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Preliminary Study on the Development of Nanoemulsions for Carbamazepine Intravenous Delivery: An Investigation of Drug Polymorphic Transition

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Carbamazepine (CBZ) is available on the pharmaceutical market as tablets, capsules, and oral suspensions, but not as a parenteral formulation for clinical use. Parenteral emulsions are a good alternative to poorly water-soluble drugs such as CBZ. In this way, four different emulsions containing 3 mg/mL of CBZ were developed, but during a period of storage, drug crystal precipitates appeared. To investigate this phenomenon, differential scanning calorimetry, infrared spectroscopy, and light microscopy were employed. The results suggested a polymorphic transition from β form to dehydrate form, resulting in drug precipitation, although the emulsions themselves remained stable for at least three months.

Keywords intravenous nanoemulsions; carbamazepine; polymorphism

INTRODUCTION

Carbamazepine (CBZ) is used for the management of epilepsy and several psychiatric diseases (Genaro, 2000; Goodman, Gilman, Hardman, & Limbird, 2001; Sweetman, 2006). The drug is available to patients as tablets, capsules, and oral suspensions (Food and Drug Administration, 2006). However, up till now, there has been no parenteral formulation commercially available, which would be desirable in cases when a parenteral administration is necessary, such as coma, swallowing problems, or emergencies.

Several investigations have been undertaken over the last 30 years with the aim to develop intravenous pharmaceutical dosage forms for CBZ delivery. The use of organic cosolvents, complexation with cyclodextrins and the incorporation of CBZ in emulsions, was investigated (Akkar & Müller, 2003; Becirevic-Lacan, Jug, Bacic-Vrca, & Cetina-Cizmek, 2002; Brewster, Anderson, Estes, & Bodor, 1997; Levy, Lockard, Green, Friel, & Martis, 1975; Loscher et al., 1995; Sznitowska, Janicki, Dabrowska, & Zurowska-Pryczkowska, 2001; Tauboll, Lindström, Klem, & Gjerstad, 1990).

Intravenous emulsions are a source of calories and essential fatty acids for patients, and for at least 50 years, their low toxicity and effectiveness for drug solubilization make them a good alternative to the delivery of poorly water-soluble drugs (Benita & Levy, 1993) such as CBZ. In recent years, three studies describe the incorporation of CBZ into preformed emulsions as a strategy for the drug parenteral administration. Sznitowska and colleagues (2001) evaluated the presence of 35 drugs, including CBZ, on the stability of parenteral fat emulsions. Formulations contained 20% or 5% soybean oil, 1.2% lecithin, and 2.2% glycerol, and were prepared by the classical method (formation of a primary emulsion at 80ºC with help of a high shear mixer, followed by high pressure homogenization). With CBZ emulsion, recrystallization was observed after autoclaving.

In another study, Becirevic-Lacan and colleagues (2002) prepared emulsions with either CBZ alone or CBZ complexed

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with hydroxypropyl-β-cyclodextrin (HPβCD) by mixing oil (isopropyl miristate), emulsifier (polysorbate 20), and water phases under speed stirring. The authors observed that the presence of HPβCD enhanced CBZ release from the emulsions. Nevertheless, the formulations presented a mean droplet size ranging from 598 nm to 1.638 μm, not being recommended for parenteral administration, and were physically stable for only one month.

Akkar and Müller (2003) proposed the use of a patented technology (SolEmuls®) that, according to the authors, promotes the localization of drugs in the interfacial area of emulsions by adding a finely dispersed drug powder (nanocrystals) into a preformed emulsion (Intralipid®) followed by high-pressure homogenization. This technology was able to incorporate 1 mg/mL of CBZ into emulsions, but the authors suggested that 3.0 mg/mL would result in an acceptable volume for intravenous bolus administration, considering the drug blood profile. The preformed emulsion employed (Intralipid®) is composed of medium chain triglycerides and egg lecithin, which comprise most of the lipophilic components employed in the currently available parenteral nanoemulsions. Drug precipitation was observed in higher drug loadings (5 mg/mL and 10 mg/mL) and no other oils and emulsifiers have been tested.

In the present work, we continued investigations in order to obtain feasible 3 mg/mL CBZ nanoemulsions by testing different oil cores and emulsifiers, as well as by employing a spontaneous emulsification process. As far as we are concerned, this method has not been investigated with the goal of developing CBZ submicron emulsions up till now.

Unfortunately, after a short period of storage, drug crystals could be observed at the bottom of the storage vials. In fact, previous literature reported the occurrence of CBZ polymorphic transitions in an aqueous environment (Kaneniwa, Yamaguchi, Watari, & Otsuka, 1984; Kaneniwa et al., 1987; Kobayashi, Ito, Itai, & Yamamoto, 2000; Krahn & Mielck, 1987; Lowes, Caira, Lotter, & Vanderwatt, 1987; Otsuka, Ofusa, & Matsuda, 2000; Ruschichelli et al., 2000; Tian et al., 2006a). Considering that no investigation concerning this phenomenon in nanoemulsions has been performed up till now, we aimed to study this event by optical microscopy, thermal analysis, and infrared (IR) spectroscopy.

MATERIALS AND METHODS

Materials

Bulk CBZ (99.1%) was purchased from Henrifarma (São Paulo, Brazil). Ultrapure water was obtained from a Milli-Q® Plus apparatus by Millipore (Billerica, USA). The excipients tested for the preparation of emulsions were soybean lecithin (Lipoid S75®) and medium-chain triglycerides (MCT), kindly gifted by Lipoid GmbH (Ludwigshafen, Germany); purified castor oil, soybean oil, and olive oil, purchased from Sigma Aldrich (Seelze, Germany); purified sesame seed oil, peanut oil, polyoxyl 35 castor oil (Etocas 35 HV®), and polysorbate 80 (Crillet 4®), kindly donated by Croda (Campinas, Brazil); and glycerol purchased from Nuclear (São Paulo, Brazil). All other reagents were of analytical grade.

Determination of CBZ Solubility in Different Oils

The selected oils were castor oil, olive oil, soybean oil, sesame seed oil, peanut oil, and MCT. An excess amount (10 mg) of CBZ was added to 5 g of each oil and kept under moderate magnetic stirring at room temperature for 24 hours. The equilibrated sample was centrifuged at 15,000 rpm for 20 minutes to separate the undissolved drug. The choice of the best oil was made by visual observation, using a qualitative criteria based on United States Pharmacopoeia's (USP) "Description and Relative Solubility" chapter (USP, 2005).

Preparation of CBZ Nanoemulsions

Preliminary tests were performed in order to obtain feasible formulations. These included the assessment of organic solvent proportions as well as the need of a cosurfactant (hydrophilic emulsifiers) in order to stabilize the emulsions. Four different formulations were prepared using different combinations of oil (castor oil or MCT) and lipophilic emulsifier (soybean lecithin or polyoxyl 35 castor oil). The formulations were named as follows: the emulsion composed by castor oil and soybean lecithin, C-L; composed by castor oil and polyoxyl 35 castor oil, C-C35; composed by MCT and soybean lecithin, MTC-L; and composed by MCT and polyoxyl 35 castor oil, MTC-C35. Polysorbate 80 was used in all formulations as hydrophilic surfactant. The final composition of emulsions was made up of 10% (w/v) oil, 4% (w/v) lipophilic surfactant, 4% (w/v) hydrophilic surfactant, 2.25% (w/v) glycerol, and up to 100% (w/v) distilled water.

The emulsions were prepared according to the method described by Yu and colleagues (1993). Briefly, CBZ was dispersed in the oil, mixed with the lipophilic emulsifier, and dissolved in an acetone:ethanol (50:50 v/v) solution (oil phase). The hydrophilic emulsifier was dissolved in water (aqueous phase). The oil phase was then slowly added into the aqueous phase under moderate magnetic stirring. The aqueous phase immediately turned milky with opalescence as a result of the nanoemulsion produced. The solvents were removed under reduced pressure. The amount of CBZ added to the preparations corresponded to 3.0 mg/mL of drug content of the final formulation. The emulsions were stored at 4°C.

Characterization of Nanoemulsions

The formulations were characterized with respect to physical appearance and mean particle size, which was assessed in a Malvern Nanosizer/Zetasizer® nano-ZS ZEN 3600 (Malvern Instruments, USA). All analyses were made in triplicate after adequate dilution in ultra-filtered purified water (0.22 μm).

Characterization of CBZ Polymorphic Transition

Drug crystal precipitates observed in the emulsions were collected after three months and dried in a vacuum desiccator for further analysis by differential scanning calorimetry (DSC), IR spectroscopy, and optical microscopy. In DSC analysis, a Shimadzu DSC-60 cell was used, and approximately 2 mg of samples were weighed out and placed in sealed aluminum pans. An empty aluminum pan was used for reference. The temperature range was 25°C to 500°C, with a heating rate of 10°C/minute in dynamic nitrogen atmosphere with the flow rate of 50 mL/minute. Fourier transform infrared (FTIR) spectra were recorded on a Perkin-Elmer Model 1600 apparatus using KBr discs in the range of 4000 cm^{-1} to 400 cm^{-1}. All results were compared to that of bulk CBZ. The morphology of the crystals was observed in a light microscopy Olympus PME 3 associated with a Moticom 1300 camera and a Moticom Images Advanced 3.1. software, which allowed the registration of the images.

RESULTS AND DISCUSSION

Solubility Study of CBZ

The solubility of CBZ in different oils was assessed in order to determine the optimal oil phase. Since CBZ is sparingly soluble in water, it is desirable to achieve its greatest solubility in the oil core of the nanoemulsion. As can be observed in Table 1, among the investigated oils, CBZ was more soluble in MCT and castor oil. In this way, these two oils were chosen for investigation.

TABLE 1
Solubility of CBZ in Various Oils at 25°C

Oil	CBZ Solubility
Castor oil	++
MCT	++
Soybean oil	+
Olive oil	+/–
Peanut oil	––
Sesame seed oil	––

++ sparingly soluble; +slightly soluble; +/– very slightly soluble; –– practically insoluble.

TABLE 2
Particle Size (nm) and Polydispersity Index Measures for CBZ Nanoemulsions (3.0 mg/mL)

Formulations	Droplet Size (nm)	Polydispersity Index (PI)
C-L	157.5 ± 1.0	0.18 ± 0.004
C-C35	209.9 ± 0.7	0.26 ± 0.012
MCT-L	113.4 ± 1.7	0.37 ± 0.048
MCT-C35	175.2 ± 4.3	0.09 ± 0.042

Characterization of CBZ Nanoemulsions

Particle size is the most important physicochemical property of emulsions because it dictates the compatibility with the parenteral route. According to Table 2, the mean droplet size of emulsions ranged from about 113 nm to 210 nm, while the polydispersity index (PI) varied from 0.09 to 0.37, in agreement with results previously reported for emulsions obtained by spontaneous emulsification procedure (Bouchemal, Briançon, Perrier, & Fessi, 2004; Yu et al., 1993). Nevertheless, after approximately one week under storage, it was possible to observe the beginning of drug precipitation at the bottom of the container. In order to have a better insight into whether drug polymorphism could have originated this phenomenon, drug crystals were investigated by DSC, IR spectroscopy, and optical microscopy.

The DSC characterization of CBZ bulk powder presented in Figure 1 shows a first event corresponding to the melting of β form (174.6°C), followed by exothermic crystallization as α polymorph (175.8°C), which subsequently melted at 190.6°C (Kobayashi et al., 2000; Koester, Mayorga, Pereira, Petzhold, & Bassani, 2003; Rustischelli et al., 2000). The DSC analysis of the precipitated crystals (Figure 1) shows that all samples have a similar behavior, but are different from the bulk

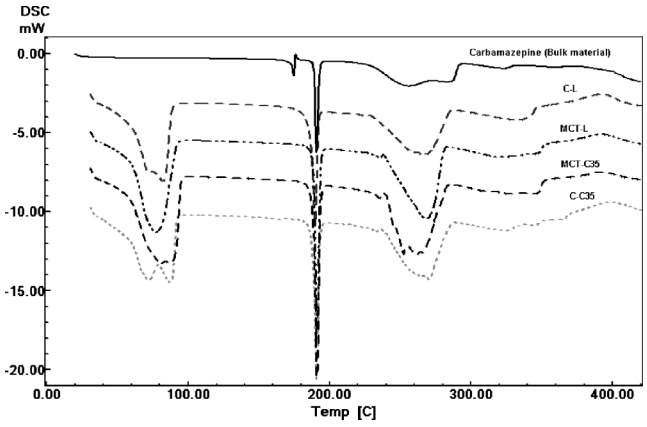

FIGURE 1. DSC thermal profiles of CBZ bulk material and precipitated crystals from emulsions at the heating rate of 10°C min^{-1}.

material. A large endothermic event can be observed between 54.1ºC and 99.4ºC, which corresponds to the water associated to the crystals. A second endothermic event, the melting point of CBZ, occurs at 190.7ºC, followed by sample degradation.

The analysis of CBZ crystals by DSC suggests that the polymorph found on the crystals would no longer be the β form previously characterized on the bulk powder but the dehydrated form (Han & Suryanarayanan, 1997; Nair, Gonen, & Hoag, 2002; Otsuka, Ofusa, & Matsuda, 1999). It is important to notice that the β form is the polymorph indicated by USP (Gosselin, Thibert, Preda, & Mcmullen, 2003; Phadnis, Cavatur, & Suryanarayanan, 1997). This polymorphic transition probably occurred because CBZ is in an aqueous environment, and it is reported that polymorphic forms α and β convert to the dihydrate under this condition (Nair et al., 2002; Otsuka et al., 1999; Tian et al., 2006a).

Since DSC may not provide enough evidence of the existence of polymorphism, even though CBZ thermal behavior has been fully reported, including the influence of the operative heating rate (Lowes et al., 1987; Nair et al., 2002; Otsuka et al., 1999; Rustichelli et al., 2000), an FTIR analysis was also performed, which corroborated the DSC results. The FTIR analysis (Figure 2) showed a sharp peak at 3460 cm^{-1} (-NH valence vibration) on the bulk material, characteristic of anhydrous CBZ. The crystal analysis demonstrated the presence of a broad peak in the OH stretching region of 3300 cm^{-1} to 3400 cm^{-1}. Bands at 3460 cm^{-1}, 1674 cm^{-1} (-CO-R vibration), 1603 cm^{-1}, and 1593 cm^{-1} (range of -C = C- and -C = O

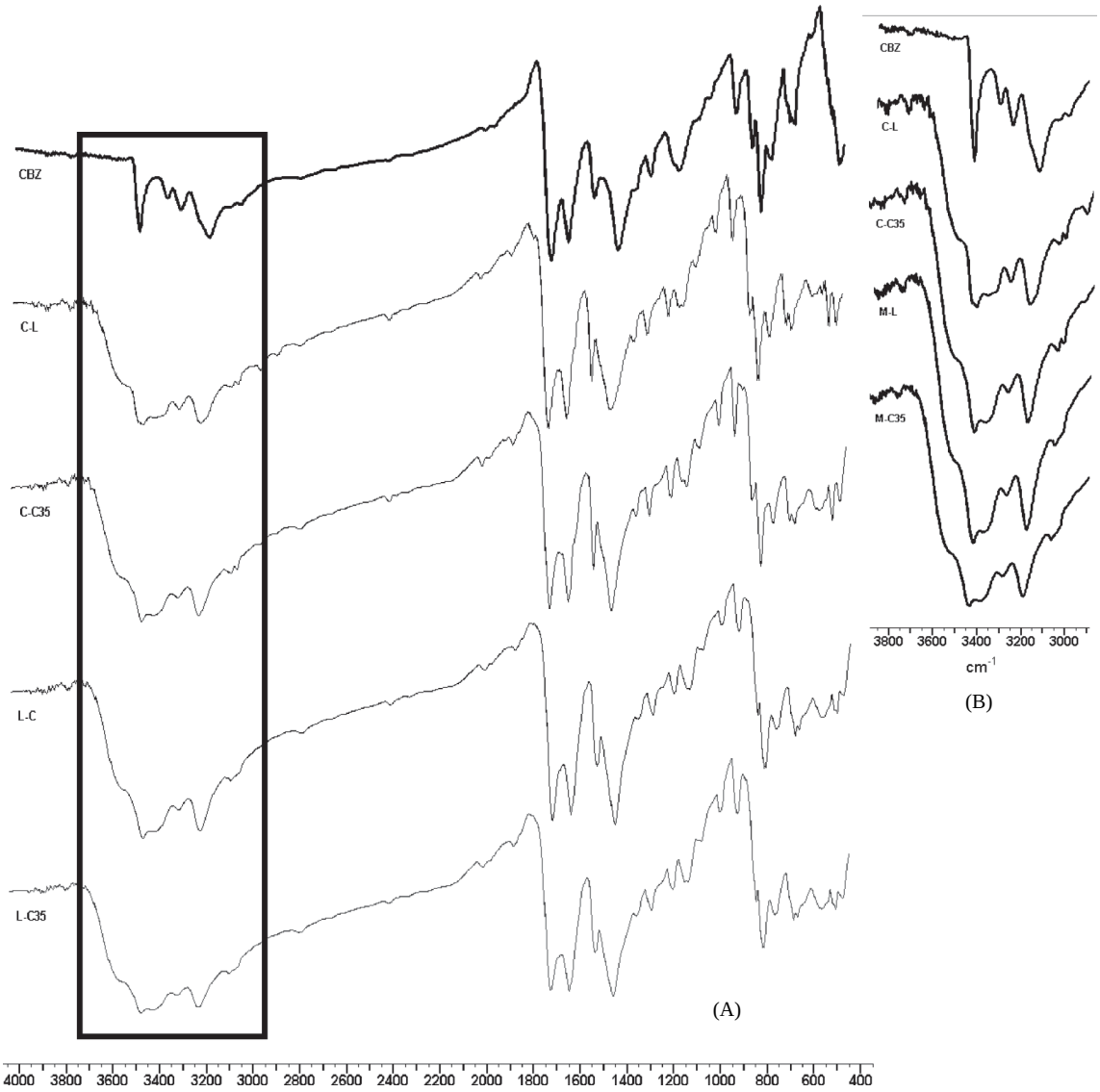

FIGURE 2. (A) FTIR spectra for the precipitated crystals from the four nanoemulsions developed and from the bulk material. (B) Enlarged OH stretching region (3000 cm^{-1}–3800 cm^{-1}).

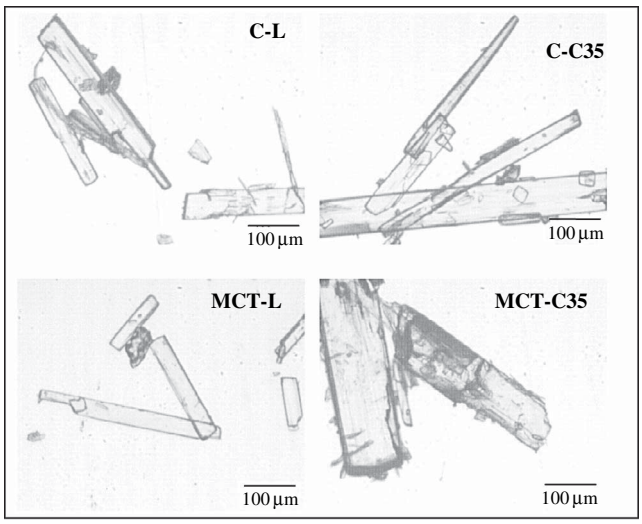

FIGURE 3. Photomicrographs of CBZ crystals from all developed nanoemulsions.

vibration and -NH deformation) could be seen in all samples (Koester et al., 2003; Ruschichelli et al., 2000; Tian et al., 2006a). The probable loss of the water associated with the crystals is observed by the broad peak on the OH stretching region. This broad peak overlaps the -NH vibration (Reffner, Seelenbinder, & Tobler, 2005).

Moreover, the photomicrographs of the crystals are shown in Figure 3 and reveal that all crystal samples have a needlelike morphology, characteristic of the dehydrated form, different from the β-polymorph, which presents a prismatic morphology (Luhtala, 1992; Murphy, Rodríguez-Cintrón, Langevin, & Kelly, 2002; Tian et al., 2006a; Tian et al., 2006b).

The results obtained in this work suggest that drug precipitation is a result of a polymorphic transition, as dihydrate is the less soluble form of CBZ and the solubility of the anhydrous CBZ is approximately twice that of its dihydrate form (Kobayashi et al., 2000; Luhtala, 1992). This polymorphic transformation may be a result of CBZ association within the oil-water interface. This is reported for drugs like CBZ, which are poorly soluble in water as well as in the registered oils (Akkar & Müller, 2003; Yu et al., 1993). Akkar and Müller (2003) attributed this preferred location to CBZ solubility as well as to the method employed (high-pressure homogenization), but according to the present results, it is suggested that a spontaneous emulsification method leads to the same phenomenon.

CONCLUSION

In conclusion, the viability of producing 3.0 mg/mL CBZ nanoemulsions was not achieved. A late-appearing drug precipitation was detected in the aqueous phase, which was characterized as the less-soluble polymorphic form of CBZ, the dihydrate. Additional studies are under development to optimize a suitable submicron emulsion for parenteral delivery of CBZ.

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