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Formulation and evaluation of sustained released niosomes containing pregabalin

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Abstract

The main objective of the present study was to encapsulate Pregabalin in niosomes for achieving prolonged release & longer duration of action. Niosomes containing Pregabalin was formulated using two surfactants such as span 40 & span 60 and evaluated for various parameters. The microscopic examination of the prepared niosomes revealed spherical small unilamellar vesicles of 80-120 nm and 250- 280 nm for F I and F II. Invitro release studies showed that the percentage amount of free drug released was 99.04% within 2.5 hours. FI showed 84.99 % of drug release within 19 hours. F II showed 93.48 % of drug release within 20 hours. Storage under refrigerated condition showed greater stability with 97.23% of drug content at the end of 3 months.

Key-Words: Pregabalin, Epilepsy, Niosomes, Thin film hydration method, *Invitro* release

Introduction

Epilepsy is a common chronic neurological disorder that is characterized by recurrent unprovoked seizures. These seizures are transient signs and / or symptoms due to abnormal, excessive or synchronous neuronal activity in the brain[1]. About 50 million people worldwide have epilepsy at any one time. Epilepsy is usually controlled, but not cured, with medication, although surgery may be considered in difficult cases. However, over 30% of people with epilepsy do not have seizure control even with the best available medications. Not all epilepsy syndromes are lifelong some forms are confined to particular stages of childhood[1]. Epilepsy should not be understood as a single disorder, but rather as a group of syndromes with vastly divergent symptoms but all involving episodic abnormal electrical activity in the brain. Pregabalin is one of the most effective drug in the treatment of epilepsy, it is an ideal second generation Anti Epileptic Drug (AED) that eliminates seizures without adverse effects.

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Niosomes are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with or without incorporation of cholesterol or other lipids[2]. They are vesicular systems similar to liposomes that can be used as carriers of amphiphilic and lipophilic drugs. Niosomes are promising vehicle for drug delivery and being non-ionic, it is less toxic and improves the therapeutic index of drug by restricting its action to target cells. In niosomes, the vesicles forming amphiphilic is a non-ionic surfactant such as Span 60 which is usually stabilized by addition of cholesterol and small amount of anionic surfactant such as dicetyl phosphate[3]. Niosomes have been used to prolong the circulation of the drugs, to alter the distribution of drugs and they offer a host of other advantages. Niosomes favour selective delivery of drugs and improves the therapeutic efficacy and reduces the severity of side effects. The need for present study is to encapsulate the drug in the niosomes vesicles for effective central nervous system drug delivery for a prolonged period of time. Thus the present studies deals with preparation methods, characterizations, factors affecting release kinetic, advantages, and applications of niosomes.

Advantages of niosomes

- The first report of non-ionic surfactant vesicles came from the cosmetic applications devised by L'Oreal[3].
- The application of vesicular (lipid vesicles and non-ionic surfactant vesicles) systems in

cosmetics and for therapeutic purpose may offer several advantages

- The vesicle suspension is water–based vehicle. This offers high patient compliance in comparison with oily dosage forms.
- They possess an infrastructure consisting of hydrophilic, amphiphilic and lipophilic moieties together and as a result can accommodate drug molecules with a wide range of solubilities.
- The characteristics of the vesicle formulation are variable and controllable. Altering vesicle composition, size, lamellarity, tapped volume, surface charge and concentration can control the vesicle characteristics.
- The vesicles may act as a depot, releasing the drug in a controlled manner.
- They are osmotically active and stable, as well as they increase the stability of entrapped drug.
- They improve oral bioavailability of poorly absorbed drugs and enhance skin penetration of drugs.
- The surfactants are biodegradable, biocompatible and nonimmunogenic.
- Niosomal dispersion in an aqueous phase can be emulsified in a non-aqueous phase to regulate the delivery rate of drug and administer normal vesicle in external non-aqueous phase.

Material and Methods

Materials

Pregabalin was a gift sample from Micro Labs (Hosur), Cholesterol was purchased from Qualigens Fine Chemicals (Mumbai), Span 40 and Span 60 was obtained from Kemphasol, all other reagents were of analytical grade.

Preparation of Niosomes[4]

Pregabalin niosomes were prepared by Thin Film Hydration Technique using Rotary flash Evaporator. The formulation code and ingredients are given in the Table 1. According to this method, accurately weighed quantity of cholesterol and non-ionic surfactant were dissolved in 10 ml of chloroform and poured into a round bottom flask. The flask was rotated at 1.5 cm above a water bath at $60^{o}C\pm 2^{0}C$ under reduced pressure, until all the organic phase evaporated and a thin layer was formed on the wall of a round bottom flask. Then accurately weighed quantity of drug was dissolved in 10 ml of water. The dried non- ionic surfactant and cholesterol film was subsequently hydrated with this drug solution and the mixture was rotated by immersing in a water bath at $60^{o}C\pm 2^{0}C$ for 1 hour until a good dispersion of mixture was obtained. The niosomes vesicles containing were subsequently formed. The suspension was then sonicated to form unilamellar vesicles.

Characterization of niosomes

Invitro Release Studies[5]

The *in vitro* releases of niosomes were studied by using simple diffusion cell apparatus. The diffusion cell apparatus consists of a glass tube with an inner diameter of 2.5 cm, open at both ends, one end of the tube is tied with Sigma dialysis membrane, which serves as a donor compartment. Niosomes equivalent to 5 mg of Pregabalin was taken in a dialysis tube and placed in 200 ml of water. The medium was stirred by using the magnetic stirrer and the temperature was maintained at $37\pm2^{0}C$. Periodically 5ml of samples were withdrawn and after each withdrawal same volume of medium was replaced. Then the samples were assayed spectrophotometrically at 215 nm using water as blank.

Determination of Drug Entrapment Efficiency[5-7]

1 ml of the sample is taken and centrifuged at 13000 RPM at $4^{0}C$ for 90 minutes using eppendorf centrifuge. Supernatant was separated without disturbing the sediment layer using micropipette. Then the supernatant layer (free drug) was diluted using PBS pH 7.4 and analysed using UV spectrophotometer.

$$\%\text{drug entrapment} = \frac{\text{Amount of drug entrapped}}{\text{Initial amount of drug}} \times 100$$

Scanning Electron Microscopy[5-7]

Niosomes were characterized by SEM (JEOL). Niosomes containing Pregabalin was taken in a cover glass and transferred on a specimen stub. Dried samples were coated with platinum alloy to a thickness of 100 A using a sputter coater. After coating, scanning was done to examine the shape and size.

Particle Size Distribution[5-7]

The size of the formulation was analyzed by using a Zetasizer, Ver. 601 (Malvern Instrument Ltd). The formulation was placed in the sample holder and the particle size was measured.

Stability Studies[5-7]

The formulated niosomes were subjected for stability studies for a period of three months. The formulated niosomes were divided into 3 portions. First portion was kept at refrigeration ($4^{0}C\pm1^{0}C$) temperature. Second portion was kept at room temperature. Third portion was kept at $40^{0}C\pm2^{0}C$, 70% ±5 %.

Results and Discussion

The SEM analysis of the prepared niosomes revealed spherical small unilamellar vesicles of 80-120 nm and 250- 280 nm for F I and F II (Figure 1 & 2). These

results revealed that the vesicle diameter complies within the niosomal size range of 100-300 nm (Table 2). The entrapment efficiency of drug in F II containing span 60 was found to be 66.50% (Table 2) which showed maximum percent drug entrapment where as those containing span 40 was found to encapsulate 48.21%. This showed that span 60 is the more suitable surfactant along with cholesterol for enhancing maximum entrapment for the drug Pregabalin. Further, the percent drug entrapment is increased by decreasing the sonication time. Therefore, the sonication time was optimized to 15 minutes and further reduction in the size by increasing sonication time was not attempted. The formulated niosomes were subjected to *in vitro* drug release using 0.1M in tubing. The amount of Pregabalin diffused was estimated spectrophotometrically at 215nm. The percentage amount of free drug released was 99.04% within 2.5 hours. FI showed 84.99 % of drug release within 19 hours. F II showed 93.48 % of drug release within 20 hours (Table 4). These results showed that niosomal pregabalin has sustained release upto 20 hours whereas free Pregabalin was released within 2.5 hours. This is because the drug is released slowly for a prolonged period of time in niosomal Pregabalin. Storage under refrigerated condition showed greater stability with 97.23% of drug content at the end of 3 months whereas storage under room temperature and at $40^0C \pm 2^0C$, RH 70 % ± 5% showed drug content of 93.92% and 84.83% at the end of three months (Table 3).

Conclusion

Niosomes containing Pregabalin was formulated using two surfactants such as span 40 & span 60 and evaluated for various parameters. From the above studies, it is concluded that Pregabalin encapsulated in niosomes showed prolonged release & longer duration of action thereby achieving sustained release.

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Table 1: Preparation of Niosomes

Formulation Code	Amount of Pregabalin	Cholesterol	Span 40	Span 60	Chloroform	water	Cholesterol – surfactant ratio	DCP
FI	10mg	30mg	30 mg	-	10 ml	10ml	1:1	1 mg
FII	10mg	30mg	-	30mg	10 ml	10ml	1:1	1 mg

Table 2: Vesicle diameter of niosomes and % drug entrapment

S/ No.	Type of formulation	Size (nm)	% Drug Entrapment
1	F 1	80-120	48.21%
2	F II	250-280	66.50%

Table 3: Stability Studies

Temperature	Amount of drug retained (%) after 3 months		
	Initial	Formulation I	Formulation II
Refrigeration ($4^0C \pm 1^0C$)	100	94.13	97.23
Room Temperature	100	90.27	93.92
$40^0C \pm 2^0C$ RH-70% ± 5%	100	80.74	84.83

Table 4: Invitro drug release studies of FI and FII

Time (hrs)	Percentage drug diffused (FI)	Percentage drug diffused (FI)
1	15.17 ± 0.50	14.29 ± 1.17
2	19.02 ± 0.61	17.83 ± 0.54
3	24.53 ± 0.55	21.95 ± 0.19
4	29.86± 0.18	24.59 ± 0.35
5	33.12± 0.11	28.40 ± 0.34
6	34.74± 0.59	30.34 ± 0.62
7	36.27± 0.07	33.83 ± 0.11
8	39.91± 1.20	38.60 ± 0.22
9	43.91± 0.98	42.25 ± 0.15
10	47.07± 0.16	48.77 ± 0.27
11	50.07± 0.34	55.11 ± 0.16
12	56.60± 0.49	60.51 ± 0.15
13	58.02± 0.43	67.86 ± 0.21
14	65.07± 0.28	74.77 ± 0.10
15	67.39± 0.81	78.47 ± 0.04
16	73.30± 0.41	83.98 ± 0.20
17	80.13± 0.12	90.42 ± 0.25
18	88.56± 0.24	91.04 ± 1.60
19	88.73± 0.24	95.49 ± 0.22
20	88.33± 0.27	95.71 ± 0.23

All the results are mean ± S.D

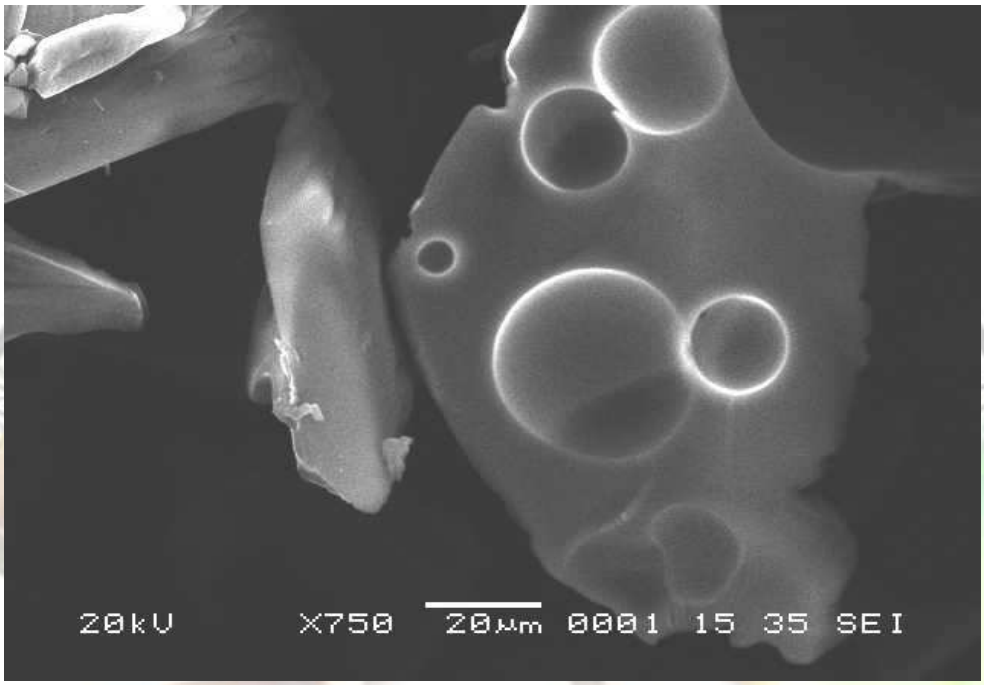

Fig. 1: SEM photograph of F-I

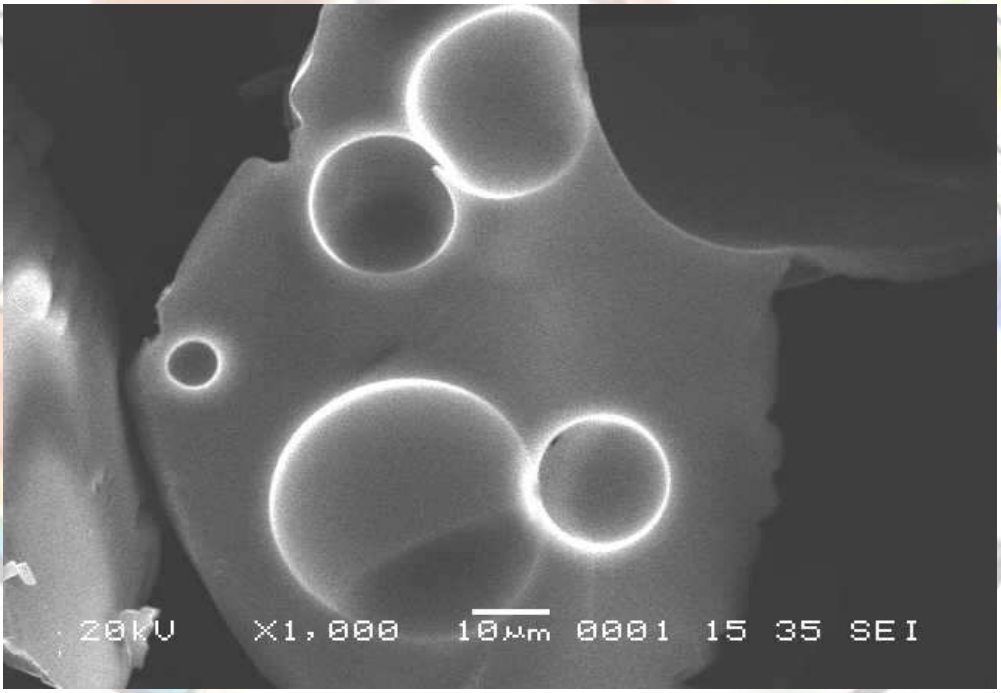

Fig. 2: SEM photograph for F-II