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Research Article

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Non Ionic Surfactant Based Vesicles (Niosomes) Containing Flupirtine Maleate as an Ocular Drug Delivery System.

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ABSTRACT

The aim of present investigation was to formulate and evaluate Niosomes, a synthetic microscopic vesicles consisting of an aqueous concentration is enclosed in a bilayer consisting of cholesterol and nonionic surfactants to improve the low corneal permeability for effective management trigeminal neuralgia. Proniosomal gels of flupirtine Maleate (centrally acting, non-opioid analgesic) were developed with span 20, span 60, span 80, tween 20 and tween 80 with cholesterol. Nonionic surfactant vesicles formed immediately upon hyadrating proniosomal gel. The morphological characteristics, entrapment efficiency, In-vitro drug release, drug release kinetic, stability studies, ocular irritation test, In vivo studies (eye wiping test) for Trigeminal neuralgia was determined. The entrapment efficiency (EE %) of flupirtine maleate was determined by centrifugation of freeze thawed vesicles followed the order span80 >span 60> span 20> tween 20> tween 80. The In vitro drug release studies showed that there was a prolong release of drug which followed Higuchi model. Niosome formed from span 80 and cholesterol is promising approach to prolong antinociception activity and improve permeation rate as compared to pure drug.

Keywords: Niosome, Flupirtine maleate, Proniosome, Trigeminal neuralgia

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INTRODUCTION

Flupirtine maleate is centrally acting; non opioid analgesic belongs to the triaminopyridine class, having unique pharmacological properties and effectively reduces chronic musculoskeletal pain, migraine and neuralgias [1]. Trigeminal Neuralgia is also known as Tic Douloureaux, is a nerve disorder that causes abrupt, searing, electric shock like facial pains, most commonly the pain involves the lower face and jaw, but symptoms may appear near the eyes, nose, ears or lips. Trigeminal neuralgia is the most unbearably painful human condition. The pain occurs because of a change in neurological structure or function due to irritation or damage of a nerve [2]. Niosome are able to prolong circulation of encapsulated drug altering its organ distribution, metabolic stability and to increase the contact time of drug with applied tissue. They offer several advantages over liposomes such as higher chemical stability, penetration enhancing properties and lower cost [3]. However, there may be problem of physical instability in niosomes dispersion during storage like vesicles aggregation, fusion, leaking or hydrolysis of encapsulated drug which affected shelf life of dispersion [4].

The present research is to develop proniosome which is liquid crystalline compact niosomes hybrid that was converted into niosomes immediately upon hydration [5].

Proniosomes is stable precursors for the preparation of niosomes. The niosomal drug delivery system have great advantage for poorly soluble drug by increasing its solubility, controlling its release and prolong its activity over period of time, Hence decreasing the frequency of administration and improving patient compliance [6]. The novelty of research work is to prepare Flupirtine Maleate loaded niosome for the effective management of trigeminal neuralgia and achieves a sustained release profile suitable for ocular delivery with enhanced efficacy, which could overcome the drawbacks of conventional drug delivery.

EXPERIMENTAL SECTION

Flupirtine maleate was obtained as gift from Optimus drugs Pvt. Ltd (India). Cholesterol was purchased from Himedia laboratories Pvt. Ltd (India). Surfactants were purchased from Merck specialities Pvt Ltd (India). Ethanol was purchased from Jiangsu Huaxi International Trade Co. Ltd (China), In-vivo drug release study were performed on Adult Wistar rats (250-300mg). Animals were housed in a standard condition of 12 hrs light/dark cycle and 22±1ºC room temperature and had freely access to food and water. Animals were treated and cared according to animal ethical guideline.

2.1 Method of Preparation

In present study niosomal formulations of Flupirtine Maleate were prepared by Proniosome method. 10 mg of Flupirtine Maleate with surfactant and cholesterol were mixed with 1 ml of absolute ethanol in a wide mouth glass vials. Then the open end of the glass vial was covered with a lid to prevent vaporization of ethanol and warmed in a water bath at 55-60ºC for 10 min when cholesterol is completely disappear, add 200 µl hot water with the help of micropipette 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. Add 6 ml of phosphate buffer 7.4 on vortex shaker to proniosomal gel and cool at room temperature. Now makeup volume of niosomal suspension with phosphate buffer to 20 ml and then observed microscopically [7].

2.2 Photomicroscopic study of niosomes

Hydration of proniosomal gel (100mg) was done by adding saline solution (0.9% solution) in a small glass vial with occasional shaking for 10 min. The dispersion was observed under ordinary light microscope 40 x magnifications. The sizes of 50 vesicles were measured using a calibrated ocular and stage micrometer fitted in the microscope [8].

2.3 Osmotic fragility

Impact of hypertonic and hypotonic solution on niosome vesicle was visualized. Niosome vesicles were coat under 0.6% NaCl solution (hypotonic) and 1.2% hypertonic solution for 10 minutes. Both the solution was visualized under light microscope [9].

2.4 Determination of entrapment efficiency of flupirtine maleate

The entrapment efficiency of Flupirtine Maleate in niosomes was determined by freeze thawing/centrifugation method. 1ml niosomal dispersion was prepared from the proniosomal gel were frozen for 24 hr at -20ºC in Eppendorf tubes. The sample were removed from the freezer let to thaw at room temperature then centrifuge at 13000 rpm for 40 min at 4ºC, then 0.1 ml supernatant was analyzed for free Flupirtine Maleate at 344 nm. The amount of entrapped drug was determined by following formula by subtracting free drug concentration from total drug concentration [10].

$$\mathbf{EE(\%)=Cc/Ct\times100}$$

Where, Cc=concentration of entrapped drug, Ct=concentration of free drug

2.5 In vitro drug release studies by “Franz diffusion cell” through Goat cornea

The In vitro drug release study of niosomes performed by Franz diffusion 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 cornea/conjunctiva membrane. The diffusion cell consists of two compartments (donar and receptor) which were placed in a 250 ml borosil beaker. The content of diffusion cell was 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 a donar and receptor cells was maintained at 37±0.5ºC with the help of thermostat. Two milliliters of each formulation was subjected to

release studies. Phosphate buffer (20 ml) pH 7.4 was placed in the receptor cell. Two milliliters sample of each formulation was transferred to the dissolution cell. One milliliter sample was withdrawn from the receptor cell at specified time intervals. 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 Flupirtine Maleate content using a UV spectrophotometer (PC based double beam Systronic UV spectrophotometer 2202) at λ max 344 nm [11].

2.6 Drug release kinetics

The drug release kinetics was studied by various kinetic models such as zero order, first order, Higuchi plot, Korsmeyer-peppas model. To study the release kinetics, data were obtained from in vitro drug release studies were plotted in various kinetic models: zero order as cumulative amount of drug release Vs time, first order as log cumulative percent of drug remaining Vs time, Higuchi model as cumulative percentage of drug release Vs square root of time. The best model was confirmed by the value of correlation coefficient near to 1.

2.7 Ocular irritation studies

Ocular irritancy test can be done on rabbit eyes, in which tear production, pupil size, redness and irritancy was observed with respect to time. Rabbit weighing 2±1 Kg of 10±2 weeks were used for ocular irritancy testing. Effect on pupil size, redness, effect on tear production and irritancy was observed after instilling to drops of niosomal suspension. Formulation was instilled in right eye, comparison was done between right and left eye. Effect on normal behavior and food, water intake was observed. Formulation was evaluated on four animals. Eye was observed just after instillation of 15 min, 1 hr and 24 hrs respectively [12].

2.8 Stability studies

The ability of vesicles to retain the drug (drug retention behavior) was assessed by keeping the niosomal formulation at two different temperature conditions i.e. refrigeration temperature (4-8ºC), room temperature (25±2ºC"). Throughout the study, niosome formulations were stored in aluminum foil sealed glass vials. The samples were withdrawn at different time intervals over a period of one month and drug leakage from the formulations was analyzed for drug content spectrophotometrically [13].

2.9 In vivo Drug Release Studies

In- vivo trigeminal neuralgia test (Nociceptive tests)/Eye wiping test was performed, 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: Animals were divided into seven groups, each group contains six animals. To compare the results obtained from control and standard group with pretreated group, the number eye wipes was first counted in response to 5M Nacl for each animal in control group. 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 and second the number of eye wiping was counted for pretreated groups of standard and all formulation groups. [14].

RESULTS AND DISCUSSION

The niosome vesicles were prepared by span 20, span 60, span 80, tween 20, tween 80 and optimized on the basis of temperature control and ratio of different excipients. The niosomes were not formed at room temperature because 55-60^{0}C temperature is needed for complete dissolution of cholesterol, and at temperature 70-85^{0}C vesicles not formed because vesicles were broken when seen in light microscope. Niosomes were formed at 55°C-60°C. Result shown in table 1.

Flupirtine Maleate loaded niosomes were swelled in hypertonic solution and shrinked in hypotonic solution, so the formulations were osmotically active prepared with different surfactants. Niosomes were subjected for photomicroscopic study for characterizing size distribution of niosomes from this study it was found that the average particle size was 3.0 ± 0.97 µm for F3 formulation. The particle size of all formulations was more than 0.1µm which revealed that niosomes were large unilamellar vesicles. After removal of unentrapped drug by centrifugation, the entrapment efficiency of all formulations was studied. From this study, it was found that the amount to drug entrapped in niosomes ranged between 58.39±0.79% for F5 formulation to 81.00±0.64% for F3 formulation. Hence,

the niosome formulated with span 80 were found to be optimum for loading maximum amount of Flupirtine Maleate in niosomal formulation. Result shown in table 2.

Table 1: Formulations of niosome containing Flupirtine Maleate

Formulation Code	Drug (mg)	Surfactant (mg)					Cholesterol (mg)	Ethanol (ml)	Temperature (°c)
		Sp 20	Sp 60	Sp 80	Tw 20	Tw 80			
F1(span20)	10	40					60	1	55°c-60°c
F2(span 60)	10		40				60	1	
F3(span 80)	10			40			60	1	
F4(tween 20)	10				40		60	1	
F5(tween 80)	10					40	60	1	

Table 2: Characterization of niosomal formulations

Formulation Code	Mean Particle size (µm)	% Entrapped Drug
F1	1.5 ± 0.70	66.00±2.65
F2	2.25 ± 1.38	75.17 ±1.17
F3	3.0 ± 0.97	81.00±0.64
F4	8.62 ± 3.14	64.17 ±1.64
F5	3.56 ± 1.31	58.39 ±0.79

Table 3: Drug release profile of all formulations

Time (hr)	% cumulative drug release				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	26.00±0.67	20.67±1.20	16.00±1.67	34.00±1.00	36.33±1.33
2	30.67±0.88	25.00±0.88	19.67±1.20	38.67±0.69	41.67±0.67
3	35.00±1.45	29.33±1.45	22.67±0.67	42.33±0.88	48.00±1.53
4	40.33±1.15	32.67±0.88	25.00±0.84	44.67±1.02	53.33±0.67
5	45.00±1.76	36.33±0.67	27.67±0.88	49.00±0.33	60.67±0.88
6	49.67±1.73	41.33±1.00	30.67±0.58	51.67±1.17	66.00±1.45
7	54.33±0.33	44.00±1.33	34.33±1.76	56.00±0.88	72.67±0.88
8	60.33±0.67	48.33±1.20	35.67±1.45	62.33±1.20	79.33±1.33
9	62.67±1.45	51.67±1.67	38.33±1.20	65.00±0.33	84.00±1.15
10	63.33±1.20	52.67±0.33	41.67±1.86	68.00±1.33	86.00±1.00
11	66.67±0.88	54.67±0.88	43.33±0.88	70.33±0.67	87.00±1.15
12	68.67±1.20	56.67±1.45	44.67±1.86	74.33±1.00	88.33±1.45

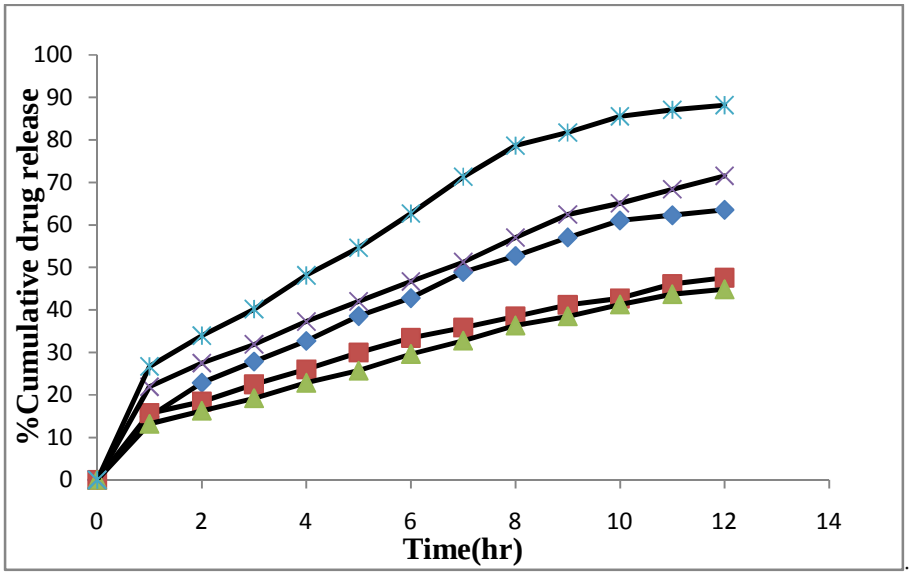

Fig. 1: Graph of %CDR v/s Time of formulations F1-F5

In vitro release study was carried out by Franz diffusion method using got cornea. From this study percentage of drug diffused into medium was evaluated. The percentage amount of drug release was calculated for all formulations. F3 gave 44.67±1.86% of drug release within 12 hours. These results showed that niosomal formulations loaded with Flupirtine Maleate have sustained release up to 12 hours. To investigate the possible mechanism of Flupirtine maleate release from the prepared niosomes, the release data were analyzed mathematically. The optimized formulation F3 was subjected to graphical treatment to assess the kinetics of drug release. The data obtained from the best formulation was fitted to various kinetic equations to determine the mechanism of drug release and release rate as indicated by higher correlation coefficient (r^2). The data were best fitted to Higuchi matrix equation for niosomal drug release with r^2 value 0.993. Further the value of n in Korsmeyer-Peppas model was less 1 indicated non-fickian transport. From this study it was found that the formulated niosome F3 was diffusion controlled.

Ocular irritation test was determined by instillation of niosomal formulation. At the point of instillation animal showed slight irritation, but no redness or any other sign of inflammation was observed in the eyes, instilled with niosomes.

Stability study of optimized Niosomal formulation (F3) at temperature (4 ± 2^0C) and room temperature at 1day, 15 day, and 30 day were determined. The % entrapment of F3 formulation was 74.28%, 72.59%, 71.88% and 74.28 %, 69.66 %, 70.80 % respectively.

In vivo Drug Release Studies ((Nociceptive tests/eye wiping test) of pretreated groups were compared with control and standard groups upto 2 hours. There was significant dose response relationship between control and standard groups with the pretreated group of formulations (measure with one way ANOVA).

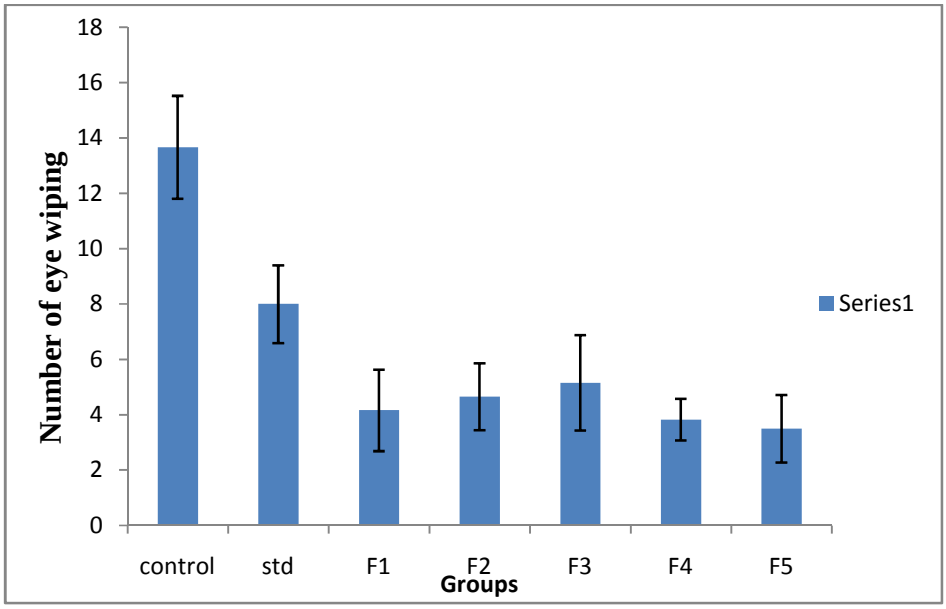

Fig. 2: Eye wiping in rats in different groups as compared to control and pure drug
**p<0.05 as compared to vehicle treated group, # p<0.05 as compared to pure drug treated group.*

CONCLUSION

Surfactants with cholesterol in the formulations prevent the metabolism of drug from the enzymatic activity at the cornea surface and improve permeability of drug. Flupirtine Maleate can be successfully formulated in niosome form by optimizing the proportion of surfactants and cholesterol as excipients and can be encapsulated for therapeutic purpose to reduce dose frequency and side effects.

From the results of characterization parameter for all the formulation we found that F3 formulation has best result for all the parameters and it is subjected to all other study also. In the present study we found formulation containing span 80(F3) have maximum entrapment i.e. 81.00±0.64%. The in vitro drug release profile shows that F3 formulation containing span80 have more controlled release profile as compare to other formulation, because %CDR (cumulative drug release) of F3 is 44.67±1.86% in 12 hours by which we conclude that formulation F3 release the drug for prolonged time in controlled manner.

The data obtained from the F3 formulation was fitted to various kinetic equations to determine the mechanism of drug release and release rate as indicated by higher correlation coefficient (r^2). The result was best fitted to Higuchi matrix equation for niosomal drug release with r^2 value 0.993. Further Korsmeyer-Peppas model is $0.5<n<1.0$ which implies that the drug follows non-fickian transport. From this study it was found that the formulated niosome F-3 was diffusion controlled.

The stability study was performed according to I.C.H. guidelines. Formulation F3 was selected on the basis of % entrapment and release profile. The results suggested that there is no significant change in drug concentration in 30 days. The results suggested greater drug loss at elevated temperature (25±2˚c) from the system as against storage at refrigerated temperature (4±2˚c). Hence it is recommended that the niosomes should be stored at refrigerated temperature (4±2˚c). Antinociceptive activity was evaluated by NaCl induced trigeminal nociception model. In this model niosomes of Flupirtine Maleate reduced number of eye wiping and nociception. *$p<0.05$ as compared to vehicle treated group, # $p<0.05$ as compared to pure drug treated group. This study indicated that Flupirtine Maleate loaded niosome is possessing prolong antinociception activity and improve permeation rate as compared to pure drug.

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