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DEVELOPMENT AND CHARACTERIZATION OF CARBAMAZEPINE LOADED NONIONIC SURFACTANT VESICLES

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ABSTRACT

The present work aims at preparing carbamazepine niosomes with combination of tweens, spans. The encapsulation of drug in vesicular system can be predicted to prolong the existence of drug in the systemic circulation and enhance penetration in to target tissues and reduce toxicity. The niosomes are prepared by combination of tween 80, span 80 using thin film hydration method. The formulation exhibit high entrapment efficiency, drug release and high stability. The carbamazepine incorporated niosomes were characterized by surface morphology, SEM analysis and entrapment efficiency of 96.89% and the *in vitro* drug release is found to be 98.10%. The optimized formulation (F_{14}) follows zero order kinetics and non-fickian diffusion. The carbamazepine niosomes made of combination of surfactants show high entrapment efficiency and increase therapeutic activity, bioavailability in treatment of epilepsy and bipolar disorder.

Key words: Carbamazepine, Non ionic surfactant, Niosomes.

INTRODUCTION

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle[1]. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes[2]. In niosomes, the vesicles forming non-ionic surfactant such as spans, tweens, which is usually stabilized by addition of cholesterol which gives the rigidity to the bilayer and results in less leaky niosomes[3]. Niosomes can entrap both hydrophilic and lipophilic drugs, either in aqueous layer or in vesicular membrane made of lipid materials[4]. 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, nonimmunogenic and biocompatible[5].

Carbamazepine (CBZ) is used in the treatment of epileptic and psychotropic disorders. It is specifically indicated in generalized tonic-clonic (grand mal) and partial (focal) seizures. It is also used in pain syndromes like trigeminal neuralgia, glasso-pharyngeal neuralgia and manicdepressive illness unresponsive to lithium. The mechanism of action of carbamazepine is stabilizes the inactivated state of sodium channels, meaning that fewer of these channels are available to subsequently open, making brain

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cells less excitable carbamazepine has also been shown to potentiate GABA receptors made up of α1, β2 and γ2 subunits. Carbamazepine is given orally in tablet or syrup form. Absorption of CBZ from the gastrointestinal tract is slow and erratic. The present work aims to improving the antiepileptic property of lipophilic drug like carbamazepine by formulating a modified release drug delivery system[6].

EXPERIMENTAL

Materials and methods

Carbamazepine were purchased from Yarrow Chem Pvt Ltd., Mumbai. Cholesterol from Sd. fine. Chemicals Limited, Mumbai. Span 20, 60, 80, tween 80 from Loba Chemie Pvt. Ltd., Mumbai. Diethyl ether, ethanol from Merck-specialities Private Limited, Mumbai.

Formulation of carbamazepine niosomes

For the formulations (F1-F14) drug, non ionic surfactant (span 20, span 60, span 80 tween 80), 100 mg cholesterol was taken to prepare niosomes by using ether injection method, thin film hydration method.

Ether injection method[7,8]

Drug, span-20, span-60, span 80 and tween 80, cholestrol were taken in prescribed ratio F1 (s20-100 mg), F2 (s60-100 mg), F3 (s80-100 mg), F4 (t80-100 mg), F5 (s80-50 mg, t80-50 mg) in a 250 mL beaker. The mixture was dissolved in diethyl ether and ethanol (8:2) solution and slowly injected through 14 gauge needle into a beaker containing carbamazepine in 10 mL phosphate buffer pH 7.4 & 1% SLS. The temperature maintained during the injection was 40-60°C. The differences in temperature between phases cause rapid vaporization of ether, ethanol resulting in spontaneous vesiculation.

Table 1: Composition of niosomal formulations of carbamazepine by ether injection method

Formulation code	Composition					
	Drug	Span 20	Span 60	Span 80	Tween 80	Cholestrol
F1	100 mg	100 mg	-	-	-	100 mg
F2	100 mg	-	100 mg	-	-	100 mg
F3	100 mg	-	-	100 mg	-	100 mg
F4	100 mg	-	-	-	100 mg	100 mg
F5	100 mg	-	-	50 mg	50 mg	100 mg

Thin film hydration method[9,10]

Drug, span 20 and span 60, span 80 tween 80 and cholesterol were taken in various ratios F6 (t80-100 mg), F7 (t80-200 mg), F8 (s80-100 mg), F9 (s80-200 mg), F10 (s60100 mg), F11 (s60-200 mg), F12 (s20-100 mg), F13 (s20-200 mg), F14 (s80-50 mg, t80-50 mg) and transferred in to a clean round bottom flask. Then chloroform and ethanol (8:2) solution was added and the flask was fixed to rotary evaporator at 50°C temp, for 20 mins under vaccum at 150 rpm. It forms a dry thin film along the sides of the R. B. flask. Carbamazepine dissolved in phosphate buffer pH 7.4 & 1% SLS and was added to the thin film and vortexed at room temperature for 20 mins which forms milky white suspension.

Table 2: Composition of niosomal formulations of carbamazepine by thin film hydration method

Formulation code	Composition					
	Drug	Span 20	Span 60	Span 80	Tween 80	Cholestrol
F6	100 mg	-	-	-	100 mg	100 mg
F7	100 mg	-	-	-	200 mg	100 mg
F8	100 mg	-	-	100 mg	-	100 mg
F9	100 mg	-	-	200 mg	-	100 mg
F10	100 mg	-	100 mg	-	-	100 mg
F11	100 mg	-	200 mg	-	-	100 mg
F12	100 mg	100 mg	-	-	-	100 mg
F13	100 mg	200 mg	-	-	-	100 mg
F14	100 mg	-	-	50 mg	50 mg	100 mg

Compatibility studies

Fourier transform infrared (FT-IR) spectrophotometer was used for infrared analysis of samples to interpret the interactions of drug with surfactants and other ingredients. The sample was used for FT-IR studies. FT-IR studies were conducted for characterization of drug in niosomes of optimised formulation. The IR spectra were recorded using Fourier transform infrared spectrophotometer. The IR spectrum of pure carbamazepine and best formulations were taken, interpreted and compared with each other.

Determination of λ_{max} and calibration curve for carbamazepine[11]

From the standard stock solution aliquots 3 mL, 6 mL, 9 mL, 12 mL and 15 mL were pipetted out into volumetric flask. The volume was made up with phosphate buffer pH 7.4 to get final concentration of 3 µg/mL, 6 µg/mq, 9 µg/mL, 12 µg/mL, 15 µg/mL, respectively. One of the above solution i.e., 3 µg/mL was selected for the determination of λ_{max}. This solution was scanned between the range of 200-400 nm from the scan. It was concluded that the λ_{max} of carbamazepine was 285 nm. The absorbance of each concentration was measured at λ_{max} 285 nm using UV-Visible spectrophotometer. Absorbance was measured at 285 nm against phosphate buffer of pH 7.4 as blank spectrophotometrically.

Characterization of niosomes

Physical appearance of niosomal suspension[12]

The prepared niosomal suspension was viewed by naked eye to characterize color and physical state of suspension. Niosomal suspension was also viewed by optical microscope at 40 X magnification, to observe crystal characteristics of suspension by spreading a thin layer of niosomal suspension on a slide and placing the cover slip on it. The appearance for each formula was checked such as color, consistency and fluidity and comparison of each one with the other.

Vesicle size analysis

Size and size distribution studies were done for niosomes. The suspension of niosomes was observed

under optical microscope at 40x magnification. The sizes of 100 vesicles were measured using a calibrated ocular and stage micrometer fitted in the optical microscope.

Vesicle morphology[13,14]

Shape and surface morphology of niosomes was studied using scanning electron microscopy (SEM). The niosomes formed were mounted on an aluminum stub with double-sided adhesive carbon tape. The vesicles were then sputter-coated with gold/palladium using a vacuum evaporator and examined with the scanning electron microscope equipped with a digital camera at 25kV accelerating voltage.

Drug encapsulation efficiency determination[13,14]

Niosomal suspension (2 mL) was placed in a glass tube in which one end is tied with dialysis membrane. The capacity of receptor compartment was 100 mL pH 7.4 phosphate buffer placed in 250 mL beaker. The membrane (dialysis membrane) was mounted between the donor and receptor compartment. A weighed amount of niosomal suspension was placed on one side of the membrane. The receptor medium taken was 100 mL of phosphate buffer of pH 7.4. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. The receiver fluid was stirred with a magnetic stirrer at a speed of 600 rpm. At each 5 min interval, 100 mL of the medium in the receptor compartment were replaced for 20 min. The Sink condition was maintained throughout the experiment. Samples withdrawn were suitably diluted and analyzed spectrophotometrically at 285 nm and the unentraped drug concentration was known by the UV spectroscopic method at 285 nm. The percentage of drug encapsulation was calculated by using the following equation.

$$EP (\%) = [(c_t - c_r)/c_t] \times 100$$

Where EP is the encapsulation percentage, c_t is the concentration of total drug, and c_r is the concentration of free drug.

In vitro diffusion study[15]

The *In vitro* release studies on niosomal suspension was performed using open ended tube, which acts like donor compartment. The capacity of receptor compartment was 100 mL pH 7.4 phosphate buffer placed in 250 mL beaker. The membrane was mounted between the donor and receptor compartment. A weighed amount of niosomal suspension was placed on one side of the membrane. The receptor medium taken was 100 mL of phosphate buffer of pH 7.4. The receptor fluid was stirred by a Teflon-coated magnetic bead fitted to a magnetic stirrer. The receiver fluid was stirred with a magnetic stirrer at a speed of 600 rpm. At each sampling interval, (1 mL) were withdrawn and were replaced by equal volumes of fresh receptor fluid on each occasion. The Sink condition was maintained throughout the experiment. Samples withdrawn were suitably diluted and analyzed spectrophotometrically at 285 nm. The percentage drug release was calculated using calibration curve of the drug in phosphate buffer of pH 7.4.

Release kinetics and mechanism of a drug release[16]

To analyze the *in vitro* release data and to determine the release mechanism various kinetic models were used. The release data of niosomes was fitted into different models like Zero order, First order, Higuchi matrix, Erosion model and Korsemeyer-Peppas models to interpret the drug release mechanism for niosomes. Based on the r-value, the best-fitted model was selected.

The results of *in vitro* release profile obtained for all the formulations were plotted in models of data treatment as follows –

- Zero-order kinetic model - Cumulative % drug release versus time.
- First-order kinetic model - Log cumulative percent drug remaining versus time.
- Higuchi's model - Cumulative percent drug released versus square root of time.
- Korsmeyer equation / Peppa's model - Log M_{t}/M_{∞} versus log time.
- Erosion model **-** cubic root of unreleased fraction of the drug versus time

Stability studies[17]

Stability studies were conducted for best formulation of carbamazepine niosomal suspension. The ability of vesicles to retain the drug (Drug Retention Behavior) was assessed by keeping the niosomal suspension at three different temperature conditions, i.e., Refrigeration Temperature (4-8°C), room temperature (25 ± 2°C) and oven (45 ± 2°C). Throughout the study, niosomal formulation was stored in aluminium foil-sealed glass vials. The stored formulation was analyzed for particle size and percent drug entrapment at 285 nm.

RESULTS AND DISCUSSION

Compatibility studies

From the FT-IR spectra of physical mixture of the drug, non-ionic surfactants, cholesterol, it was observed that the peak of major functional groups of carbamazepine, which were present in the spectrum of pure drug were present.

The presence of peaks at 507 (C-N-C bending), 514 (C-N-C bending), 624 (NH_2 Wagging), 647 ((C=O) in plane bending), 1384 (NH_2 rocking), 1436 (C=C stretching), 1489 (NH in plane bending/C=C stretching), 1677 (NH_2 scissoring/C=O stretching), 3162 (C-H stretching), 3340 (N-H asymmetric stretching), 3466 cm^{-1} (O-H stretching) were characteristic to the pure carbamazepine. IR spectrum of physical mixture of optimized formulation of drug revealed that there was no appreciable change in position and intensity of peak with respect to IR spectrum of pure carbamazepine. IR analysis revealed that there was no known chemical interaction between drug and surfactant, cholesterol.

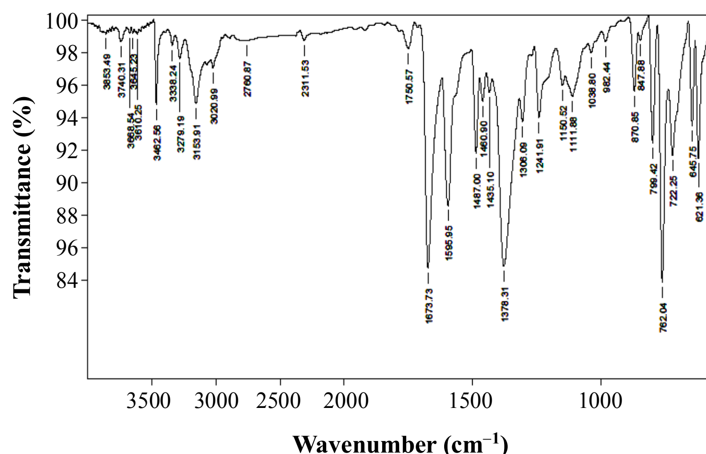

Fig. 1: FT-IR spectrum of pure drug carbamazepine

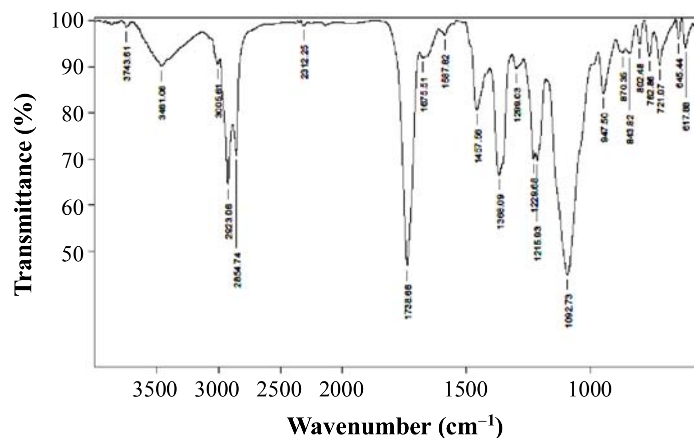

Fig. 2: FT-IR spectrum mixture of carbamazepine and excipients

Calibration curve of carbamazepine

Standard plot of carbamazepine was plotted as per the procedure in experimental methods and its linearity was shown. The standard graph of carbamazepine shows good linearity with R^2 value of 0.999, which indicates that it obeys beer's-Lamberts Law in the concentration range of 3-15 µg/mL.

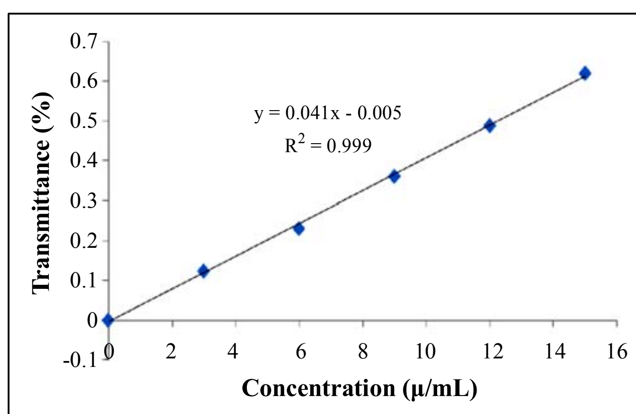

Fig. 3: Calibration curve of carbamazepine

Vesicle morphology and SEM analysis

The morphology of niosomes was studied using scanning electron microcopy. SEM images of carbamazepine revealed that the niosomes were spherical in shape and discrete with sharp boundaries having large internal aqueous space. SEM images of niosomes produced from optimized formulation shown in Fig. 5. The optical microscopy images of the niosomes from different formulations are shown Fig. 4.

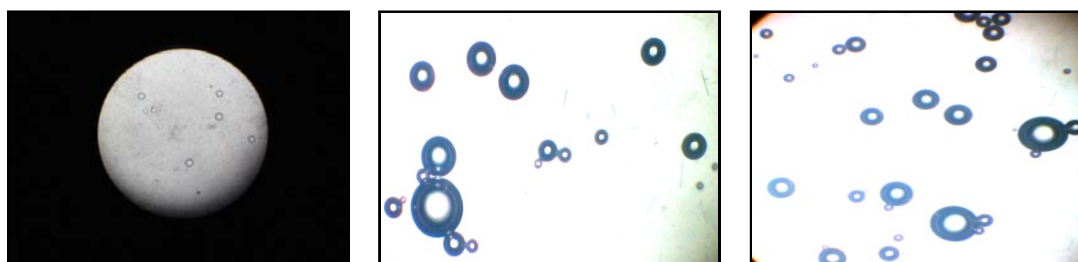

Fig. 4: Optical microscopic images of niosomes in formulations

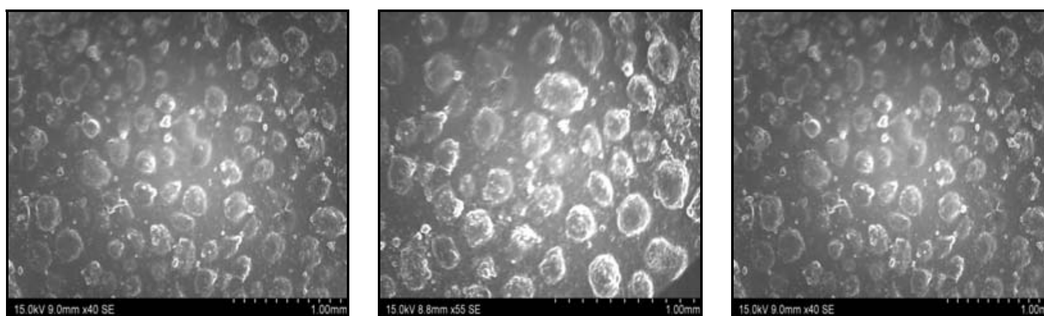

Fig. 5: SEM images of optimised formulation

Vesicle size analysis

Results of Vesicle size of Carbamazepine niosomes are presented in Table 3 and 4. This indicated that Vesicle formed with combination of Span 80 & tween 80, by thin film hydration is smaller in size than vesicle formed with other individual surfactants. Vesicles prepared by thin film hydration method were small in size compared to vesicles prepared with ether injection method, due to rotation applied during thin film hydration method.

Table 3: Size distribution of Niosomes prepared by ether injection method (F1-F5)

Size range (um)	Number of niosomes				
	F1	F2	F3	F4	F5
Below 0.1	18-20	13-15	11-13	4-6	15-17
0.1-3.52	3-6	4-6	3-5	18-23	4-6
Above 3.52	65-70	68-74	70-74	60-63	70-73

Table 4: Size distribution of niosomes prepared by thin film hydration method (F6-F14)

Size range (um)	Number of niosomes								
	F6	F7	F8	F9	F10	F11	F12	F13	F14
Below 0.1	3-6	4-6	4-6	3-5	3-8	3-6	4-6	3-6	4-8
0.1-3.52	18-22	13-15	20-22	14-16	13-16	18-24	13-15	11-14	16-19
Above 3.52	65-70	68-74	60-63	68-74	72-76	65-70	68-74	72-74	62-65

Drug encapsulation efficiency

Encapsulation efficiency of niosomes formulations ranged from 45.6% to 96.89% the drug encapsulation efficiency of all 14 formulations.

As shown in Table 5, encapsulation efficiency of niosomes formed from Span 80 & tween 80 was found high compared with niosomes prepared from span 20, span 60, span 80, tween 80 Niosomes prepared by thin film hydration method were having greater encapsulation efficiency compared to niosomes prepared by ether injection method. Most of the surfactants used to make nonionic surfactant vesicles have a low aqueous solubility. As the surfactant content of the formulation increased, the encapsulation of drug also increased. The formulations (F6-F14) containing 1:1:1, 1:0.5: 0.5:1 ratio of drug : surfactant: cholesterol prepared by thin film hydration method shows high encapsulation efficiency compared to the formulations

(F1-F5) containing 1:1:1, 1:0.5: 0.5:1 ratio of drug :surfactant: cholesterol prepared by ether injection method.

Table 5: Size distribution and entrapment of niosomes

Formulation code	Entrapment efficiency (%)	Particle size ( )
F1	45.6	4.4
F2	57.3	4.6
F3	62.8	4.6
F4	68.4	3.8
F5	72.1	4
F6	94.43	3.8
F7	95.58	4
F8	93.86	3.8
F9	95.97	4
F10	83.5	4.2
F11	89.1	4.3
F12	72.2	4.4
F13	80.4	4.6
F14	96.89	3.5

In vitro drug diffusion profile of niosomal formulations

The niosomal formulations of carbamazepine (F1-F14) were characterized for their drug permeation study using open ended tube through an artificial membrane. Drug diffusion study of all the formulations was carried out using Phosphate buffer of pH 7.4 for 12 hrs at 37 ± 0.5^{o}C with 600 rpm speed. Samples were withdrawn at regular intervals (0 1, 2, 3, 4, 6, 8, 10, 12). At every interval, 5 mL of sample was withdrawn, after appropriate dilution the sample solutions were analyzed at 285 nm for carbamazepine by using UV-Visible spectrophotometer. The cumulative percentage of carbamazepine released from niosome formulations containing different non-ionic surfactants were reported. The cumulative percentage release of carbamazepine from niosome formulations containing non-ionic surfactant span 20, span 60, span 80, tween 80, formulations F1-F5 prepared by ether injection method released 89.23%, 90.3%, 93.6%, 94.1%, 93.12% in 12 hrs, respectively.

The cumulative percentage release of carbamazepine from niosome formulations containing non-ionic surfactant span 20, span 60, span 80, tween 80 & combination of span 80, tween 80 in different ratios were reported the formulations F6-F14 containing span 20, span 60, span 80, tween 80 (F6, F8, F10, F12 -1:1:1, F7, F9, F11, F13, -1:2:1) span 80, tween 80 F14 (1:0.5:0.5:1) prepared by thin film hydration method released 94.98%, 96.97%, 92.2%, 94.87%, 88.87%, 90.2%, 87.62%, 89.9%, 98.10% in 12 hrs, respectively.

From the *in vitro* release data, it may be concluded that the formulation F14 (containing drug, Span 80, tween 80 (non-ionic surfactant), cholestrol in 1:0.5:1 ratio) prepared by thin film hydration method was selected as best formulation as the drug release from these formulation is 98.10 ± 0.19 in 12 hrs.

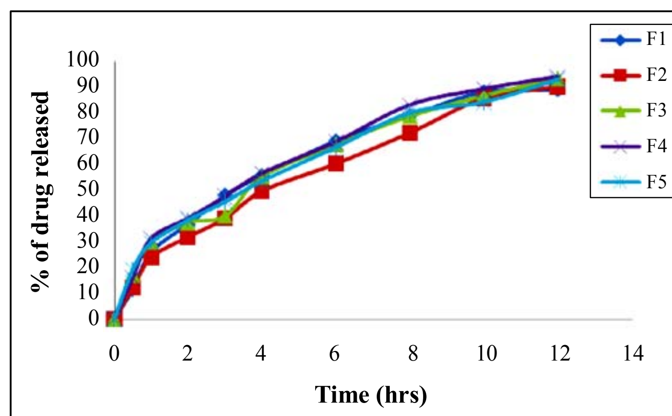

Fig. 6: Percent drug release profile of carbamazepine niosomal formulations for F1 to F5

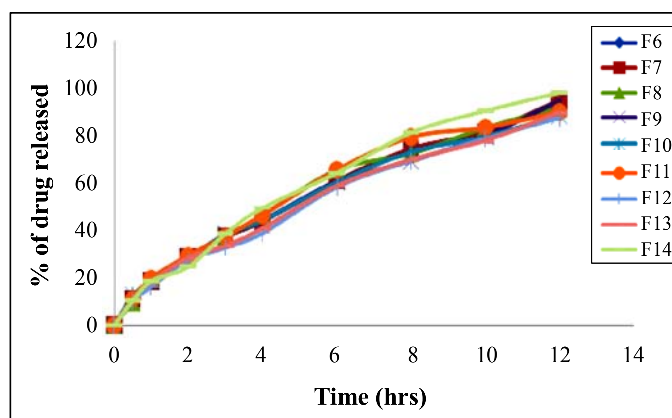

Fig. 7: Percent drug release profile of carbamazepine niosomal formulations for F6 to F14

Drug release kinetics

In order to elucidate the mode and mechanism of drug release and release rate kinetics of the dosage form, the *in vitro* drug release data obtained from niosomal formulations in Phosphate buffer of pH 7.4 were fitted into various kinetic models. The kinetic and the release mechanisms were estimated by regression plots for zero order, first order, Higuchi model, Erosion model and Kores Meyer Peppas model. When the R^2 values of regression plots for first order and zero order were considered, it is evident that the drug release from all carbamazepine formulations follow zero order kinetics.

The R^2 values of all the formulations greater for Higuchi model. So all the formulations in this study were best expressed by higuchi's classical diffusion equation. The linearity of plot indicated that the release process was diffusion controlled. To further confirm the exact mechanism of drug release, the data was incorporated in to Kores Meyer Peppas model and the mechanism of drug release was indicated according to the value of exponent 'n'. All the niosomal formulations follows non-fickian diffusion.

Stability studies

The niosomal dispersion showing highest entrapment efficiency (F14) was stored in three different temperatures 4 ± 2°C, 25 ± 2°C, 45 ± 2°C. The encapsulation efficiency and vesicle size was again calculated after 2 months.

Table 6: Correlation coefficient values carbamazepine niosomal formulations F1-F14

Formulation code	Correlation coefficient values of various release kinetics					
	Zero	First	Higuchi	Erosion	Peppas	
	R^2	R^2	R^2	R^2	R^2	N
F1	0.959	0.988	0.984	0.889	0.991	0.559
F2	0.978	0.986	0.993	0.873	0.992	0.561
F3	0.965	0.968	0.988	0.879	0.972	0.491
F4	0.966	0.980	0.992	0.844	0.991	0.473
F5	0.966	0.970	0.995	0.887	0.992	0.473
F6	0.983	0.892	0.970	0.922	0.982	0.686
F7	0.976	0.873	0.974	0.921	0.990	0.740
F8	0.959	0.935	0.983	0.889	0.983	0.738
F9	0.959	0.934	0.979	0.886	0.982	0.686
F10	0.971	0.933	0.986	0.889	0.990	0.740
F11	0.969	0.962	0.986	0.884	0.992	0.738
F12	0.976	0.935	0.961	0.869	0.978	0.783
F13	0.980	0.950	0.970	0.887	0.981	0.786
F14	0.969	0.892	0.990	0.884	0.986	0.717

Table 7: Stability studies of optimized formulation (F14)

S. No.	Temp. (°C)	Initial		After 2 months	
		Vesicle size	Encapsulation efficiency	Vesicle size	Encapsulation efficiency
1	4-8	3.52 ± 0.43	96.89 ± 0.56	3.5 ± 0.67	96.89 ± 0.46
2	25 ± 2	3.52 ± 0.43	96.89 ± 0.56	4.12 ± 0.34	90.32 ± 0.56
3	45 ± 2	3.52 ± 0.43	92.14 ± 0.56	4.72 ± 0.95	86.73 ± 0.32

CONCLUSION

The present investigation was concerned with the design and evaluation of niosomes of carbamazepine, which after administration were expected to prolong the bio availability, and reduces the toxicity associated problems. UV spectrophotometric analysis of carbamazepine was studied. carbamazepine showed maximum absorption at wavelength 285 nm in pH 7.4 phosphate buffer. The value of correlation coefficient was found to be 0.999, which showed linear relationship between concentration and absorbance. Preformulation study for drug surfactant compatibility by FTIR gave conformation about their purity and showed no interaction between drug and selected surfactant Various formulations were developed by using non ionic surfactants (span 20, span 60, span 80, tween 80) by ether injection method, thin film hydration with the incorporation of cholesterol as membrane stabilizer. Developed niosomes were evaluated for size and shape, surface morphology, drug entrapment efficiency, *in vitro* drug release studies, stability properties. The best entrapment efficiency and the best *in vitro* drug release profile were achieved by formulation F14,

the drug release (98.10%) upto 12 hr. For the optimized formulation, based on R^2 values the best formulation (F14) follows zero order & Peppas model and suggests the release occurs by diffusion mechanism, based on "n" value it follows non fickian diffusion mechanism. Most of the vesicles are spherical in shape, size range is about (3-4.6 μm) prepared by ether injection method and (3.5-4.6 μm) prepared by thin film hydration method. A high % of carbamazepine can be encapsulated in the vesicles (89-98.10%) prepared by thin film hydration method. Niosomal preparation was stable at refrigeration temperature (4°C). Concentrations of non-ionic surfactants such as Span 20 and span 6o, span 80, tween 80 might influences the drug release pattern of all formulations. From above these studies, it was concluded that, carbamazepine was successfully encapsulated into niosomes. Vesicles prepared by thin film hydration method (F14) showed best result in terms of encapsulation efficiency and *in vitro* drug release.

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