



## EVALUATION OF ACECLOFENAC NIOSOMES PREPARED BY VARIOUS TECHNIQUES

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### ABSTRACT

The current investigation were aimed to develop sustain release formulation of aceclofenac niosomes in order to minimize gastrointestinal disturbances and to provide better therapeutic effect. Aceclofenac niosomes was prepared by different techniques namely Ether injection method (F<sub>1</sub>), Ethanol injection method (F<sub>2</sub>), Sonication method (F<sub>3</sub>), Thin film hydration (F<sub>4</sub>) and Reverse phase evaporation technique (F<sub>5</sub>) using 1:1:1 ratio of drug, cholesterol and surfactant (Span 60). The formulations were optimized from the above methods with respect to vesicle shape, particle size, entrapment efficiency, drug content, compatibility studies and *in-vitro* drug release. The well spherical shaped niosomes were obtained in all the methods. FT-IR Spectra shows the drug and excipients were compatible. The *in-vitro* release studies indicates that all the formulations exhibits retarded release for 24hrs but the thin film hydration method and reverse phase evaporation method was found to be most satisfactory with respective to niosomes particle size, drug entrapment efficiency, *in-vitro* drug release and its release mechanism was followed by zero order kinetics  $r^2=0.9948$  and  $0.9913$ .

**Keywords:** Niosomes, span 60, Aceclofenac, Cholesterol and Chloroform.

### INTRODUCTION

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<sup>1</sup>. The drug is having narrow therapeutic index, short biological half-life (about 4 h) as well as two third (70-80%) of dose is excreted by renal transport and it makes aceclofenac dosing frequency more than once a day. Niosomes are non-ionic surfactant vesicles, with or without incorporation of cholesterol or other lipids with subsequent hydration in aqueous media. This vesicular system are similar to liposomes which are acting as carriers for both hydrophilic and lipophilic drugs, made up of either aqueous layer or vesicular membrane made of lipid materials<sup>2</sup>. They are less toxic and improve the therapeutic index of drug by restricting its action to target cells. As this dosage form would reduce the dosing frequency, hence better patient compliance can be achieved. In niosomes, the vesicles forming amphiphile is 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 cholesterol concentration were influences the drug entrapment efficiency, the optimum quantity of cholesterol were taken for preparing the niosomal formulations. The present work was to investigate the influence of various preparation techniques on 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.

### MATERIALS AND METHODS

Aceclofenac was obtained as a gift sample from Emcure Pharmaceuticals Pvt.Ltd, Pune. Span 60, Cholesterol, Chloroform, Ethanol, Sodium hydroxide, Potassium dihydrogen phosphate was obtained from Loba Chemie Pvt. Ltd., Mumbai.

#### Methods of preparation

Aceclofenac niosomes were prepared by the following methods using 1:1:1 ratio of drug:cholesterol:surfactant.

#### Modified ether injection method

In this method cholesterol (100 mg) and span 60 (surfactant 100 mg) at 1:1 ratio were dissolved in 10 ml of chloroform and was injected slowly at a rate of 0.25 ml/min through 16 gauges needle into 15 ml of hydrating solution phosphate buffer pH 7.4 containing aceclofenac (100 mg) as the aqueous phase and the solution was stirred on magnetic stirrer by maintaining the temperature at 60°C<sup>3</sup>.

#### Ethanol injection method

The ethanol injection method was similar to modified ether injection method where instead of chloroform, ethanol was used as an organic solution for dissolving the cholesterol and span 60. It has been reported that small unilamellar vesicles can be prepared by this method<sup>4</sup>.

#### Sonication method

The aqueous phase phosphate buffer solution pH 7.4 containing aceclofenac (100 mg) was added to the chloroform containing mixture of span 60 and cholesterol



at 1:1 ratio in a scintillation vial and was subjected to bath sonication, maintained the temperature at 60°C for 3 minutes to produce small and uniform size niosomes<sup>5</sup>.

#### Thin film hydration method

In this method cholesterol (100 mg) and span 60 (100 mg) at 1:1 ratio were dissolved in chloroform, and the solvent was evaporated at room temperature, using rotary vacuum evaporator (Buchi). The thin layer of cholesterol and surfactant mixture was formed on the round bottom flask. The aqueous phase phosphate buffer solution pH 7.4 (15 ml) containing aceclofenac was added to the round bottom flask at 70°C and shaken for about 15 min which results in good dispersion of the mixture and the niosomes were formed<sup>6</sup>.

#### Reverse phase evaporation method<sup>7</sup>

In this method cholesterol and span 60 at 1:1 ratio were dissolved in 10ml of chloroform. An aqueous phase phosphate buffer solution pH 7.4 (5 ml) containing drug was added to the above organic solution and the resulting two phases were sonicated at 50°C for 5 min. The emulsion was formed, from which the organic solvent was removed slowly at 40°C under low pressure using a rotary vacuum evaporator until the thin film was formed around inside the flask wall. The resulting film was hydrated with 10ml of phosphate buffer solution pH 7.4 to produce niosomal suspension and kept on water bath at 60°C for 10 min to yield niosomes.

#### Characterization of niosomes

##### Vesicle diameter

It was determined by using an optical microscope with a calibrated eyepiece micrometer. About for 100 niosomes the vesicle diameters were measured individually for all the formulations. Its average value was determined, their size range and mean diameter were calculated.

##### Entrapment efficiency

In this method the measured quantity of niosomal suspension (sample) was taken into a glass tube where the cellulose membrane was securely tied on one side. The glass tube containing sample was suspended in 100ml phosphate buffer (pH 7.4) medium, which was stirred slowly on a magnetic stirrer<sup>6</sup>. The untrapped aceclofenac was diffused from the niosomal suspension through the cellulose membrane. At every hour entire medium (100 ml) was replaced with fresh medium (for about 4-5 h) till the absorbance value was reaching a constant range indicating no drug were available in untrapped form. The percentage entrapment efficiency was calculated by using following equation. The drug concentration present in the medium was determined by measuring the absorbance in UV spectrophotometer (Techcomp UV 2310) at 274 nm.

**Entrapment efficiency** = (Amount of entrapped drug / Total amount of drug) X 100

#### Drug content

Niosomes preparation equivalent to 40 mg of aceclofenac was taken into a volumetric flask and were lysed with 100 ml of propan-1-ol by continuous shaking. From this solution 1 ml was withdrawn and it was subsequently diluted with phosphate buffer (pH 7.4). The sample absorbance was measured in spectrophotometer (Techcomp UV 2310) at 274nm and the drug content was calculated from the calibration curve.

#### Fourier – Transform Infrared Spectroscopy (FT – IR)

The drug and excipient interactions were investigated by FTIR spectroscopy. Cholesterol, span60, aceclofenac and physical mixture, were taken for the study by compressing into a pellets using KBr. The prepared pellets were kept in a sample holder and scanned from 4000 cm<sup>-1</sup> to 400 cm<sup>-1</sup> in FTIR (Spectrometer model 2500) Spectrometer.

#### In-Vitro Drug Release

The release of aceclofenac from niosomal formulations were determined by using membrane diffusion technique. The niosomal formulation equivalent to 40 mg of aceclofenac was placed in a glass tube of diameter 2.5 cm with an effective length of 8 cm which was tied with previously soaked cellulose membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 100 ml of phosphate buffer (pH 7.4), acting as a receptor compartment. The whole assembly was fixed in such a way that the lower end of tube containing suspension was just touching (1-2 mm depth) the surface of diffusion medium. The temperature of receptor medium was maintained at 37 ± 5°C and was agitated at the speed of 100 rpm using magnetic stirrer. Aliquots of 5ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed at 275 nm in double beam UV-VIS spectrophotometer using phosphate buffer (pH 7.4) as blank.

#### Drug release kinetic data analysis

Release mechanism of aceclofenac from the prepared niosomal formulations was studied. This data was then treated to study the best linear fit for the following equations

Percent release =  $Kt$  ... zero order

Percent release =  $Kt^{0.5}$  ... Matrix (Higuchi matrix)

Percent unreleased =  $Kt$  ... Hixson-Crowell.

Amount of drug release at time 't' =  $Ktn$  ...Peppas-Korsmeyer

Amount of drug release at time t

Percent 'r' is the percentage of drug release at time 's', 'k' is release rate constant and 'n' is the diffusion coefficient, which is indicative of the transport mechanism and can be used to characterize different release mechanism.



**RESULTS AND DISCUSSION**

In the present study, non-ionic surfactant span 60 along with cholesterol was used in equal ratios for the development of aceclofenac niosomes. Aceclofenac is a non-steroidal anti-inflammatory drug used in osteoarthritis, rheumatoid arthritis and spondylitis. Non-ionic surfactants, a cheap, non-toxic biodegradable material has recently gained wide popularity for development of better drug delivery systems.

Aceclofenac loaded niosomes were prepared with the aid of various techniques which include the ratio of drug: cholesterol: surfactant in 1:1 ratio.

The well spherical shaped niosomes were obtained in all the methods. The size of niosomal vesicles were depends on the method of preparation. It was observed that span 60 (HLB 4.7) were produced niosomes with larger mean vesicle diameter 10.2 (µm) in Modified ether injection method (F<sub>1</sub>) as compared to Thin film hydration method (F<sub>4</sub>) where the mean vesicular diameter was found to be 3.30 (µm). From the microphotographs of formulation it was evident that the niosomes were discrete and round in shape.

FT-IR Spectrum of aceclofenac (fig.1) and physical mixture of drug, cholesterol and span 60 (fig.2) were recorded. The FT-IR spectrum of pure aceclofenac shows absorption at 743.47 cm<sup>-1</sup> (C-H bending), 1253.88 cm<sup>-1</sup> and 1346.48 cm<sup>-1</sup> (C-N stretching), 1450.06 cm<sup>-1</sup> (O-H bending) and 1716.85 cm<sup>-1</sup> (C=O stretching). These peaks were also found in physical mixture, indicating the compatibility of drug among with ingredients.

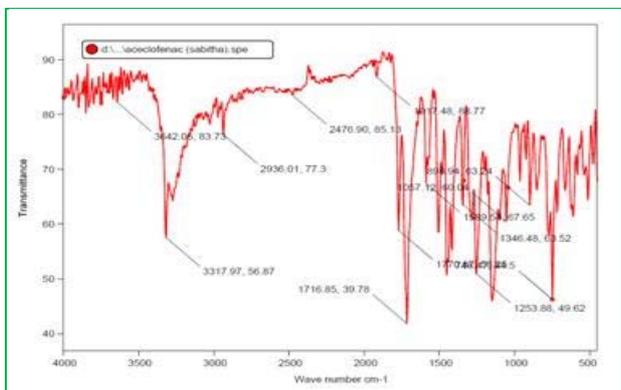


Figure 1: FTIR Spectrum of Aceclofenac

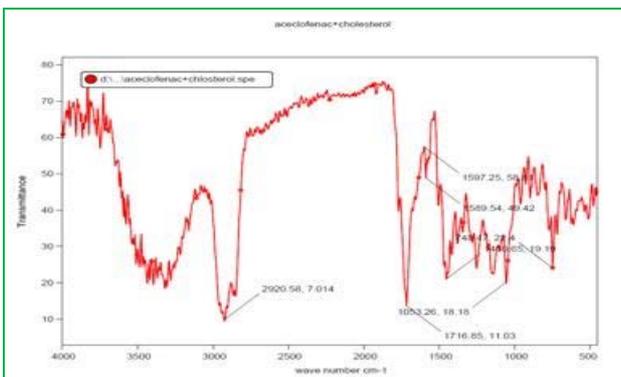


Figure 2: FTIR Spectrum of physical mixture (Aceclofenac, Cholesterol, Span 60)

The drug encapsulation in niosomal formulations were listed in table 1. It shows that entrapment efficiency was good in reverse phase evaporation method (F<sub>5</sub> formulation) and thin film hydration method (F<sub>4</sub> formulation) and was found to be 92 and 90 % whereas it was 87, 69 and 71 % for F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> formulations respectively, all these formulation were subjected to drug content estimation.

The drug content in prepared niosomal formulations were listed (Table-1), shows that drug content was in the range between 78 % to 95 %. Among the formulations made by different method thin film hydration techniques were produced maximum drug content of 95 % (F<sub>4</sub>) followed by ether injection method 88 % (F<sub>1</sub>) and reverse phase evaporation method 86 % (F<sub>5</sub>).

The drug release studies were conducted for all the five formulations and were found to have a linear release (fig.3) and provide approximately 70 to 80 % releases within a period of 24 h. As compared to other batches the drug release was found to be well slower for F<sub>4</sub> and F<sub>5</sub> (70% and 71%) where it was prepared by thin film hydration method and reverse phase evaporation method. With respect to vesicle diameter and percentage entrapment efficiency both the above method was almost showed similar results. The formulation prepared by sonication method exhibits drug release of 86 % at 24 h where the method might produce small, unilammellar vesicles by which the drug can be released and sustained to the moderate range as compared to other methods (fig.4).

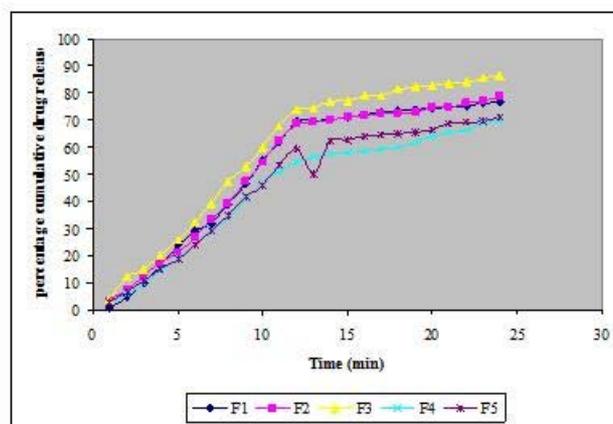


Figure 3: In-vitro drug release of aceclofenac niosomes (F<sub>1</sub>-F<sub>5</sub>)

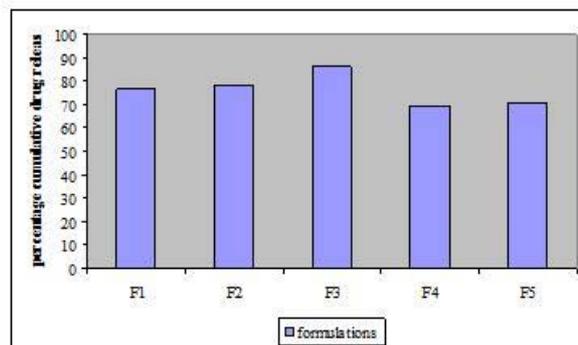


Figure 4: Comparative in-vitro drug release of aceclofenac niosomes (F<sub>1</sub>-F<sub>5</sub>) at 24 hrs.

The  $T_{50}$  % values were calculated for all the formulation, by which it may be possible to predict efficiency of the technique. Maximum time of 11hrs was taken by the  $F_4$  formulation to release 50 % of drug followed by 10 h 30 min for  $F_5$  whereas  $F_1$ ,  $F_2$  and  $F_3$  it takes 8 to 9 h to release 50 % of drug.

## CONCLUSION

These indicates that, among the 5 techniques thin film hydration and reverse phase evaporation techniques were found to sustain the drug release rate for more than 24 h. Release characteristics were determined and found to be zero order whose correlation value was 0.9948 and 0.9913 for  $F_4$  and  $F_5$  formulations.

**Table 1:** Characterization of niosomes

Formulation code	Method Used	Vesicle Diameter ( $\mu\text{m}$ )	Entrapment Efficiency (%)	Drug content (%)	% Cumulative drug release
$F_1$	Ether injection method	10.2	86.89	88.33	76.51
$F_2$	Ethanol injection method	3.57	68.53	78.43	78.73
$F_3$	Sonication method	3.02	71.33	82.44	86.30
$F_4$	Thin film hydration technique	3.30	89.52	94.66	69.52
$F_5$	Reverse phase evaporation method	3.17	91.70	86.40	70.98

Thus niosomes were prepared in 1:1:1 ratio of drug, cholesterol and surfactant. The effective method was identified in developing a novel drug delivery system of aceclofenac niosome which have significant impact towards sustaining the drug for more than 24hrs where its therapeutic indications can be improved for various clinical conditions. Further the formulations were evaluated for stability studies in order to develop the stable product.

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