

FORMULATION AND *IN VITRO* EVALUATION OF NIOSOMES CONTAINING OXCARBAZEPINESAKTHIVEL M*¹, KANNAN K², MANAVALAN R², SENTHAMARAI R¹¹Periyar College of Pharmaceutical Sciences, Tiruchirappalli, Tamilnadu. ²Annamalai University, Annamalai Nagar, Chidambaram, Tamilnadu. Email: sakthi00767@yahoo.com

Received: 03 Apr 2012, Revised and Accepted: 07 Jun 2012

ABSTRACT

Niosomes are the novel vesicular drug delivery system by which we can achieve the constant plasma drug concentration for the extended period of time. They are nonionic surfactant vesicles that have potential applications in the delivery of hydrophobic and hydrophilic drugs. Oxcarbazepine is one of the most effective drugs used in the treatment of Epilepsy. The objective of the present study is to treat epilepsy with Oxcarbazepine niosomes. Oxcarbazepine niosomes were prepared by thin film hydration method using span60 in order to achieve prolonged circulation time and sustained release. The prepared niosomes were evaluated for size, shape, degree of drug entrapment, drug content and stability studies. *In vitro* drug release studies were performed and drug release kinetics was evaluated using linear regression method. From this study it was observed that the formulation F-II showed satisfactory particle size 230-275nm, entrapment efficiency 58.87% and *in vitro* release 78.08% for the period of 16 hours. Thus the niosomal formulation could be a promising delivery system for Oxcarbazepine with improved anticonvulsant activity, stability and sustained drug release profile.

Keywords: Oxcarbazepine, Span 60, *In vitro* studies, Niosomes.

INTRODUCTION

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle¹. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes². In niosomes, the vesicles forming amphiphile is a non-ionic surfactant such as span 60 which is usually stabilized by addition of cholesterol which gives the rigidity to the bilayer and results in less leaky niosomes³ and small amount of anionic surfactant such as dicetyl phosphate⁴. Niosomes can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials⁵. Niosomes behave *in vivo* like liposome prolonging the circulation of entrapped drug and altering its organ distribution. Niosomes also exhibit special characteristics such as easy handling and storage. Surfactant forming niosomes are biodegradable, non-immunogenic and biocompatible⁶.

Oxcarbazepine is one of the most effective drugs in the treatment of epilepsy⁷. The objective of this study is to treat epilepsy with Oxcarbazepine niosomes, since it is an ideal second generation Anti-Epileptic Drug (AED) that eliminates seizures without any adverse effects⁸. It has a short biological half-life of 1-5 hours and requires frequent administration for a prolonged period of time. Generally Oxcarbazepine tablet is available in the market as 150-600mg tablets which are administered for twice a day. In severe conditions, dose can be increased upto 1200 mg. Niosomes were prepared by thin film hydration technique⁹. The present study involves the preparation and characterization of Oxcarbazepine entrapped niosomes and evaluated for improved drug carrier qualities of the niosomes.

MATERIALS AND METHODS

Materials

Oxcarbazepine was obtained as a gift sample from Micro Labs, Hosur. Cholesterol and Spans were purchased from Qualigens fine chemicals Ltd, Mumbai. All other materials used in the study were of Analytical Grade.

Formulation of oxcarbazepine niosomes

Oxcarbazepine niosomes were prepared by Thin Film Hydration Technique using Rotary flash Evaporator⁵. Weighed quantity of cholesterol and surfactant were dissolved in chloroform and methanol mixture (1:1 v/v) taken in a round bottom flask. The flask was rotated in rotary flash evaporator at 100 rpm for 20 minutes in a thermostatically controlled water bath at 60°C± 2°C. The flask was rotated at 1.5 cm above the water bath under reduced pressure (10-15mm mercury) until all the organic phase evaporated and a slimy layer was deposited on the wall of a round bottom flask^{9,10}. To the thin dry lipid formed, Oxcarbazepine solution was added previously dissolved in 10ml of phosphate buffer saline pH 7.4 and the flask was rotated again at the same speed and temperature as before but without vacuum for 30minutes for lipid film removal and dispersion^{11,12}. The niosomal suspension so formed was then transferred to a suitable glass container and sonicated for 30minutes using bath sonicator in an ice bath for heat dissipation^{13,14}. The sonicated dispersion was then allowed to stand for about 2 hours at room temperature to form niosomes. The formulation was sterilized by passing into 0.2µm membrane filter. Each batch was prepared three times and stored in refrigerator.

Table 1: Formulation of oxcarbazepine niosomes

S. No.	Ingredients	Quantity Used in mg		
		F - I	F- II	F- III
1	Oxcarbazepine	10	10	10
2	Cholesterol	10	10	10
3	Span 40	30	-	-
4	Span 60	-	30	-
5	Span 80	-	-	30
6	Chloroform (ml)	10	10	10
7	Methanol (ml)	10	10	10
8	Phosphate Buffer Saline pH 7.4 (ml)	10	10	10

Optical Microscopy

The microscopic methods used are Bright field, phase contrast microscope useful in evaluating the vesicle size and the size distribution of vesicles. Vesicular dispersion appropriately diluted is

wet mounted on a haemocytometer and photographed with a phase contrast microscope. The negatives are projected on a piece of calibrated paper using a photographic enlarger at X 1250¹⁵. The best formulation is shown in the Figure no: 1

Determination of particle size and shape

The particle size and shape of niosomes were viewed and photographed using Scanning Electron Microscope. Oxcarbazepine niosomes were transferred to a cover glass. Then it was mounted on a specimen stub. Dried samples were coated with platinum to a thickness of 100Å using Hitachi vacuum evaporator. Coated samples were viewed and photographed in HITACHI S- 3000 H, SEM operated at 20 Kv¹⁶. The best formulation is shown in the Figure no: 2.

Removal of unentrapped drug from Niosome

The unentrapped drug Oxcarbazepine from niosome was removed by dialysis method. Niosome suspension was placed in 3cm x 8cm long dialysis bag whose molecular weight cut off was 12,000. The dialysis bag was then placed in 250 ml beaker containing phosphate buffer saline of pH 7.4 with constant stirring by means of a magnetic stirrer. Dialysis was carried out for 24 hour by replacing the buffer with fresh for every 3 hours⁵.

Percentage drug entrapment

Niosomes vesicles containing Oxcarbazepine were separated from unencapsulated drug by dialysis. Niosomal preparation of 0.5 ml was taken after dialysis. To this 0.5 ml of 10% triton X - 100 was added and incubated for 1 hour^{17,18}. The triton X-100 was added to lyse the vesicles in order to release the encapsulated Oxcarbazepine¹⁹. Then it was diluted with phosphate buffer saline solution (pH 7.4) and filtered through whatmann filter paper. The filtrate was measured spectrophotometrically at 255 nm using phosphate buffer 7.4 and triton X - 100 mixture as blank. From the absorbance value, the concentration of drug in mcg/ml was found using the standard curve

$$\text{Entrapment Percentage} = \frac{\text{Entrapped Drug}}{\text{Total Drug added}} \times 100$$

Drug content analysis

1ml of niosome preparation was taken in a 100ml volumetric flask. 2ml of acetone was mixed and volume was made up with phosphate buffer pH7.4. Samples were filtered through whatmann filter paper number 40 and diluted with PBS pH7.4. Drug content was determined spectrophotometrically at 255nm²⁰.

Determination of particle size analysis (size distribution)

Niosomes were subjected to analysis by Particle Size Analyzer (Microtrac - Bluewave, USA)¹⁶. The best formulation is shown in the Figure no: 3

In vitro release studies

The *in vitro* release of niosomes was studied by using simple diffusion cell apparatus^{21,22}. The diffusion cell apparatus consists of a glass tube with an inner diameter of 2.5cm, open at both ends, one end of the tube is tied with Sigma dialysis membrane, which serves as a donor compartment^{23,24}.

Niosomes equivalent to 5mg of Oxcarbazepine was taken in a dialysis tube and placed in 200ml of phosphate buffer pH7.4. The medium was stirred by using the magnetic stirrer and the temperature was maintained at 37±2°C. Periodically 5 ml of samples were withdrawn and after each withdrawal same volume of medium was replaced. Then the samples were assayed spectrophotometrically at 255nm using phosphate buffer pH7.4 as blank. The releases of all formulation were compared with pure Oxcarbazepine solution. The invitro drug release for formulation FI, FII, FIII, in shown in the Figure no: 4

Statistical Analysis

All experiments were repeated thrice, the average values were taken and standard deviation was calculated.

Release kinetics

To investigate the possible mechanism of Oxcarbazepine release from the prepared niosomes, the release data were analyzed mathematically according to the following models:

Zero order- $Q = K_0 t^{25}$.

First order- $\text{Log } Q = \text{Log } Q_0 - K_1 t / 2.303^{26}$.

Higuchi- $Q_t = K_H t^{1/2}^{26}$.

Korsmeyer - Peppas- $Q_t/Q_\infty = K t^{n27}$.

Hixson- Crowell- $Q_0^{1/3} - Q_t^{1/3} = K_{HC} t^{28}$.

Where, Q is the amount of drug release at a time (t) and K is the rate constant.

Stability studies

The stability studies for best Oxcarbazepine niosomes formulation was carried out as per ICH guides for 3 months^{29,30}. Formulated niosomes were divided into 3 groups. One group was kept at refrigeration (4±2°C). The second group was kept at room temperature (25±2°C). The third group was kept at 40±2°C and 60±5% RH. Every month 1 ml of formulation was withdrawn and analyzed for drug content. The drug release studies for formulation F-II was also performed before and after 3 months at various temperatures. The best formulation is shown in the Figure no: 5 and 6.

RESULTS AND DISCUSSION

Optical microscopy

Microscopic method used one Bright field phase contrast microscope useful in evaluating vesicle size and size distribution. From this study, it was found that numerous spherical vesicles are formed in F-II (formulation containing Span 60), compared to other two formulations.

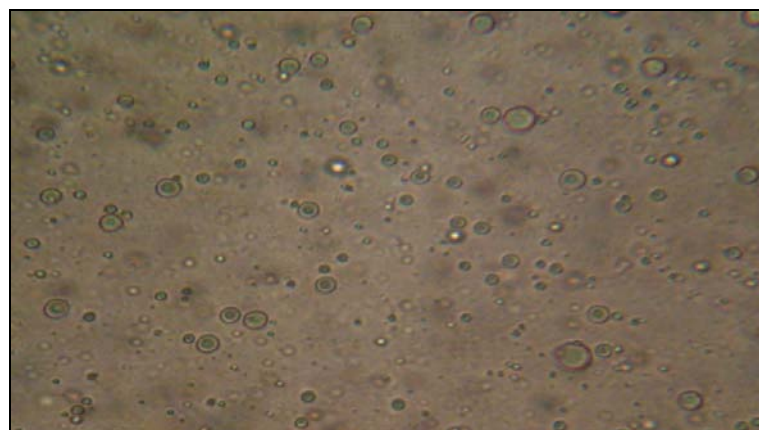


Fig. 1: Optical Microscopy of oxcarbazepine niosomes of F-II formulation

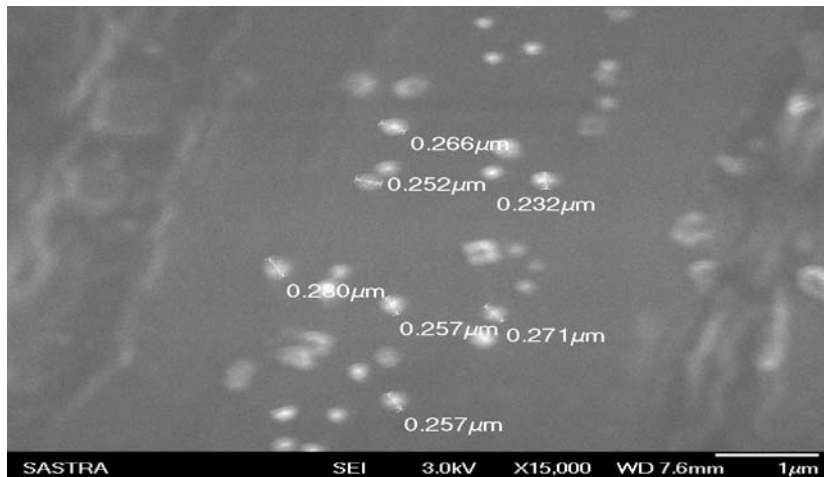


Fig. 2: SEM Photo of oxcarbazepine niosomes of F-II formulation

Particle size distribution of niosomes

The particle size distribution of Oxcarbazepine niosomes were viewed and photographed using HITACHI-S-3000 scanning electron microscope operated at 20 Kv. From this study it was found that in shape and size ranged from 230 to 275nm for formulation (F-II). The particle size of other formulations differs due to variation in the composition of the formulation.

Removal of Untrapped Drug from Niosomes

As the amount of surfactant increased, the amount of dialyzed Oxcarbazepine was also increasing 1:1 ratio indicates that concentration of surfactant used should be optimum so that more amount of drug can be in the encapsulated form for an extended release. Among all the formulations, the dialyzed quantity of formulation F-II (Cholesterol: Span60:: 1:1) was maximum. The result indicated more amount of Oxcarbazepine in an encapsulated form.

Determination of percentage drug entrapment

After the removal of untrapped drug by dialysis, the entrapment efficiency of all the formulations was studied. The various factors like lipid concentration, drug to lipid ratio and cholesterol content may change the entrapment efficiency. From this study, it was found that the entrapment efficiency of drug in F-II formulation containing Span 60 was found that 58.87% which showed maximum percentage drug entrapment. Hence, the niosome formulated with span 60 were found to be optimum for loading maximum amount of Oxcarbazepine in niosomal formulation.

Determination of particle size analysis

Niosomes were subjected to particle size analyzer for characterizing size distribution of niosomes from this study it was found that the average particle size was 270nm for F-II formulation.

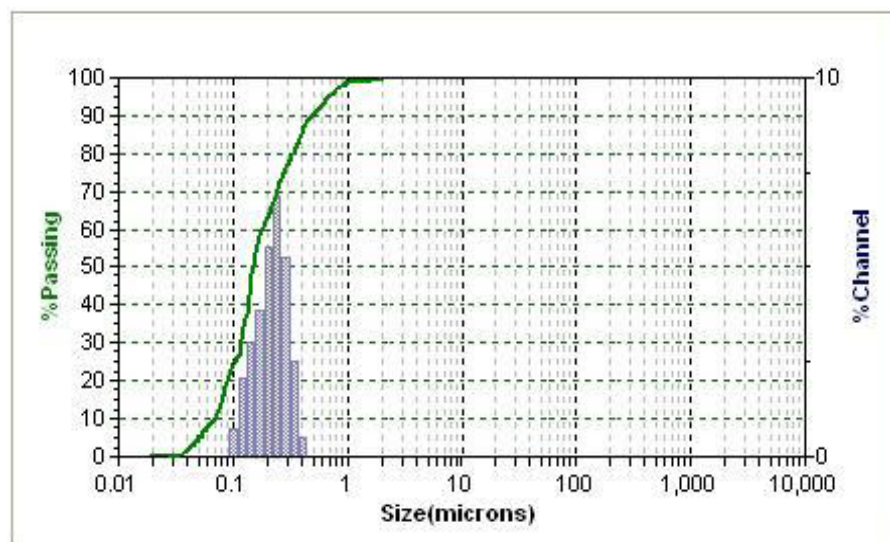


Fig. 3: Particle size distribution of Oxcarbazepine niosomes of F-II formulation

In vitro drug release

In vitro release study was carried out by diffusion method using Sigma dialysis membrane. From this study percentage of drug diffused into the medium was evaluated.

The percentage amount of free drug released was $99.04 \pm 0.86\%$ within 2.5 hours. F-I showed $70.83 \pm 0.81\%$ of drug release within 10 hours. F-II showed $78.04 \pm 0.32\%$ of drug release within 16 hours

and F-III gave $71.25 \pm 0.67\%$ of drug release within 12 hours. These results showed that niosomal Oxcarbazepine has sustained release up to 16 hours whereas free Oxcarbazepine was released within 2.5 hours. This is because the drug is released slowly for a prolonged period of time in niosomal Oxcarbazepine. Also, F-II containing span 60 showed higher releases when compared to F-I containing span 40 and F-III containing span 80. Therefore, formulation F-II is selected for further studies like release kinetics and stability studies.

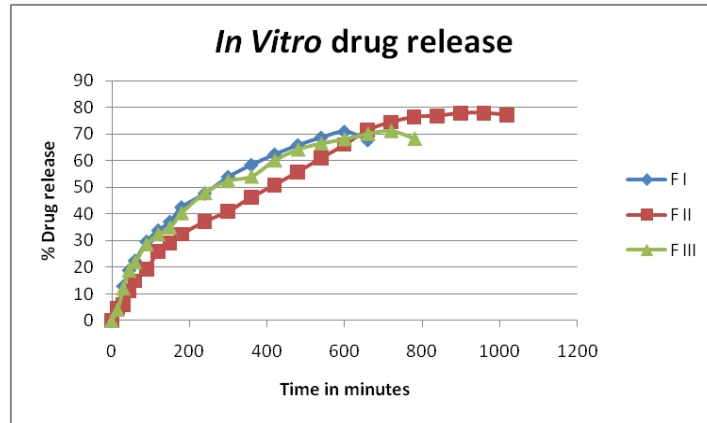


Fig. 4: *In vitro* Drug release for Formulation F-I, F-II, F-III

Kinetics of drug release

The optimized formulation F-II 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's equation for niosomal drug with r^2 value 0.988 compare to r^2 value of 0.932 and 0.9786 for zero order and first order respectively. Further Korsmeyer-Peppas model indicated a good linearity ($r^2=0.989$) and 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-II was diffusion controlled.

Stability studies

A stability study for the best niosomes formulation was carried out as per ICH guidelines for 3 months. Leakage of drug from the prepared niosomes was analyzed in terms of percentage drug retained. At refrigerated condition the niosomal formulation F-II showed $95.74 \pm 0.41\%$ at $(4 \pm 2^\circ\text{C})$. At room temperature ($25 \pm 2^\circ\text{C}$) niosomal formulation F-II showed $93.74 \pm 0.72\%$ and at $40 \pm 2^\circ\text{C}$ and $75 \pm 5\% \text{RH}$ niosomal formulation F-II showed $90.82 \pm 0.41\%$. Further the drug release profile was also found to be good in all the three temperature conditions. From this study it was found that storage under refrigerated condition showed greater stability.

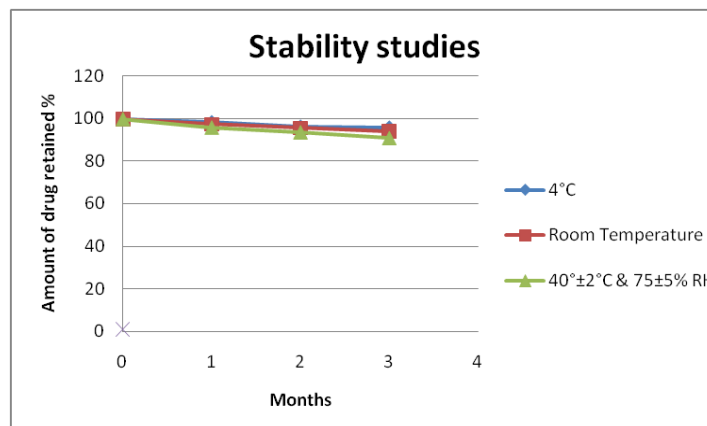
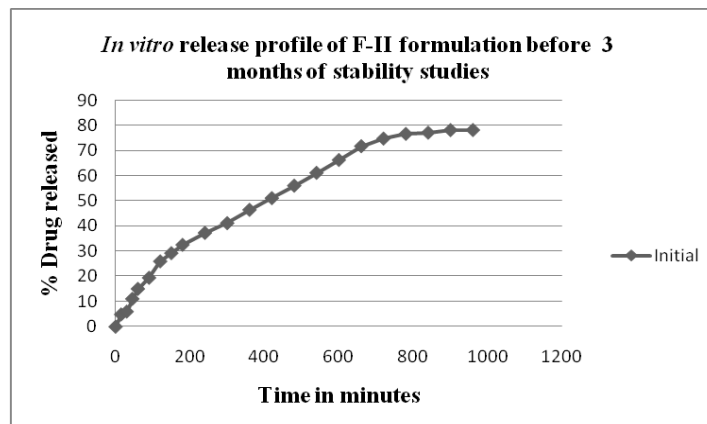


Fig. 5: Drug content profile of F-II during Stability studies



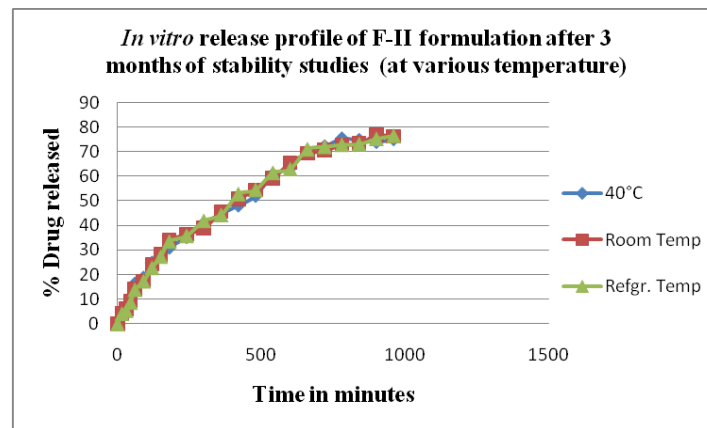


Fig. 6: *In vitro* release profile of F-II during Stability studies

CONCLUSION

Niosomes containing Oxcarbazepine were formulated using different surfactants such as span 40, span 60 and span 80 and evaluated for various parameters. From the above studies, it can be concluded that Oxcarbazepine niosomes encapsulated with non-ionic surfactants in the formulation F-II showed prolonged release and longer duration of action thereby achieving sustained release. The optimized formulation F-II was found to follow diffusion controlled release pattern. Thus the prepared niosome could be promising delivery system for Oxcarbazepine with sustained drug release profiles.

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