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Formulation, stability and degradation kinetics of intravenous cinnarizine lipid emulsion

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ABSTRACT

Cinnarizine was loaded in the lipid emulsion to develop an intravenous formulation with good physical and chemical stability. High-pressure homogenization was used to prepare the lipid emulsion. The factors influencing the stability of cinnarizine lipid emulsion, such as different drug loading methods, pH, temperature, sterilization methods and sterilization time were monitored by high-performance liquid chromatograph. The degradation of cinnarizine in aqueous solution and lipid emulsion both followed apparent first-order kinetics. A possible degradation mechanism was postulated by the bell-shaped pH-rate profile of cinnarizine. Localization of the drug in the interfacial lecithin layer significantly improved the chemical stability of cinnarizine and its stabilizing mechanism was thoroughly discussed and proved. The activation energy of cinnarizine in lipid emulsion was calculated to be 51.27 kJ/mol which was similar to that in aqueous solution. This indicates that the stabilizing effect of the drug carrier on cinnarizine was not an alteration of the degradation reaction. In addition, shelf-life of cinnarizine in lipid emulsion was estimated to be 1471.6 days at 4 °C, which is much longer compared with 19.8 days in aqueous solution. The final products were stable enough to resist a 121 °C rotating steam sterilization for 15 min.

1. Introduction

Cinnarizine (CN), a cerebral blood flow promoter (Fig. 1), is widely used for the treatment of cerebral apoplexy, cerebral arteriosclerosis and post-traumatic cerebral symptoms (Godfraind et al., 1982; Singh, 1986). However, the optimum method of administering the drug has not been fully established, since cinnarizine has poor aqueous solubility and chemical instability. The currently commercially marketable dosage forms are tablets and capsules. Both of these exhibit low and erratic oral bioavailability (Ogata et al., 1986). Several intravenous dosage forms, such as water soluble CN salt solution and CN–β-cyclodextrin inclusion complex solution, have been investigated to overcome these problems (Wang, 2006; Zhang et al., 2004). However, there are two main problems associated with their use. On one hand, the injectable dosage forms may cause venous irritation because the pH value of the solution must be adjusted acid (pH < 4) to enhance the solubility of CN. On the other hand, none of these can effectively reduce the degradation of CN in solution.

To ensure both high solubility and stability of CN, a suitable carrier is needed. Lipid emulsions are firstly used in parenteral nutrition. But, significantly, their advantages in terms of the reduction in irritation or toxicity of the incorporated drug and the possibility of sustained release and targeted delivery of the drug to various organs make them excellent vehicles (Gettings et al., 1998; Singh and Ravin, 1986). The lipid emulsion, which consists of an interior oil phase and an exterior water phase, is prepared by incorporating drugs into the interior oil phase and the oil–water interfacial film. This means that the drug with poor aqueous solubility can be easily loaded and the hydrolysis of unstable drugs can be minimized (Floyd, 1999). All these excellent characterizations of the emulsion may contribute to investigation of intravenous CN and make the lipid emulsion be a appropriate carrier for CN.

In our study, cinnarizine lipid emulsion (CLE) was initially designed and prepared by optimizing the formulations. We carried out a detailed investigation of the degradation of CN in different oils and the effect of the amount of lecithin on the chemical stability of CLE in order to reduce the degradation of CN during the initial stages. Considering that degradation of CN occurs in both the oil and water medium, we tried to localize the drug in the interfacial lecithin layer of the emulsion to isolate the drug from water or oil medium. This was achieved by the method ever used for the solubilization of poor soluble drug (Davis and Washington, 1988; Lance et al., 1995). Then, other factors influencing the stability of CLE were investigated to evaluate the feasibility of using CLE for intravenous injection. It was reported previously that CN is very labile in aqueous systems following an investigation of the degradation kinetics of CN in acidic solutions (pH < 4) (Tokumura et al., 1985). However,

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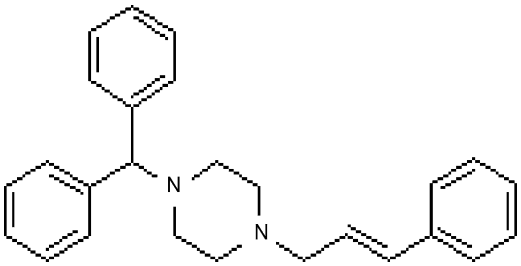

Fig. 1. Structure of cinnarizine.

the range of pH values was too narrow to include a suitable pH range for injection (pH 4–9). So, the degradation kinetics of CN in aqueous solution were supplemented by providing a wider range of pH values (3.12–9.44) to find the most appropriate pH value and postulate a possible degradation mechanism. At the same time, a kinetic study of CLE was also carried out in order to investigate the effect of drug carrier on the degradation of CN and estimate the shelf-life of the product. Until now, no injectable preparation of CN has been reported which can withstand autoclaving sterilization (Jia et al., 2007; Wang, 2006; Zhang et al., 2004). Our study concentrated on a formulation which could withstand a 121 °C rotating steam sterilization for 15 min to improve the safety of the product and reduce the cost of the industrial process.

2. Methods

2.1. Materials

The following materials were purchased from the sources in brackets: cinnarzine (Shanghai Xiandai Hashen Pharma Ltd. Co., Shangqiu, China), medium-chain triglyceride (MCT) (Lipoid KG, Ludwigshafen, Germany), egg lecithin (EPIKURON 170, PC72%, Degussa Food Ingredients, Germany), long-chain triglyceride (LCT) (Tieling Beiya Pharmaceutical Co., Tieling, China), Poloxamer 188 (Pluronic F68®) was purchased from BASF AG (Ludwigshafen, Germany), Tween 80 for parenteral use (Shenyu Medicine and Chemical Industry Ltd. Co., Shanghai, China), and glycerol (Zhejiang Suichang Glycerol Plant, Zhejiang, China). All chemicals and reagents used were of analytical or chromatographic grade.

2.2. Preparation of CLE

Firstly, CN together with egg lecithin was dissolved in oil which consisted of LCT and MCT, and heated at 80 °C to obtain a clear oil phase. Then, the aqueous phase consisting of glycerin, sodium oleate, Tween 80, F68 and EDTA was also heated to 80 °C and agitated until uniformly dissolved. Finally, the water phase was added slowly to the oil phase with high speed shear mixing (ULTRA RURRAX®IKA® T18 basic, Germany) at 10,000 rpm for 5 min to obtain coarse emulsion. The pH was adjusted to 8.50 with 0.1 mol/l NaOH or HCl, and the volume made up with purified water to 100 ml. After that, the coarse emulsion was subjected to high-pressure homogenization (Niro Soavi NS10012k homogenization, Via M. da Erba, 29/A-43100 PARMA, Italy) at 700 bar for eight cycles to get the final emulsion. The temperature of the entire homogenization process was maintained at 40 °C. Then, the CLE was sealed in vials, and rotated in a 121 °C water steam bath for 15 min.

2.3. Characterization of CLE

The particle size was measured by photon correlation spectroscopy (PCS, dynamic light scattering, DLS) with a Nicomp™ 380 Particle Sizing system (SantaBarbara, USA). The system is sensitive to particles ranging from 3 nm to 3 μm in size and provides two parameters, a mean diameter and the standard deviation (S.D.). The two parameters are usually used to evaluate the particle size distribution of CLE. Samples were diluted 1:5000 with purified water immediately before measurement at 25 °C. In addition, the microscopic assessment was carried out on using a Motic DMBA 450 microscope (MoticChina Group Co. Ltd., Beijing, China). Emulsion samples were investigated without diluted using an oil immersion with 100-fold magnification; typically 20 microscopic fields were analyzed for the detection of microns, the size of which was greater than 3 μm, particularly those drug crystals.

The Nicomp™ 380 system was also used to determined zeta potential of the CLE by electrophoretic light scattering (ELS) technique. Samples were diluted 1:5000 with purified water adjusted to the same pH as the CLE using 0.01 mol/l HCl or NaOH beforehand. The determination was performed at 25 °C.

The pH values of CLE were measured using a pH meter (Leici®, Shanghai Precision Science Instrument Ltd., Shanghai, China) fitted with a microelectrode at room temperature (25 ± 2 °C).

The entrapment efficiency (EE) of CLE was determined by measuring the concentration of CN in the dispersion phase. The CLE was subject to a Hitachi ultracentrifuge operated at 50,000 rpm for 1.5 h at 4 °C. Polyallomer tubes were used and their bottoms were pricked after centrifugation with a syringe needle to collect the aqueous phase. The amount of CN in the aqueous phase was estimated by high-performance liquid chromatography (HPLC) and the EE was calculated according to the following equation (Ferezou et al., 1994; Groves et al., 1985):

$$\mathrm{EE}\,(\%) = \frac{C_{\mathrm{total}}V_{\mathrm{total}} - C_{\mathrm{water}}V_{\mathrm{water}}}{C_{\mathrm{total}}V_{\mathrm{total}}} \times 100$$

2.4. HPLC analysis

The HPLC system consisted of an L-7100 pump, an L-7400 UV detector, an L-7200 autosampler (Hitachi Company, Japan) and a HiQ sil C18W column (250 mm × 4.6 mm, 5 μm, Kya Tech Co., Japan). The mobile phase consisted of methanol–water–triethanolamine–glacial acetic acid (73:27:0.4:0.6) at a flow rate of 1.0 ml/min, modified from the literature (Guo et al., 2006). The wavelength of the UV detector was set at 254 nm and the injection volume was 10 μl.

2.5. Analysis of cinnarizine and its degradation products

One milliliter of emulsion (1 mg/ml) was diluted to 100 ml with methanol, vortexed, and then centrifuged (LG10-2.4A, Beijing) for 10 min (4000 rpm). The supernatant was collected and filtered through a 0.45-μm membrane. The filtrate was subjected to HPLC analysis to estimate the amount of cinnarizine and degradation products. The calibration graph of CN ranging from 1.2 to 60.0 μg/ml was linear and described by the following equation: $y = 28{,}591x - 6033.4$ ($r = 0.9999$). The recovery of the CN evaluated at three concentrations was from 99.3% to 102.3%. The intra-day accuracy of the method for cinnarizine ranged from 98.4% to 100.9%, while the intra-day precision ranged from 0.7% to 1.5%. The inter-day accuracy ranged from 96.8% to 102.4%. The precision and accuracy of the method were both well consistent with analysis requirement.

2.6. The degradation kinetics of cinnarizine in the aqueous solution

A pH 6.8 phosphate buffer was prepared according to USP 31 (United States Pharmacopeia 31). The buffer was divided into aliquots and the pH adjusted with NaOH or H_3PO_4 to pH 3.12, 4.66,

5.19, 6.32, 6.84, 7.32, 8.29, and 9.44. CN was dissolved in these buffers to obtain solutions of 6.62×10^{-3} mol/l. Each buffer containing 6.62×10^{-3} mol/l CN was placed in a 10 ml ampule, which was then sealed and kept in a water bath at 80 °C. Samples were withdrawn at intervals of 0, 0.5, 1, 2, 4, 8, 12, 24 h and cooled immediately to terminate the reaction before HPLC analysis. The residual CN content was calculated by a calibration curve prepared in advance.

2.7. Stability study

2.7.1. The degradation of cinnarizine in different oils

CN was dissolved in different oils by agitating in a 60 °C water bath. Then, the oils which contain 1% CN were sealed in vials and put in a 60 °C temperature constant oven. The samples were withdrawn at intervals of 0, 5 and 10 days and allowed to the room temperature. The concentrations of CN in oils at different time point were determined by HPLC. And the degradation of CN in different oils was evaluated by the changes of the CN concentration.

2.7.2. Effect of the drug loading method

In our study, two methods were investigated to load CN in the CLE. The methods were as follows:

- *Method A*: CN was dissolved in the oil phase in which the lecithin was dispersed thoroughly in advance.
- *Method B*: CN and lecithin were dissolved in dehydrated alcohol. After dissolving uniformly, the dehydrated alcohol was evaporated by stirring on a water bath at 80 °C until the weight of remaining lecithin–drug mixture was constant. Then, the mixture was dispersed in oil for further processing.

The following steps for preparing the CLEs were consistent with Section 2.2. The final products were placed in an 80 °C water bath. The samples were withdrawn at appropriate intervals and cooled immediately to terminate the reaction then analyzed by HPLC. The concentration of CN was calculated by a calibration prepared beforehand.

2.7.3. Effect of temperature on degradation rate of CN in aqueous solution and lipid emulsion

CN was dissolved in water to give a 0.5 mg/ml solution at pH 8.50. At the same time, 1 mg/ml CLE was prepared according to Section 2.2 at the same pH. Both of these were kept in a water bath at 40, 60, 80, and 90 °C. Samples were collected at intervals of 0, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, and 120 h and cooled immediately for determination by HPLC. The physical appearances of the CLE were examined at the different time intervals.

2.7.4. Effect of the thermal stability method

The CLE containing 1.0 mg/ml CN was prepared according to Section 2.2. The samples were sterilized under different conditions involving rotating in a 115 °C water steam bath for 30 min, rotating in a 121 °C water steam bath for 8 min, and rotating in a 126 °C water steam bath for 3 min. Then, the corresponding samples, before and after sterilization, were analyzed by HPLC. Their particle sizes and zeta potentials were also determined.

2.7.5. Effect of the thermal sterilization time

The CLE with the concentration of 1.0 mg/ml was prepared as described in Section 2.2. The samples were rotated in a 121 °C water steam bath for different sterilization time of 8, 10, 12, 15, 20 and 25 min. Then, all the samples were analyzed by HPLC. And the physical stability of CLE was evaluated by physical appearances, particle sizes and pH values.

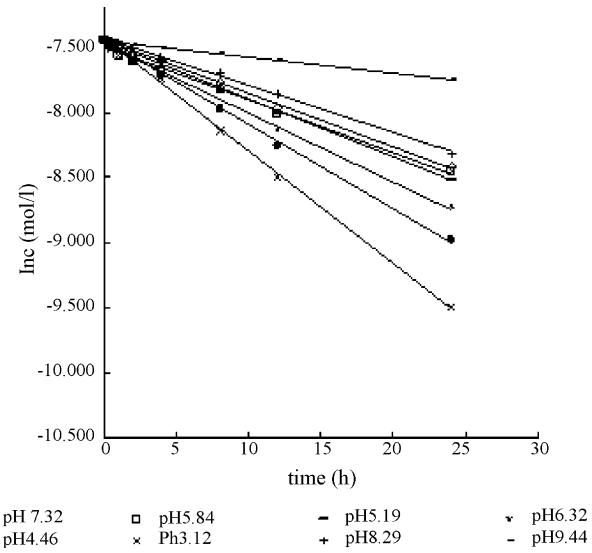

Fig. 2. Pseudo-first-order plots of the degradation of CN in buffer solutions over a range of pH values (3.12–9.44) at 80 °C.

2.7.6. Long-term stability investigation

A new batch of optimum CLE was prepared and stored at 25 ± 2 and 4 ± 2 °C, which was close to the particle storage condition. At pre-determined time intervals, samples were removed and allowed to the room temperature. Their physical and chemical stability were evaluated by physical appearance, particle size distribution, pH value, entrapment efficiency and drug remaining. The study was performed in triplicate.

3. Results and discussion

3.1. The degradation kinetics of cinnarizine in aqueous media

The degradation of CN in buffered solutions at 80 °C over a range of pH values (3.12–9.44) was monitored by HPLC. Fig. 2 shows the relationship between concentration of CN on a natural logarithmic scale and the time at a series of pH values. The ln *C* (concentration) versus time was linear at all pH values following pseudo-first-order kinetics. And the relationship between the degradation rate constant (k) and the pH values is exhibited in Fig. 3. It can be observed from Fig. 3 that the degradation of CN was extremely rapid at pH 3.12–7.32 exhibiting an increase in the curve. While, as the pH value increased from 7.32 to 9.44, the corresponding degradation rate of CN significantly decreased.

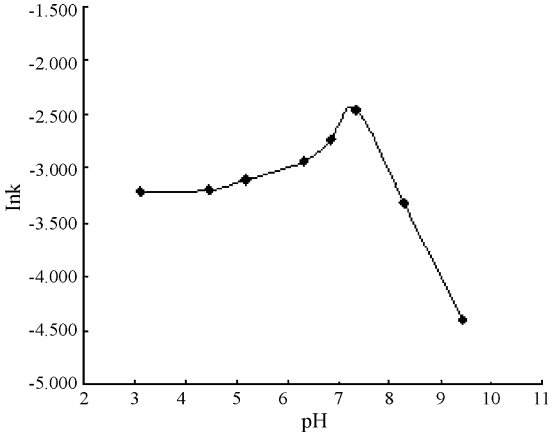

Fig. 3. Rate-pH profile for the degradation of CN in buffers at 80 °C.

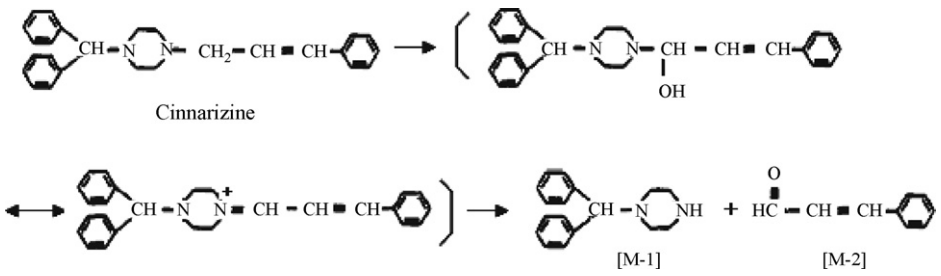

Fig. 4. The degradation pathway of CN in buffers (pH 3.12–9.44).

This profile appears a bell shape, which is hard to interpret as the kinetics of the ionization constants of the reactants. An expression of kinetics of the reaction is more reasonable at this time. This behavior has been recognized in a number of reactions and may be described as a change in the rate-determining step of the reaction with changing pH. A change in the rate-determining step implies the presence of at least two steps and one intermediate in the reaction (Mollica et al., 1969). Until now, no intermediate can be isolated and observed by chromatographic or spectroscopic analysis. However, it can be inferred that a cationic imine intermediate may be exist in the series of degradation reactions. The cationic imine intermediate, R–N^+=CH–R which upon dehydration of hydroxymethylamine, R–N–CHOH–R was subjected to the Schiff base formation, hydrolysis reactions and decomposed to the degradation products. The degradation pathway of CN in buffer solutions (pH 3.12–9.44) is presumed to be that exhibited in Fig. 4.

Two pieces of evidence made the existence of the cationic imine intermediate reasonable: the ln *k*–pH profile of CN was similar to that observed for a hydrolysis of a Schiff base (Cordes and Jencks, 1962). Beside, l-(diphenylmethyl) piperazine (M1) displayed in Fig. 4 was previously reported as a degradation product of CN (Allewijn, 1968).

The results obtained above suggest that the pH must be higher than 7.32 and as basic as possible to reduce the degradation of CN. Considering the allowed pH value range for injection (4–9), we finally selected the pH value of 8.50 to minimize the degradation of CN.

3.2. Preparation investigations of CLE

3.2.1. Oil phase composition in CLE

LCTs as the oil phase of lipid emulsion have been used in clinical situations for over 30 years. However, they still have some side effects, such as immune dysfunction, accumulation in reticuloendothelial cells and deposition of adipochrome in liver or lung after long-term use. Alternative oil mixtures containing MCTs may reduce the toxicity associated with pure LCT-based lipid emulsions and may also provide more stable all-in-one admixtures (Driscoll et al., 2000; Smyrniotis et al., 2001).

Hence, the 10% (w/v) oil phases of LCT, MCT, the mixture of LCT and MCT at ratios of 7.5:2.5, 5:5, 2.5:7.5 in CLEs were employed to investigate the effect of different oil phases of CLE on physicochemical stability (other components in all the formulations were the same as in Section 2.2). The CLEs with different oil phases were evaluated with regard to physical appearance, particle size distribution and entrapment efficiency as shown in Table 1. According to Table 1, there were a lot of oil drops on the surface of the CLE with Formulation 5. Formulation 4 was better, but after 3-month storage at room temperature, some oil drops were also seen on the surface of CLE. This might be because that excess MCT in the oil phase resulted in an increase in the mean particle size and standard deviation or even oil drops. The EE of each formulation was almost unchanged suggesting that the oil phase did not have a significant effect on EE.

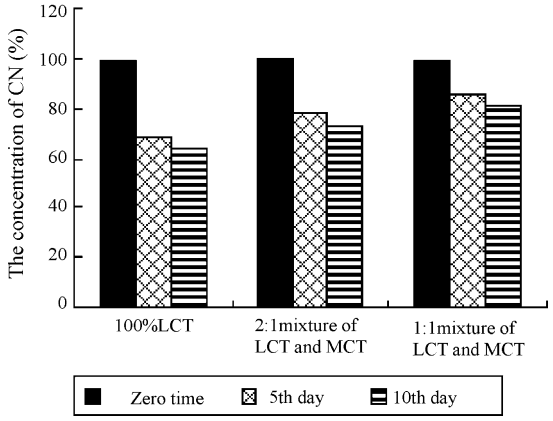

Fig. 5. The degradation of CN in different oils at 60 °C during 10 days.

Based on these results, the choice of a suitable oil phase was limited to LCT and the mixture of LCT and MCT at ratios of 7.5:2.5 and 5:5.

In addition, considering CN may be also liable to degrade in oils, the degradation of CN in different oils was accelerated by being stored at 60 °C and evaluated by the change of the CN concentration during 10 days. According to Fig. 5, it suggests that the degradation of CN also occur in the oil. However, as the amount of MCT in the oil phase increased, the degradation of CN reduced. It can be considered that the addition of MCT in the oil phase of CLE may contribute to the reduction in the degradation of CN. Therefore, taking account to both of the desired characterization and chemical stability of CLE, a 5:5 mixture of LCT and MCT seems to be the best choice.

Table 1
The effect of oil phase composition and oil phase ratios on the characterization of CLE.

	LCT:MCT	Physical appearance after sterilization	Particle size distribution (nm)	Entrapment efficiency (%)	Physical stability
F1	10:0	Good	151.2 ± 58.28	98.3	Stable for 12 months
F2	7.5:2.5	Good	157.5 ± 52.12	99.1	Stable for 12 months
F3	5:5	Good	154.7 ± 51.36	98.7	Stable for 12 months
F4	2.5:7.5	Good	169.8 ± 78.58	98.9	Oil drops after 3 months
F5	0:10	Visible supernatant oil drops	Not determined	ND	Creaming after 4 months

F, Formulation; ND, not determined.

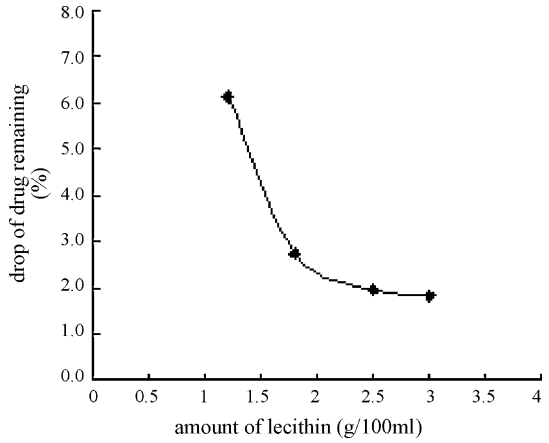

Fig. 6. The reduction of the concentration of CN in emulsion with different amounts of lecithin at 60 °C during 5 days.

3.2.2. Emulsifier composition in CLE

The choice of the emulsifier is critical for the formulation of the lipid emulsion. It is well known that emulsification of the emulsion depends on the emulsifiers used, and the emulsification process significantly influences the stability of the emulsion (Yamano and Seike, 1983). In the following, the emulsifiers were selected with respect to the stability of the CLE, and the amount of each emulsifier selected was investigated alongside other emulsifiers at a constant level.

As far as the various emulsifiers on the market are concerned, lecithin should always be the first choice due to its excellent biocompatibility. On one hand, lecithin as a main emulsifier protects the system acting as a solubilizing agent for added drugs or by creating a steric and electrostatic barrier at the interphase (Muchtar et al., 1991). On the other hand, it is a key factor for maintaining the chemical stability of CN in emulsion (illustrated in Section 3.3.1). The Formulations from I to IV in Table 2 show that when the amount of lecithin below 1.8% (w/v), the emulsifying capacity is not enough to stabilize the surfactant film. Therefore, to ensure the physical stability of CLE, the amount of lecithin must be above 1.8% (w/v). In addition, the effect of different amounts of lecithin on the reduction of CN content can be observed in Fig. 6. As the amount of lecithin increased from 1.2% to 2.5% (w/v), the degradation of CLE during 5 days was reduced sharply. However, since the amount of lecithin was above 2.5% (w/v), the degradation of CLE did not markedly change. It means that a small amount of lecithin (<2.5%) cannot encapsulate the drug entirely and some drug transfer into the oil phase inducing the degradation of CN. Hence, to ensure the chemical stability of CLE, the amount of lecithin should be above 2.5% (w/v). Formulation IV containing 3% lecithin would be the best choice because of its minimum degradation and excellent physical characterization.

However, a single emulsifier of egg lecithin was not sufficient to maintain the stability of the CLE. As the Formulations V and VI displayed in Table 2, the CLE without addition of Tween 80 or Pluronic F68 cannot undergo a few months storage at room temperature, since the phospholipids are too hydrophobic to form spontaneously the zero curvature lipid layer required for the formation of balanced emulsion. Hence, in order to form phospholipids-based emulsion, it is necessary to adjust the HLB of phospholipids and to inhibit their tendency to form lamellar liquid crystalline phases. This can be achieved by using auxiliary emulsifiers like Tween 80 (HLB 15) (Moreno et al., 2003; Morey et al., 2004). Pluronic F68 as an even stronger emulisifier, can stabilize the newly created interface immediately. Besides, it is superior to that derived from the use of other nonionic emulsifiers, including Tween 80, upon autoclaving. They explained the results on the basis of the high cloud point of F68, resulting in more resistance against dehydration during autoclaving and subsequently no emulsifier damage (Eccleston, 1992; Floyd, 1999). Hence, the combination of the two emulsifiers above is effective and essential for the stability of CLE. Based on the formulation screening in Table 2, the amount of the Tween 80 and F68 was fixed to be 0.2% (w/v) and 0.2% (w/v).

A small amount of sodium oleate can also stabilize the surfactant film by increasing the absolute value of the zeta potential. For disperse systems, a zeta potential maintained in the range −20 to −45 mV could produce suitable electronic repulsion among the particles (Yu et al., 2008), and the emulsion with a larger negative surface charge can undergo less coalescence during steam sterilization by providing adequate electrostatic repulsive force (Chansiri et al., 1999). Referring to the Formulations VIII, IV, IX and X in Table 2, it is obvious that the incorporation of sodium oleate makes an important contribution to increase the absolute value of the zeta potential. But excess sodium oleate will induce more oil drops in the system. So, the amount of sodium oleate was fixed to be 0.03% (w/v).

3.2.3. The effect of the high-pressure homogenization process

High-pressure homogenization has advantages of being simple to carry out and use on an industrial scale, consequently, it has been developed and used extensively to prepare emulsions, lipid microspheres, and liposomes. According to Washington and Davis (1988) and Bock et al. (1994), the most important homogenization parameters for controlling droplet size are homogenization pressure, temperature and duration (number of cycles). In this investigation, the homogenization temperature was controlled to be 40 °C by a circulator in order to avoid disrupting droplets overprocessed according to Bock's study (Bock et al., 1994). In Fig. 7(A), six different homogenization pressures were involved to prepare the CLEs while maintaining the other conditions constant. The figure shows that the particle size and S.D. value reduced as the homogenization pressure increased from 500 to 700 bar, but above 700 bar, the values almost do not change. Therefore, to avoid overprocessing, the homogenization pressure is chosen as 700 bar. In Fig. 7(B), five homogenization cycles were investigated. Likewise, when the homogenization cycle is up to eight, excess cycles do not change the particle size and S.D. value anymore. Consequently, the final choice of the homogenization cycle is eight times.

3.3. Stability investigation

3.3.1. The effect of the drug loading method

Our research used two methods to load the drug in lipid emulsions and these are described in detail as method A and method B in Section 2.7.2. The lipid emulsions prepared by the method A and method B (CLE A and CLE B) were placed in an 80 °C water bath to accelerate the degradation of CN. The samples were withdrawn at intervals of 0, 1, 2, 4, 8, 12, 24, 48, 72, and 118 h, and their concentrations were analyzed by HPLC.

In Fig. 8, the CLE A and CLE B plots of ln C (concentration) versus time both gave good straight lines suggesting pseudo-first-order degradation kinetics. And the rate constants gained from the slopes could be used to calculate the half-lives ($T_{0.5}$) of the CLE A and CLE B. The equation for determining the half-lives is as follows:

$$T_{0.5} = \frac{0.693}{k}$$

The results show that the half-life of CLE B (78.1 days) was much longer than that of CLE A (6.3 days) at 80 °C. This suggests that method B markedly reduces the degradation of CN in emulsion. The mechanism can be explained as follows.

The main difference between the two methods is that where the drug dispersed. The method A as a principle method is used to load

Table 2
Effects of the different emulsifiers on the characterization of CLE (the amount of each emulsifier used was investigated on the premise of other emulsifiers being at a constant level).

	Lecithin (w/v, %)	Tween 80 (w/v, %)	F68 (w/v, %)	Sodium oleate (w/v, %)	Physical appearance after sterilization	Particle size distribution (nm)	ζ-potential (mV)	Physical stability
FI	1.2	0.2	0.2	0.03	Visual oil drops	ND	ND	Creaming after 3 months
FII	1.8	0.2	0.2	0.03	Good	174.2 ± 60.81	−24.56	Creaming after 6 months
FIII	2.5	0.2	0.2	0.03	Good	159.2 ± 52.32	−26.75	Stable for 12 months
FIV	3.0	0.2	0.2	0.03	Good	146.4 ± 47.16	−25.96	Stable for 12 months
FV	3.0	0	0.2	0.03	Visual oil drops	ND	ND	Creaming after 1 month
FVI	3.0	0.2	0	0.03	Bulk oil drops	ND	ND	Creaming after 3 months
FVII	3.0	0.2	0.4	0.03	Good	142.8 ± 47.57	−24.78	Stable for 12 months
FVIII	3.0	0.2	0.2	0	Good	ND	−6.92	Creaming after 2 months
FIX	3.0	0.2	0.2	0.06	Visual oil drops	ND	−28.45	Creaming after 1 month
FX	3.0	0.2	0.2	0.10	Bulk oil drops	ND	−30.21	Creaming after a few days

F, Formulation; ND, not determined.

the drug in the oil phase and interfacial film, while the method B aims at retaining the drug within the interfacial lecithin layer. Most of the drugs susceptible to hydrolysis were usually loaded by method A in the emulsion to protect itself from water. However, it is not enough to prevent degradation of CN effectively because the degradation of CN also occurs in the oils (discussed in Section 3.2.1). So, to minimize the degradation, the drug must be prevented from the oil or water medium as far as possible.

The method B involved the dissolution of excess lecithin and CN in dehydrated alcohol, removal of the dehydrated alcohol by evaporation and then using the lecithin–drug mixture for the production of the emulsion. In this process, the drug molecules were embedded in the lipophilic group of the phospholipids molecules to form a tight combination with phospholipids. This combination may result in the localization of the drug in the lecithin layer of the CLE. Besides, it can be concluded that this behavior must be a physical embedment rather than a chemical combination, because the activation energy of CN in emulsion (calculated in Section 3.3.2) seems similar to that in aqueous solution. This indicates that the stabilizing effect of the drug localization on CN was not a result of an alteration in the degradation reaction itself.

To support this theory, further experiments are needed to confirm that no drug disperse in oil phase or water phase. Firstly, the EEs of the CLE A and CLE B were determined as 98.5% and 98.7%. The EE is a parameter to evaluate the percent of the amount of the drug dispersed in the oil phase and interfacial oil–water layer. The same high entrapment efficiencies suggest that little drug dispersed in the water phases of the CLE A and CLE B.

As the oil phase and the interfacial oil–water layer cannot be separated, the amount of the drug in the oil phase is not able to be determined directly. However, it can be estimated by the degradation degree of CN in the oils. Referring to Section 2.7.1, the oils with different drug loading methods were put in a 60 °C temperature constant oven and withdrawn at intervals of 0, 5 and 10 days. The concentrations of CN in oils at different time points were determined by HPLC. The results exhibited in Fig. 9 were consistent with deduced schematic models of CLE A and CLE B. When the drug is

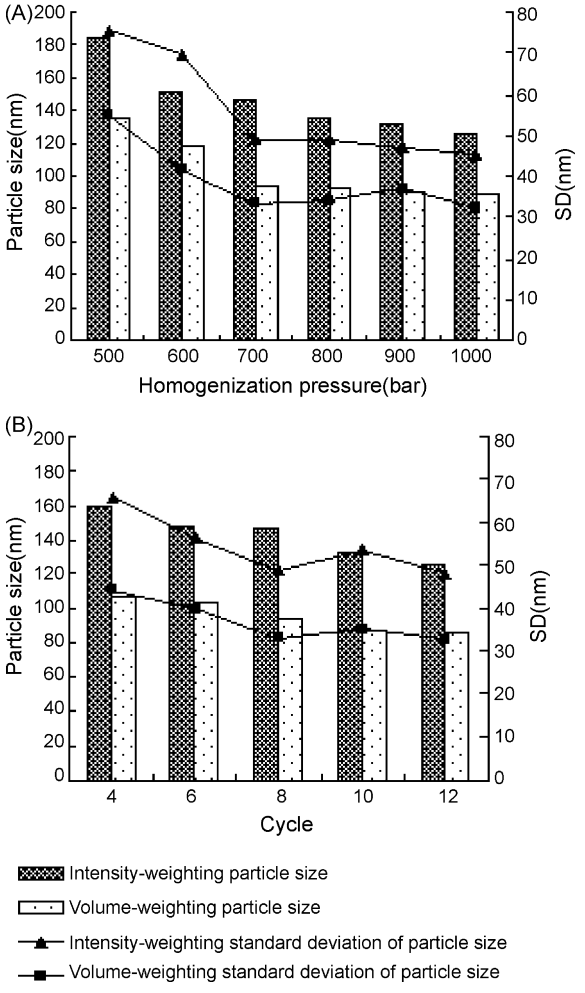

Fig. 7. (A) Influence of different homogenization pressures on the particle size distribution of CLEs (cycle number was 8) and (B) influence of different cycle numbers on the particle size distribution of CLEs (homogenization pressure was 700 bar).

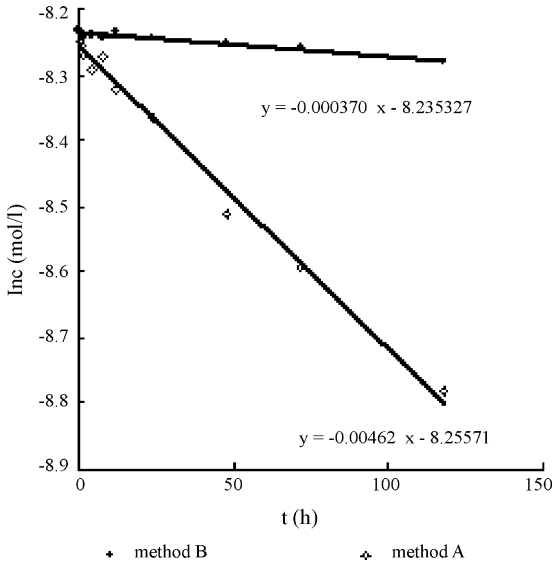

Fig. 8. The effect of the drug loading methods on the content of CN in CLE at 80 °C.

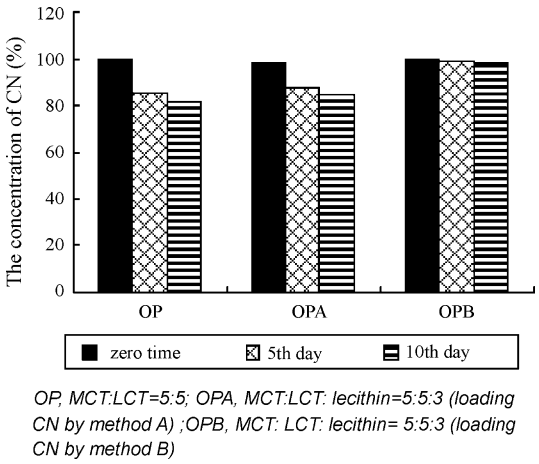

Fig. 9. The degradation of CN in the oil phase with different drug loading methods at 60 °C over 10 days.

loaded by method A, the stability of CN in the oil improved slightly. This must be attributed to the localization of a part of the drug in the lecithin layer. Nevertheless, when the method B was used, the degradation of CN in the oil was reduced significantly with only a 1.4% decrease in the content of CN at 60 °C after 10 days. Therefore, it can be concluded that almost no drug was dispersed in the oil phase either.

In summary, the results above can prove that the CN is successfully localized within the lecithin membrane by method B.

3.3.2. Effect of temperature on the degradation rate of CN in aqueous solution and lipid emulsion

The degradations of CN in aqueous solution and emulsion with a pH of 8.50 at 40, 60, 80, and 90 °C were monitored by HPLC to evaluate the effect of the drug carrier on the kinetics of CN.

It was found that there were oily materials sticking to the wall of the tube at the surface of CLE at 80 and 90 °C at 120 h. The reason for this phenomenon might be as follows: the surface water of CLE evaporated more quickly than that internally inducing CLE to become dehydrated to give an oil component at high temperatures. This may affect the degradation kinetics of CLE. Therefore, test samples were collected within 120 h.

The solution and emulsion plots of ln C (concentration) versus time gave good straight lines at four different temperatures indicating pseudo-first-order degradation kinetics. The influence of temperatures on the rate constants (k) could be expressed in natural logarithmic form (ln) by the Arrhenius equation:

$$\ln k = -\frac{E_a}{RT} + \ln A$$

where A is the frequency factor, E_a is the activation energy, R is the universal gas constant, and T is the absolute temperature. Fig. 10 shows Arrhenius plots of the degradation of CN in aqueous solution and lipid emulsion at different temperatures and the equations fitting for the k values. The activation energies calculated from the slopes were 53.28 and 51.27 kJ/mol in aqueous solution and emulsion, respectively. The activation energy of CN in emulsion seems similar to that in aqueous solution. This indicates that the stabilizing effect of the drug carrier on CN was not a result of an alteration in the degradation reaction itself. The frequency factors (A) of CN in aqueous solution and emulsion were calculated to be 2.45×10^6 and 1.58×10^4. The shelf-life ($T_{0.9}$) is a parameter to forecast the period a product can be stored without loss of potency. It is described as the time taken for 10% of the product at a given temperature. The following equation was used for determining the shelf-life of CN at

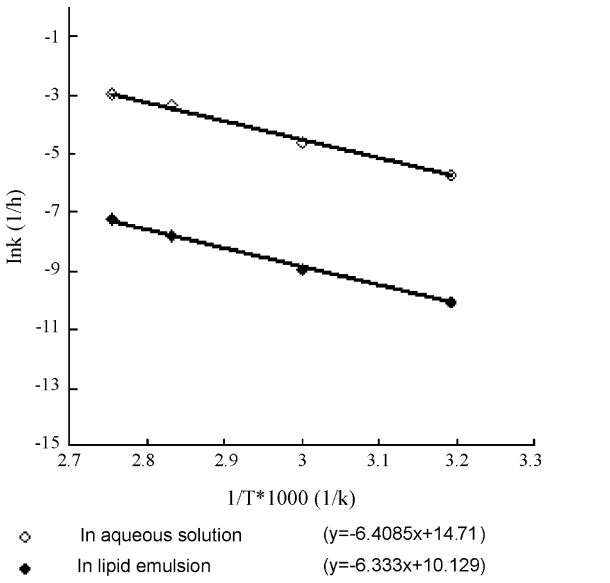

Fig. 10. Arrhenius plot based on the rate constants of CN in aqueous solution and emulsion measured at different temperatures at pH 8.50.

25 and 4 °C:

$$T_{0.9} = \frac{0.105}{k}$$

Since k could be obtained from the Arrhenius formulation, it was easy to calculate the shelf-life of CN in aqueous solution at 25 and 4 °C and this was 3.9 days and 19.8 days. While the shelf-life of CLE at 25 and 4 °C was found to be 258.4 days and 1238.4 days. From the results above, it can be concluded that the $T_{0.9}$ of CN in emulsion was clearly prolonged compared with that in aqueous solution. The CLE was stable at room temperature, and its shelf-life could be extended significantly if stored at 4 °C.

3.3.3. Effect of thermal sterilization methods and thermal sterilization time

Several thermal sterile methods were used, including rotating autoclave in a 115 °C water steam bath for 30 min (i), rotating autoclave in a 121 °C water steam bath for 8 min (ii), and rotating autoclave in a 126 °C water steam bath for 3 min (iii) to optimize the thermal sterile conditions. To quantify the effectiveness of thermal sterilization, the F_0 value of all the selected thermal sterile methods above were about 8, where F_0 was used to express the time required to kill a particular organism under specified conditions. Table 3 provides the study results.

According to Table 3, the CLE could not withstand the sterilization condition (iii) as shown by the appearance of oil drops on the surface of the CLE. The CLE sterilized with methods (i) and (ii)

Table 3
The physical appearance, particle size distribution, zeta potential and the content of CN after different thermal sterilization methods.

	Before sterilization	(i)	(ii)	(iii)
Physical appearance	Good	Good	Good	Visual oil drops on surface
Particle size distribution (nm)	157.5 ± 58.81	158.1 ± 52.12	152.0 ± 61.31	ND
Zeta potential (mV)	−26.32	−26.45	−27.12	ND
Residual content (%)	99.4	99.5	99.2	ND

ND, not determined.

Table 4
Evaluation of the CLE autoclaving in a 121 °C rotating water steam bath for different time.

	0 min	8 min	10 min	15 min	20 min	25 min
Physical appearance	Good	Good	Good	Good	Good	Good
pH value	8.51	8.49	8.48	8.45	8.33	8.12
Particle size distribution (nm)	157.5 ± 58.81	152.0 ± 61.31	156.8 ± 52.72	157.7 ± 53.63	148.9 ± 58.15	159.8 ± 62.76

Table 5
Characterization of optimum CLE during 12 months investigation at 25 ± 2 and 4 ± 2 °C.

Characterization of CLE	0 time	Storage at 25 ± 2 °C for 12 months	Storage at 4 ± 2 °C for 12 months
Physical appearance	Good	Good	Good
pH value	8.49	8.23	8.31
The existence of drug crystal	NF	NF	NF
Particle size distribution (nm)	158.9 ± 48.56	168.3 ± 45.87	162.5 ± 51.84
Entrapment efficiency	99.2	97.8	98.5
Zeta potential	−26.34	−21.54	−24.12
Drug remaining	100.1	98.4	99.2

NF, not found.

possessed good physical and chemical stability. It is well known that provided that the sterilization effects are similar, the method with the higher sterilization temperature and shorter sterilization is better for maintaining the stability of the drug on the account of the lower activation energy. Therefore, the 121 °C rotating steam sterilization was the best among all the methods examined.

Based on the results obtained from different sterilization methods, the CLE needs further investigation in a 121 °C rotating water steam bath for different time (8, 10, 15, 20, and 25 min) to find the most appropriate sterilization time. The physical and chemical stability parameters of the CLE before and after sterilization are shown, respectively, in Table 4 and Fig. 11.

Table 4 shows that the CLEs sterilized for different time remain stable with regard to their physical appearance and particle size distribution. However, the pH value of the CLEs fall on heating slightly, as a result of glyceride and phosphatide hydrolysis, and this liberates free fatty acids (Alison, 1999). This phenomenon cannot be avoided during the thermal sterilization. Since the change of the pH value is slight, it will not affect the stability of the CLE.

Referring to Fig. 11, there was almost no degradation of CN in emulsion within 15 min. After the sterilization time increased to 20 min, the content of CN reduced. This can be attributed to the hydrolysis of lecithin at high temperature. As the lecithin hydrolyzed, its capacity to encapsulate the drug weakened, resulting in the degradation of CN. It is suggested that 15 min is a critical time for maintaining the stability of CLE during the steam sterilization. Hence, 15 min was selected as the most suitable thermal sterilization time.

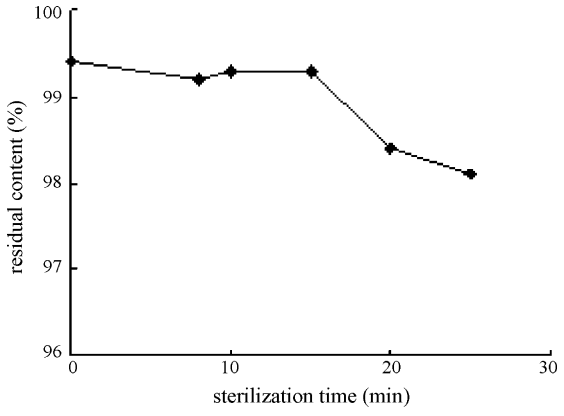

Fig. 11. The content of CN determined at different times during rotating sterilization in a 121 °C water steam bath.

Considering of both of the safety and stability of CLE, the most appropriate sterilization condition was sterilization in a 121 °C rotating water steam bath for 15 min.

3.3.4. *Long-term stability investigation*

Though we can estimate the period of validity of CLE by the shelf-life which calculated from the data about the kinetics of CLE, the CLE still needs undergoing the practical condition and duration to provide the actual values. So, the long-term stability of sterilized CLE was investigated at 25 and 4 °C during 12 months and evaluated by physical appearance, particle size distribution, pH value, entrapment efficiency and drug remaining. In addition, because the microns which were greater than 3 μm cannot be detected by Nicomp™ 380 Particle Sizing system (SantaBarbara, USA), a Motic DMBA 450 microscope (MoticChina Group Co. Ltd., Beijing, China.) was used to detect the drug crystals in the system. The final results were provided in Table 5.

Based on Table 5, the mean diameters of all the samples were controlled in the range of 100–200 nm and no drug crystal or oil droplet was observed by light microscopy. This could guarantee an ameliorated tissue tolerance, uptake and transfer, no vascular blockage and no foreign body (Constantinides, 1995). Beside, the good chemical stability of CLE was confirmed by the unchanged CN concentration. Other characterizations of CLE did not change significantly either. Hence, it can be concluded that CLE is an excellent intravenous administration system, which can undergo the real storage conditions for 12 months. The study of the long-term stability is still ongoing.

4. Conclusion

In conclusion, the main difficulty associated with the study was the poor chemical stability of CN. This problem can be solved by loading the drug in the lecithin layer in the lipid emulsion. Formulations for intravenous CLE were developed by considering the physical and chemical stability, and the optimum one consisted of cinnarizine 0.1% (w/v), MCT 5% (w/v), LCT 5% (w/v), egg lecithin 3% (w/v), Tween 80 0.2% (w/v), F68 0.2 (w/v), glycerol 2.5% (w/v), sodium oleate 0.03% (w/v) and EDTA 0.02% (w/v). The factors influencing the chemical stability of CLE, such as the choice of oil phase, the amount of lecithin, the drug loading method, the pH, the temperature and sterilization conditions, were discussed thoroughly in this paper. The study demonstrates that CLE can resist rotating sterilization in a 121 °C water steam bath and be stored at 4 °C with a shelf-life of 1471.6 days.

The final product is superior in chemical stability to the aqueous solution of CN demonstrating the feasibility of and using it in clinical applications producing it on an industrial scale.

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References

Alison, G.F., 1999. Top ten considerations in the development of parenteral emulsions. Pharm. Sci. Technol. Today 4, 134–143.
Allewijn, F.T., 1968. The distribution of cinnarizine and its metabolites in the rat. Life Sci. 7, 989–994.
Chansiri, G., Lyons, R.T., Patel, M.V., Hem, S.L., 1999. Effect of surface charge on the stability of oil/water emulsions during steam sterilization. J. Pharm. Sci. 88, 454–458.
Bock, T.K., Lucks, J.S., Kleinebudde, P., Mueller, R.H., Mueller, B.W., 1994. High pressure homogenisation of parenteral fat emulsions—influence of process parameters on emulsion quality. Eur. J. Pharm. Biopharm. 40, 157–160.
Constantinides, P.P., 1995. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. Pharm. Res. 12, 1561–1572.
Cordes, E.H., Jencks, W.P., 1962. On the mechanism of Schiff Base formation and hydrolysis. J. Am. Chem. Soc. 84, 832.
Davis, S.S., Washington, C., 1988. Drug emulsion. European Patent 0 296 845 A1, 22 June.
Driscoll, D.F., Bacon, M.N., Bistrian, B.R., 2000. Physicochemical stability of two different types of intravenous lipid emulsion as total nutrient admixtures. J. Parenter. Enteral Nutr. 24, 15–22.
Eccleston, G.M., 1992. Emulsions. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology, vol. 5. Marcel Dekker Inc., pp. 137–188.
Ferezou, J., Nguyen, T.L., Leray, C., Hajri, T., Frey, A., Cabaret, Y., Courtieu, J., Lutton, C., Bach, A.C., 1994. Lipid composition and structure of commercial parenteral emulsions. Biochim. Biophys. Acta 1213, 149–158.
Floyd, A.G., 1999. Top ten considerations in the development of parenteral emulsions. Pharm. Sci. Technol. Today 4, 134–143.
Gettings, S.D., Lordo, R.A., Feder, P.I., Hintze, K.L., 1998. A comparison of low volume, draize and in vitro eye irritation test data. II. Oil/water emulsions. Food Chem. Toxicol. 36, 47–59.
Godfraind, T., Towse, G., Nueten, J.M., 1982. Cinnarizine a selective calcium entry blocker. Drugs Today 18, 27–42.
Groves, M.J., Wineberg, M., Brain, A.P.R., 1985. The presence of liposomal material inphosphatide stabilized emulsions. J. Disper. Sci. Technol. 2, 237–243.
Guo, P., Liu, Y.Z., Rong, X.Y., Nie, X.Y., 2006. Determination of cinnarizine and related substances by HPLC. J. Hebei Univ. Sci. Technol. 27, 214–215.
Jia, Z.R., Sun, Y.H., Wang, D.M., He, Z.G., 2007. Studies on free-drying liposomes encapsulating cinnarizine–cyclodextrin complexes. Chin. J. Pharm. 5, 298–305.
Lance, M.R., Washington, C., Davis, S.S., 1995. Structure and toxicity of amphotericin B/triglyceride emulsion formulations. J. Antimicrob. Chemother. 36, 119–128.
Mollica, J.A., Rehm, C.R., Smith, J.B., 1969. Hydrolysis of hydrochlorothiazide. J. Pharm. Sci. 58, 635.
Moreno, M.A., Ballesteros, M.P., Frutos, P., 2003. Lecithin-based oil-in-water microemulsions for parenteral use: pseudo ternary phase diagrams, characterization and toxicity studies. J. Pharm. Sci. 92, 1428–1437.
Morey, T.E., Varshney, M., Flint, J.A., Seubert, C.N., Smith, W.B., Bjoraker, D.G., Shah, D.O., Dennis, D.M., 2004. Activity of microemulsion-based nanoparticles at the human bio-nano interface: concentration-dependent effects on thrombosis and hemolysis in whole blood. J. Nanoparticle Res. 6, 159–170.
Muchtar, S., Levy, M.Y., Saring, S., Benita, S., 1991. Stability assessment of a fat emulsion prepared with an original mixture of purified phospholipids. STP Pharm. 1, 130–136.
Ogata, H., Aoyagi, N., Kaniwa, N., Ejima, A., Sekine, N., Kitamura, M., Inoue, Y., 1986. Gastric acidity dependent bioavailability of cinnarizine from two commercial capsules in healthy volunteers. Int. J. Pharm. 29, 113–120.
Singh, B.N., 1986. The mechanism of action of calcium antagonists relative to their clinical applications. Br. J. Clin. Pharmacol. 21, 109–212.
Singh, M., Ravin, L.J., 1986. Parenteral emulsions as drug carrier systems. J. Parenter. Sci. Technol. 40, 34–41.
Smyrniotis, V.E., Kostopanagiotou, G.G., Arkadopoulos, N.F., Theodoraki, K.A., Kotsis, T.E., Lambrou, A.T., Vassiliou, J.G., 2001. Long-chain versus medium-chain lipids in acute pancreatitis complicated by acute respiratory distress syndrome: effects on pulmonary hemodynamics and gad exchange. Clin. Nutr. 20, 139–143.
Tokumura, T., Ichikawa, T., Sugawara, N., Tatsuishi, K., Kayano, M., Machida, Y., Hoshida, H., Nagai, T., 1985. Kinetic of degradation of cinnarizine in aqueous solution. Chem. Pharm. Bull. 33, 2069–2072.
Wang, D.K., 2006. Water soluble cinnarizine salt and its injection form. CN Patent 1847230-2006-10-18.
Washington, C., Davis, S.S., 1988. The production of parenteral feeding emulsions by microfluidizer. Int. J. Pharm. 44, 169–176.
Yamano, Y., Seike, N., 1983. Stability of emulsion prepared with soybean phospholipids and transfer of phospholipid to separated phases. Nippon Shokuhin Gakkaisshi 30, 618–623.
Yu, Y.L., Lu, Y., Tang, X., Cui, F.D., 2008. Formulation, preparation and evaluation of an intravenous emulsion containing Brucea javanica oil and Coix Seed oil for anti-tumor application. Biol. Pharm. Bull. 31, 673–680.
Zhang, T.H., Zhou, C.S., Liu, Y., 2004. Cinnarizine powder injection containing HPCD, its solid preparation, and preparation method. CN Patent 1515260A-2004-7-28.