International Journal of Pharmaceutics 425 (2012) 53–61

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

In vitro and *in vivo* evaluation of a simple microemulsion formulation for propofol

Guiling Li[a,*], Yating Fan[b], Xinru Li[b], Xiaoning Wang[b], Yanfang Li[c], Yan Liu[b,*], Mei Li[a]

[a] *Institute of Medicinal Biotechnology, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100050, China*
[b] *Department of Pharmaceutics, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China*
[c] *Central Lab, Peking University Third Hospital, Beijing 100191, China*

ARTICLE INFO

Article history:
Received 30 January 2011
Received in revised form
15 December 2011
Accepted 7 January 2012
Available online 14 January 2012

Keywords:
Propofol
Microemulsion
Safety
Pharmacological effect

ABSTRACT

The aim of the present study was to develop an oil-free o/w microemulsion, composed of pluronic F68, propylene glycol and saline, which solubilized poorly soluble anesthetic drug propofol for intravenous administration. The ternary diagram was constructed to identify the regions of microemulsions, and the optimal composition of microemulsion was determined by *in vitro* evaluation such as globule size upon dilution and rheology. The droplet size of the diluent emulsion corresponding to oil-in-water type ranged from 200 to 300 nm in diameter. Stability analysis of the microemulsions indicated that they were stable upon storage for at least 6 months. Hemolysis percent of propofol microemulsions was lower than that of commercial lipid emulsion (CLE) at 4 h. Acute toxicity test showed that median lethal dose of propofol microemulsion was the same as that of CLE. No significant difference in time for unconsciousness and recovery of righting reflex was observed between the prepared microemulsions and CLE. In conclusion, microemulsion would be a promising intravenous delivery system for propofol.

1. Introduction

Propofol (2,6-diisopropylphenol) has gained increasing popularity in anesthesia. Induction of anesthesia with propofol is rapid, and maintenance can be achieved by continuous infusion or by intermittent bolus doses. Its greatest advantage is the rapid recovery, even after long periods of anesthesia. A particularly low incidence of postoperative nausea and vomiting is also observed (Langley and Heel, 1988; Trapani et al., 1998). Propofol is formulated as 1% (w/v) of oil/water emulsion (Diprivan®, Zeneca UK) composed of soya bean oil, glycerol and purified egg phosphatide for intravenous use in clinic. However, some side-effects associated with the emulsion-based formulation of propofol, such as the risk of hyperlipidemia in patients under long-term use, the risk of bacterial contamination after exposure to air, pain on injection, poor physical stability, potential for embolism and the phase separation by concomitant use of other drugs, e.g., lidocaine (Bennett et al., 1995; Masaki et al., 2000; Park et al., 2003; Prankerd and Stella, 1990; Tan and Onsiong, 1998), have been reported. In view of the clinical importance of propofol, the alternative formulations such as microemulsions (Kim et al., 2007; Lee et al., 2008; Morey et al., 2006), inclusion complex (Trapani et al., 1998) and polymeric micelles (Ravenelle et al., 2008a,b) have been developed to improve its solubility. Among them, as a monophasic, thermodynamically stable and transparent (or slightly translucent) dispersion (Aboofazeli et al., 2000), microemulsion has been recently attracted much attention in pharmaceutical research areas. Several propofol microemulsion formulations which with potential perspective were investigated in which novel surfactants such as sodium caprylate and polyethylene glycol 660 hydroxystearate were selected as an emulsifier except for pluronic F68. However, sodium caprylate, an ionic surfactant, might cause hemolysis as occurred *in vitro* with higher concentrations (EC_{50} of 213 mM) (Morey et al., 2004). Besides, change in pH will affect the ratio of ionic to nonionic form, which altered surface activity itself and further some important properties (e.g., phase diagram, free drug concentration and so on) of microemulsion. As for polyethylene glycol 660 hydroxystearate, it has not been approved by Food and Drug Administration (FDA). In addition, there was no report for safety, such as hemolysis, vein irritation and anaphylaxis. Thus, there still remains a need to develop a well proven propofol microemulsion formulation that can solubilize propofol efficiently and overcome the aforementioned drawbacks.

This study presented a new microemulsion formulation consisting of Pluronic F68, propylene glycol and saline which were proved to be safe in clinic and used in commercial injectable formulations. It was expected to manifest increased drug solubility, improve the safety and maintain the efficacy of propofol. Optimal microemulsion formulation was selected regarding reduced amount of surfactant, resistance to dilution, smaller globule size and appropriate rheology. The physicochemical properties of microemulsion system, such as *in vitro* drug release and stability,

* Corresponding authors. Tel.: +86 10 63150697; fax: +86 10 63150697.
E-mail addresses: liguiling99@gmail.com (G. Li), yanliu@bjmu.edu.cn (Y. Liu).

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doi:10.1016/j.ijpharm.2012.01.011

were investigated in detail. In addition, safety such as hemolysis, acute toxicity and *in vivo* pharmacological effect thereof were also evaluated.

2. Materials and methods

2.1. Materials

Propofol was purchased by Zhongke Taidou Chemical Co., Ltd. (Shandong, China). Pluronic F68 was obtained from Yunhong Chemical Co., Ltd. (Shanghai, China). Propylene glycol was purchased from Nanjing Chemical Reagent Co., Ltd. (Jiangsu, China). Propofol commercial lipid emulsion (CLE) injection was provided from Guorui Pharmaceutical Co. (Sichuan, China).

Male Sprague-Dawley (SD) rats (weighing 200 ± 20 g), New Zealand rabbits (weighting 2.0–2.5 kg) and Kunming mice (weighing 18–22 g) were obtained from Animals Center of Peking University Health Science Center. All care and handling of animals were performed with the approval of Institutional Authority for Laboratory Animal Care of Peking University.

2.2. Phase diagram studies

Safe and biocompatible nonionic surfactants, Pluronic F68, and a cosurfactant, propylene glycol (PG), were chosen to form microemulsions. Pseudoternary phase diagrams of oil, surfactant/cosurfactant, and saline were developed in order to formulate microemulsion delivery systems for propofol (oil phase). Briefly, propofol was dissolved by PG in certain weight ratios (from 1:1 to 1:10), and a certain amount of pluronic F68 dissolved in saline was added under magnetic stirring. The weight ratio (K_m) of pluronic F68 to PG varied from 1:9 to 20:1 (w/w), and the weight ratio of pluronic F68 to saline was 3:1. Then, each mixture was titrated with saline under magnetic stirring at room temperature. Following the addition of aliquot of water phase, the mixture was visually examined for transparency. The points from clear to turbid and turbid to clear were designated as emulsion and microemulsion, respectively. The physical states were represented on a pseudo-ternary phase diagram with one axis representing saline, one representing propofol and the third representing surfactant–cosurfactant mixture (S_m) with a certain amount of saline dissolving pluronic F68.

2.3. Electric conductivity

The electric conductivity (κ) of the selected microemulsions containing propofol (1.9%, w/w_{water}), pluronic F68 (38.5%, w/w_{water}), PG (38.5%, w/w_{water}) and water, was measured as a function of weight fraction of water using a DDSJ-308 conductivity meter (Shanghai REX Instrument Factory, China) with a DJS-1 electrode. The dependence of conductivity on the amount of water was carried out by dropwise addition of aqueous phase into the selected mixture at 25 °C.

2.4. Preparation of microemulsion for propofol

A certain amount of propofol was dissolved in PG, then the propofol solution was dropped into 20% (w/w) Pluronic F68 saline solution at room temperature. After agitation with a magnetic stirrer, these components combined to form microemulsions. The propofol microemulsion was filtered through a 0.45 μm filter (Millex-GV, Millipore, USA) thereafter to strain off the insoluble particles. Finally, it was poured into glass vials under nitrogen atmosphere, and autoclaved for 30 min at 100 °C. A clear, colorless and homogeneous microemulsion of propofol was obtained.

2.5. Globule size determination

The globule size and its distribution (characterized by polydispersity index, PDI) of the formulations were measured using dynamic light scattering (DLS) (Malvern Zetasizer ZEN3500, UK). All measurements were performed at a wavelength of 532 nm with a scattering angle of 173° at 25 °C after diluting the dispersion to an appropriate volume with saline.

2.6. Rheology

The rheological properties of the propofol microemulsions were studied with viscometer (DV-II+, Brookfield, USA). All measurements involved shear stress, shear rate and apparent viscosity, made in triplicate, were performed in a temperature-controlled environment at 37 °C and the range of rotational speed was ranged from 15 to 200 rpm. The shear rate (γ) in s^{-1} and the viscosity (η) in centipoises (mPa s) were determined from the instrument readings and fitted to the power law constitutive equation (Tung, 1994): $\eta = m\gamma^{n-1}$. The two dimensionless quantities: the consistency index (m) and the flow index (n) characteristic for each formulation were obtained. If $n = 1$ the formulation exhibits Newtonian behavior while if n is less than 1, the formulation corresponds to shear thinning flow. The lower the value of n the more shear thinning the formulation (Chang et al., 2002; Copetti et al., 1997; Owen et al., 2000).

2.7. High-performance liquid chromatography (HPLC) analysis

High-performance liquid chromatography (HPLC) analyses were performed with a Shimadzu LC-10A (Shimadzu, Kyoto, Japan) apparatus equipped with a Shimadzu SPD-10A UV detector and a Shimadzu LC-A pump. A reversed phase column (Diamonsil C-18, 4.6 mm × 250 mm, Dikma Technologies, China) in conjunction with a security guard cartridge was eluted with a mobile phase composed of methanol and deionized water (8:2, v/v). The mobile phase was pumped at a flow rate of 1.0 mL/min and the column temperature was set to 25 °C. Calibration curves were prepared using mobile phase as the solvent and were linear ($r^2 > 0.999$) over the range of concentrations of interest. The results of analysis method validation indicated that the coefficient of variation of the inter-day and intra-day precision of the quality control samples ranged from 0.27% to 1.71% and accuracy ranged from 98.1% to 102.1%. To determine drug content in microemulsions, the propofol microemulsion was diluted with mobile phase to appropriate concentration before HPLC analysis.

2.8. In vitro release

One milliliter of propofol microemulsion as well as commercial lipid emulsion as control containing a certain amount of propofol was placed into a dialysis bag with molecular weight cutoff of 3 kDa, respectively, and then the dialysis bag was immersed into a flask containing 30 mL of release medium (pH 7.4 phosphate buffer solution) with 30% (v/v) ethanol (sink condition) which was kept in a constant temperature shaking water bath at 37 °C and 100 rpm. At appropriate intervals, 1 mL aliquots of the release medium were withdrawn and immediately replaced with an equal volume of fresh medium. Based on the amount of released drug determined by HPLC, the cumulative release percentage of propofol was calculated.

2.9. Stability assessment

Chemical and/or physical stability of propofol microemulsion was assessed under long-term storage conditions (20 ± 2 °C/60 ± 5% RH). Propofol microemulsions were subdivided

into 5-mL glass vials with Teflon-coated butyl stoppers and aluminum crimped tops under nitrogen atmosphere, respectively, and the vials were stored upright for long-term storage. At various time points, content of propofol quantified by the HPLC method and pH were determined. Besides, physical changes including mean globule size, PDI and flow index (n) were monitored as mentioned above. The CLE was also tested as control.

2.10. Hemolytic test

The hemolytic potential of the developed microemulsions was evaluated according to an *in vitro* method (Reed and Yalkowsky, 1985) using fresh rabbit whole blood obtained from arteria cruralis. Initially, 20 mL of rabbit blood were put into a clean and dry beaker stirred with a glass stick to remove fibrinogen and were placed into centrifugal tube. Thereafter normal saline was added into the centrifugal tube. After mixing, the resulted mixture was centrifuged for 15 min (2500 rpm). Once the upper clean liquid was removed, normal saline was added. The mixture was centrifuged again and precipitates (red cells) were rinsed again and again up to that upper clean liquid got transparent. Finally, 2% erythrocyte standard dispersion was obtained by adding adequate amount of normal saline to the tube. The sample solutions and the erythrocyte suspensions were incubated separately at 37 °C for 20 min before mixing. Then they were mixed to a final erythrocyte volume percentage of 1% and incubated at 37 °C in a bath shaker. Control samples of 0% lysis (in saline) and 100% lysis (in double-distilled water) were employed in all experiments. After vortex, the tubes were incubated at 37 °C and observed microscopically from 30 min to 4 h. The absorption of the resulting supernatant was determined at 545 nm (Shimadzu, UV2450, Japan) against a blank sample. The mean value of three measurements using different samples was recorded.

2.11. Acute toxicity studies

To evaluate the acute toxicity of propofol formulations, the median lethal dose (LD_{50}) of both propofol microemulsion and CLE, was calculated. Male Kunming mice weighing 18–22 g were housed under normal conditions with free access to food and water. Fifty mice were randomly divided into five groups ($n = 10$). Propofol emulsions diluted with normal saline to variable concentration were injected via tail vein at the different doses, respectively. Mice were observed for fifteen days in all groups, and the number of mice surviving was recorded. The LD_{50} was calculated using the Bliss method.

2.12. Anesthetic action

Male Sprague-Dawley rats at the beginning of the experiments, were provided with standard food and tap water ad libitum, and exposed to alternating 12 h periods of light and darkness. Temperature and relative humidity were maintained at 22 °C and 60%, respectively. Food and water were freely available, and the animals were acclimatized for more than 7 days before use.

Eighteen animals were randomly divided into two groups. Rats in each group were injected intravenously (single bolus in the lateral tail vein) with their respective formulations such as emulsions diluted from propofol microemulsions and CLE at a single dose of 10 mg/kg. The end of injection was taken as time zero ($t = 0$). After each administration, the time to loss of locomotor activity was recorded for each animal. This was defined as the time at which the animal lost the capability to regain ventral recumbency once manually placed on its side. Rats were maintained in dorsal or lateral recumbency during evaluation, and the time to regain locomotor activity was recorded. Following recovery from anesthesia, animals were returned to their respective cages and offered food. Finally, the rats were humanely sacrificed.

2.13. Statistical analysis

All data were shown as mean ± SD. The difference between groups was analyzed using the Student t-test. p-Value less than 0.05 was considered as statistically significant.

3. Results and discussion

The ideal immediate-release injectable intravenous formulation is aqueous and isotonic with respect to body fluids such as saline, glucose, and dextran. If the drug is not soluble in water, a common approach is to increase the solubility by changing the solution pH and/or adding a water-soluble organic solvent (i.e., a cosolvent). These water-soluble organic solvents used in intravenous formulations include ethanol, glycerol, PEG 400, and propylene glycol. The upper limits of these solvents were 20% for ethanol and 50% for the others (Strickley, 2004). In addition, solubilization techniques for injectable formulations, similar to those used in oral formulations, are applied, including the use of mixed aqueous/organic cosolvents, organic solvent mixtures, cyclodextrin complexation, emulsion, liposome formulations, nano-formulations, polymeric micelles, and combinations of techniques (Akers, 2002; Gaucher et al., 2005; Kipp, 2004; Strickley, 1999; Sweetana and Akers, 1996; Wang and Kowal, 1980).

Propofol is a unique compound in comparison to other intravenous anesthetics. In the molecule structure of propofol, the benzene ring and the isopropyl side groups are highly lipophilic. This high lipophilicity ($\log P = 4.16$) (Thompson and Goodale, 2000) suggests that propofol can mix only with lipophilic substances or organic solvents. Nearly all low-molecular-weight organic solvents with which propofol is freely miscible are not useful due to the fact that vehicles for the clinical delivery of anesthetics should be devoid of toxic side effects and of sedative and anesthetic properties (Baker and Naguib, 2005).

Microemulsions, first introduced by Hoar and Schulman (1943), are isotropically clear, thermodynamically stable dispersions of two immiscible liquids, such as oil and water, that are stabilized by a relatively large amount of an emulsifier, commonly in conjugation with a co-emulsifier (Ho et al., 1996). In general, the interfacial tension of microemulsions is ultralow ($\sim 10^{-2}$ mN/m), and the small size of the droplets in microemulsions, less than the wavelength of light, is responsible for their transparency (Bhargava et al., 1987). In the case of our experiment, propofol, a poorly water-soluble drug, was solubilized in microemulsions primarily composed of PG (co-emulsifier) and Pluronic F68 (emulsifier). These emulsions had small globule diameters ranging from 20 to 80 nm, which, as noted for such small particles in solution, resulted in the solutions being clear (Kwon, 2003).

3.1. Phase diagram analysis

Pseudo-ternary diagrams are equilateral triangles that describe the compositional phase behavior of a system. The vertices represent pure component or two components, the edges denote two-component systems, and the interior represents all three components in the system. These diagrams can be used to note the physical boundary conditions of oils and surfactants necessary to formulate thermodynamically stable microemulsions. Fig. 1 presents the pseudo-ternary phase diagrams with various weight ratios (K_m) of Pluronic F68 to PG. The transparent region and the rigid region on the phase diagram represent the microemulsion and a conventional emulsion based on visual observation, respectively.

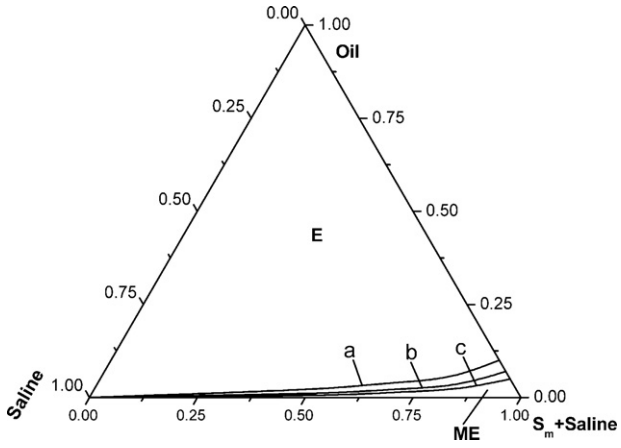

Fig. 1. Pseudo-ternary phase diagrams composed of propofol, pluronic F68, propylene glycol and saline. K_m, mass ratio of pluronic F68 (emulsifier) to propylene glycol (co-emulsifier). (a) 20, (b) 10 and (c) 5; S_m, the mixture of surfactant and cosurfactant; *E*, crude emulsion; ME, o/w microemulsion.

It was shown that, as the K_m increased, the area of the microemulsion region increased, a result that was consistent with that of a previous report (Zhang et al., 2004). At the same time, the boundary lines moved toward the oil apex (propofol) as with larger K_m values indicating that larger K_m values meant more amount of pluronic F68 relative to PG in S_m might enhance the solubility of propofol. That is to say, more amount of pluronic F68 might incorporate more oily drug (propofol) in systems.

Generally, the dilutability of microemulsions with water without causing precipitation of the incorporated drug is a major concern for their use in the clinic (Kiepert, 1989; Lawrence and Rees, 2000). Thus, we attempted to fully characterize the chosen system. The results indicated that a greater amount of Pluronic F68 should be added into the microemulsion system to solubilize more propofol and keep the system dilutable, as shown in Fig. 1. However, this high amount of Pluronic F68, resembling other polymeric carrier materials used in delivery system, might lead to a series of problems such as increased viscosity and decreased safety of the microemulsion. Based on these considerations, several dilutable microemulsions with various contents of Pluronic F68 and PG were prepared and are referred to as formulations I–V (Table 1). Propofol (1%) was incorporated into these systems to determine the optimum ratios of the components for these microemulsions.

3.2. Electric conductivity

Electrical conductivity (κ) measurements provided structural information mainly on possible transitions upon dilution. Fig. 2 showed the variation of electrical conductivity as a function of water weight fraction. With increasing water content, the electrical conductivity changed according to three successive stages: The linear increase was due to the formation of aqueous microdomains ascribed from the partial fusion of clustered inverse microdroplets. The phenomenon suggested that a W/O microemulsion was formed in lower water content region, which was attributed to the occurrence of a percolation transition (Caboi et al., 1997; Eicke et al., 1999; Mays, 1997; Peyrelasse and Boned, 1990; Riter et al., 1997).

Table 1
Compositions (%, w/w_{saline}) of the studied propofol microemulsions.

Formulation	I	II	III	IV	V
Propofol	1	1	1	1	1
Pluronic F68	20	20	20	15	10
PG	60	40	20	20	20

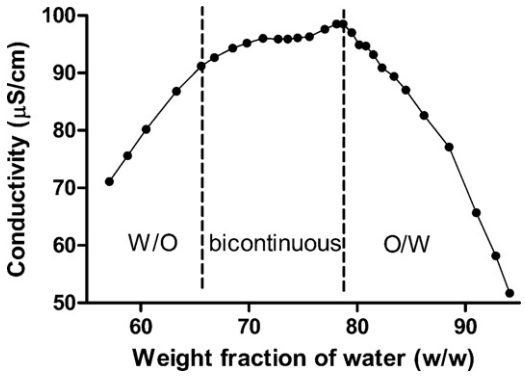

Fig. 2. The electric conductivity κ of the microemulsion system composed of propofol/pluronic F68/propylene glycol/water (1/20/20/52) as a function of weight fraction of water.

In the initial stage, these W/O droplets embedded in nonconducting oil phase, were isolated from each other below percolation threshold, and hence contributed very little to the conductance. As the weight fraction of water was over the percolation threshold, some of these conductive droplets began to contact and colloid each other and formed clusters (Moulik and Ray, 1994; Vollmer et al., 1984). The number of such clusters increased sharply above the percolation threshold, leading to the observed increase of electrical conductivity. The next nonlinear curve increase indicated that the medium underwent further structural transitions and a bicontinuous microstructure was formed (Lada et al., 1989; Uvray and Gelbart, 1994), owing to the progressive growth and interconnection of the aqueous microdomains. The final decrease of κ with increase of water content corresponded to the appearance of water-continuous emulsion type media (Djordjevic et al., 2004). That is, an O/W emulsion formed at high water content. The final decrease of κ merely resulted from the fact that the concentration of the O/W emulsion droplets was progressively diluted with water. The κ measurements (Fig. 2) revealed an expected behavior with aqueous phase dilution. It was illustrated that three different microstructure including W/O, bicontinuous, and O/W types were presented. The transitions from water-in-oil to bicontinuous domains and finally to oil-in-water microdroplets occurred at which the conductance slope changed and the boundaries of each structure were easily detected by the changes in the slope of the κ values.

3.3. Effect of dilution on globule size

In any emulsion, stability is demonstrated by the maintenance of the globule size distribution (GSD) within defined limits (i.e., no growth in the extreme population of large-diameter fat globules). Alterations in the GSD indicate a change in the stability of the lipid-based injectable emulsion. The collapse of an emulsion system is manifested by the fusion of droplets that ultimately separate from the dispersed phase as enlarged fat globules via a process known as coalescence (Driscoll, 2006). From a clinical perspective, globule size limits for lipid-based injectable emulsions are most important, as they ultimately reflect the safety of the formulation. Fat globules with a size of greater than 5–6 μm are thought to put patients at risk for fat embolism (Illum and Davis, 1982; Kanke et al., 1980). United States Pharmacopeia (USP) also specifies a physiologically relevant dimension of 5 μm. Consequently, changes in the globule size during dilution and storage were evaluated for propofol microemulsions.

Fig. 3 shows the effect of dilution on the globule size of propofol microemulsions containing different amounts of Pluronic F68 and PG. The results indicated that the extent of the dilution took a pronounced effect on the globule size for all of the prepared

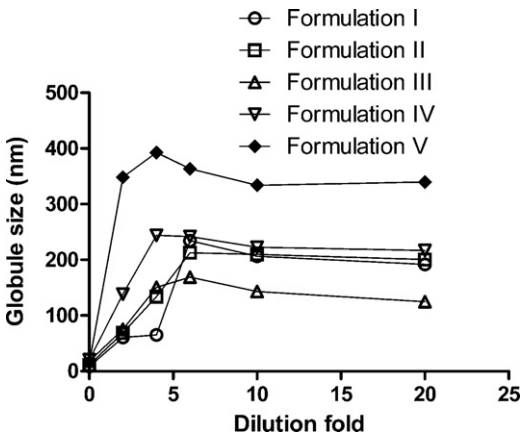

Fig. 3. The effect of dilution fold on globule size of propofol microemulsions. Values plotted are mean ± standard deviation ($n = 3$).

microemulsions. For all microemulsions without dilution appearance was clear and colorless. When saline solution was added, the samples became turbid, and the globule size gradually increased, most likely indicative of phase transformation emulsification. It should be noted that according to the data from Table 1, the weight fraction of water for formulations (I–V) with above 2-fold dilution was over 78% (w/w), indicating that all diluent samples corresponding to all time point as shown in Fig. 3, presented O/W emulsion. Hence, it was significative for globule size to reflect the change of formulation upon dilution.

Specifically, the globule size increased with increasing dilution volume when the dilution fold was lower than 6 for all formulations, especially for formulations IV and V. Moreover, no phase inversion, the globule size for all of the formulations decreased slightly suggesting no phase transformation, which might be attributed by deaggregation of smaller particles or increase in surface tension at the droplet/vehicle interface related with reordering of surfactant (Pluronic) at the droplet surface. These results demonstrated that the content of Pluronic F68 was a vital factor affecting the globule size upon dilution as compared with PG in these microemulsion formulations.

The variation of the globule size for all samples upon ten-fold dilution with saline over time was also evaluated. As shown in Fig. 4, the growth rate of globule size was enhanced by lowering the content of Pluronic F68. The samples with 20% (w/w_{saline}) Pluronic F68 (formulations I–III) upon dilution exhibited better stability with respect to the globule size, and the mean globule size ranged from

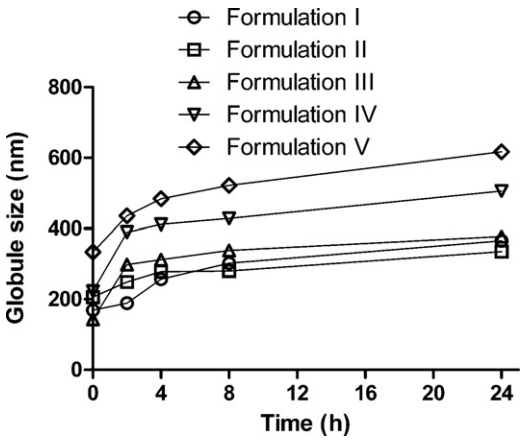

Fig. 4. The effect of storage period on globule size of propofol microemulsions. Values plotted are mean ± standard deviation ($n = 3$).

Table 2
Power law parameters of propofol microemulsions.

Formulation	m (mPa s^n)	n
II	2.215	0.978
III	2.088	0.992
IV	2.198	0.987
V	2.120	1.003

200 to 300 nm within 24 h after dilution, suggesting that the particle of the propofol emulsion might be stable in circulation and, more importantly, that the particles could pass through capillaries (5–7 μm) without causing emboli (Driscoll et al., 1995; Singh and Ravin, 1986) as long as the propofol emulsion was administrated within 24 h after dilution.

Formulation III exhibited better properties after dilution and was therefore selected as the optimal microemulsion for the subsequent studies.

3.4. Rheology

Due to the high concentration of Pluronic F68 in the propofol microemulsion system, rheology measurements such as viscosity and rheological behavior were investigated. The viscosity of the propofol microemulsion increased from ~50 mPa s to ~250 mPa s as the concentration of Pluronic F68 increased from 10% to 20% (w/w_{saline}), and n was close to 1, indicating that typical Newtonian flow was obtained (Chang et al., 2002; Copetti et al., 1997; Owen et al., 2000), as described in Table 2. Moreover, the increase in PG content led to an increase in the viscosity of the system, a result that was with that of a previous report (Miller and Drabik, 1984), which might be attributed to an increased content (weight ratio) of Pluronic F68 in the aqueous phase due to decreased weight ratio of water. It was shown in Fig. 5 that as compared with CLE without dilution, formulations II–V containing relatively higher concentrations of Pluronic F68 or PG exhibited lower viscosities (below 20 mPa s) when diluted with saline, and all samples presented Newtonian flow (data not shown).

The decrease in viscosity for formulations I~V diluted with saline indicated that the water became the outer phase and O/W emulsions were formed. The reduction in the viscosity with the increase in water weight fraction above 0.65 (as shown in Fig. 2) which seemed to be related with compositional and structural effects derived from the interfacial packing, might be attributed to the fact that high water content caused a decrease in the interdroplet interactions or reduced the hydrophobic interaction of the

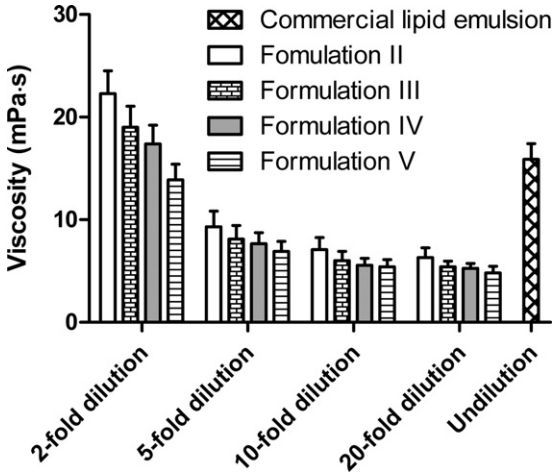

Fig. 5. The effect of dilution extent on viscosity of propofol formulations upon dilution with saline. Values plotted are mean ± standard deviation ($n = 3$).

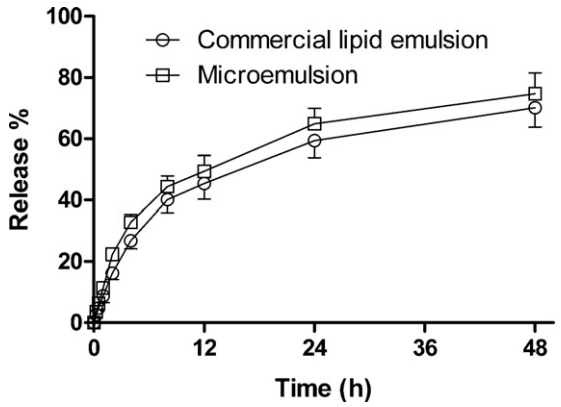

Fig. 6. *In vitro* release of propofol from microemulsions and commercial lipid emulsions. Values plotted are mean ± standard deviation ($n = 3$).

surfactant tails (Fanun, 2010). Moreover, the relatively low viscosity values indicated that the microemulsions formulated were composed of individual spherical droplets or bicontinuous structures and no anisometric aggregates were present (Djordjevic et al., 2004; Moulik and Paul, 1998; Suratkar and Mahapatra, 2000). The results also suggested that propofol microemulsions with higher concentrations of Pluronic F68 or PG upon dilution were easy to prepare and should facilitate administration in the clinic.

3.5. In vitro release

The ability of microemulsions to deliver propofol was examined by determining the drug release rate. CLE (1%, w/v propofol) was used as the control. As shown in Fig. 6, approximately 20% of propofol was released within the first 2 h, and almost 72% of propofol was released within 48 h. Moreover, no burst release occurred for any sample during the initial period. That is to say, similar drug release characteristics for the developed microemulsion (formulation III) and CLE were observed, indicating that the presence of oil in CLE did not obviously affect the release behavior of the drug. This finding might be attributed to several factors contributing to drug release from emulsions, such as the droplet size, the PEG chain length of surfactant, the interaction between the oil and the drug molecules, and the drug loading content (Chung et al., 2001; Hung et al., 2007; Kandadi et al., 2011; Li et al., 2011).

3.6. Stability

The current edition of USP and its accompanying monograph detail the desirable physical and chemical characteristics of lipid-based injectable emulsions required to meet pharmacopeial standards. Destabilization of these emulsions via coalescence is the inevitable outcome of these thermodynamically unstable dosage forms. In a similar way, in microemulsion systems, physical instability is indicated by changes in the droplet size and PDI. Besides, chemical instability is indicated by oxidation and hydrolysis of the oil or emulsifier, a change in the emulsion pH, and an increase in the free fatty acid component or acidity of the oil. Additionally, the chemical stability of any incorporated drug must be monitored (Floyd, 1999).

The long-term stability of the developed microemulsion (formulation III) was evaluated by monitoring the microemulsion for six months. Table 3 showed the representative parameters used to evaluate the physico-chemical stability of propofol formulations. No remarkable variation for any parameter, including mean globule size, PDI, viscosity, pH and content of remaining propofol, was observed for the propofol microemulsion. For the lipid emulsion CLE, the globule size increased remarkably due to thermodynamic instability.

Emulsions or microemulsions intended for intravenous use should have an extremely small droplet size and be highly stable. Any large droplets in the circulation that might lodge in the pulmonary capillaries and potentially lead to an embolism (Ilium et al., 1982). Further, the clearance of large fat globules produced under storage was extremely accelerated, and they most likely accumulated in RES organs, where they could cause increased oxidative stress and possible organ injury (Driscoll, 2006). Previous animal data (Driscoll et al., 2008; Hamawy et al., 1985) and human data (Jensen et al., 1990; Seidner et al., 1989) suggested that the liver is the principal organ injured by the infusion of unstable lipid-based injectable emulsions, as originally described by Geyer (1960).

As shown in Table 3, CLE, intravenous feeding emulsions, had much smaller globule size being of the order of 100–300 nm. However, the emulsions were significantly polydispersed (thermodynamic unstable) system, and a small number of particles larger than 1 μm in the formulations could be detected during the storage lifetime, and exceed clinically safe limits (Han et al., 2001). For comparison, the preparation of microemulsions composed of large amount of Pluronic F68 as an emulsifier required no special equipment (homogenizer), and the globule size (300–400 nm) below pharmacopeial upper limits (5 μm) for propofol emulsion upon dilution was acceptable in the case of our study. It has been suggested that propofol microemulsions formulated with Pluronic F68 as an emulsifier and without lipids such as triglycerides might not induce the abovementioned embolism and the toxicity connected with the prejudice in plasma clearance of the infusion.

Table 3 also shows that the pH of CLE decreased during storage, which could be related to the oxidation of lipids. Of note was that the emulsions released additional small quantities of free fatty acids during heat sterilization and after preparation due to the hydrolysis of phospholipids and soybean oil. This fatty acid release was to be expected as a result of the hydrolytic degradation of long-chain triglycerides into their constituent free fatty acids over time. Accompanying the fatty acid release was a decrease in the pH that acted to destabilize the emulsion. Because propofol emulsions were not buffered, the pH could decrease over time. As the pH decreased, the stabilizing anionic electrostatic charge conferred to the droplets by the phospholipids moieties was reduced, and as the phospholipids moieties became less ionized, the emulsion was destabilized (Driscoll, 2006). In the extreme case, an increasingly acidic environment typically occurred at a pH of 3.2 (Washington, 1990) eventually neutralized the electrostatic charge residing on

Table 3
Stability of propofol formulations at long-term storage condition (mean ± SD).

	Microemulsions		Commercial lipid emulsions	
	0	6	0	6
Mean globule size (nm)	69.3 ± 2.1	66.4 ± 2.8	81.3 ± 3.1	82.6 ± 2.3
PDI	0.20 ± 0.05	0.21 ± 0.09	0.19 ± 0.05	0.19 ± 0.09
Viscosity (mPa s)	57.9 ± 3.6	58.2 ± 2.4	11.3 ± 3.2	13.1 ± 2.4
pH	6.18 ± 0.25	6.09 ± 0.14	6.24 ± 0.11	5.53 ± 0.19
Content remaining (%)	100.9 ± 1.5	99.5 ± 2.8	99.6 ± 1.5	98.1 ± 2.6

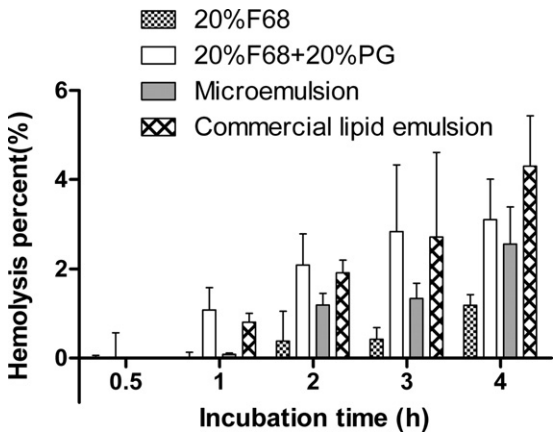

Fig. 7. Variation of hemolysis percent of propofol formulations and adjuvent samples with incubation time in the erythrocytes at 37 °C. Values plotted are mean ± standard deviation ($n = 3$).

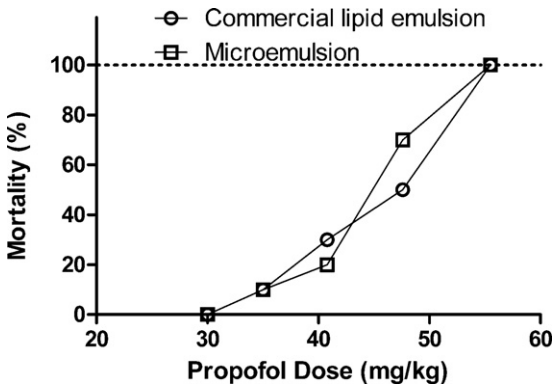

Fig. 8. Mortality of mice in acute toxicity test of propofol CLE and microemulsion by once intravenous administration in acute toxicity test ($n = 10$).

the lipid droplets. Even with seemingly modest reductions in the proposed pharmacopeial pH range of a lipid-based injectable emulsion formulation, the detrimental effects on stability could be seen. These results indicated that the propofol microemulsion exhibited much improved physical and chemical stability compared to the commercial lipid emulsion.

3.7. Hemolysis test

Hemolytic activity has been suggested as a toxicity screen *in vitro*, serving as a simple and reliable measure for estimating the membrane damage caused by formulations *in vivo* (Jumaa et al., 1999). The erythrocyte membrane damage induced by the formulations containing Pluronic F68 and/or PG was studied as a function of time. Complete hemolysis was observed for the positive control at 30 min, resulting in a red clear-diaphanous solution and with no surviving erythrocytes at the bottom of the tube. Erythrocytes precipitated to the bottom of the tubes containing saline (negative control) and dispersed after shaking during the 24 h observation period.

Propofol CLE induced a certain extent of hemolysis, as shown in Fig. 7. It has been reported lipid emulsions made of soybean lecithin exhibited hemolytic activity probably related to the presence of impurities in soybean lecithin, which might be transferred to the erythrocytes by collisions or by diffusion through the aqueous space (Quirion and St-Pierre, 1991). However, the percentage of hemolysis induced by above two formulations was below 5% before 4 h indicating that the hemolysis caused by the interaction between erythrocytes and propofol formulations could be neglected, thereby demonstrating the safety of these formulations. The results could also act as a guide for the administration of propofol microemulsions in clinical studies.

In addition, we also investigated the effect of adjuvants on hemolysis. As shown in Fig. 7, Pluronic F68 did not show any hemolytic activity before 60 min, whereas 20% Pluronic F68 + 20% PG showed hemolytic activity beginning at 30 min and increasing with time, indicating that the addition of PG led to a remarkable increase in the hemolytic activity induced by the microemulsion. This effect could be related to the ability of Pluronic F68 to form an additional layer around the emulsifier mixed-film, reducing the direct contact of PG with the cell membrane, leading to a reduction in toxicity (Forster et al., 1988; Weingarten et al., 1991).

The hemolytic power of the surfactant was affected by the sizes of the hydrophilic and hydrophobic moieties (Vinardell and Infante, 1999). Therefore, the hemolytic behavior appeared to depend not only on the hydrophobic character of the amphiphile but also on other factors such as the volume of the headgroup, which in turn depended on the chemical structure of the hydrophilic head as proposed by other reports (Bielawski, 1990; Isomaa et al., 1986; Zaslavsky et al., 1978). Pluronic F68 is a highly purified form of a nonionic block copolymer. It lowered blood viscosity and decreased red blood cell (RBC) aggregation by inhibiting weak hydrophobic adhesion (Gibbs and Hagemann, 2004). In addition, "surface coating" of the emulsion by Pluronic F68 would greatly reduce the hemolytic activity of propofol-loaded emulsions toward erythrocytes due to cell surface modifications that could not be compensated by the steric hindrance at the surface of the emulsion. Consequently, the RBCs were stabilized and restored to their normal, non-adhesive state (Gibbs and Hagemann, 2004). In this manner, Pluronic F68 protected the cells from injury due to weak hydrophobic adhesion. Thus, Pluronic F68 took advantages over the most common surfactants such as polysorbate 80 and Cremophor EL, and it was feasible for formulations containing higher concentrations of Pluronic F68 to enhance the solubility of other lipophilic drugs.

3.8. Acute toxicity

The acute toxicities of the developed microemulsion and CLE were also evaluated. As shown in Fig. 8, the LD_{50} and 95% confidence interval of the microemulsion were 44.35 mg/kg and 41.26–47.56 mg/kg, respectively. The corresponding LD_{50} of CLE was 44.15 mg/kg, and the 95% confidence interval was 42.65–45.67 mg/kg. These results demonstrated that there was no remarkable difference in the toxic effects between the two formulations.

In addition, acute and long-term studies evaluating the toxicological effects of Pluronic F68 have been conducted in mice, rats, guinea pigs, dogs, and rabbits using intravenous, intraperitoneal, subcutaneous, intracutaneous, oral, and topical routes of administration (www.cytrx.com, 2003 May 15). The results of these studies suggested that Pluronic F68 did not cause a high degree of toxicity, a result that appeared to be independent of the route of administration, the duration of exposure, and the dose. It was therefore inferred that the acute toxicity of the developed microemulsion might be induced by the drug and not by Pluronic F68.

3.9. Pharmacological effect

The mean time values for the loss and re-appearance of locomotor activity are plotted in Fig. 9 for the developed microemulsion (formulation III) and CLE. Following administration of the microemulsion and CLE, animals rapidly lost locomotor

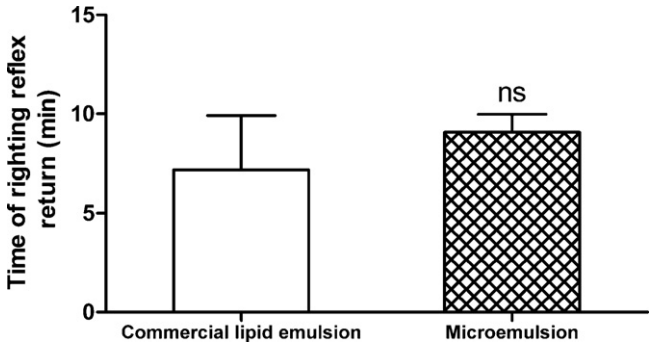

Fig. 9. Sleep-recovery study results. The dose was 10 mg/kg (onset of sleep was less than 0.5 min). Values plotted are mean ± standard deviation ($n = 9$); ns: $p > 0.05$ vs. the commercial lipid emulsion.

activities within 0.5 min. On average, both groups of animals given propofol formulations regained their locomotive activities after 8 min. The time intervals for the animals to lose and regain locomotor activity were not significantly different for both of propofol formulations ($p > 0.05$). Results from this preliminary pharmacodynamic study in rats suggested that microemulsions took very similar pharmacological effects to those of CLE. Based on these data, it might be inferred that similar release characteristics for the two formulations ensured the similar partitioning of the drug to the site of action (e.g., the central nervous system) in rats, hence resulting in the similarity in the pharmacological effects observed.

In sum, these results indicated that the propofol microemulsion system has several advantages over commercial lipid emulsion with respect to preparation, globule size, viscosity, physical and chemical stability. More importantly, the propofol microemulsion exhibited pharmacological effects similar to those of CLE. Before this propofol microemulsion can be used in the clinic, more detailed studies on these microemulsions need to be conducted. These studies should investigate the storage stability over a longer period (~24 months) and should include other safety tests including long-term toxicity experiments, vessel irritation tests, and anaphylaxis tests.

4. Conclusion

The microemulsion delivery system for propofol was successfully prepared. Except for the excellent physicochemical stability, microemulsion offered the convenience of administration. In addition, the intravenous safety investigation proved that microemulsion was safe as an intravenous injection, and the same pharmacological effect for microemulsion and CLE was shown. Therefore, microemulsion delivery systems were a promising system for propofol, which supported further investigations of this system for *in vivo* and clinical situations.

Acknowledgments

We would like to acknowledge the support of this work by the National Major Science and Technology Project of China (Pharmaceutical Research for Microbial Medicine and Biopharmaceutics, 2009ZX09301-003-8-2).

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