**Development of nanostructured lipid carrier for improvement of solubility of poorly soluble drug**

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**Abstract**

Carbamazepine is used in the treatment of epilepsy, but it is having limitations such as low solubility leading to lower oral bioavailability. Aiming to improve its poor solubility and oral bioavailability, Carbamazepine NLCs were prepared by high speed homogenization followed by ultrasonication and in vitro evaluated. The prepared NLCs were tested for encapsulation efficiency (EE%) and in vitro drug release. EE% lied between 75 % and 86.2%. In vitro release data revealed sustained release behavior and about 24% of the drug released after 3h. Release kinetics of prepared NLCs revealed that all NLCs follow zero order reaction.

**Introduction**

Epilepsy is a disease characterized by recurrent seizures, which are nothing but episodes of paroxysmal neural discharges (1). Twice or thrice the mortality was observed in people facing epilepsy when compared to regular population (2). Most of the newly developed molecules for treatment of epilepsy are suffering from variability in absorption that limits their therapeutic efficacy, which can be attributed to their change in physicochemical properties. Most of the drug delivery to brain has been limited mainly due to the lower solubility. Carbamazepine is recorded in Biopharmaceutical Classification system as class II which indicates low solubility and high permeability for the drug molecules. As the drug is having low solubility, it dissolute very poorly so it delays the absorption that indicates the rate of dissolution is the controlling step for absorption (3,4).

Lipid-based drug delivery systems are expected as promising oral carriers because of their potential to increase the solubility and improve oral bioavailability of poorly water-soluble and/or lipophilic drugs (5). The first generation of lipid nanoparticles, the so-called solid lipid nanoparticles (SLN), is composed by an aqueous dispersion of nanoparticles with a solid lipid matrix that is stabilized by one or more surfactant layer. However, SLN presented some shortcomings, such as limited drug loading capacity and potential for drug expulsion during storage. Therefore, this leads to a need of create the second generation of lipid nanoparticles, the nanostructured lipid carriers (NLC). In contrast to SLN, NLC dispersions are formed by a blend of solid lipid with liquid lipid, which provides a higher payload and prevents drug expulsion during storage (6,7). This higher drug encapsulation is attributed to the differences in the structures of the solid and liquid lipids and then formation of a perfect crystal is distorted. Thus, the mixture accommodates the active in molecular form or in amorphous clusters (8). Additionally, NLCs promote oral absorption of encapsulated drug via selective uptake through lymphatic route or payer’s patches (9). Larger lipid nanoparticles accumulate at the injection site, and the drug is slowly released from the nanoparticles. The free drug can enter the blood circulation via pores on the walls of the capillaries. Smaller lipid nanoparticles (<0.1 µm) can easily access the lymphatic capillaries and concentrate in regional lymph nodes[1].Thus, based on these advantages, NLCs could be developed as a carrier for lymphatic drug delivery by subcutaneous administration because they have improved physicochemical properties compared with other lipid-based nanocarrier systems (10).

The objective of the present work was to load Carbamazepine into NLCs in order to enhance solubility and hence bioavailability. Stearic acid was chosen as solid lipids while oleic acid and castor oil were liquid lipids. Hydrophilic surfactants (SDS) was investigated while span 60 was the lipophilic emulsifier.

# Materials and methods

#  Materials

Carbamazepine castor oil, SDS, Stearic acid and oleic acid were purchased from Sigma-Aldrich (Germany).

# Methods

# *Compatibility between lipid components*

A compatibility screening of liquid lipids with solid lipid were performed. Bulk lipid was weighed accurately into glass vials and heated up to 100°C and mixed with liquid lipids. The mixtures were checked after 1 h, immediately after solidification and after 24 h. Mixtures creating one single phase only were selected for preparation step.

# *Preparation of Carbamazepine loaded NLCs*

The NLC was prepared by a modified method of high speed homogenization followed by ultrasonication. The lipid and aqueous phases were prepared separately. The solid lipid/liquid lipid phase consisted of 10% (w/v) Stearic acid, and oleic acid or castor oil and 0.5% Span 60 as the lipophilic emulsifier, while the aqueous phase consisted of distilled water and 2% hydrophilic emulsifier (SDS). Carbamazepine was positioned in the lipid phase. Both phases were heated separately to 85°C for 10 min. The aqueous phase was added drop wise to the molten lipid phase and mixed using a high-speed homogenizer at 10,000 rpm for 10 min. The mixture was further treated using a bath-type sonicator for 10 min at 50 W. The obtained dispersion cooled at room temperature. The formulations are depicted in Table 1.

**Table 1**. Compositions of different formulations.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ***Code*** | ***Solid lipid(2%)*** | ***Liquid lipid (8 %)*** | ***Aqueous surfactant (2 %)*** | ***Span 60*** |
| NLC 1 | Stearic acid | Oleic acid | SDS | 0.5% |
| NLC 2 | Stearic acid | Castor oil | SDS | 0.5% |

# *Drug encapsulation efficiency*

The entrapment efficiency (EE) is defined as the ratio of the amount of Carbamazepine encapsulated in the lipid core to that of the total Carbamazepine added to the dispersion during NLC preparation. The proportion of non-encapsulated Carbamazepine was determined by centrifugation precipitation method. The pH value of the obtained NLC dispersion was adjusted to 1.20 to form the NLC aggregation by adding 0.1N of hydrochloric acid. Then the precipitate was precisely separated by centrifugation at 15,000rpm for 15min followed by filtration through 0.45µm filter. The filtrate was diluted appropriately with ethanol and analyzed by UV visible spectrophotometer at 285nm. The encapsulation efficiency of Carbamazepine was then calculated according to the following equation (1):

$\%E=\left(AD-FD/AD\right)×100$ , (1)

where %E = the encapsulation efficiency in percentage, AD = the amount of added drug during NLC preparation and FD = is the amount of free drug in the supernatant after centrifugation.

*In vitro drug release studies*

The in vitro drug release experiments were performed during 3 h by the dialysis bag diffusion technique. The release profile Carbamazepine from NLCs formulations were compared with the conventional suspension in 3% CMC. The bag was mounted between the donor and the receptor compartments. The receptor medium consisted of 500 ml of phosphate buffer pH 7.4. The stirring rate and temperature was 300 rpm and37 ◦C respectively. At appropriate intervals, 2ml aliquots of the receptor medium were withdrawn and immediately replaced within equal volume of fresh buffer. The amount of drug released was determined UV spectrophotometrically.

*In vitro drug release kinetics*

The data obtained from the experiments were analyzed by means of personal computer to establish the order of the drug release. The release data were analyzed using the following linear regression equations.

A) Ct = CoKt for zero order kinetics.

B) Log Ct = - Kt/2.303 + log Co for first order kinetics

C) Q = Dt (2A-Cs) 1/2Cs for Higuchi model (Higuchi, 1962).

Where

Q is the amount of drug released per unit area at time t,

D is the drug diffusion coefficient in the matrix,

A is the total amount of drug present in the matrix per unit volume, and Cs is the drug solubility in the matrix. Stating the proper mode of release is based on the correlation (r) for parameters involved, were the highest correlation coefficient represent the actual mode of the release.

# Results and discussion

# Compatibility test and formulations

Castor oil and oleic acid seemed compatible with Stearic acid without phase separation.

**Encapsulation efficiency**

The entrapment efficiency of Carbamazepine within the different prepared nanostructured formulations was found to vary between 75 % and 86.2 % (Table 2). It is clear that oleic acid containing NLC had higher EE% than that of castor oil. This may be attributed to complete solubility of Carbamazepine in oleic acid which in turn leads to massive crystal order disturbance and leaves enough space to accommodate drug molecules, thus leading to improved drug entrapment efficiency (11).

**Table 2**: EE% of NLCs

|  |  |  |
| --- | --- | --- |
| ***Code*** | ***Liquid lipid (8 %)*** | ***EE%*** |
| NLC 1 | Oleic acid | 86.2 |
| NLC 2 | Castor oil | 75 |

**In vitro drug release studies**

From the data listed in table (3) and figure (1), it is clear that Carbamazepine in its NLC forms exhibit a higher in-vitro release as compared with corresponding conventional suspension.

**Table (3):** In vitro drug release from different formulations

|  |  |
| --- | --- |
| Time (min) | Release of Carbamazepine (mg %) |
| NLC1 | NLC2 | Suspension |
| 0 | 0 | 0 | 0 |
| 30 | 3.5 | 5 | 2 |
| 60 | 7.5 | 8.1 | 3.5 |
| 90 | 10.9 | 11.2 | 4.9 |
| 120 | 15.9 | 18 | 5.3 |
| 150 | 22 | 21.8 | 7 |
| 180 | 23.9 | 24.6 | 10 |

**Figure (1):** In vitro drug release from different formulations

**In vitro drug release kinetics**

From the data listed in table (4), it is clear that Carbamazepine in its NLC forms exhibit a zero order release kinetics while conventional suspension behaves as first order.

**Table (4):** In vitro drug releasekinetics from different formulations

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Formula | Kinetic order or model | Intercept(a) | Slope(b) | Correlation(r) | Rate Constant (k) | t1/2(min) |
| NLC1 | **Zero**FirstDiff. | 7.8011.984-11.54 | 0.471-6.3 | 0.9981-0.99690.9904 | 0.4714-0.0076.30 | 106-9762.9 |
| NLC2 | **Zero**FirstDiff. | 14.691.95-5.5 | 0.492-6.58 | 0.9993-0.99680.9922 | 0.492-0.0086.588 | 101.5-81.157.59 |
| Suspension | Zero**First**Diff. | 8.501.991-15.7 | 0.58-7.83 | 0.9782-0.98370.9789 | 0.581-0.0097.83 | 86-71.540.7 |

**Conclusion**

NLCs have evolved as second-generation lipid nanocarriers which retain the advantages of SLN but unlike SLN they exhibit good physical stability and higher drug loading. Inview of this, NLCs of carbamazepine were successfully fabricated by high shear homogenization followed by sonication. The high shear homogenization followed by sonication technique successfully yielded nanostructured lipid carriers with EE% of 75% and 86.2%. In vitro release data revealed sustained release behavior and about 24% of the drug released after 3h. Release kinetics of prepared NLCs revealed that all NLCs follow zero order reaction.

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