

Research Article

ISSN 2320-4818
JSIR 2014; 3(3): 337-341
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Received: 27-02-2014
Accepted: 28-06-2014

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Formulation and *in-vitro* evaluation of Niosomes of Aceclofenac

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Abstract

The present research work was to formulate and evaluate the site specific delivery of aceclofenac niosomes in order to overcome the problem of GI discomfort and to produce a better therapeutic response. Niosomes of Aceclofenac were formulated by an Ether injection method using different concentrations of drug, cholesterol and surfactant (Span 60). The formulations were evaluated from the various methods like vesicle shape, particle size, entrapment efficiency, drug content, compatibility studies and *in-vitro* drug release. Ether injection method was found to be most satisfactory with respect to niosomes particle size, drug entrapment efficiency, *in-vitro* drug release and its release mechanism was followed by krommeyer Peppas method $R^2=0.9840$.

Keywords: Niosomes, Span 60, Aceclofenac, Cholesterol, Methanol, Diethyl ether.

Introduction

Niosomes or non-ionic surfactant vesicles are microscopic lamellar structures formed on an admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media.^{1,2}

Niosomes are novel vesicular Drug delivery system by which we can achieve the constant plasma drug concentration for the extended period of time.³

Aceclofenac belongs to non-steroidal anti inflammatory drug (NSAID) is considered to be the first-line drug in the symptomatic treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The drug is having a narrow therapeutic index, short biological half-life (about 4 h) as well as two third (70-80%) of the dose is excreted by renal transport and it makes aceclofenac dosing frequency more than once a day. As this dosage form would reduce the dosing frequency. In niosomes, the vesicles forming amphiphile are a nonionic surfactant such as span 60 which is usually stabilized by addition of cholesterol. Cholesterol (CH) is one of the common additives included in the formulation in order to prepare stable niosomes and it is also the essential component in niosomes formulation. Cholesterol was known to abolish the gel to liquid phase transition of niosomes systems, which could be able to effectively prevent leakage of drug from niosomal vesicle.

Since the cholesterol concentration were influencing the drug entrapment efficiency, the optimum quantity of cholesterol was taken in preparing the niosomal formulations.

The present work was to investigate the influence of various preparation techniques on the formulation of aceclofenac niosomes by using span 60 and cholesterol followed by evaluating the parameters such as drug content, entrapment efficiency, particle size, shape, and in-vitro drug release and drug release kinetics.⁴

Materials and Methods

Materials

Aceclofenac was obtained as a gift sample from G Laboratories, Karnal. Span 60 Loba chemie pvt.ltd. Mumbai India, Cholesterol Qualigens Fine Chemicals, Sodium hydroxide RFCL limited, Sodium dihydrogen phosphate Merck Specialties Private limited, Methanol RFCL Limited, Diethyl ether Thermo Fisher Scientific India Pvt.Ltd.

Method

Modified Ether injection Method: Cholesterol and surfactant were dissolved in 8 ml diethyl ether mixed with 2 ml methanol containing weighed quantity of aceclofenac. The resulting solution was slowly injected using a micro syringe at a rate of 1ml/min into 10 ml of hydrating solution phosphate buffer (pH 7.4). The solution was stirred continuously on magnetic stirrer and the temperature was maintained at 60-65°C. As the lipid solution was injected slowly into the 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.^{5,6}

Composition of surfactant and cholesterol for preparation of niosomes

Table 1: Formulation amount for different batches

Code	Span 60	drug	cholesterol
ACE-1	50	110	50
ACE-2	50	110	55
ACE-3	50	110	30
ACE-4	50	110	20

Characterization of niosomes

Vesicle diameter

Vesicle size measured by using optical microscope with a calibrated eyepiece micrometer. The vesicle size of 100 niosomes is measured individually for all batches and its mean value is calculated.^{7,8}

Drug content

Niosomal suspension equivalent to 10mg taken in a volumetric flask of 100 ml and volume was made up by phosphate buffer pH7.4 after that 1ml of this mixture was diluted to 10ml by phosphate buffer pH 7.4 and the percentage drug content was observed at 275nm using uv spectrophotometer.^{9,10}

Percentage drug entrapment (PDE)

The entrapped aceclofenac within niosomes was determined after removing the untrapped drug by dialysis. The dialysis was carried out by taking niosomal dispersion in dialysis bag, which was dipped in a beaker containing 400 ml of PBS with a pH of 7.4 the beaker was placed on a magnetic stirrer run for 4 h with a speed of 80-120 rpm. Then, the solution inside the receptor compartment was studied for untrapped aceclofenac at 275 nm using a UV spectrophotometer. The PDE in the niosomes was calculated from the ratio of the difference of the total amount of drug added and the amount of untrapped drug detected, to the total amount of drug added.^{11,12}

In-vitro Drug release

The releases of aceclofenac from niosomal formulations were determined using membrane diffusion technique. The niosomal formulation equivalent to 75mg of aceclofenac was placed in a Dialysis membrane of diameter 2.5cm with an effective length of 8cm that acts as a donor compartment. The membrane 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 membrane containing a suspension was just touching (1-2mm deep) the surface of diffusion medium. The temperature of the receptor medium was maintained at 37±10°C and agitated at 100rpm speed using magnetic stirrer. Aliquots of 5ml sample were withdrawn periodically and after each withdrawal same volume of the medium was replaced. The collected samples were analysed at 274nm in Double beam

UV-VIS spectrophotometer using phosphate buffer (pH 7.4) as blank.^{13,14}

Stability Studies

Optimized formulation preserved at refrigerated temperature (4-8±1°C) and room temperature (25±2°C) for 30days. After 30days, shape, % drug remaining and % entrapment efficiency of vesicles were measured. The results were compared with the initial shape, %drug remaining and % entrapment efficiency of both samples.¹⁵

Drug release kinetics data analysis

The release data obtained from various formulations were studied further fitness of data in different kinetic models like Zero order, Higuchi's and peppa's. In order to understand the kinetic and mechanism of drug release, the result of in-vitro drug release study of noisome were fitted with various kinetic equation like zero order (equation 1) as cumulative % release vs. time, higuchi's model (equation 2) as cumulative % drug release vs. square root of time. r2 and k values were calculated for the linear curve obtained by regression analysis of the Above plots

$$C = K_0t \dots\dots\dots (1)$$

Where k0 is the zero order constant expressed in units of concentration/time and t is time in hours.

$$Q = KHt^{1/2} \dots\dots\dots (2)$$

Where, KH is higuchi's square root of time kinetic drug release constant. To understand the release mechanism in-vitro data was analyzed by peppa's model (equation 3) as log cumulative % drug release vs. log time and the exponent n was calculated through the slope of the straight line.

$$Mt / M_{\infty} = b t^n \dots\dots\dots (3)$$

Where Mt is amount of drug release at time t, M∞ is the overall amount of the drug, b is constant, and n is the release exponent indicative of the drug release mechanism. If the exponent n= 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have near 1.0 then it is Non-Fickian diffusion.¹⁶⁻¹⁸

Result and Discussion

In vitro release of all batches: Release of Drug from ACE-4 was evaluated through Dialysis membrane to study the

pattern of Drug Release. The mechanism of Drug Release was found to be non Fickian (anomalous) diffusion governed by peppa's model (shown in Fig.1).

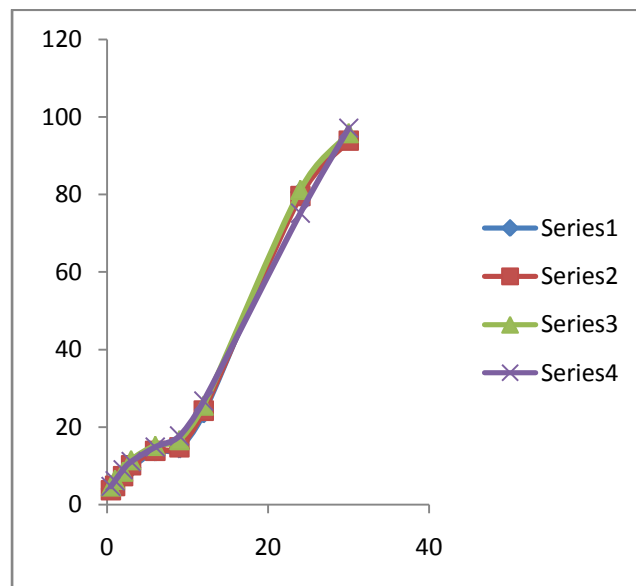


Figure 1: *In vitro* release comparison curve of all formulations

Table 2: Kinetic Release study of various Formulations

Formulation code	Krosmeier-peppas		Diffusion technique
	R ²	n	
ACE-1	0.9773	1.32	non-Fickian
ACE-2	0.9793	1.30	non-Fickian
ACE-3	0.9763	1.247	non-Fickian
ACE-4	0.9840	1.246	non-Fickian

Formulation ACE-4 has a highest Percentage Drug Release (R2 =0.9840) value and follows drug release by Peppas model.

Particle Size Analysis

From each batch about 100 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 shown in Fig. 2

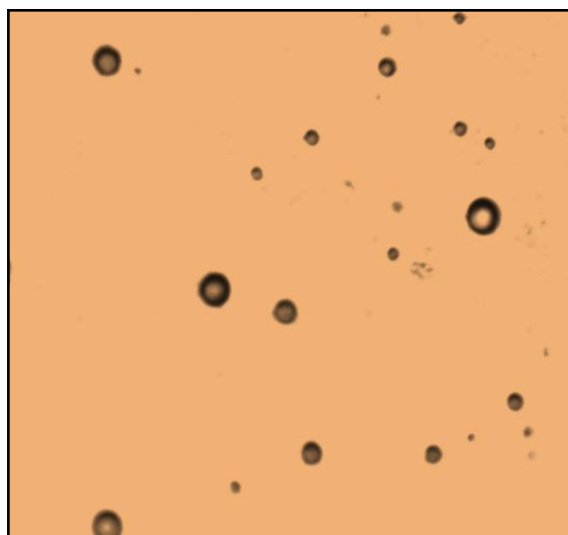


Figure 2.1a: Morphology of Niosomes

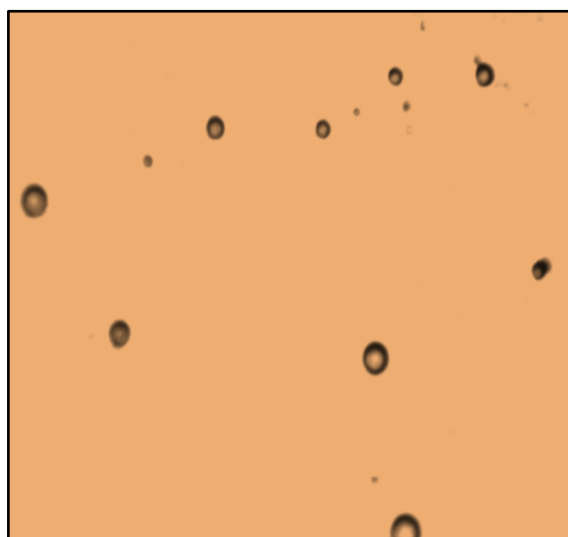


Figure 2.1b: Morphology of Niosomes

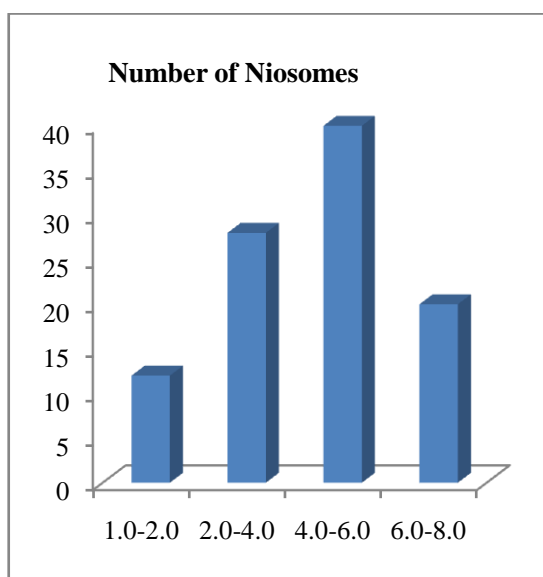


Figure 2.1c: Size distribution of Niosomes

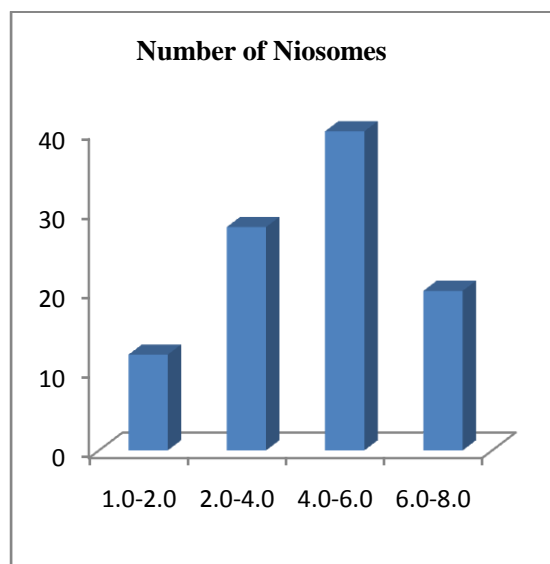


Figure 2.1c: Size distribution of Niosomes

Stability Study

Stability Analysis

Stability of Niosomal dispersion was carried out for 30 days at 2-8°C and Room temperature. Response obtained for different parameters niosomal dispersion during stability period (Shown in Table-3).

Table3: Stability Analysis

Formulation	Refrigerated temperature (2-8°C) Vesicle shape %EE	Room temperature Vesicle shape %EE
Fresh sample	Spherical shape 90.85	Spherical shape 90.85
After 1 month	Spherical shape 89.05	Spherical shape 85.39
Result	Drug remaining 98%	Drug remaining 93%

Characterization of Niosomes

Drug content was determined for all Niosomal formulations in triplicates. Entrapment efficiency was determined for all niosomal formulations in triplicate. In Vitro release of aceclofenac from niosomes was studied using dialysis tube method. Particle size was estimated. after all the characterizations it was found that ACE-4 was better Niosomal formulation than others (Shown in Table-4).

Table 4: Characterization of Formulations by various Evaluation Methods

Code	%EE	Invitro release	Partical size	Drug content
ACE1	83%	93%	4.57um	98%
ACE2	87%	94%	4.36um	89%
ACE3	87%	95%	4.99um	92%
ACE4	90%	97%	4.89um	98%

Conclusion

These indicate that, among the 4 formulations found to sustain the drug release rate for more than 24 h. Release characteristics were determined and found to be krosmeier Peppas method whose correlation value was 0.9840 and 0.9913 for ACE-4

Acknowledgement

I would like to thank G Laboratories, Karnal India for providing the gift sample of Aceclofenac, Loba chemie Pvt.Ltd. Mumbai India for Span 60 and Qualigens Fine Chemicals for cholesterol.

Conflict of Interest

We declare that there is no conflict of interests regarding the publication of this paper.

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