,11and 12h. At each time immediately after the removal of the sample, the medium was compensated with fresh phosphate buffer (pH 7.4). The samples were analyzed for Naproxen content using a UV spectrophotometer (PC based double beam Systronic UV spectrophotometer 2202) at λmax 330.1nm.[11, 12]

Release kinetic study

To investigate the possible mechanisms of Naproxen release from the prepared Niosomes, the release data were analyzed mathematically according to the following models.[12]

Zero order equation

$$Q_t = Q_0 + K\,t$$

First order equation

$$\text{Log } Q_t = \log Q_0 + K\,t/2.303$$

Higuchi order equation

$$Q = K\sqrt{t}$$

Where,

Q is the amount of drug released at a time (t) and K is the rate constant.

The cumulative percent release of Naproxen for all the batches was calculated and the data analyzed for release kinetics.

Skin Irritation Test

For this permission from ethical committee had been taken issued by the working laboratory on Wistar rats of weighing 200-250g after being examining proper protocols as per the experiments, which was attached in the last & for which Skin irritation test was done with selected niosome performed on male Wistar rats. Wistar rats were divided in to two groups: 1- Untreated animal and 2- Niosome treated animal. A single dose of 10µl of naproxen niosome was applied to the left ear of rat with the right ear as a control. The development of erythematic was monitored for 14 days.[13]

Histopathology examination of skin specimens

Dorsal skin of mice was treated with niosome. After 24 hrs, mice as sacrificed and skin sample from treated and untreated area were taken. Each skin sample was stored in buffered formalin solution. The skin samples were cut into vertically in different sections. Each section was dehydrated using ethanol, embedded in paraffin for fixing and section of 50 µ and stained with hematoxylene and Eosine. Skin samples were taken then observed under light microscope (Olympus) fitted with camera and compared with control samples. The light microscopy was performed at 40 X magnification.

***In vitro* skin permeation**

The skin permeation of naproxen from niosome formulations was determined by using Franz (vertical) diffusion cell. The Wistar rat (7–9 weeks old) skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The

donor compartment was filled with the niosome formulation. A 20 ml pH 7.4 phosphate buffer was used as receptor medium to maintain a sink condition. The available diffusion area of cell was 2 cm^2. The receptor compartment was maintained at 37±2°C and stirred by a magnetic bead at 500 rpm. At appropriate time intervals, 2 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh receptor solution. The samples were analyzed by UV-spectrophotometer for naproxen contents.[9]

The cumulative amount of Naproxen permeated into the receptor compartment was plotted against time to obtain a percentage permeation profile. The steady state flux, Jss (mcg/cm^2/h), was calculated from the slope of the linear portion of the plot of the cumulative amount permeated versus time and expressed as:

$$J_{ss} = dM/dt$$

Where,

M is the cumulative amount of Naproxen permeated through skin and t is experimental time

Enhancement ratios were calculated according to the following expression:

$$ER = J_{enh} = J_{ctrl}$$

Where,

J_{enh} is the enhanced flux with application of proniosomal formulation and J_{ctrl} is the flux of drug from the control formula.[14]

***In vivo* study (animal model)**

Acetic acid-induced vascular permeability

According to a modification of the method of Whittle (1964), acetic acid-induced vascular permeability test was performed. Mice were divided into groups of three. One hour after oral administration of vehicle (saline), or naproxen (120 mg/kg), 0.1 ml/10 g body weight of 1% Evans blue solution was injected intravenously in each mouse. Thirty minutes later, 0.1 ml/10 g body weight of 0.7% acetic acid in saline was intraperitoneally injected. Thirty minutes after the administration of acetic acid, the mice were killed by cervical dislocation. The peritoneal cavity was washed with 10 ml of saline and the solutions were collected in test tubes. The concentration of Evans blue in the peritoneal cavity was measured by the absorbance at 630 nm in a Spectrophotometer. The vascular permeability was represented in terms of the absorbance (*A*630) which leaked into the cavity. Percent (%) inhibitions of inflammation were determined by following formula.[15]

(%) inhibition of Inflammation = Abs of control group – Abs of test group / Abs of control group X 100

***In- vivo* trigeminal neuralgia test (Nociceptive tests)**

Eye wiping test: the animals were placed on a 50 x 50 cm table for 10 min habituation period. One drop (50 µl) of 5M Nacl solution was put into right eye of the animal. When the concentration was irritant the animals immediately began to wipe eye with ipsilateral forepaw, and the number of eye wipes was counted during 30 second. Testing protocol: to compare the result obtained from two different test in same subjects, the number eye wipes was first counted in response to 5M Nacl for each animal. All rats showed wiping reaction to applying 5M Nacl into their eyes. The wiping behavior was performed with ipsilateral forepaw, and was obviously different from grooming activity.[13] The effect of drug from the maxima possible effect was calculated according to the following formulas:

%MPE= (post drug wipe count - pre drug wipe count / 0-pre drug wipe count) X100

Biostatical interpretation

All data analyzed by one way ANOVA followed by Benferroni's test.

RESULTS & DISCUSSION

Entrapment efficiency and particle size

Table.2: Entrapment efficiency and Particle size of Niosomes

Formulation Code	Entrapment Efficiency (%)	Particle Size (µM)
F1	75.24	3.93±1.81
F2	74.59	3.81±1.82
F3	67.55	4.41±1.80
F4	74.02	3.84±1.82
F5	**65.91**	**4.11±1.83**

The entrapment efficiency was performed to estimate the actual amount of drug being entrapped. Maximum percent drug entrapped in F1 and lowest percent in F5. Increases in the concentration of cholesterol did not show any influence in entrapment efficiency. Amount of drug not increases for increasing the entrapment shown in table no.2

Particle size was performed by ocular light microscope the average of the niosome was found between the ranges of 3.81 – 4.14 µm (Table 2). The main factor affecting the size of niosome is cholesterol and HLB of surfactant. F5 having highest average Particle size. Noisomes are spherical in shape.

Optimization of Process Variable

The preparation procure was accordingly optimized and validated on the basis of following process variable

Table 3 Effect of temperature

Temperature	Cholesterol	Tween-80(mg)	Tween-20(mg)	Drug	% Entrapment
40^{O} C	50	100	100	50	Not formed
Above 80^{0} C	50	100	100	50	Color change

Table.4: Effect the concentration of cholesterol and drug

Cholesterol	Tween-80(mg)	Tween-20(mg)	Drug	% Entrapment
50	100	100	100	68.26
100	100	100	50	69.03

Excipients and Active Compatibility Studies by FT-IR Spectroscopy

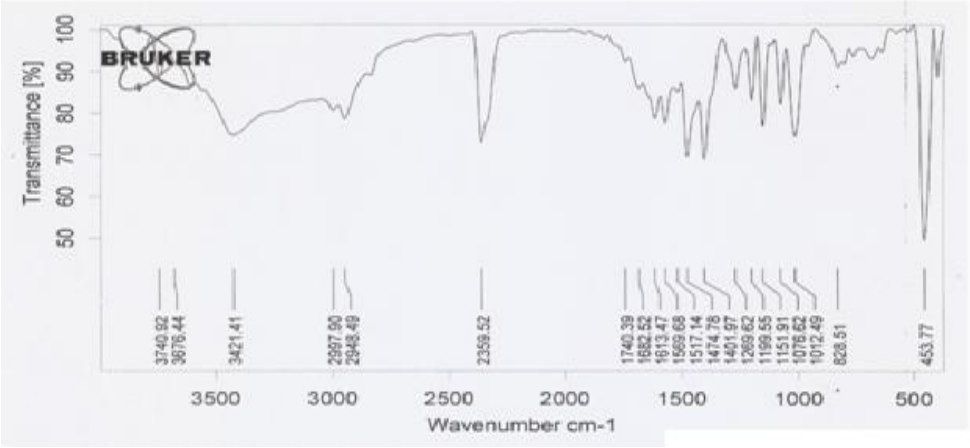


Figure 4: IR Spectra of Naproxen

By comparing IR spectra of pure drug and drug with excipients it was observed that the drug is compatible with selected surfactant and co surfactants.

Table.5: Interpretation of FTIR spectra of naproxen

Bonds	Standard(cm^{-1})	Observed(cm^{-1})
O-H stretching	3600-3500	3421.41
C=O stretching	1750	1740.39
C-O stretching	1100	1076.62
C=C stretching	1650-1550	1569.68

Comments for compatibility: All IR spectra of pure compound and its composition with excipients in formulation are same. This indicates that there was no structural change caused by excipients.

***In Vitro* release study**

Table 6: % Cumulative drug release of niosome

Time	F1	F2	F3	F4	F5	Controlled group
0	0	0	0	0	0	0
1	7.44	6.83	6.51	5.14	6.4	6.61
2	12.23	13.25	11.26	10.82	13.42	15.62
3	20.20	19.65	17.18	17.04	19.50	28.24
4	28.46	27.69	24.30	22.72	26.84	45.21

5	35.65	35.22	31.70	29.49	31.72	63.89
6	41.50	42.85	37.33	37.06	36.29	78.09
7	48.15	50.44	42.67	43.90	40.26	97.01
8	52.41	57.68	46.23	47.61	44.22	
9	57.20	61.00	50.08	52.48	50.02	
10	61.72	64.87	53.93	56.54	53.68	
11	66.51	67.45	57.78	61.68	56.73	
12	**70.24**	**71.98**	**62.52**	**65.20**	**60.39**	

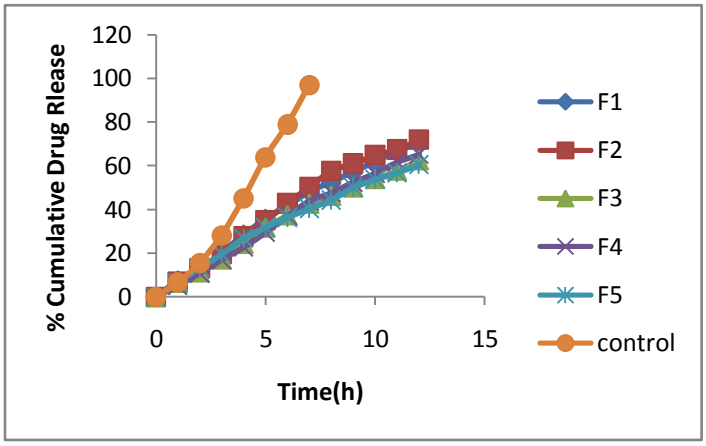


Figure.5: %CDR Vs TIME graph for naproxen release

Release Kinetic order

Table.7 Value of rate constant (k) and coefficient of regression (R^2)

Formulation	Zero order		First order		Higuchi order	
	K	R^2	K	R^2	K	R^2
F1	2.48	0.988	0.030	0.995	20.30	0.949
F2	2.33	0.983	0.028	0.992	20.80	0.942
F3	3.12	0.987	0.040	0.997	18.06	0.948
F4	2.90	0.993	0.036	0.995	18.84	0.934
F5	**3.30**	**0.986**	**0.042**	**0.998**	**17.45**	**0.962**

Under perfect sink condition, the drug release rate depends on concentration of cholesterol and surfactant. Drug release behavior of Naproxen was studied in phosphate buffer (pH 7.4) at 37±2°C. The curve was obtained after plotting the cumulative amount of drug released from each formulation against time. Formulation F2 (71.98%) showed maximum release while other formulation showed less amount of drug release in 12h. Formulation F5 has highest coefficient of regression (R^2 =0.998) value and follows drug release by first order model.

To predict the release pattern of Naproxen from Niosomal formulation batches (F-1 to F-5) correlation coefficient and rate constant (Table-7) was calculated for zero order, first order and higuchi order kinetics. The study of drug release kinetics showed that majority of the formulations governed by first order kinetic model.

Skin irritation study

F1 formulation was use for performing skin irritation test. Erythema was not seen when animal observed for 14 days. It indicates there is no irritation produce by formulation.

***In-vitro* skin permeation**

The amount Naproxen Niosomes by skin permeation were similar, but much higher than that from the free drug (Figure. 6 and Table 8).The formulation of control group was Naproxen suspended in the same solvent of proniosome formulation. Flux of Tween20 and Tween80 Naproxen Niosomes were similar, but much higher than free drug formulation (control) (Table 9). Surfactant in formulation always acts as a permeation enhancer; however, the enhancement ratio of Niosomes was shown in (Table 9) F3 formulation show maximum permeation flux of Naproxen and control formulation show minimum permeation. (Table .9) the figure 5 shows the permeation of drug was enhanced by surfactant present in Niosomes.

Table.8: Cumulative amount release of niosome by skin permeation

Time(Hrs)	Cumulative F1	Amount of F2	Drug F3	Release F4	Change(µg) F5	Control
0	0	0	0	0	0	0
1	20.83	22.21	28.81	17.77	24.84	19.16
2	40.10	39.49	47.39	40.62	42.99	28.33
3	64.33	61.71	76.20	63.47	60.19	41.66
4	80.20	79.81	101.30	92.24	80.26	52.50
5	105.26	97.91	119.88	111.71	100.32	66.66
6	117.80	116.83	144.05	128.64	117.52	75.00
7	138.68	138.23	158.92	144.72	131.86	91.66
8	159.57	157.15	178.43	165.03	149.06	104.16
9	182.96	181.01	200.74	184.49	161.48	120.00
10	201.34	204.05	213.75	203.96	182.50	135.83
11	221.39	220.51	226.76	221.73	218.81	151.66
12	246.46	242.72	237.91	242.89	236.01	167.50

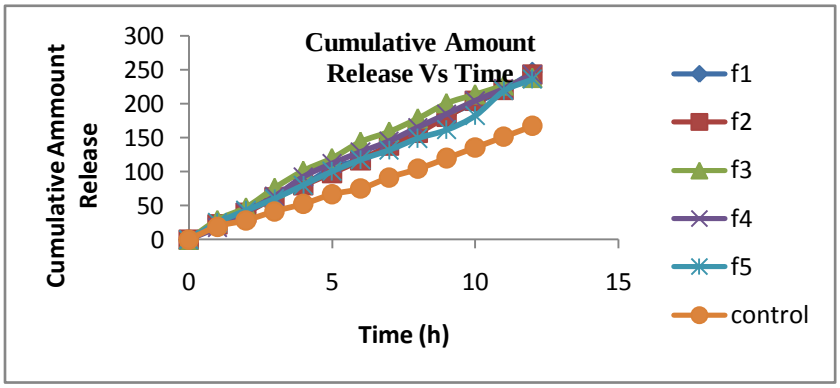


Figure 6 : % cumulative amount of drug release of naproxen by skin permeation

Table 9: Flux and Enhancement ratio of niosome

Sr. No.	Formu. Code	Flux(µg/cm^2/h)	Enhancement Ratio
1	F1	20.32 ± 0.53	1.46
2	F2	20.14 ± 0.73	1.44
3	F3	23.36 ± 2.43	1.67
4	F4	20.72 ± 1.29	1.48
5	F5	19.94 ± 1.82	1.43
6	Control	13.91 ± 1.72	-

***In vivo* study**

Vascular permeability assay is a model first stage inflammatory reaction. In vascular permeability assay, mediators of inflammation, released following stimulation, leads to dilation of arterioles and venules and increased vascular permeability (Eun-Hee Park, 2007). Naproxen at the oral doses of 120 mg/kg showed an inhibition of 43.73% in vascular permeability. From this finding, it is assumed that acute inflammatory effect of naproxen arises from its protection on the release of inflammatory mediators at the first stage. Result of Vascular permeability studies revealed that Niosome show significant ($p<0.05$) inhibition of inflammation.

Table 10: Treatment of the groups and leakage of Evans blue dye (*A*630)

Group.	Treatment	Abs. of Evans blue
1	1% Evans blue + 1% AA	0.129
2	1% Evans blue	0.041
3	1% Evans blue + 1% AA + Niosome	0.070[a]

The innervations of the cornea is provided by a relatively small number of primary sensory neurons located in the trigeminal ganglion. The majorities corneal fiber (poly model nociceptors) activate by near noxious mechanical energy, but they are respond to heat, exogenous chemical irritants and to a large variety of endogenous chemical mediator released by damage corneal tissue. Therefore, these extensive nociceptive innervations of cornea make it suitable for studying pain and pain killer drug in trigeminal system. Trigeminal nucleus responds vigorously when applied hypertonic solution of 5M Nacl in corneal surface. Naproxen dose 50 µl show 51.55 % (%MPE) decrees in number of eye wiping.

Acetic acid vascular permeability model

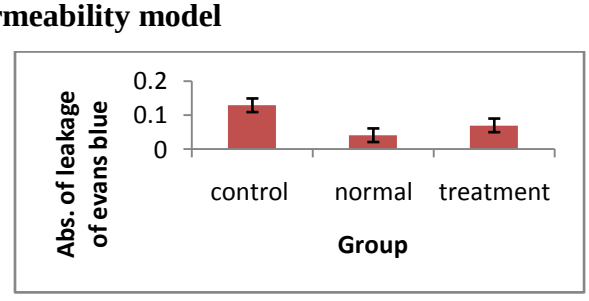


Figure 7: Vascular permeability of Evans blue dye in mice in different groups

Table 11:- Comparison group of different of mice

Comparison	Difference in mean	P<0.05
1 vs3	0.088	Yes
1 vs2	0.059	Yes
2 vs3	0.029	Yes

Trigeminal neuralgia test (Nociceptive tests)

Table 12:- Average eye wiping

Group	Treatment	Eye wiping (avg.)
1	Control (5M Nacl)	5.16
2	Treatment	2.50[a]

a- p < 0.05 as compared to control group

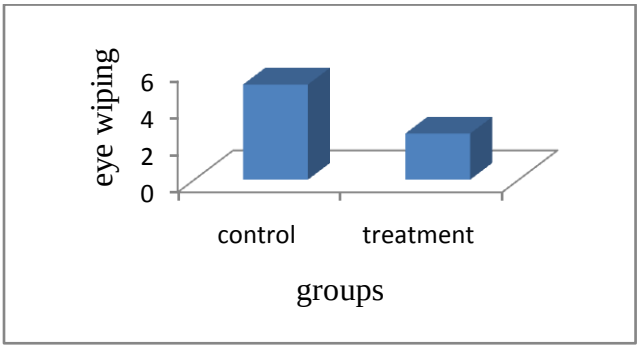


Figure 8:- Eye wiping in rats in different groups

CONCLUSION

Naproxen niosome was prepared by "Niosome formulate from proniosome". The niosome was characterized by entrapment efficiency, particle size analysis, skin irritation, skin permeation test for all the batches of niosome. F2 formulation subjected for skin irritation test. Concentration of twen 80 increases the entrapment efficiency of noisome increase. The *in-vivo* release was carried out for naproxen content by using dissolution cell obtained in to the laboratory, the cumulative amount release from formulations varied and it is depend of the concentration of the surfactant used. Release kinetic study model performed for all formulation and indicate that first order release kinetic model was followed. In vitro skin permeation of naproxen was performed and obtained the amount of naproxen was permeated is higher than the control (free) drug. The permeation flux was higher than free drug. The surfactant used for formulation enhances the permeation flux of formulation. The anti inflammatory activities of naproxen niosome were evaluated by Acetic acid-induced vascular permeability model and eye wiping test for trigeminal neuralgia test. Formulation F2 and F4 was used for *in-vivo* study. It concludes that formulation reduced the vascular permeability 45.73% in comparison to the vehicle or control group of mice. %MPE in eye wiping test is 51.55% obtained.

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