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Carbamazepine parenteral nanoemulsions prepared by spontaneous emulsification process

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Abstract

Carbamazepine (CBZ), a widely used anticonvulsant drug, is a poorly soluble drug with no parenteral treatment available for patients. This study was aimed at developing a nanoemulsion for CBZ intravenous delivery. The spontaneous emulsification method was used to prepare different formulations containing 2 mg/mL CBZ. Likewise, a 2^2 full factorial experimental design was applied to study the influence of two independent variables (type of oil and type of lipophilic emulsifier) on emulsion physicochemical characteristics. The nanoemulsions were evaluated concerning droplet size, zeta potential, viscosity, drug content and association to oily phase. The formulation, which presented the best characteristics required for intravenous administration was selected and refined with respect to the lipophilic emulsifier content (increase from 5% to 6% of soy lecithin). This formulation was characterized and kept its properties in a satisfactory range over the evaluated period (3 months), i.e. droplet size around 150 nm, drug content around 95% and zeta potential around −40 mV. The transmission electron microscopy revealed emulsion droplets almost spherical in shape with an amorphous core, whereas the *in vitro* release profile assessed by dialysis bags demonstrated a release kinetics square root time dependent, with 95% of ca. having been released within 11 h.

Keywords: Carbamazepine; Nanoemulsions; Intravenous; Spontaneous emulsification; Factorial design

1. Introduction

Carbamazepine (CBZ), 5*H*-dibenz(*b*,*f*)azepine-5-carboxamide (Fig. 1), is a widely used antiepileptic agent which has been effective in the therapy of psychomotor seizures and trigeminal neuralgia for 40 years (Genaro, 2000; Goodman et al., 2001; Sweetman, 2006). Due to its poor water solubility (<200 μg/mL), the drug has a slow and irregular gastrointestinal absorption (Lund, 1994; Kobayashi et al., 2000; Sethia and Squillante, 2002). To date, CBZ is only available in the pharmaceuticals market in tablets (immediate release, controlled release and chewable), capsules and oral suspensions (FDA, 2006a). No parenteral formulation is offered to patients, which could prove quite helpful in cases such as emergencies, coma, swallowing problems, among other circumstances.

Over the last three decades, some authors have investigated strategies to administer CBZ to the intravenous route, such as applying co-solvents (Levy et al., 1975; Tauboll et al., 1990) and complexation with hydroxypropyl-β-cyclodextrin (HPβCD) (Löscher et al., 1995; Brewster et al., 1997; Löscher and Hoenack, 1997). Nevertheless, these approaches are of limited success, since solvents can be dose limiting due to their toxicity, and few products based on complexation with cyclodextrins are available on the pharmaceuticals market (Thompson and Chaubal, 2002; Chaubal and Roseman, 2006).

Recently, the preparation of submicron emulsions has emerged as a promising alternative for CBZ intravenous administration (Sznitowska et al., 2001; Becirevic-Lacan et al., 2002; Akkar and Müller, 2003). Emulsion formulations offer an appealing alternative for the administration of poorly water soluble drugs due to their effectiveness for drug solubilization, potential for improved efficacy and anticipated patient acceptance and compliance due to the reduced side effects (Constantinides et al., 2004). Moreover, lipid emulsions have

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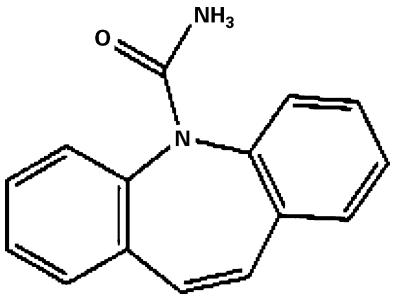

Fig. 1. Structure of carbamazepine.

been used for at least 30 years in patients requiring parenteral nutrition (Washington, 1996; Floyd, 1999).

Concerning lipid emulsions and CBZ, the use of classical methods (Sznitowska et al., 2001; Becirevic-Lacan et al., 2002) and a patented technology, which uses high-pressure homogenization (SolEmuls®) (Akkar and Müller, 2003) have been already reported in the literature. The development of parenteral nanoemulsions through spontaneous emulsification and the testing of different oils and emulsifiers comprise another strategy, which is currently under investigation by our research group.

In the spontaneous emulsification method, the addition of the solvent–oil solution results in the emulsification of the oily phase into nanodroplets, due to some kind of interface instability originating from rapid diffusion of the solvent across the interface and decrease of the interfacial tension (Vitale and Katz, 2003; Bouchemal et al., 2004). This method is receiving increased attention and is interesting for formulation studies as it is easy to perform in laboratory scale, does not require sophisticated equipment nor the use of high temperature, and generally leads to the formation of small droplet size formulations, among other advantages. Droplet size and distribution are strongly affected by the nature of the solvent used during the process (Bouchemal et al., 2004), and considering its toxicity, those belonging to Class II or III from European Pharmacopoeia should be preferred.

In a previous investigation, the solubility of CBZ in different oils used in parenteral formulations was studied (medium chain triglycerides, castor, soybean, olive, peanut, sesame seed oils). Its solubility was higher in castor oil followed by medium chain triglycerides (MCT), and CBZ nanoemulsions were prepared with these oils through spontaneous emulsification (Kelmann et al., 2007a).

A factorial design is frequently used for planning this type of research as it provides the maximum amount of information, yet requires the least number of experiments (Sucker, 1971; Montgomery, 1991). Factorial design enables all factors to be varied simultaneously, allowing for the quantification of the effects caused by independent variables as well as the interactions between them. Factors might represent a continuous quantity or categories, and optimization can be performed through sequential experiments.

In the present study, a preliminary factorial design was proposed to investigate the influence of the category's type of oil and type of lipophilic emulsifier, with the intention of developing CBZ nanoemulsions through spontaneous emulsification. In a second step, the formulation, which presented the best characteristics required for intravenous administration were selected and refined so as to improve its physicochemical stability.

2. Materials and methods

2.1. Materials

The CBZ reference standard (99%) was obtained from Sigma–Aldrich (São Paulo, Brazil). CBZ raw material was purchased from Henrifarma (São Paulo, Brazil). Ethanol and acetone were purchased from Vetec (São Paulo, Brazil). Excipients used in the preparation of nanoemulsions were: soybean lecithin (Lipoid S75®) and medium chain triglycerides (MCT), kindly donated by Lipoid GmbH (Ludwigshafen, Germany); purified castor oil, purchased from Sigma–Aldrich (Seelze, Germany); polyoxyl 35 castor oil (Etocas 35 HV®) and polysorbate 80 (Crillet 4®), donated by Croda (Campinas, Brazil) and glycerol purchased from Nuclear (São Paulo, Brazil). All other reagents were of analytical grade. Water used in the preparation of formulations was distilled whereas in HPLC analyses water was obtained from a Milli-Q® Plus apparatus (Millipore, Billerica, USA).

2.2. Determination of CBZ solubility in oils

The solubility of CBZ in castor oil, MCT and a mixture at the ratio of 1:1 (w/w) of both oils was determined. An excess amount of CBZ was added to both oils and kept under moderate magnetic stirring for 24 h to reach equilibrium. The sample was centrifuged at 15,000 rpm for 20 min, an aliquot of the supernatant was diluted with methanol and CBZ content was assayed by high-performance liquid chromatography (HPLC).

2.3. High-performance liquid chromatographic analysis

The HPLC apparatus consisted of a Shimadzu LC-10A system (Kyoto, Japan) equipped with a model LC-10AT pump, an SPD-10AV variable-wavelength detector (set at 286 nm), an SCL-10Avp system controller and a Rheodyne 7125 injection valve with a 20 μL loop. The stationary and mobile phases used included a C18 Polaris cartridge (150 mm × 4 mm i.d., 5 μm particle size, 100 Å pore diameter, end-capped) and methanol:water (70:30 v/v), respectively. The mobile phase was pumped in an isocratic flow for 6 min followed by an acetonitrile gradient for elution of the lipid components. The method was validated for CBZ assay, according to ICH guidelines (ICH, 2005), with respect to specificity, linearity ($R^2 > 0.999$), precision (intra-day R.S.D. < 0.44 and inter-day R.S.D. < 1.21%), and accuracy (recoveries between 99.4% and 102.1%) (Kelmann et al., 2007b).

2.4. Preparation of formulations

2.4.1. Preparation applying a factorial design

Two factors and their influence on the nanoemulsion properties (particle size, polydispersity index, zeta potential, viscosity, drug content, and drug association) were evaluated using a 2^2 full

Table 1
Variables and levels of 2^2 factorial design for preparation of CBZ nanoemulsions

Variables	Levels[a]
(A) Oil phase composition[b]	Castor oil (+) Castor oil:MTC (1:1) (−)
(B) Lipophilic emulsifier[c]	Soybean lecithin (+) Polyoxyl 35 castor oil (−)

[a] Variable in higher level (+) and variable in lower level (−).
[b] Final oil concentration: 10.0% (w/v).
[c] Final lipophilic emulsifier concentration: 5.0% (w/v).

factorial design. The investigated factors were: type of lipophilic emulsifier and type of oily phase (Table 1). The design required a total of four experiments (Table 2) and the different formulations were prepared in triplicate to estimate experimental error. Analysis of variance (ANOVA) and Tukey's pairwise comparisons were performed at a significance level of $p < 0.05$. To perform the statistical analysis of the data, the Design Expert® software (Stateese, Minneapolis, USA) was used.

The emulsions were prepared by spontaneous emulsification according to the following briefly described procedure (Yu et al., 1993; Bouchemal et al., 2004): the drug is mixed with the oil (castor oil or a mixture of castor oil:MCT 1:1 (w/w)). The lipophilic emulsifier (soybean lecithin or polyoxyl 35 castor oil) is dissolved in a solution of acetone:ethanol (50:50, v/v) and added to the oil:drug dispersion, resulting in the oily phase. The hydrophilic emulsifier (polysorbate 80) is dissolved in water forming the aqueous phase. For adjustment of isotonicity, 2.5% (w/v) of glycerol was added to the aqueous phase. The oily phase is then slowly added into the aqueous phase under moderate magnetic stirring, forming the nanoemulsion. Solvents and most of the water are removed under reduced pressure resulting in a formulation concentration of 10% (v/v) of the initial volume from the aqueous phase. The emulsions were stored at $4 \pm 2\,^{\circ}C$. The amount of hydrophilic and lipophilic surfactants was fixed to 4.0% and 5.0% (w/w) respectively, and the drug content was 2.0 mg/mL at the final emulsion. The pH was adjusted to 7.0 with a NaOH 0.1 M solution. The formulations were named as follows: the emulsion made up of castor oil and soybean lecithin (C-L); that made up of castor oil and polyoxyl 35 castor oil (C-C35); that made up of castor oil:MCT and soybean lecithin (M-L); that made up of oil:MCT and polyoxyl 35 castor oil (M-C35). Blank formulations were also prepared. All formulations had their physicochemical characteristics evaluated.

Table 2
Experimental arrangement

Combination[a]	Coded units	Composition	
		A	B
(1)	M-C35	−	−
A	C-C35	+	−
B	M-L	−	+
AB	C-L	+	+

[a] A: oily phase composition; B: lipohilic emulsifier; (1) lower level to all variables.

2.4.2. Refinement of the selected formulation

Based on the characteristics required for parenteral administration (mainly mean droplet size and viscosity), as well as on the physical stability after 3 months of storage, the best combination of oil and lipophilic emulsifiers was selected and changed according to the increase in the lipophilic emulsifier content (from 5.0% to 6.0%, w/w). This formulation was named M-L-6.

2.5. Characterization of CBZ nanoemulsions

All formulations were characterized with respect to physical appearance, particle size, zeta potential, viscosity, drug content and association with the emulsion oily phase. Most analyses were repeated after 3 months. The refined formulation was also evaluated regarding these parameters plus morphology and *in vitro* release.

2.5.1. Particle size analysis

The mean particle size and polydispersity index were measured at 25 °C by photon correlation spectroscopy (PCS) using a Malvern Nanosizer/Zetasizer® nano-ZS ZEN 3600 (Malvern Instruments, USA). Each 15 μL sample was diluted in 10 mL of ultrapure water. The analysis was performed three times to determine mean values.

2.5.2. Zeta potential measurement

The zeta potential was measured by electrophoretic mobility using Malvern Nanosizer/Zetasizer® nano-ZS ZEN 3600 (Malvern Instruments, USA). All analyses were done in triplicate and each 15 μL sample was diluted in 10 mL of NaCl (1 mM) ultra-filtered (0.22 μm).

2.5.3. Viscosity

The rheological measurements were carried out in an AVS 350 viscometer (Schott-Geräte, Hofheim, Germany) equipped with a capillary tube Ic (viscosimeter constant, $k = 0.035$). Seven milliliters of each emulsion (adjusted to $25 \pm 0.1\,^{\circ}C$) were poured into the filling tube and transferred to the capillary tube by gentle suction. The time was recorded, in seconds, for the liquid to flow from the upper to the lower mark in the capillary tube. All formulations were analyzed in triplicate.

2.5.4. Drug content and association with the emulsions

The amount of CBZ in the emulsions was assayed by the HPLC method as previously described. A 1.0 mL volume of emulsion was diluted in methanol and the resulting solution was injected in the HPLC.

Free CBZ in the nanoemulsions was determined by measuring the non-incorporated drug present in the aqueous phase after ultrafiltration/centrifugation (Michalowski et al., 2004) using Microcon® centrifugal filter devices (100,000 NMWL; Millipore, USA). The CBZ associated with emulsions was calculated from the difference between the total and the free drug concentrations.

2.5.5. Microscopic studies

A drop of the refined CBZ emulsion was placed on a carbon-coated copper grid (200 mesh) overlayed with 1% formwar in chloroform, stained by 2.0% uranyl acetate aqueous solution and examined by transmission electronic microscopy using a JEM-1200 EXII, instrument (JEOL, Tokyo, Japan).

2.5.6. In vitro release study

The release of CBZ from the refined nanoemulsion was evaluated using the dialysis technique. Dialysis bags (Spectra/Por® membrane MWCO 100,000 Spectrum, USA) were soaked in filtered diffusion medium (Phosphate buffer pH 7.4) for 24 h and kept under refrigeration until use. One milliliter of the emulsion was placed in each dialysis bag ($n = 3$), then sealed at both ends with medicell clips (Spectrum, USA), and placed at the bottom of the dissolution vessels. The study was carried out in a VK 7000 multi-bath dissolution tester (Varian, USA), at $37 \pm 0.5\,^{\circ}C$ using dissolution apparatus USP II with an agitation speed of 50 rpm. Aliquots of 2 mL were withdrawn from the dissolution medium at designed intervals for 11 h. An equivalent volume of fresh medium was added to maintain the volume of the dissolution medium at 400 mL, which in turn ensures the sink condition. For comparison purposes, bulk CBZ and water at the same content and volume were placed in the dialysis bags, and the profile of CBZ released was assessed by the procedure described above.

The samples were analyzed by the previously described HPLC method, whose parameters were re-validated with respect to this performance study (ICH, 2005). In this way, the specificity was evaluated by analyzing a blank nanoemulsion submitted to the same test conditions and linearity range was widened (2–10 μg/mL; $R^2 > 0.999$). The release kinetics of CBZ from the nanoemulsion was assessed by first order ($\ln Q_t = \ln Q_0 - k_1 t$), zero order ($Q_t = Q_0 + k_0 t$) and simplified Higuchi ($Q_t = k_H t^{1/2}$) equations. The mathematical evaluation was performed considering the points up to 90% of CBZ release, since those precede the beginning of the profile plateau.

3. Results and discussion

3.1. Solubility determination in tested oils

The drug solubilization in the oily phase is a prerequisite to exploiting the nanoemulsion advantages. In previous studies only the use of MCT (Akkar and Müller, 2003), isopropyl miristate (Becirevic-Lacan et al., 2002) and soybean oil (Sznitowska et al., 2001) are described for CBZ nanoemulsion development. Likewise, emulsion stability problems or drug precipitation were also reported. Two studies using the combination of castor oil and MCT (1:1 w/w) revealed that this mixture led to a decrease in the castor oil viscosity and interfacial tension of the oily phase (Jumaa and Müller, 1998, 1999). In this way, the use of castor oil and a mixture of castor oil and MCT seemed to be a promising and previously uninvestigated approach.

The solubility study results revealed that CBZ is much more soluble in castor oil than in MCT. The castor oil:MCT (1:1, w/w) mixture reported better results than only MCT, but even applying this strategy, the solubility of CBZ was found to be low (Fig. 2) as compared to tetrazepam (~45 mg/g in a 1:1 castor oil:MCT mixture), a drug which was previously investigated with respect to the development of intravenous emulsions (Jumaa and Müller, 2001). This low solubility brings as a consequence a reduced drug association to the oily phase and dictated a higher incorporation of hydrophilic and lipophilic emulsifiers, which were used in permitted concentrations for the parenteral route, according to the inactive ingredient list approved by the U.S. Food and Drug Administration (FDA, 2006b).

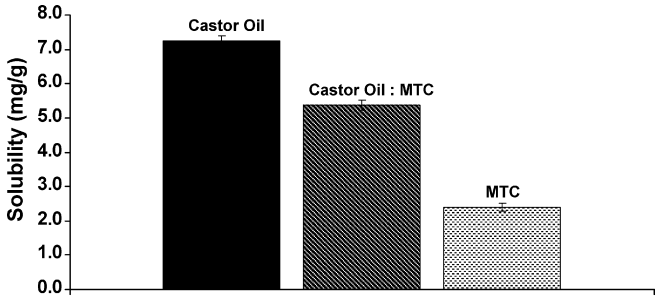

Fig. 2. CBZ solubility in castor oil, MCT:castor (1:1) oil mixture and MCT.

3.2. Preparation of CBZ parenteral emulsions

After preparation, all formulations presented a milky aspect with apparently low viscosity, which was further measured. The emulsions prepared with soybean lecithin presented a more yellowish appearance compared to those formed by polyoxyl 35 castor oil, which were almost blank. Formulations were stored under 4 °C; after 24 h, their characterization was performed.

The main feature common to all emulsions is their strict droplet size, which must be in a nanometer range. Therefore, particle size analysis was performed to confirm whether the resultant emulsions were indeed nanoemulsions. Polydispersity index gives information on the deviation from the average size, and values up to 0.250 are acceptable for parenteral emulsions (Müller et al., 2004). As can be seen in Fig. 3, photon correlation spectroscopy indicated that the average size of the emulsions is the range of 150–212 nm with a low polydispersity index, not significant (ANOVA, $p \leq 0.05$). The mean particle size of emulsions was smaller when soybean lecithin was used as a lipophilic emulsifier but these results are not significantly different either (ANOVA, $p \leq 0.05$). In general, emulsions containing the smallest globules (usually 200–500 nm) tend to be the most physically stable (Floyd, 1999).

Emulsifiers act not only as a mechanical barrier but also through the formation of a surface potential (zeta potential), which can produce repulsive electrical forces among approaching oil droplets and thus hinders coalescence (Benita and Levy, 1993; Floyd, 1999). The more negative the zeta potential, the greater the net charge of the droplets, and the more stable the emulsion is (Han et al., 2001). Zeta potential values lower than −30 mV generally indicate a high degree of physical stability (Wang et al., 2006). The results of CBZ emulsion zeta potential measurements presented in Fig. 4 showed a negative surface charge. This was calculated at approximately −35 mV for formulations prepared with soybean lecithin and −5 mV

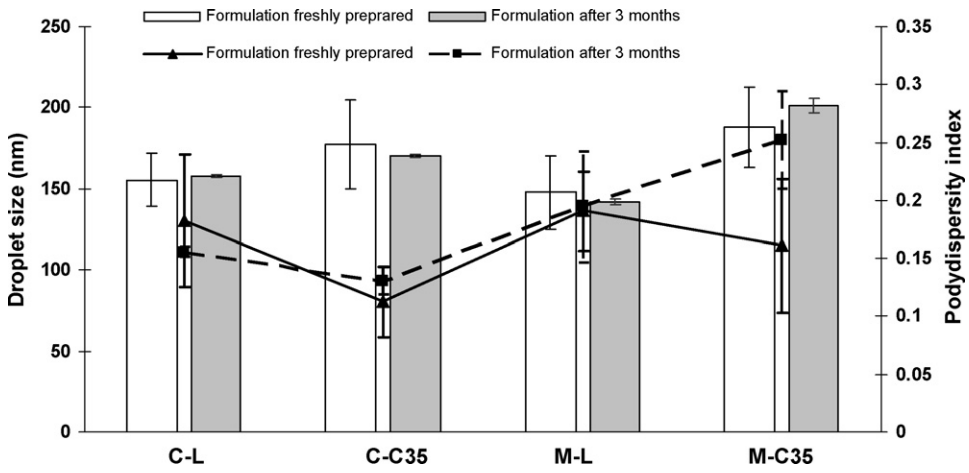

Fig. 3. Results of mean droplet size (±S.D.) and polydispersity index values (±S.D.) of CBZ nanoemulsions after 24 h (blank columns and triangles) and 3 months (grey columns and squares) of preparation. C-L: emulsion composed by castor oil and soybean lecithin; C-C35: composed by castor oil and polyoxyl 35 castor oil; M-L: composed by castor oil:MCT and soybean lecithin; M-C35: composed by oil:MCT and polyoxyl 35 castor oil.

for formulations with polyoxyl 35 castor oil. It is known that lecithin has high negatively charged phospholipids, which can produce emulsions with a high zeta potential (Benita and Levy, 1993). The formulations prepared with polyoxyl 35 castor oil, a non-ionic emulsifier, presented a negative zeta potential most likely due to the release of fatty acids from the castor oil portion. The results suggested that the emulsions composed of soybean lecithin have sufficient charge and mobility to inhibit aggregation of oil droplets, unlike of those made with polyoxyl 35 castor oil as lipophilic emulsifier. In fact, after 3 months in storage, the formulations prepared with polyoxyl 35 castor oil presented a slight creaming phenomenon, therefore, formulations prepared with lecithin seemed to be preferred. It should be noted that a comparison of the zeta potential data with the particle size results showed, in general, that a decrease in particle sizes of emulsions was accompanied by a decrease in negative surface charge values. It should be pointed out that blank emulsions particle size and potential zeta were also evaluated (data not shown). The results showed no significant differences when compared to the CBZ emulsion, which suggests that CBZ does not influence the droplet size.

The droplet size and zeta potential are the most representative parameters in the control of emulsions stability. To evaluate the emulsion stability, these aspects were monitored over 3 months. After this period, these parameters still met the requirements for i.v. administration. The emulsions prepared with the castor oil:MCT mixture presented a reduction in mean droplet size, as can be observed in Fig. 3. This may be the result of a good particle stabilization, since the castor oil presents a high amount of free fat acids that can act as co-surfactants producing an oil phase with superior properties (Yamagushi et al., 1995). The polydispersity index (Fig. 3) and zeta potential (Fig. 4) remained practically unchanged for all formulations ($p \leq 0.05$).

Viscosity measurements are not usually described for parenteral drug delivery emulsions, but this is a relevant characteristic as viscous emulsions are usually painful to the patient during i.v. administration. This parameter depends, among others, on the nature of the surfactant (Nielloud et al., 1996). As can be observed in Table 3, the formulations prepared with soybean lecithin presented a significantly higher viscosity values ($p \leq 0.05$), especially C-L formulation. Jumaa and Müller (2001) suggested that viscosities higher than 3.9 mPa s might be not attractive for parenteral administration.

As can be also observed in Table 3, HPLC analysis revealed a drug content ranging from approximately 90.4% to 101.4%. The CBZ content of C-L emulsion was statistically different

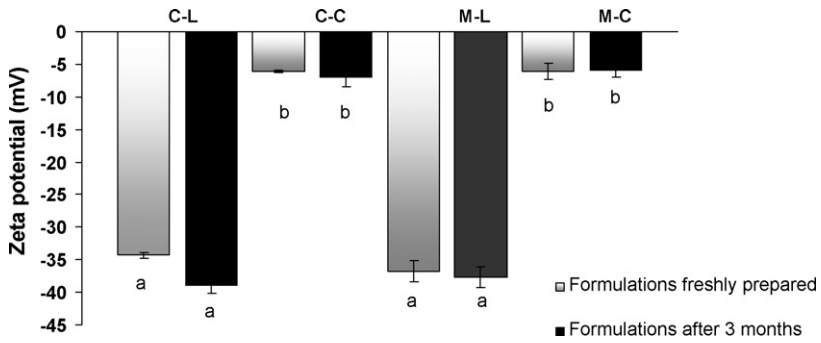

Fig. 4. Zeta potential results of CBZ nanoemulsions. Means with same letter are not significantly different (ANOVA, Tukey test, $p \leq 0.05$). Grey columns: measures (±S.D.) after 24 h. Black columns: measures (±S.D.) after 3 months. C-L: emulsion composed by castor oil and soybean lecithin; C-C35: composed by castor oil and polyoxyl 35 castor oil; M-L: composed by castor oil:MCT and soybean lecithin; M-C35: composed by oil:MCT and polyoxyl 35 castor oil.

Table 3
Results of viscosity, drug content and drug association of CBZ nanoemulsions

	Formulations			
	C-L	C-C35	M-L	M-C35
Viscosity (mPa s)	9.51 ± 0.31 c	2.85 ± 0.20 a	4.97 ± 0.49 b	2.75 ± 0.11 a
Drug content (%)	90.35 ± 2.4 a	101.03 ± 1.7 b	101.36 ± 1.0 b	98.38 ± 1.5 b
Drug association (%)	89.79 ± 0.41 a	89.51 ± 0.32 a	91.38 ± 0.45 a	88.94 ± 0.38 a

Each value represents the mean ± S.D. ($n = 3$). Means in line with the different letters are not significantly different (ANOVA, Tukey test, $p \leq 0.05$). C-L: emulsion composed by castor oil and soybean lecithin; C-C35: composed by castor oil and polyoxyl 35 castor oil; M-L: composed by castor oil:MCT and soybean lecithin; M-C35: composed by oil:MCT and polyoxyl 35 castor oil.

from the other formulations ($p \leq 0.05$). Since a reference parenteral dosage form was absent, a drug content limit from 90% to 110% for CBZ tablets was considered (USP, 2005). In this manner, the results may be judged to be satisfactory. Additionally, approximately 90% of the total CBZ content was found in the inner phase of the emulsions. There is no significant difference ($p \leq 0.05$) among CBZ association efficiencies in the various formulations.

The polynomial equations for droplet size, polydispersity index, zeta potential, viscosity, drug content, and drug association are presented in Eqs. (1)–(6), respectively, where y is dependent variable, A the type of oil, B type of lipophilic emulsifier and AB the interaction between the two factors.

$$y = 166.67 - 0.67A - 15.50B + 4.83AB \quad (1)$$

$$y = 0.18 - 0.01A - 0.01B + 1.67E - 003AB \quad (2)$$

$$y = 20.88 - 0.56A - 14.18B + 0.49AB \quad (3)$$

$$y = 4.88.67 - 1.17A - 2.21B + 1.13AB \quad (4)$$

$$y = 99.77 - 2.08A - 1.93B + 3.42AB \quad (5)$$

$$y = 90.39 - 0.21A - 1.12B + 0.075AB \quad (6)$$

By the manner described by Montgomery (1991), the response value of the effects could be calculated. Main effects alone do not have much meaning when they are involved in significant interactions. Those interactions are the key to getting the optimal conditions. The results obtained from the interactions are shown in Fig. 5. From the AB interaction on drug content, droplet size, viscosity and polydispersity index, it is showed that when the emulsifier (variable B) is used at high level (soybean lecithin) and the variable A is used at low level (mixture of oils), a better response is observed. No significant effect could be seen in AB interaction on zeta potential and association efficiency results.

Considering all evaluated statistical parameters and physical stability, the M-L emulsion seemed to be the most promising formulation. The use of castor oil:MCT mixture as oily phase makes the formulations less viscous while soybean lecithin gives a highly negative zeta potential to the emulsion, which contributes to the formulation stability when compared to those prepared with polyoxyl 35 castor oil. Therefore, M-L emulsion would be preferred.

However, after 3 months, the mean drug content of all formulations decreased to approximately 51%. This phenomenon occurred most likely due to a polymorphic transition of CBZ. It has been reported that CBZ has some polymorphic forms and that in an aqueous environment they convert to the dehydrated form, the less soluble form of CBZ (Nair et al., 2002; Otsuka et al., 1999; Kelmann et al., 2007a; Tian et al., 2006). In order to overcome this drawback, an increase in the lipophilic emulsifier content (6.0%, w/w) was proposed as a first strategy to improve the physicochemical stability of M-L emulsion, that is, to refine this formulation.

3.3. Characterization of the refined formulation

Emulsion M-L-6 characteristics were evaluated with respect to droplet size, polidispersity index, zeta potential, drug content (at 0 and 3 months), morphology and *in vitro* drug release. As can be observed in Table 4, the results were considered satisfactory and after 3 months, the evaluated characteristics still met the requirements for parenteral administration, with no precipitation being visualized.

The results of transmission electron microscopy can be observed in Fig. 6. The figure reveals that the lipid emulsion droplets were almost spherical in shape and has an amorphous core. The results corroborate with the droplet size analysis, showing that droplets are present in the nanometer range, varying from approximately 100 to 250 nm.

Table 4
Results of the characterization of M-L-6 CBZ nanoemulsion

Storage time (days)	Characterization			
	Droplet size (nm)	Polydispersity index	Zeta potential (mV)	Drug content (%)
0	148.47 ± 7.04	0.211 ± 0.04	−39.68 ± 1.61	95.25 ± 1.52
90	153.00 ± 3.27	0.200 ± 0.01	−40.10 ± 1.71	93.76 ± 7.9

Each value represents the mean ± S.D. ($n = 3$). C-L: emulsion composed by castor oil and soybean lecithin; C-C35: composed by castor oil and polyoxyl 35 castor oil; M-L: composed by castor oil:MCT and soybean lecithin; M-C35: composed by oil:MCT and polyoxyl 35 castor oil.

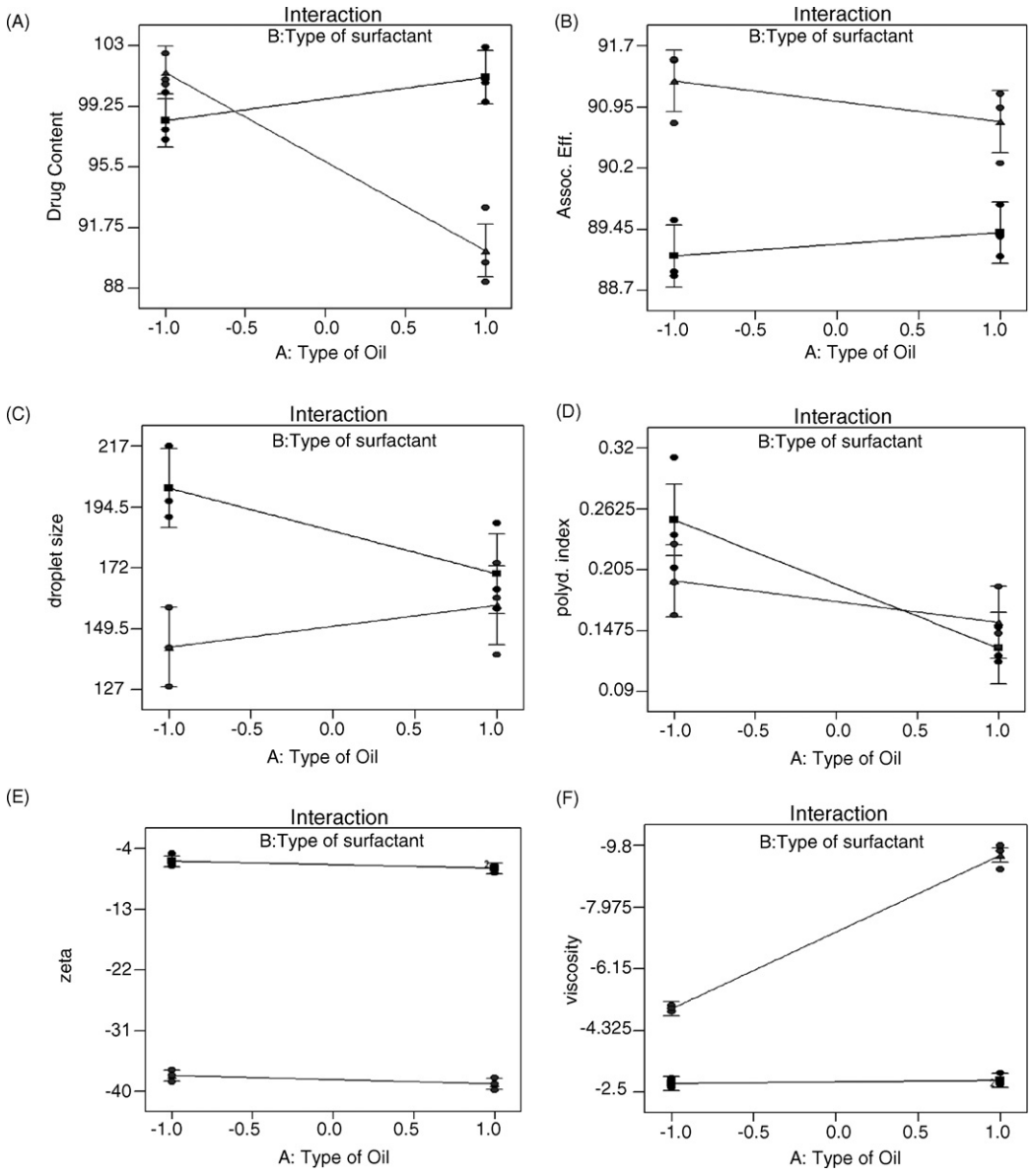

Fig. 5. Plots of AB interactions; (A) drug content, (B) association efficiency, (C) droplet size, (D) polydispersity index, (E) potential zeta and (F) viscosity. Darker line: variable B at low level; slighter line: variable B at high level.

Finally, a preliminary evaluation of CBZ release behavior from the refined nanoemulsion was performed. The mean cumulative% CBZ released versus time plot for nanoemulsion and drug freely dispersed in water inside the dialysis bag is presented in Fig. 7. As can be observed, the release kinetics of CBZ from the nanoemulsions is slower than that of the free drug, and both can be considered prolonged. Moreover, both release kinetics seem to be a square root time dependent. Higuchi provided the model which best characterized these release profiles ($R^2 > 0.99$ for plotting the nanoemulsions, and $R^2 > 0.98$ for plotting the free drug) and described drug release as a diffusion process based on Fick's law (Costa and Lobo, 2001). Kinetics, as well as the prolonged drug release observed *in vitro* can be explained by the fact that CBZ diffusion from the oily core and interface is hindered by the aqueous medium, which acts as a barrier to drug transport due to its very low solubility in water. In the same way, the free drug presents a behavior, which is typical from a dispersed system, considering a low aqueous soluble drug. In the case of the nanoemulsion, such sustained delivery is frequently reported in the literature (Schultz et al., 1997; Constantinides et al., 2000; Abrol et al., 2005) and may occur *in vivo*; however, one can expect the foreignness of nanoemulsions to increase drug uptake by the living tissues due to oil droplet phagocytosis (Grassi et al., 2000; Abrol et al., 2005). It is worth mentioning that the dialysis membrane also accomplishes a physical separation of the nanoemulsions from the CBZ released to the medium and may be in part responsible for this feature. This technique is criticized by some authors for not mimicking *in vivo* conditions and release mechanism for parenteral route (Henriksen et al., 1995; D'Souza and DeLuca, 2006). Thus, all data concerning

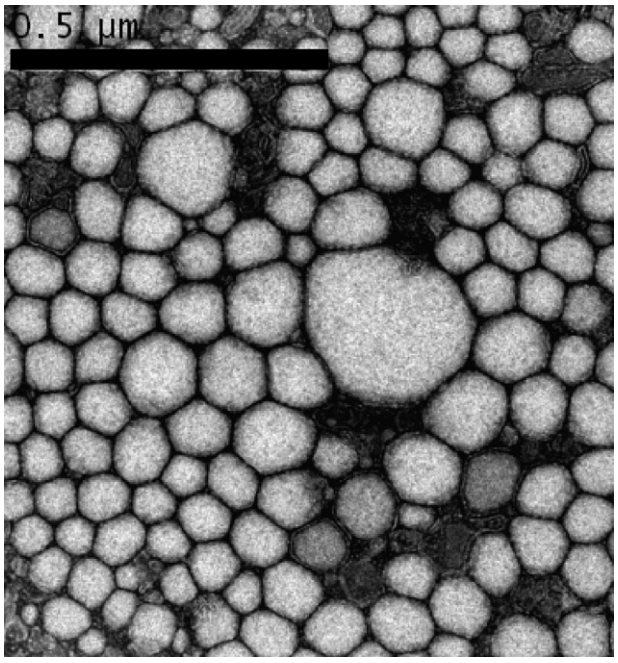

Fig. 6. Transmission electron photomicrograph of the M-L-6 CBZ nanoemulsion.

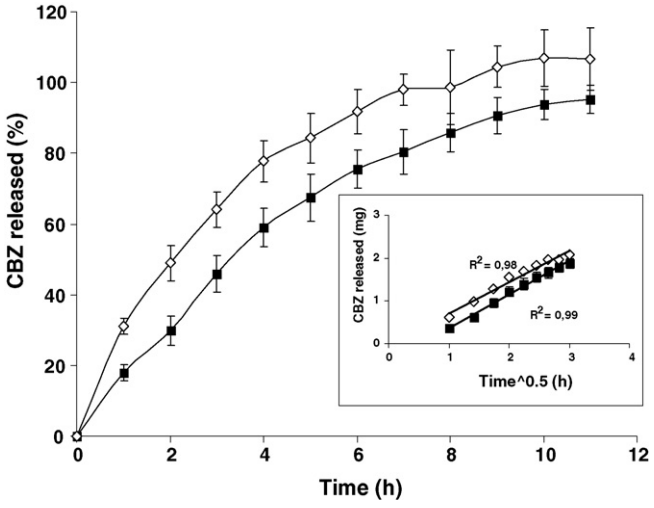

Fig. 7. *In vitro* release profiles of M-L-6 nanoemulsion (dark squares) and CBZ aqueous dispersion (open triangles) from dialysis bag in phosphate buffer pH 7.4. Mean ± S.D.; $n = 3$. Detail: Higuchi plot.

drug release from nanoemulsions should be interpreted with care and further test conditions for evaluating CBZ release should be investigated.

4. Conclusions

The factorial design was a beginning instrument in selecting the mixture of castor oil:MCT (1:1, w/w) and soybean lecithin as the best oily phase and lipophilic emulsifier, respectively, in order to prepare 2 mg/mL CBZ nanoemulsions by spontaneous emulsification. Nevertheless, a reduced drug content after 3 months led to the refinement of formulation by increasing soybean lecithin content to 6.0% (w/w). The physicochemical characteristics, such as drug content, zeta potential, and droplet size, were evaluated after 24 h and 3 months of preparation, demonstrating the feasibility of this emulsion for the i.v. route. The morphological evaluation confirmed the formation of an emulsion in the nanosize range. An *in vitro* release study was also performed, revealing a release kinetics, which fits the Higuchi model.

The stability of this refined formulation will be further assessed and the need for optimization will be complemented by sequential factorial designs. Upon obtaining successful results, preclinical studies are to be undertaken and other *in vitro* test conditions may be investigated in the attempt to establish an *in vitro–in vivo* correlation.

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