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PREPARATION AND EVALUATION OF NIOSOMES CONTAINING ACECLOFENAC

S. SRINIVAS, Y. ANAND KUMAR*, A.HEMANTH, M.ANITHA
V.L.College of Pharmacy. Manik prabhu temple road, Raichur. 584103. India

Aceclofenac is a drug with narrow therapeutic index and short biological half-life. This study was aimed at developing and optimizing niosomal formulation of aceclofenac in order to improve its bioavailability. In evaluation study the effect of the varying composition of non ionic surfactant and cholesterol on the properties such as encapsulation efficiency, particle size and drug release were studied. Moreover, the release of the drug was also modified and extended over a period of 72 h in all formulations. NSF-3 emerged as the most satisfactory formulation in so far as its properties were concerned. Further, release of the drug from the most satisfactory formulation NSF-6 was evaluated through dialysis membrane to get the idea of drug release. The mechanism of dug release was governed by Peppas model.

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1. Introduction

Niosomes or nonionic surfactant vesicles are microscopic lamellar structures formed on admixture of nonionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media [1]. In niosomes, the vesicles forming amphiphile is a nonionic surfactant such as span 60 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate[2]. Niosomes can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials. It is reported to attain better stability than liposomes. It can prolong the circulation of the entrapped drugs. Because of the presence of nonionic surfactant with the lipid, there is better targeting of drugs to tumour, liver and brain. It may prove very useful for targeting the drug for treating cancers, parasitic, viral and other microbial disease more effectively [3].

Aceclofenac belongs to non steroidal anti- inflammatory drugs (NSAIDs)[4-5]. It works by blocking the action of cyclooxygenase. Cyclooxygenase is involved in the production of various chemicals in the body, some of which are known as prostaglandins. Prostaglandins are produced in response to injury or certain diseases and would otherwise go on to cause pain, swelling and inflammation. Aceclofenac is used to relieve pain and inflammation in arthritic conditions. All the drugs in this group reduce inflammation caused by the body's own immune system and are effective pain killers. But it has several drawbacks such as narrow therapeutic index, short biological half-life. These factors necessitated niosomal formulation for aceclofenac. As this dosage form would reduce the dosing frequency, hence better patient compliance. The present study was aimed with the formulation of niosomes of aceclofenac followed by the evaluating parameters such as drug content, entrapment efficiency, particle size, shape, size distribution and *in vitro* drug release

* Corresponding author: neeru241586@yahoo.co.in

2. Materials and methods

Aceclofenac was obtained from Rantus Pharmaceuticals Hyderabad, India; Spans were obtained from Rolex chemical industries, Mumbai. Diethyl ether Methanol, potassium dihydrogen phosphate, sodium hydroxide was obtained from S.D.Fine chemicals. All ingredients used were of analytical grade.

2.1. Evaluation of raw materials

Identification and standardization of drug and other excipients were carried out as per the official procedures mentioned in respective monographs.

2.2. Preparation of Niosomes

Niosomes containing aceclofenac were prepared by modified ether injection technique using nonionic surfactant (span 60, span 20) and cholesterol at different concentrations. Cholesterol and surfactant were dissolved in 6ml diethyl ether mixed with 2ml methanol containing weighed quantity of aceclofenac. The resulting solution was slowly injected using microsyringe at a rate of 1ml/min into 15 ml of hydrating solution phosphate buffer (pH 7.4).The solution was stirred continuously on magnetic stirrer and temperature was maintained at 60-65ºC. As the lipid solution was injected slowly into aqueous phase, the differences in temperature between phases cause rapid vaporization of ether, resulting in spontaneous vesiculation and formation of niosomes. Different batches of niosomes were prepared in order to select an optimized formula as per general method described above and proportion of surfactant and cholesterol for the preparations of niosomes is given in Table 1 and optimized niosomal formula are given in Table 2.

Table 1: composition of surfactant and cholesterol for preparation of niosomes.

Sr.No	Code	Surfactant	Drug: Surfactant: Cholesterol	Weight taken (mg)		
				Drug	Surfactant	Cholesterol
1	NSF-1	S60	1:1:1	200	200	200
2	NSF-2		1:1.5:1	200	300	200
3	NSF-3		1:2:1	200	400	200
4	NSF-4	S20	1:1:1	200	200	200
5	NSF-5		1:1.5:1	200	300	200
6	NSF-6		1:2:1	200	400	200

Table 2: Optimized formula for the noisome preparation.

Sr. No	Formula	NSF-3	NSF-6
1	Cholesterol	200mg	200mg
2	Span 60	400mg	-----
3	Span 20	-----	400mg
4	Aceclofenac	200mg	200mg
5	Methanol	2ml	2ml
6	Diethyl ether	8ml	8ml
7	Phosphate buffer 7.4	15ml	15ml

2.3. Drug Entrapment efficiency of niosomes

Entrapment efficiency of niosomes was determined by exhaustive dialysis method. The measured quantity of niosomal suspension was taken into a dialysis tube to which osmosis cellulose membrane was securely attached on one side. The dialysis tube was suspended in 100ml phosphate buffer (pH 7.4), which was stirred on a magnetic stirrer. The unentrapped drug was separated from the niosomal suspension into the medium through osmosis cellulose membrane. At every hour entire medium (100ml) was replaced with fresh medium (for about 9-12h) till the absorbance reached a constant reading indicating no drug is available in unentrapped form. The niosomal suspension in the dialysis tube was further lysed with propane-1-ol and estimated the entrapped drug by UV spectrophotometric method at 274nm. The entrapment efficiency was calculated using following equation.

$$\text{Entrapment efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Total amount of drug}} \times 100$$

2.4. Drug content

Niosomes preparation equivalent to 40mg of aceclofenac was taken into a standard vol flask. Then they were lysed with100ml of propane-1-ol by shaking. Then 1ml of this was subsequently diluted with phosphate buffer (pH 7.4). The absorbance was measured at 274nm and calculated drug content from the calibration curve.

2.5. Particle size and shape analysis

Particle size analysis was carried out using an optical microscope with a calibrated eyepiece micrometer. About 200 niosomes were measured individually, average was taken and their size distribution range and mean diameter were calculated. Further microphotographs of optimized niosomes were taken by using 9 megapixel Sony DSC-W110 digital camera. The histogram for particle size distribution and particle size are shown in Figure 1a, 1b and the microphotographs are shown in Fig. 2a, 2b.

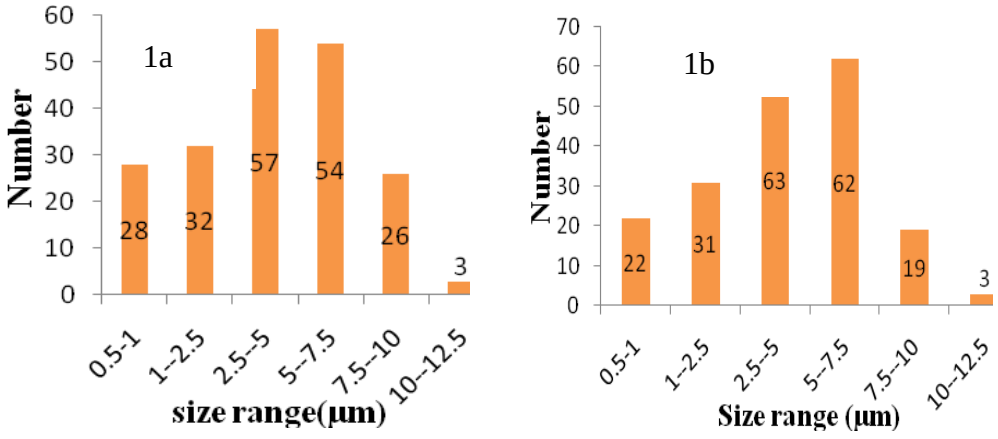

Fig. 1a,1b: Size distributionn of NSF-3 and NSF-6

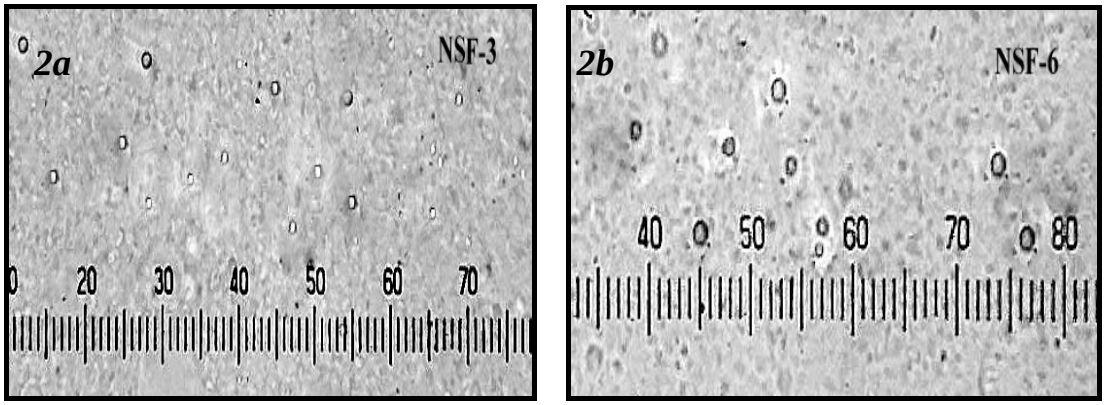

Fig.2a,2b: Microphotographs of of NSF-3 and microphotograph of NSF-6.

Table 3. Evaluation parameters of niosomes.

Sr. No	Formulation	Entrapment efficiency	Mean particle size µm±SD
1	NSF-1	85.2%±0.51	4.83±0.35 µm.
2	NSF-2	87.2%±0.43	4.50±0.49µm.
3	NSF-3	88.4%±0.46	4.47±0.51 µm.
4	NSF-4	94.02%±0.39	4.75±0.46µm
5	NSF-5	95.35%±0.28	4.31±0.71µm.
6	NSF-6	96.07%±0.35	4.22±0.47µm.

(Mean ± S.D., n=3)

2.6 *In vitro* release studies

The release of aceclofenac from niosomal formulations were determined using membrane diffusion technique [6-7]. The niosomal formulation equivalent to 40mg of aceclofenac was placed in a glass tube of diameter 2.5cm with an effective length of 8cm that was previously covered with soaked osmosis cellulose membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 100ml of phosphate buffer (pH 7.4), which acted as receptor compartment. The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touching (1-2mm deep) the surface of diffusion medium. The temperature of receptor medium was maintained at 37 ± 10^0C and agitated at 100rpm speed using magnetic stirrer. Aliquots of 5ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analysed at 274nm in Double beam UV-VIS spectrophotometer using phosphate buffer (pH 7.4) as blank.

3. Results and discussion

Among the various methods, Modified ether injection method is widely used to prepare niosomes. The entrapment efficiency of niosomes prepared at varied concentration of surfactants: aceclofenac, keeping cholesterol concentration constant are shown in Table 3.

Drug content was determined for all niosomal formulations in triplicate. The drug content was found to be uniform with low SD and CV<2.The sizes of the niosomes are measured using an optical microscope with calibrated eyepiece micrometer. From each batch about 200 niosomes were measured for the diameter. The average vesicular size of niosomes of all the batches was measured in the range of 4.22±0.47µm to 4.83±0.35 µm. The result suggested that niosomes

prepared were of uniform size and spherical in shape as shown in microphotographs Figures 1a, 1b and 2a and 2b.

In all the niosomes prepared with spans, as the concentration of surfactant increased drug entrapment efficiency increased. The encapsulation efficiency of niosomes is governed by the ability of formulation to retain drug molecules in the aqueous core or in the bilayer membrane of the vesicles. Cholesterol improves the fluidity of the bilayer membrane and improves the stability of bilayer membrane in the presence of biological fluids such as blood/plasma. Among the spans, span 60 having high phase transition temperature (gel to liquid transformation) and having critical packing parameter (CPP) ranging from 0.5 to 1 entrap drug molecule without any cholesterol. The only drawback of span 60 vesicles was rapid leakage of drug from the vesicle because of high phase transition temperature. A small concentration of about 10-20% cholesterol was optimum to get stable vesicle by abolishing the phase transition temperature resulting in stable niosomes avoiding drug leakage. *In Vitro* drug release of various formulations of niosomes are shown in Figure 3.

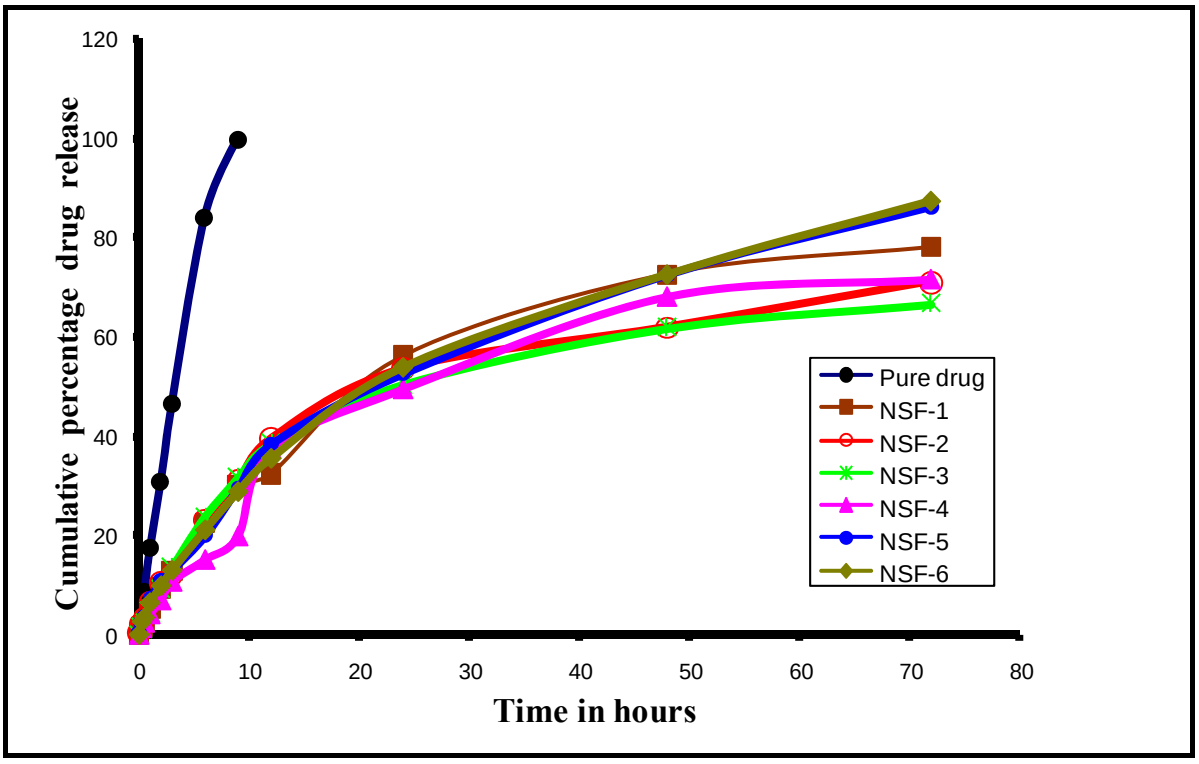

Fig. 3. In vitro dissolution profiles aceclofenac niosomes (NSF1-NSF6), compared with pure aceclofenac.

To ascertain the drug release mechanism and release rate, data of the above formulations were model fitted using PCP Disso V3.0 dissolution software. The models selected were Zero order, Higuchi Matrix, Korsemayer Peppas. The regression coefficient values for all these models are shown in Table 4. In all the cases the best fit model was found to be Peppas with 'n' value between 0.65 to 0.73 suggesting the non fickian (anomalous) release mechanism for the drug i.e., erosion followed by diffusion controlled.

The study of drug release kinetics showed that majority of the formulations governed by Peppas model. The curve was obtained after plotting the cumulative amount of drug released from each formulation against time. Formulation NSF-6 (87.21%) showed maximum release while other formulation showed less amount of drug release in 72h.Formulation NSF-6 has highest correlation coefficient (r =0.9796) value and follows drug release by Peppas model.

Table 4: Data showing in vitro drug release model fitting kinetic parameters of NSF1-NSF6

Formulation code	Cumulative % drug released vs time (zero order)		Cumulative % drug released vs square root of time(Higuchi's)		Log cumulative % drug retained vs time (Kosermeyer's peppas)		
	R	K	R	K	Slope(n)	R	K
NSF-1	0.8548	1.3787	0.9826	10.0491	0.7382	0.9875	5.0062
NSF-2	0.8167	1.2383	0.9822	9.1386	0.6662	0.9856	5.7979
NSF-3	0.8001	1.1893	0.9788	8.8249	0.7208	0.9796	4.8856
NSF-4	0.9546	1.0852	0.9866	7.5596	0.6853	0.9957	4.1239
NSF-5	0.9058	1.4206	0.9935	10.1617	0.6607	0.9946	6.1578
NSF-6	0.9131	1.4299	0.9937	10.196	0.6534	0.9967	6.2592

4. Conclusions

The present study demonstrated the successful preparation of Aceclofenac niosomes and their evaluation. Formulation NSF-6 showed high entrapment efficiency (96.07%±0.35), particle size (4.22±0.47µm) and drug release (87.21%) over 72 h. Hence it was considered to be good niosomal formulation with greater bioavailability.

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