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IN VITRO DRUG RELEASE PROFILE OF ACECLOFENAC NIOSOMES FORMED WITH DIFFERENT RATIO'S OF CHOLESTEROL USING SORBITAN ESTERS

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ABSTRACT

Niosomes are non-ionic surfactant vesicles are microscopic lamellar structures formed on the admixture of non-ionic surfactant of alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. Niosomes were formed using sorbitan esters (span 20, 40, 60 and 80) and cholesterol in different molar ratios. Acelofenac niosomes are formed using ether injection method. The so formed niosomes were evaluated for their *in-vitro* drug release and the best formulation with sustained drug release was formed with span-60. The mechanism of drug release was governed by peppas.

Key words: Aceclofenac, Sorbitan esters, Cholesterol, Ether injection method.

INTRODUCTION

Novel drug delivery system aims at providing some control, whether this is of temporal or spatial nature, or both, of drug release in the body[1-3]. Novel drug delivery attempts to either sustain drug action at a predetermined rate or by maintaining a relatively constant, effective drug level in the body with concomitant minimization of undesirable side effects. 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. Niosomes can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials. It can prolong the circulation of the entrapped drugs[4]. Because of the presence of nonionic surfactant with the lipid, there is better targeting of drugs to tumor, liver and brain. It may prove very useful for targeting the drug for treating cancers, parasitic, viral and other microbial disease more effectively[5-8].

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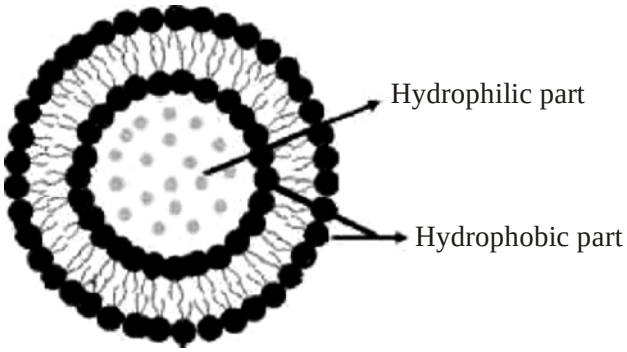

Fig. 1: Structure of niosome

The aim of the present study was to prepare stable niosomes of aceclofenc for analgesic and anti-inflammatory use, which has got advantages over conventional dosage forms. Vesicles were prepared with the help of chemically stable surfactants, ie. Sorbitan esters (span) using cholesterol as a stabilizing agent. The formed Niosomes were characterized for their in-vitro release profile in phosphate buffer. Aceclofenac is a phenyl acetic acid derivative and has been shown to have potent analgesic and antiinflammatory activities, due to its preferential cox-2 blockade, it has better safety than conventional NSAIDs with respect to adverse effects on gastrointestinal and cardiovascular system[9-10].

EXPERIMENTAL

Materials and method

Aceclofenac was a gift sample from Dr. Reddy's Laboratories, Hyderabad. Cholesterol (Moly Chem. Pvt. Ltd., Mumbai), diethyl ether (Fisher Scientific, Mumbai), methanol (Thermo Fischer Scientific, Mumbai) and all the other materials used in this research were of Pharmacopeial grade.

Development of UV spectroscopic method

Stock solution was prepared by dissolving 100 mg of accurately weighed aceclofenac sodium in 100 mL of methanol. 1 mL of this solution was pipetted into 100 mL of volumetric flask and diluted to 100 mL with phosphate buffer 7.4 solution. From this solution of aceclofenac was subsequently diluted with phosphate buffer pH 7.4 to obtain a series of dilutions containing 2, 4, 6, 8 and 10 ug/mL of aceclofenac sodium, respectively. The absorbance of these solutions was measured in Elico-SL 159, UV-Vis Spectrophotometer

at 274 nm using phosphate buffer of pH 7.4 as blank (Table 1, Fig. 2).

Table 1: Calibration curve for the estimation of aceclofenac sodium

Concentration (μg/mL)	Absorbance
0	0
2	0.054
4	0.109
6	0.157
8	0.213
10	0.260

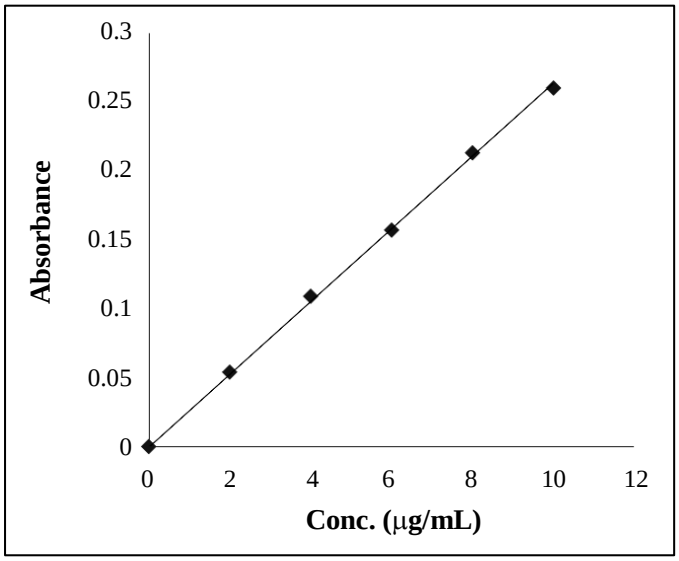

Fig 2: Calibration curve for the estimation of aceclofenac sodium

Niosomes were prepared by modified ether injection method. Niosomes containing aceclofenac sodium were prepared by modified ether injection technique using nonionic surfactants (spans and tweens) and cholesterol at different concentrations. Cholesterol and surfactant were dissolved in 6 mL diethyl ether mix with 2 mL methanol, which previously

containing weighed quantity of aceclofenac sodium. Then, the resulting solution was slowly injected using micro syringe at a rate of 1 mL/min into 15 mL of hydrating solution (phosphate buffer pH 7.4). The solution was stirred continuously on magnetic stirrer and temperature is maintained at 60-65^{o}C. As the lipid solution was injected slowly into aqueous phase, the differences in temperature between phases cause rapid vaporization of ether results in spontaneous vesiculation and formation of niosomes.

Table 2: Formulation code of aceclofenac prepared with sorbitan ester and cholesterol

S. No.	Formulation code	Drug : Surfactant : Cholesterol
1	A_1	1 : 1 : 1
2	A_2	1 : 1 : 2
3	A_3	1 : 1 : 3
4	A_4	1 : 1 : 4

RESULTS AND DISCUSSION

Average particle size determination

Particle size analysis was carried out using an optical microscope (compound microscope) with a calibrated eyepiece micrometer. About 100 niosomes were measured individually, average was taken and their size distribution range, mean diameter were calculated (Table 3, Fig. 3).

Table 3: Particle size, drug content, and entrapment efficiency of different aceclofenac formulations

S. No.	Formulation code	Average particle size (µm)	% of drug content	% of entrapment efficiency
1	A_1	1.4	87.5	73.19
2	A_2	1.52	85.35	77.4
3	A_3	1.6	83.75	80.51
4	A_4	1.72	81.57	86.22

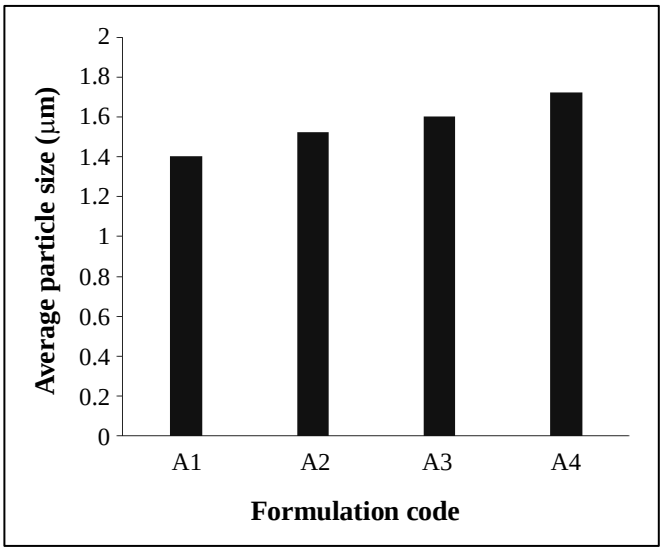

Fig. 3: Particle size distribution of different aceclofenac formulations

FTIR studies

The compatibility between pure drug and surfactants, cholesterol were detected by FTIR spectra obtained on Perkin-Elmer 1600 series USA. FTIR spectrum of aceclofenac showed N-H bond at 3317 cm^{-1}, strong aromatic CH=CH stretching at 3030, 2935 cm^{-1} and

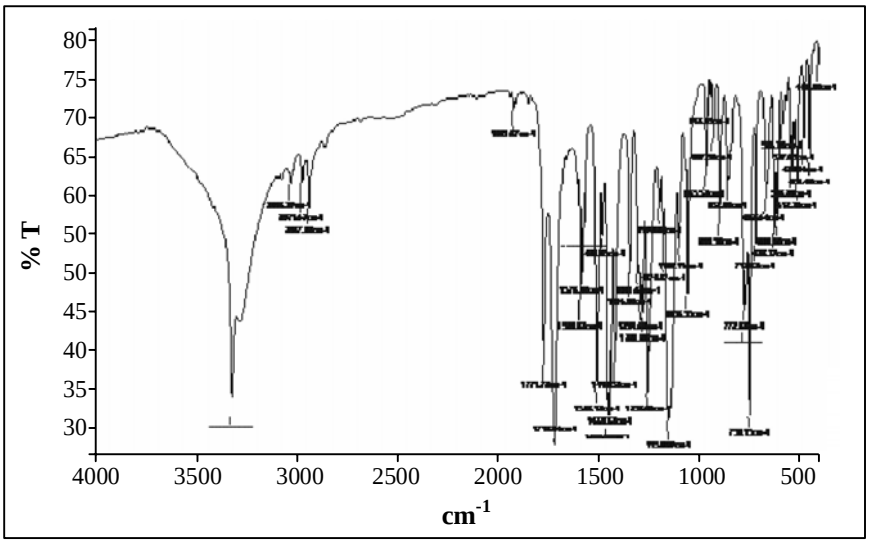

Fig 4: FTIR spectra of aceclofenac sodium

C=O absorption band of carbonyl group at 1720 cm^{-1}. The absorption bands of di, tri-substituted benzene were exhibited at 852, 773 and 750 cm^{-1}. The cholesterol showed the presence of hydroxyl absorption at 3423 cm^{-1}, strong aromatic CH=CH stretching at 2935 cm^{-1} and strong C=O of carboxylic ester is noticed at 1745 cm^{-1}. All the characteristic peaks of drug was shown in the formulation. So, there was no interaction between the drug and polymer (Figs. 4 to 6).

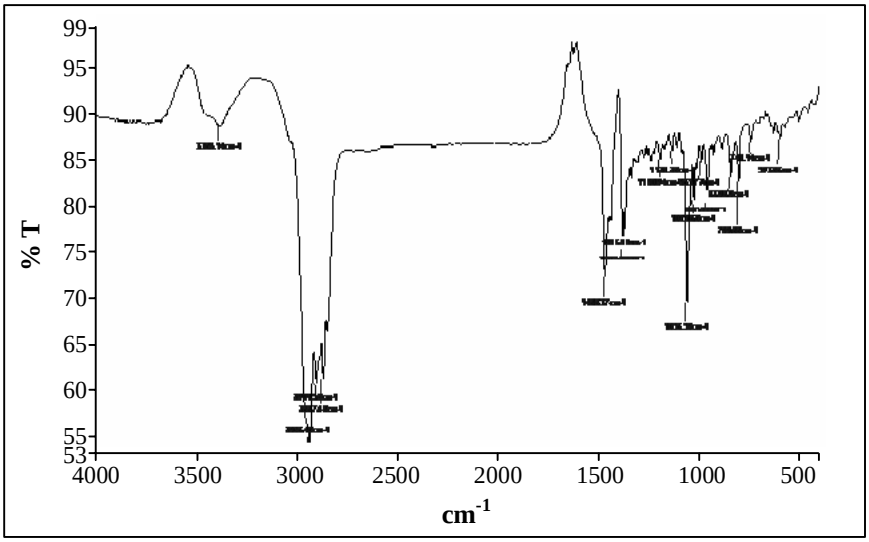

Fig. 5: FTIR Spectra of aceclofenac-cholesterol (1 : 1)

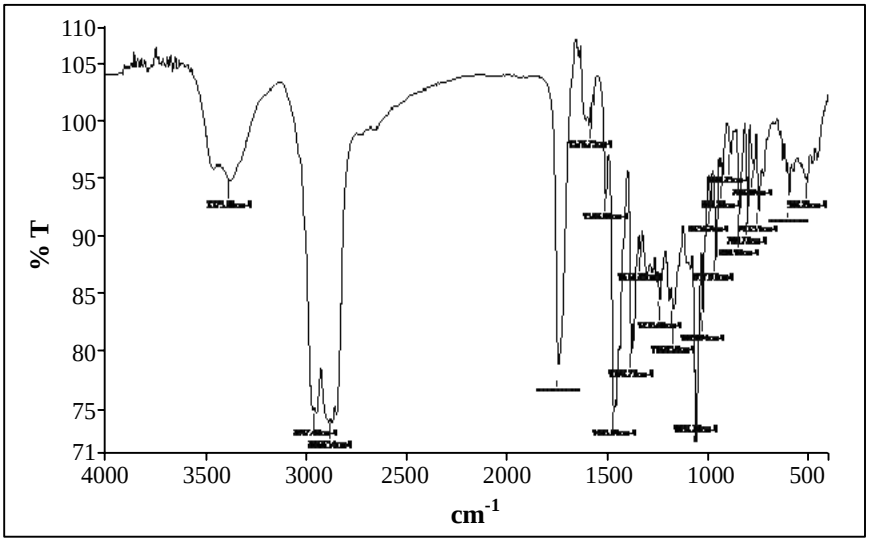

Fig 6: FTIR Spectra of aceclofenac-cholesterol-span 60 (1 : 1 : 4)

Drug content

Niosomes suspension equivalent to 40 mg was taken into a standard volumetric flask were lysed with 100 mL of propane-1-ol by shaking. Then 1 mL of this solution is diluted to 10 mL with phosphate buffer 7.4. The absorbance was measured at 274 nm and calculates drug content from the calibration curve (Fig. 7).

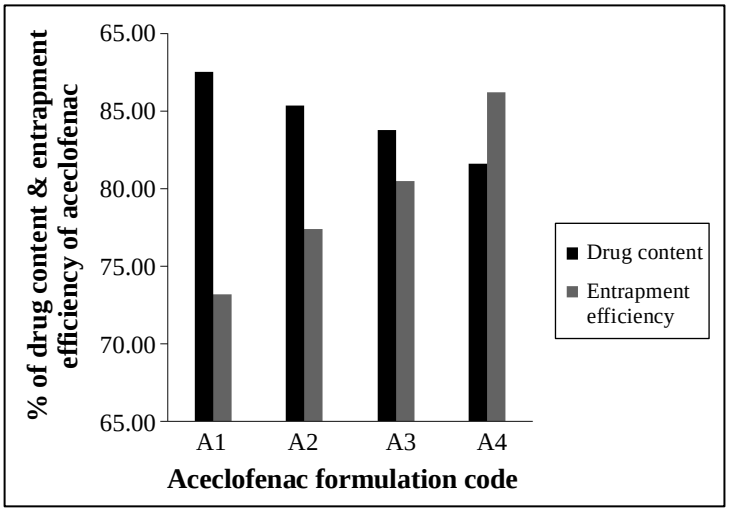

Fig. 7: Drug content of niosomes

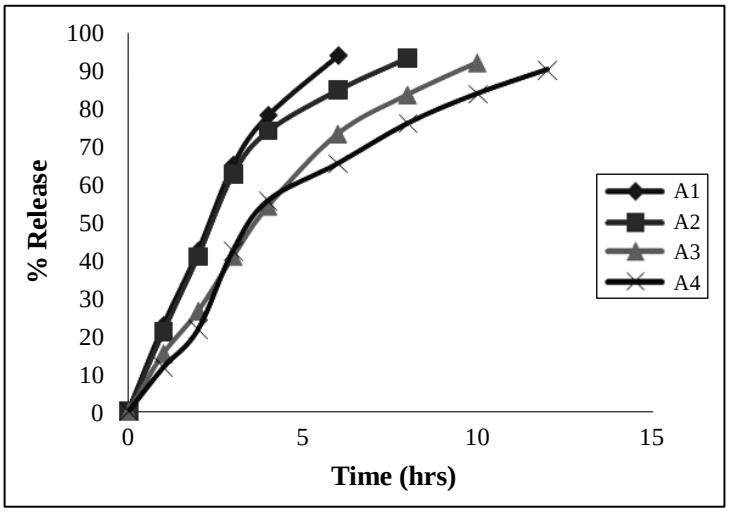

Fig. 8: *In vitro* release studies of different aceclofenac formulations

Entrapment efficiency

Separation of unentrapped drug from the niosomal formulation was done by exhaustive dialysis method. The measured quantity of niosomal suspension taken into a dialysis tube was suspended in 100 mL phosphate buffer pH 7.4, which was stirred on a magnetic stirrer. The unentrapped drug was separated from the niosome suspension into the medium through osmosis cellulose membrane. At every hour whole medium i.e. 100 mL was replaced with fresh medium (for about 9-12 hours) 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 estimates the entrapped drug by UV-spectrophotometric method. Calculate the entrapment efficiency by using following equation.

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

In all the niosomes prepared with spans, as the concentration of surfactant increases drug entrapment efficiency increases. A small concentration of about 10-20% cholesterol was optimum to get stable vesicle by abolishing the phase transition temperature results in stable niosome with avoiding drug leakage. As the concentration of cholesterol increases above the optimum concentration, there is a decrease in entrapment efficiency and the drug released rapidly.

In vitro release studies

The release of aceclofenac sodium from niosomal formulations were estimated by membrane diffusion technique. The niosomal formulation equivalent to 40 mg of aceclofenac sodium was placed in a glass tube having a diameter 2.5 cm with an effective length acts as a donor compartment. The glass tube was placed in a beaker containing 100 mL of phosphate buffer pH 7.4, which acts as receptor compartment.

The whole assembly was fixed in such a way that the lower end of the tube containing suspension was just touched (1-2 mm deep) the surface of diffusion medium. The temperature of receptor medium maintained at 37 ± 10^{o}C and the medium was agitated at 100 rpm speed using magnetic stirrer. Aliquots of 5 mL sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analysed at 274 nm in Elico-SL 159, UV-Vis Spectrophotometer using Phosphate buffer 7.4 as blank. In all the cases it was found that at the completion of 3 hours, 40 to 60 percent of drug was released its mainly due to initial bursting of improper niosomes in

formulations and at the start of 4 hours the release was found to be steady because stable niosomes retain and the release was extended up to 12 hours (Table 4).

Table 4: *In vitro* release studies of different aceclofenac formulations

S. No.	Time (hr)	% of drug release			
		A_1	A_2	A_3	A_4
1	0	0	0	0	0
2	1	22.73	21.06	15.33	11.69
3	2	42.52	41.02	26.61	21.69
4	3	65.20	62.77	41.04	42.56
5	4	78.36	74.20	54.37	55.84
6	6	94.12	85.07	73.53	65.63
7	8	_	93.57	83.88	76.25
8	10	_	_	92.40	84.14
9	12	_	_	_	90.46

Kinetics

To ascertain the drug release mechanism and release rate, data of the above formulations were model fitted using different models like zero order, first order, Higuchi matrix and Korsemayer Pepps. The models selected were Zero order, Higuchi Matrix, Korsemayer Peppas. The regression coefficient values for all these models are shown in Table 7. In all the cases the best fit model was found to be Peppas with 'n' value between 0.60 to 0.79 suggesting the non fickian (anomalous) release mechanism for the drug i.e., erosion followed by diffusion controlled.

Table 5: *In vitro* drug release parameters of different aceclofenac formulations

Formulation code	Zero order	First order	Higuchi	Korsemayer Peppas	
	R^2	R^2	R^2	R^2	N
A1	0.966	0.694	0.913	0.608	0.791
A2	0.943	0.728	0.875	0.785	0.601
A3	0.971	0.694	0.929	0.789	0.608
A4	0.988	0.673	0.942	0.803	0.642

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 A4 (94.26%) showed maximum release while other formulation showed immediate release of drug in 12 h. Formulation A4 has highest correlation coefficient (r = 0.803) value and follows drug release by Peppas model (Table 5).

CONCLUSION

For all the niosomal formulations the release was extended for 12 hours and better dissolution profiles were found with niosomes prepared with 1 : 1 : 4 ratio of span 60.

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