Carbamazepine loaded mucoadhesive solid lipid nanoparticle: Formulation and in-vitro characterization

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ABSTRACT

Background: By the oral route of administration, bioavailability of Carbamazepine is low and variable. In the present study, Carbamazepine-loaded solid lipid nanoparticles (SLNs) were prepared with the aim to enhance the uptake of CBZ to brain via intranasal delivery.

Materials and methods: SLNs were prepared by modified emulsification–diffusion technique and evaluated for particle size, zeta potential, drug entrapment efficiency, in vitro drug release. The SLNs developed were used to develop thermosensitive and mucoadhesive gel by using carbopol and Pluronic F127. The gels were evaluated in vitro.

Results: The optimized SLN was characterized for % EE (90.63 ± 0.48), drug content (20.59 ± 0.11), mean particle size (331.4 nm), zeta potential (-32.6±2.6) and PDI (0.473). In-vitro release showed 62.2% CBZ release in 20 hrs. Furthermore, shelf life predicted for the optimized formulation was 0.78 years.

Conclusions: The studies demonstrate that the formulation can be tested and used for nasal route for improved delivery of CBZ.

Keywords: Solid lipid nanoparticle (SLN), carbamazepine, surfactant, mucoadhesive, nasal delivery.

INTRODUCTION

Nanotechnology is the study, design, creation, synthesis, manipulation, and application of functional materials, devices, and systems through control of matter at the nanometer scale. It encompasses the production and application of physical, chemical and biological systems at submicron level as well as the integration of resulting nanostructures into larger systems (Falzarano et al., 2013). Over the past two decades, there has been a marked improvement in our understanding of the underlying etiology and treatment of central nervous system (CNS) disorders (Misra et al., 2003). However, many of the drugs used to treat these disorders lack an effective means for crossing the blood–brain barrier (BBB) (Alam et al., 2014). The BBB presents a great obstacle to the transport of exogenous substances into the brain. Thus, various approaches like BBB disruption (osmotic and biochemical), drug manipulation (prodrug, lipophilic analogs, chemical drug delivery, carrier mediated drug delivery, and receptor/vector-mediated drug delivery) and alteration in the route of administration, including intracebroventricular, intrathecal, and olfactory pathways (intranasal route) are used for the targeting of drugs to the brain (Illum, 2002). In the present scenario, the intranasal route to bypass the BBB is explored, as this route provides a novel, practical, simple and non-invasive approach to bypass the blood brain barrier and reduces the systemic exposure and thus systemic adverse effects. Drug after intranasal administration reaches the olfactory epithelium region of the nasal mucosa that acts as a gateway for substances
proteins which results into maximal in vivo residence (surfactants) are selected after studying interaction with lipid. To obtain a better bio-distribution, stabilizers

The general criterion of lipid selection for the development of SLNs is drug solubility in a particular lipid. To obtain a better bio-distribution, stabilizers (surfactants) are selected after studying interaction with proteins which results into maximal in vivo residence time. But stabilizers, if not bounded chemically are loosely attached to the colloidal surfaces. Also, their micellar network is concentration dependent and it is expected that after sufficient dilution they may leave the surface. On the other hand chemical and enzyme-catalyzed processes are the main forces which may lead to degradation of stabilizers. The enzymatic degradation of such polymers depends on a number of factors (apart from those related to the polymer chemical structure) like, temperature, pH, electrolyte concentration, presence of enzyme inhibitors, presence of other organic compounds (e.g., urea), cations (certain systems), and other co-solutes. Thus, when the stearic hindrance by the stabilizers is minimized around colloidal particles (e.g., solid lipid nano particles), it is the core of particle (lipid in case of SLN) which interacts with body fluids. So, an attempt has been made here to study the preferential adsorption of a protein over different solid lipids available. Once lipid is without the coatings of stabilizers, it is its inherent affinity of lipids with body fluid / proteins that allow it to remain dispersed within body. Otherwise it may precipitate out. For the above hypothesis, following experimental set up was run.

1.5 grams of selected lipids (lipids showing maximum drug solubility) were passed through sieve number 80 and stored in desiccators. In a 15 mL glass syringe (inverted) lipids were put by blocking the nozzle with a filter paper of same internal diameter and putting a cap also. 10 mL of freshly prepared BSA solution (2 mg/mL) was added into the syringe. The assembly was left undisturbed at room temperature (25 °C) for an hour. Then the cap was removed and samples were collected, samples were analyzed by UV spectrophotometer (Shimadzu, UV 1601) using a calibration plot (R²= 0.996) made with BSA at 279 nm.

**Selection of surfactant**

A high surfactant concentration favors a lower particle size, a narrower particle size distribution and a better long-term stability of lipid nanoparticles but simultaneously increases the toxicological potential. Hence, a balance needs to be found to have sufficient surfactant present to ensure a small particle size and a good physical stability of the carrier system as well as avoiding free surfactant in the formulation as binding surfactants to the surface of lipid nanoparticles markedly reduces the toxicological potential. In this section we select only the type of surfactant, strength will be optimized in previous section. To select the surfactant (or combination), placebo SLNs were made and percentage transmittance were determined. % Transmittance was also determined after 3 days to see the effect of stabilizers on particle growth (size ripening). Stearic acid and...
compritol E ATO (1:1 ratio) was taken as a lipidic phase. Organic aqueous phase ratio was taken 10:50. Stabilizers selected (in strength of 4%) were of high HLB values, considering the formation of o/w type of emulsion.

**Preparation of solid lipid nanoparticle (SLN)**

The solid lipids (Stearic acid, Compritol 888, Compritol E ATO and their combinations) were dissolved in small quantity of dichloromethane; bath sonicated for 2 minutes and total volume was made up to 10 mL. Sodium taurocholate was taken as a co-surfactant to generate sufficient negative charge over the SLNs and was taken in a fixed quantity (0.5% w/v). Poloxamer 188 was taken as a stearic stabilizer in a variable quantity (2-5% w/v). Organic phase was then added drop wise (with the help of syringe) into aqueous surfactant solutions and simultaneously it was given high energy with the help of probe sonicator for total 3 minutes with a pause of 10 seconds after every minute. Ratio of organic to aqueous phase was taken 10:50. The colloidal suspension was then filtered through Whatman filter papers and left for stirring (1500 rpm) for 4 hrs to remove the chloroform. Higher stirring rates don’t significantly change the particle size, but slightly improve the polydispersity index. The suspension was frozen for 24 h in a Lyph-lock apparatus and then freeze dried (Dry winner, DW-8-85 HetoHolten, Denmark) for 12 h. Mannitol was (1% w/v) added as a cryoprotectant. A batch of optimized formulation was also freeze dried without cryoprotectant for different characterizations.

**Characterization of SLN**

SLN based on single lipids exhibits limited drug payloads and drug expulsion from the crystal lattice as the lipids have a tendency to re-crystallize in due course of time. During re-crystallization, the surface area of the particle would increase remarkably which add instability to the colloidal system. Cryoprotectors are space holders which prevent the contact between discrete lipid nanoparticles. Furthermore, they interact with the polar head groups of the surfactants and serve as a kind of ‘pseudo hydration shell’ (Mobley and Schreier, 1994). Following characterizations were performed for the developed SLNs.

**Particle size and zeta potential (ζ) analysis**

Particle size was determined using a photon correlation spectrometer (PCS; Zetasizer-1000 HAS, Malvern Instruments, UK) based on the laser light scattering phenomenon, which analyzes the fluctuations in light scattering. Freeze dried powders were dispersed in deionized water and 0.1 N HCl in a drop-wise manner at 25°C under gentle shaking and filtered through 0.22 μm membrane filter in order to eliminate multi-scattering phenomenon and experimental errors. Average particle diameter ($A_{ava}$), polydispersity index ($p$) and zeta potentials (ζ) were recorded. The values were calculated from electrophoretic mobility using Henry’s equation:

$$U_z = \frac{2\varepsilon \eta f(K\alpha)}{3\eta}$$

Where, $U_z$ = Electrophoretic mobility  
$\varepsilon$ = Dielectric constant  
$\eta$ = Viscosity  
$f(K\alpha)$ = Henry’s function

All measurements were done in triplicate using disposable polystyrene cuvettes (Malvern Instruments, UK).

**% Entrapment efficiency**

The % entrapment efficiency (% EE) of CBZ into SLN was determined by ultra filtration method using centrifugal filter tubes with a molecular weight cut-off of 30 kDa (Amicon Ultra, Millipore, Ireland). The concentration of CBZ in SLN and in the ultra-filtrate (free drug) was analyzed by the method described in previous section. R of CBZ was observed at 8.25 min. The E.E. was calculated using the following equation:

$$\%\, EE = \frac{\text{Total amount of CBZ - Amount of free CBZ}}{\text{Total amount of CBZ}} \times 100$$

Possible lipid interferences with CBZ were also investigated by comparing the standard curves of drug alone and in the presence of lipids. The differences observed between the standard curves were within the experimental error, thus inferring that no lipid interference occurred.

**Determination of residual solvent content**

The residual solvent content in samples were quantified by GC-MS system [Agilent 7890A series (Germany)] equipped with split-split less injector and CTC-PAL auto sampler attached to an apolar HP-5MS capillary column (30 m x 0.25 mm i.d. and 0.25 μm film thickness) and fitted to a mass detector. Carrier gas flow rate (Helium) was 1mL/min, split: split less ratio 1:100, injector temperature was 70 °C, detector temperature 250 °C, while column temperature was kept at 60 °C for 2 min followed by linearly programming from 70 to 230 °C (at rate of 5 °C/min), and then kept isothermally at 230 °C for 2 min. Transfer line was heated at 280 °C. Split ratio was kept at 1:100. Mass spectra were acquired in El mode (70 eV); in m/z range 30-400. The amount of sample was injected through head space. The residual solvent of the
formulation were identified by comparison of their mass spectra to those from Wiley 275 and NIST/NBS libraries, using different search engines.

**Lipid stability by GC-MS study**

For successful formulation of drugs in SLN, the chemical stability of excipients used for particle production is a prerequisite. Chemical analysis of lipids was performed by gas chromatography using a GC-MS system [Agilent 7890A series (Germany)] equipped with split-split less injector and CTC-PAL auto sampler as described previously (Radomska et al., 1999). Determination was performed via the corresponding methyl esters of the fatty acids of the lipids. Transmethylation of the lipids was made according to the method described (Garces and Mancha 1993). In order to separate the lipid from the SLN dispersions, the formulations were centrifuged at 17,000 rpm for 30 min. 50 mg of the obtained lipid phase were mixed with methyleating mixture containing methanol/toluene/dimethoxypropan/H2SO4 (39:20:5:2, by volume) in tubes with teflon caps. The quantity of methyleating liquid was 3.3 mL, heptane was added to a total volume of 5 mL. This mixture was incubated in a water bath at 80 °C for 90 min. The tubes were cooled to room temperature and shaken, two phases were formed. The upper phase contained the fatty acid methyl esters. The fatty acid composition was analyzed using a silica capillary column with high polarity (Supelkowax™10, 30 m×0.32 mm ID, 0.25 μm film thickness, Supelco, Bellefonte, USA). It operated at a constant temperature. The temperature of the injector and the detector (FID) were 200 °C and 220 °C, respectively. The carrier gas was helium at the flow rate of 1.5 mL/min.

**Development of mucoadhesive gel incorporating SLN**

In order to substantially enhance the sustain drug release profile; SLNs were further converted into gels. Carbopols are known to have mucoadhesive properties and is reported to have highest bioadhesion potential (Repka and McGinity 2001). Poloxamer 407 (Pluronic F127) has been used as a thermo-sensitive polymer which shows excellent water solubility, good drug release characteristics, low toxicity and irritation, and has compatibility with other excipients (Zaki et al., 2007). It was carried out for a period of three months. The samples on the plate were noted visually. The residence time of the gel on the plate was studied for following parameters;

**Measurement of SOL-GEL transition temperature (TSOL-GEL)**

In a 20 mL transparent vial containing a magnetic bar in 5 mL gel was placed in a water bath, which was heated at a rate of 2 °C/min with constant (150 rpm) stirring. When the magnetic bar stopped moving due to gelation, the temperature displayed on the thermistor was determined as the gelation temperature.

**In vitro drug release kinetics**

Despite the limits of in vitro dissolution profiles, we may at least extrapolate and predict the relative rates of dissolution in vivo based on the controlled in vitro data. Dissolution studies were performed according to our earlier reported method (Chopra et al., 2007). In brief, dissolution studies were performed using the USP XXVIII, paddle-rotating method (Electrolab dissolution tester, Electrolab, India) at 37 °C ± 0.5 °C and 75 rpm using two dissolution media: phosphate-buffered saline (PBS) containing 1 % Tween 80. The gels were first packed into a dialysis bag and placed into the dissolution media. Dissolution studies were carried out in triplicate, maintaining the sink conditions for all the formulations. The level of CBZ in the media was estimated using the developed HPTLC method. The cumulative % drug release was calculated.

**In vitro bioadhesion study**

Mucoadhesion studies were carried out by slightly modifying a published method (Nakamura et al., 1996). Briefly, an agar plate (1 %, w/w) was prepared in pH 4.5 citrate-phosphate buffer. Small amount of gel was placed at the center of plate. After 5 min, the agar plate was attached to a USP disintegration test (model 1901, Electronics India, India) apparatus vertically and moved up and down (10 ± 1 cycle per minute) in pH 4.5 citrate phosphate buffer at 37 ± 1°C. The sample on the plate was immersed into the solution at the lowest point and was out of the solution at the highest point. The residence time of the gel on the plate was noted visually.

**Accelerated stability studies for determination of shelf life**

It was carried out for a period of three months. The optimized thermo sensitive and mucoadhesive gel (G4) was evaluated for different parameters like physical appearance, pH, drug content uniformity, in vitro bioadhesion and in vitro drug release profile. Since the temperature chosen for the study was high enough to
make its consistency rigid, the accelerated stability studies were carried out by storing the samples (15 g) in collapsible aluminum tube at 40 ± 2 °C, 50 ± 2 °C and 60 ± 2 °C. Tubes were also stored at 2-8 °C as control samples. Samples were withdrawn at intervals of initial (zero day), 30 days, 60 days, and 90 days. For proper withdrawal of samples, tube were cut in a crosswise manner, opened and observed visually and for analysis. For the estimation of drug in the sample, developed HPTLC method was used. The logarithms of percent of drug remained in gels was plotted against time, slope of which was used to calculate degradation rate constant using the formula;

$$\text{Slope} = -\frac{K}{2.303},$$

where $K$ = degradation rate constant.

An Arrhenius plot was drawn for the formulation plotting logarithms of K values against inverse of the absolute temperatures and straight lines were obtained. $K_{25}$ was calculated using the above graph. Shelf life (at room temperature) of the formulation was calculated using below mentioned formula;

$$t_{10\%} = \frac{0.1054}{K_{25}}$$

RESULTS AND DISCUSSION

Selection of lipids

Interaction with bovine serum albumin

Result obtained with the study is shown in Fig. 1. Total volume of sample recovered was less than the volume of BSA used. Hence, the amount of BSA adsorbed by the 1.5 gm of lipid was determined which was in the order of Compritol E ATO > Compritol 888 ATO > Stearic acid > Precirol Cetyl palmitate > Gelucire (39/01) > Gelucire (50/13). Thus, lipids selected for further studies were Compritol E ATO, Compritol 888 ATO, and Stearic acid.

Preferential interaction of BSA with lipoidal colloid particles is well supported by literature (Almeida and Souto 2007). Like, a double hydrogen-bonding mechanism for stearic acid binding to BSA has been described in literature (Ge et al., 1990). The ability of the acid to form two hydrogen bonds apparently fixes it more rigidly in the protein, preventing rotation about either single hydrogen bond. A double-hydrogen bonding mechanism is most consistent with the formation of a salt bridge between the negatively charged carboxylate of the acid and either a positively charged guanidino group of arginine, or the positively charged omega-amino groups of two lysine residues. On the other hand, Compritol 888 ATO (glyceryl behenate) is chemically a mixture of mono-, di- and triglycerides of behenic acid (C22). It is also expected to interact with BSA by formation of some hydrogen bonds.

![Figure 1: Differential interaction of BSA with different lipids available. Differential affinity is observed in mg of BSA adsorbed per gm of lipid. Results are given on average scale, omitting standard deviation.](image1)

Selection of surfactant

A nanosuspension with high % Transmittance may be correlated with smaller particle size and maximum adsorption of surfactants over colloidial particles. Maximum transmittance was observed with Poloxamers, 82.6 % with Poloxamer 407 and 81.3 % Poloxamer 188. But for further studies a $S_{mix}$(5 % P 188 + 0.5 % Na Taurocholate) was chosen because the combination gave negligible decrease in % Transmittance after 3 days (Fig. 2).

![Figure 2: % transmittance of a model SLN suspension using different surfactant (s). Values were taken just after preparation and after 3 days.](image2)

Particle size and zeta potential ($\zeta$) analysis

Obtained zeta potentials, exhibit sufficient stability of the SLNs. Lipid particles are generally negatively charged and colloidal system is found to be stable when $\zeta$ ($>30\text{mV}$) due to electrostatic repulsion (Levy et al., 1994). This is valid only in case of sole electrostatic stabilization. Electrostatic stabilization and stearic stabilization effects are additive. Hence for the development of SLN we have taken a combination of surfactants (Poloxamer and sodium taurocholate). It has also been suggested that without steric stabilization, a colloidal system would not be stable even if $\zeta$ value is greater than 30 mV (Tadros and Vincent, 1980). A certain thickness of polymer layer is
necessary (>10 nm) for efficient and complete stearic stabilization (Washington, 1996). According to the theory of DLVO, system could be regarded as stable if the electrostatic repulsion dominated the attractive Van der Waals forces. Non ionic surfactants contribute to the stability by offering stearic stabilization while ionic surfactants contribute by generation of electric double layer. Increasing amount of stearic stabilizer (P 188) was also found to increasing zeta potential values (Table 1). Non ionic surfactants couldn't ionize like ionic surfactants but demonstrated its role in zeta potential. The reason might be due to molecular polarization and the adsorption of emulsifier molecule on the charge in water, it was absorbed to the emulsifier layer of particle/water interface and electric double layer similar to ionic was found. Like in the case of P 188, less polar poly (propylene oxide) chain were dissolved and segregated into a hydrophobic micelle core surrounded by a soft “brush” of highly hydrated flexible poly (ethylene oxide) chain. Average size of the particles and their size distribution are also given in Table 1. Mean particle size was bigger when stearic stabilizer was used alone. In the given set of experiments, we achieved a size range of 250.2-546.9 nm which is acceptable in case of nasal drug delivery. From the table it is evident that with the increment of surfactant, size of the particle is falling sharply. Using lipidic mixture had also a positive effect on decreasing the size of the particle. The difference in polarity of the molecules (stearic acid Vs Compritol) should have a minor influence on the particle size as the interfacial phenomena are supposed to be dominated by the properties of the emulsifier.

**Table 1.** Details of different trials and evaluation of SLNs formed in terms of % entrapment efficiency (% EE), mean particle size, particle distribution index (PDI) and change in Gibb's free energy ($\Delta G^{298} _{sol}$) at room temperature.

<table>
<thead>
<tr>
<th>Trials</th>
<th>Lipid (gms)</th>
<th>Surfactant (% w/v)</th>
<th>% EE</th>
<th>Drug content</th>
<th>Mean particle size (nm)</th>
<th>Zeta potential (mV)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 1</td>
<td>CT E ATO 5% Plxr188 + 0.5% Sod.Trchl</td>
<td>84.55± 0.08</td>
<td>17.68 ± 0.01</td>
<td>250.2</td>
<td>-41.3±1.8</td>
<td>0.292</td>
<td></td>
</tr>
<tr>
<td>T 2</td>
<td>CT E ATO 3% Plxr188 + 0.5% Sod.Trchl</td>
<td>83.23 ± 0.24</td>
<td>17.68 ± 0.03</td>
<td>285.3</td>
<td>-37.1±2.6</td>
<td>0.264</td>
<td></td>
</tr>
<tr>
<td>T 3</td>
<td>CT 888 3% Plxr188 + 0.5% Sod.Trchl</td>
<td>83.91± 1.03</td>
<td>17.92 ± 0.24</td>
<td>503.1</td>
<td>-36.6±2.2</td>
<td>0.538</td>
<td></td>
</tr>
<tr>
<td>T 4</td>
<td>CT 888 5% Plxr188 + 0.5% Sod.Trchl</td>
<td>81.21 ± 0.62</td>
<td>17.13 ± 0.67</td>
<td>467.6</td>
<td>-39.8±0.8</td>
<td>0.468</td>
<td></td>
</tr>
<tr>
<td>T 5</td>
<td>CT 888 + CT E ATO 3% Plxr188 + 0.5% Sod.Trchl</td>
<td>87.38 ± 0.31</td>
<td>19.74 ± 0.07</td>
<td>306.4</td>
<td>-37.4±1.7</td>
<td>0.295</td>
<td></td>
</tr>
<tr>
<td>T 6</td>
<td>CT 888 + CT E ATO 4% Plxr188 + 0.5% Sod.Trchl</td>
<td>86.90 ± 0.32</td>
<td>18.76 ± 0.07</td>
<td>309.1</td>
<td>-42.5±0.9</td>
<td>0.466</td>
<td></td>
</tr>
<tr>
<td>T 7</td>
<td>SA + CT E ATO 2% Plxr188 + 0.5% Sod.Trchl</td>
<td>88.01 ± 0.83</td>
<td>20.46 ± 0.20</td>
<td>546.9</td>
<td>-35.4±1.4</td>
<td>0.561</td>
<td></td>
</tr>
<tr>
<td>T 8</td>
<td>SA + CT E ATO 3% Plxr188 + 0.5% Sod.Trchl</td>
<td>88.18 ± 0.20</td>
<td>± 20.13 ± 0.04</td>
<td>311.0</td>
<td>-39.7±3.3</td>
<td>0.421</td>
<td></td>
</tr>
<tr>
<td>T 9</td>
<td>SA + CT E ATO 4% Plxr188 + 0.5% Sod.Trchl</td>
<td>86.67 ± 0.32</td>
<td>18.73 ± 0.08</td>
<td>325.9</td>
<td>-43.8±4.1</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>T 10</td>
<td>SA + CT 888 3% Plxr188 + 0.5% Sod.Trchl</td>
<td>90.63 ± 0.48</td>
<td>± 20.59 ± 0.11</td>
<td>331.4</td>
<td>-32.6±2.6</td>
<td>0.473</td>
<td></td>
</tr>
<tr>
<td>T 11</td>
<td>SA + CT 888 4% Plxr188 + 0.5% Sod.Trchl</td>
<td>87.52 ± 0.55</td>
<td>± 19.02 ± 0.13</td>
<td>381.4</td>
<td>-36.7±1.8</td>
<td>0.430</td>
<td></td>
</tr>
<tr>
<td>T 12</td>
<td>SA + CT 888 5% Plxr188 + 0.5% Sod.Trchl</td>
<td>88.66 ± 0.09</td>
<td>± 18.74 ± 0.12</td>
<td>408.9</td>
<td>-40.3±3.2</td>
<td>0.603</td>
<td></td>
</tr>
</tbody>
</table>

**% Entrapment efficiency**

The outcome of % EE was in accordance with the results obtained from XRD and DSC studies i.e. as we break the crystalline lattice of solid by developing a mixture, we could accommodate more amount of drug. Highest $d$ values were noted with mixtures of SA: CT E ATO and SA: CT 888 which translated into maximum entrapment of drug in the lattice.
Enthalpy (ΔH), which is an indirect estimation of crystallinity of the material, was lowest in case of these two mixtures. Thus, we may funnel out a conclusion that by above given method we may develop substantial imperfections in a carrier which results into higher drug loading. Considering the different parameters T 10 was selected as an optimized SLN.

**Determination of residual solvent content**

Dichloromethane was found to be absent in formulations. Recommended limit of ICH Q3C guideline (ICH, 1997) in case of Dichloromethane is 600 ppm. And the residual amount limit in USP XXIII is 500 ppm.

**Lipid stability by GC-MS study**

After keeping the SLN at room temperature for 3 months, lipid stability was performed. Data obtained are given in Table 2. There is no major decomposition in any case. This could be attributed to the fact that surfactant concentration was low and incubation time was small.

**Table 2.** The percentage of the lipid content in SLN formulations (initially and after 3 months) of the incubation at 25 °C.

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Lipid content (initially) in %</th>
<th>Lipid content (after 3 months) in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T 1</td>
<td>99.32</td>
<td>98.6</td>
</tr>
<tr>
<td>T 2</td>
<td>100.0</td>
<td>99.3</td>
</tr>
<tr>
<td>T 3</td>
<td>98.16</td>
<td>96.43</td>
</tr>
<tr>
<td>T 4</td>
<td>97.3</td>
<td>96.7</td>
</tr>
<tr>
<td>T 5</td>
<td>96.42</td>
<td>96.2</td>
</tr>
<tr>
<td>T 6</td>
<td>98.44</td>
<td>97.32</td>
</tr>
<tr>
<td>T 7</td>
<td>100.0</td>
<td>98.7</td>
</tr>
<tr>
<td>T 8</td>
<td>96.7</td>
<td>95.2</td>
</tr>
<tr>
<td>T 9</td>
<td>98.6</td>
<td>96.4</td>
</tr>
<tr>
<td>T 10</td>
<td>99.2</td>
<td>97.5</td>
</tr>
<tr>
<td>T 11</td>
<td>100.0</td>
<td>98.5</td>
</tr>
<tr>
<td>T 12</td>
<td>100.0</td>
<td>97.6</td>
</tr>
</tbody>
</table>

**Measurement of SOL-GEL transition temperature (T sol-gel)**

The gelation temperature is defined as the point where the elasticity modulus is half way between the values for the solution and for the gel. Carbopol has been reported to interact strongly with low molecular weight drugs and causes precipitation (Blanco-Fuente et al., 2002). However, the physicochemical status of drug or SLN may not affect gelling temperature significantly. The drug was entrapped inside the lipid core and hence not available for any interaction. Different gelation temperatures are given in Table 3. With increase of fraction of P 407, gelation temperature was falling down. Even at higher concentration it may happen at 4 °C. Gelation phenomenon is a result of body centered cubic packing of spherical micelles. Temperature plays an important role in the micelle formation through temperature dependent hydration of the ethylene oxide units. Water is a good solvent for PEO as well as PPO chains of polymer at low temperatures. However, at higher temperature the solubility of PPO is reduced and micelle formation occurs. At low temperatures in aqueous solutions, a hydration layer surrounds P 407 molecules. However, when the temperature is raised, the hydrophilic chains of the copolymer become desolvated as a result of the breakage of the hydrogen bonds that had been established between the solvent and these chains. This phenomenon favors hydrophobic interactions among the polyoxypropylene domains, and leads to gel formation.

**Table 3.** Gelation temperature of different compositions of gel.

<table>
<thead>
<tr>
<th>Code</th>
<th>Components (% w/v)</th>
<th>Gelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>G 1</td>
<td>0.2% CP 934, 15% P 407</td>
<td>41</td>
</tr>
<tr>
<td>G 2</td>
<td>CBZ-SLN (1% w/v), 0.2% CP</td>
<td>40</td>
</tr>
<tr>
<td>G 3</td>
<td>CBZ-SLN (1% w/v), 0.2% CP</td>
<td>38</td>
</tr>
<tr>
<td>G 4</td>
<td>CBZ-SLN (1% w/v), 0.2% CP</td>
<td>35</td>
</tr>
</tbody>
</table>

At low pH (about pH 3) the carbopol chains exist in a spiral coiled form, exhibiting a relatively low viscosity. With the progression of pH, the carboxyl groups of the Carbopol become ionized, causing an increased repulsion of negative charges. It leads the molecular structure to unwind and a gradual rise in viscosity.

**In vitro drug release kinetics**

The release behavior (Fig. 3) could be helpful in predicting the gel’s performance. Highest release (72.4 % in 20 hrs) was achieved by G2, followed by G3 (66.6 % in 20 hrs) and then G4 (62.2 % in 20 hrs). A faster release of CBZ expected in vivo because of the presence of salts and proteins. Error bars have been removed from the figure to make the data more understandable. All the three formulations were showing a sustained release profile. One unexpected phenomena observed in the formulations were an initial burst release, which was not expected considering the presence of drug in solid lipidic core and then dispersed in gel matrix. Since we had allowed stirring the final formulation for hours, some amount of drug must have oozed out from the lipidic core and dispersed in gel matrix. So, during release study, this fraction of drug was responsible for burst release. With progression of time, all the formulations achieved a sustained release profile. Concentration of polymer was also found to have role in release profile. With increasing polymer (P 407) concentration (15%–18%), drug release was found to be decreasing. More concentrated gels were
reported to dissolve at a slower rate than less concentrated ones because of the decreased water diffusion through the gel. Dissolution of polymer is actually a controlling factor in drug release but it is not the only factor also, because the surface is eroding at a constant rate. It is actually a two step process. Other than burst release, the drug molecule first oozes out from the solid lipid core then diffuse through gel matrix. Hence considering the gelation temperature and in vitro drug release, G4 was chosen for further evaluation.

CONCLUSIONS

The present research work proposed a lipid nanoparticulate drug delivery system (SLNs) for intranasal delivery of CBZ. SLNs were prepared by the emulsification technique and evaluated for particle size, particle size distribution (PDI), zeta potential, entrapment efficiency, in vitro release. All measurements were found to be in an acceptable range. In vitro drug release was found to be 62.2% of CBZ in 20 hrs, indicating a controlled and sustained release profile of CBZ-SLNs gel. It was concluded that CBZ-SLNs could be an effective drug delivery system for effective landing of CBZ to brain via nose.

CONFLICT OF INTEREST

None declared.

REFERENCES


Ge MT, Rananavare SB, Freed JH. ESR studies of stearic acid binding to bovine serum albumin. Biochimica et biophysica acta. 1990;36(3):228-36.


Levy MY, Schutze W, Fuhrer C, Benita S. Characterization of diazepam submicron emulsion


