Kinetically stable propofol emulsions with reduced free drug concentration for intravenous delivery

ARTICLE *in* INTERNATIONAL JOURNAL OF PHARMACEUTICS · MARCH 2015

Impact Factor: 3.65 · DOI: 10.1016/j.ijpharm.2015.03.057 · Source: PubMed

2 AUTHORS, INCLUDING:

Robert Damitz
University of Florida
5 PUBLICATIONS **11** CITATIONS

SEE PROFILE

International Journal of Pharmaceutics 486 (2015) 232–241

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

Kinetically stable propofol emulsions with reduced free drug concentration for intravenous delivery

Robert Damitz, Anuj Chauhan *

Department of Chemical Engineering, University of Florida, Gainesville, FL 32611-6005, United States

ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 24 February 2015
Accepted 27 March 2015
Available online 31 March 2015

Keywords:
Propofol
Emulsion stability
Excipients
Partition coefficient
Surfactant
Interfacial phenomena

ABSTRACT

Purpose: Intravenous injections of propofol emulsions are accompanied by pain likely due to the interaction of the dissolved drug with endothelial cells of the vasculature. It is commonly hypothesized that reducing the aqueous phase concentration of propofol could reduce pain.
Methods: To minimize the propofol concentration in the aqueous phase, we developed stable oil-in-water emulsions with excipient oil mixtures that have an increased partition coefficient for propofol. We then explored the emulsion stability by measuring size distributions after extended durations of shelf storage and also after freeze–thaw cycling. The effects of oil type, surfactant and salt concentration on emulsion stability were also explored.
Results: Small chain oils like ethyl butyrate exhibit high drug partitioning but poor stability, while larger molecules such as soybean oil exhibit lower partitioning but excellent emulsion stability. Emulsions with mixtures of soybean oil and ethyl butyrate are stable for longer than a year, resistant to freeze–thaw cycling, and reduce aqueous drug concentrations of propofol twofold compared to pure soybean oil emulsions.
Conclusions: Oil-in-water emulsions of propofol formulated with mixtures of ethyl butyrate and soybean oil are kinetically stable and significantly reduce the aqueous phase drug concentration making them promising candidates for future propofol therapies.

1. Introduction

Propofol (2,6-diisopropylphenol), a common anesthetic, is formulated as an oil-in-water (O/W) emulsion and administered intravenously. One commercially-available formulation of propofol known as Diprivan® is prepared with 1% propofol dissolved in 10% soybean oil stabilized with 1.2% egg lecithin surfactant and 0.005% sodium EDTA as a preservative (all w/v) (Thompson and Goodale, 2000). Diprivan® has some disadvantages including thermodynamic instability, limited shelf life, risk of hypertriglycemia after injection (Baker and Naguib, 2005; Driscoll et al., 2002; Hulman, 1995; Knibbe et al., 2002). However, Diprivan® is most notable for causing significant patient pain on injection which is partially attributed to the free drug concentration (Baker and Naguib, 2005; Lee, 2010; Sim et al., 2009), or the portion of drug which dissolves in the emulsion aqueous phase. A more recent formulation known as Propofol Lipuro® has been observed to reduce the free drug concentration up to 30% (Yamakage et al., 2005) with an excipient oil mixture of 5% long-chain triglycerides (LCT) and 5% medium-chain triglycerides (MCT). Propofol Lipuro® is reported to have reduced incidence of pain on injection (Ozawa et al., 2005; Sundarathiti et al., 2007), but 37% of patients still reported pain after injection (Larsen et al., 2001). These observations suggest that pain on injection can be decreased by further decreasing the aqueous drug concentration of the propofol formulation.

The free drug concentration of an emulsion system is driven by its solubility equilibrium. Propofol is poorly soluble in water at 150–180 μg/mL (Altomare et al., 2003 Trapani et al., 1996). While a majority of the drug in Diprivan® is encapsulated in the oil phase, propofol concentrations in the aqueous phase have been observed as high as 14.8 μg/mL (Lee, 2010; Sim et al., 2009; Yamakage et al., 2005). After injection, the aqueous phase drug concentration makes immediate contact with the vasculature while the encapsulated drug must first diffuse out of the emulsion droplets. It is well established that sterically-hindered phenolic compounds such as propofol are biological membrane irritants (Hayashi et al., 1999), thus the drug in the emulsion aqueous phase can cause significant tissue irritation and damage. This hypothesis is supported by studies with propofol microemulsions which are a

* Corresponding author. Tel.: +1 352 392 2592; fax: +1 352 392 9513.
E-mail address: Chauhan@che.ufl.edu (A. Chauhan).

http://dx.doi.org/10.1016/j.ijpharm.2015.03.057
0378-5173/

thermodynamically-stable subclass of emulsions with greatly reduced interfacial tension and droplet sizes (<100 nm) due to higher surfactant to oil ratios (Spernath and Aserin, 2006; Date and Nagarsenker, 2008; Bagwe et al., 2001; Li et al., 2012; Morey et al., 2006; Ryoo et al., 2005). Microemulsions were initially considered attractive candidates for propofol delivery, but poor results were seen with elevated pain levels on injection of microemulsion propofol (Hasani et al., 2012; Lee et al., 2011; Morey et al., 2006; Sim et al., 2009). Elevated pain with microemulsion propofol is attributed to greater aqueous phase drug concentration of 83.9 μg/mL (Sim et al., 2009).

Despite causing less pain on injection, macroemulsions (or simply emulsions) have limited shelf life and increased risk for complications after injection (Driscoll et al., 2002; Hulman, 1995). Emulsions are subject to several destabilizing mechanisms including gravimetric settling, flocculation, coalescence, Ostwald ripening, creaming, and finally phase separation each with unique driving forces and mechanisms. However, several strategies can be employed to increase emulsion stability. Smaller emulsion droplets are less susceptible to the more destructive mechanisms of creaming, settling, and phase separation (McClements, 2007). Nanoemulsions are a distinct classification of thermodynamically unstable emulsions which achieve kinetic stability when their droplet size is reduced with high shear mixing. Both commercial formulations of propofol listed above are classified as nanoemulsions.

In addition, excipient and surfactant selection also has strong effects on the resulting emulsion stability. Certain surfactants are more effective at stabilizing some oil compounds but have little effect on other oils. Some surfactants, for example the nonionic Pluronics, can provide strong rigidity to the emulsion interface resulting in minimal surface deformation during collisions between neighboring droplets (Gregory, 1995; Tadros, 2006). Electrostatics and DLVO theory suggest that electrostatic repulsion between droplets provides an energy barrier which deters neighboring droplets from approaching (Derjaguin and Landau, 1941; Verwey and Overbeek, 1948; Tadros, 2006). Additives can also be used to modify density or viscosity of each phase to resist gravimetric settling or reduce droplet collisions (McClements, 2007). Thus, there is a very broad scope of emulsion design, and it is challenging to design a shelf-stable emulsion.

Therefore, the goals of this study are to reduce the free drug concentration in propofol emulsion formulations while maintaining acceptable emulsion stability. We also investigate the dominant forces behind emulsion kinetic stability before finally presenting an improved propofol formulation.

2. Materials and methods

Propofol USP was donated by Albemarle Corporation (Baton Rouge, LA) and Diprivan® was kindly provided by Nanomedex, Inc. (Middleton, WI). Generally regarded as safe (GRAS) excipient oils including soybean oil, olive oil, ethyl butyrate, isopropyl myristate, isopropyl palmitate, and octanoic acid were all obtained from Fisher Scientific (Hampton, NH). Food grade extra virgin olive oil was purchased at the local Publix grocery store (Lakeland, FL). All oils were used as received. Dulbecco's phosphate buffered saline (PBS), sodium caprylate, Pluronic F68, Tween 80, and Brij 78 were obtained from Sigma–Aldrich (St. Louis, MO). Sodium stearate was obtained from Alfa Aesar (Ward Hill, MA).

2.1. Reducing the free propofol concentration in emulsions

2.1.1. Equilibrium partition coefficients in pure excipient oils

Partition coefficients of propofol in different oils were measured by first equilibrating a mixture of propofol, water, and oil with mass fractions f_{drug},f_{aq}, and f_{oil}, respectively, then measuring the concentration of propofol in the aqueous phase. The drug loading was kept constant at 1%, while oil loadings were chosen to be 5 or 15% (all w/w). Additional experiments were performed at 10% excipient oil loading for ethyl butyrate and soybean oil because of the major focus on these oils in this work. The mixtures of drug, oil, and water were vigorously mixed for three days under high magnetic stirring (900 rpm). Following mixing, 5 mL of each mixture was pipetted into a borosilicate test tube which was then placed into a polypropylene centrifuge tube. The samples were centrifuged for three cycles of 1 h each at about 3000 rpm. Both oil and aqueous samples were carefully collected without disturbing the interface and analyzed for propofol and excipient oil concentration using high performance liquid chromatography (HPLC, Waters Acquity). A 50% water and 50% acetonitrile (v/v) mobile phase was used at 1 mL/min flow. Propofol peaks eluted through the 4 μm C18 column at approximately 4.5 minutes. The partition coefficient was obtained from a mass balance, *i.e.*,

$$M_{drug} = V_{aq}c_f + KV_{oil}c_f \quad (1)$$

where K is the oil–water partition coefficient, c_f is the aqueous drug concentration, M_{drug}, V_{aq} and V_{oil} are the mass of oil and volumes of water and oil, respectively in the system. The mass balance yields the following equation for K:

$$K = \frac{f_{drug} - (f_{aq}c_f/\rho_{aq})}{f_{oil}c_f/\rho_{oil}} \quad (2)$$

where ρ_{aq} and ρ_{oil} are the densities of water and oil, respectively.

2.1.2. Validation of aqueous phase drug concentration in emulsions

The aqueous phase concentration of several emulsions was measured using a dialysis method. A volume of emulsion containing 10% w/w soybean oil, 1% propofol and between 1 and 5% Pluronic F68 surfactant was placed in a well-rinsed 12–14 kDa MWCO dialysis bag (Fisher Scientific). The dialysis bag was then suspended into isotonic dialysis media at a 5:1 ratio of dialysis media to emulsion (v/v). We used a solution of 2.25% (w/w) glycerol in DI water as dialysis media which matched the osmotic pressure of emulsion samples. Care was taken to ensure that the dialysis bags did not leak into the dialysis media. Samples were taken from the dialysate at several time intervals to obtain transient free drug concentration data, and a final free drug concentration was observed when equilibrium was reached. Dialysate samples were analyzed with HPLC using an identical method for propofol concentration.

2.1.3. Equilibrium drug partitioning in mixtures of excipient oils

An optimal emulsion design may include a mixture of excipient oils. If mixing is ideal, drug partitioning in oil mixtures can be estimated based on the partition coefficients of the drug in each oil type. To explore this, we measured the drug partitioning in binary mixtures of soybean oil and ethyl butyrate with 1% drug loading and 10% total excipient oil loading (w/w). The relative fractions of excipient oil were varied between 100% ethyl butyrate and 100% soybean oil. These experiments were also repeated by replacing soybean oil with olive oil. The same procedure used to measure equilibrium partitioning of single excipient oils was followed.

2.2. Evaluating the stability of emulsion formulations

2.2.1. Emulsion preparation

We prepared emulsions with 1% propofol USP, 10% of various GRAS oils (soybean oil, olive oil, ethyl butyrate, isopropyl myristate, isopropyl palmitate, and octanoic acid), various concentrations of

nonionic surfactants (Pluronic F68, Tween 80, and Brij 78), and various concentrations of sodium stearate (all w/w). 1% drug and 10% excipient oil was selected as the experimental basis for direct comparison to Diprivan® and Propofol Lipuro®. In some cases, salt was added to explore ionic effects and glycerol was added to control osmolarity without increasing solution ionic strength. The compositions of various emulsions explored here are tabulated in Supplemental data section, where the experiments focusing on a specific ingredient or parameter are grouped together. For each formulation, the excipient oil(s) and drug were added in their proper mass ratios to 20 mL glass vials and mixed until homogenous. Concentrated stock surfactant solutions were separately prepared. 15% Pluronic F68 was dissolved in DI water with magnetic stirring. Tween 80 and Brij 78 were used as received. Sodium stearate has limited aqueous solubility at room temperature; therefore a stock solution of 0.1% sodium stearate was prepared in DI water and stirred at approximately 65 °C. The concentrated surfactant solutions, glycerol, and any other desired component were added to the oil and drug mixture and diluted with DI water (qs).

These oil and water mixtures were then agitated under high shear using an ultrasonic probe sonicator (Fisher Scientific Sonic Dismembrator Model 100) at an instrument setting of 8–10 for between 10 and 30 min. The root mean square power for this setting varied from 13 to 18 W. Vials were suspended in a bath of cool water during sonication to maintain sample temperature. The sonicator probe tip was placed just below the oil–water interface to maximize the mixing of the two phases. This process resulted in the formation of a homogenous milky white emulsion.

2.2.2. Stability criteria

Emulsions were evaluated for their stability in three ways. Emulsions were first observed visually for any destabilization due to creaming. If the emulsion resisted creaming for several days, changes in the emulsion droplet size distribution were then measured using dynamic light scattering (DLS) after several freeze–thaw cycles and at extended shelf life. Stable emulsions should exhibit no creaming and no changes to the droplet size distribution after freeze–thaw cycling and at long shelf lives.

To explore each emulsion's stability to creaming, about 10 mL of each emulsion was placed in a 20 mL vial and left undisturbed on a lab bench. Creaming is an instability mechanism where lower density oil droplets rise to the top of the system forming a cream layer with a more clear serum layer below. The emulsions were photographed periodically with a digital camera (Panasonic DMC FH25), and the photographs were analyzed using image processing software (ImageJ) to quantify the degree of creaming. The height of each emulsion phase was measured in number of pixels, and the percentage serum was calculated from the serum height divided by the total emulsion height (McClements, 2007). The time at which creaming was first observed was used as a measure of each emulsion's stability, and the time at which the emulsion had completely creamed was noted if applicable. A creaming time longer than two years is desired to be considered a commercially viable pharmaceutical emulsion.

Emulsions which showed no creaming for over 1 month were then evaluated for changes in droplet size after several freeze–thaw cycles and at long shelf lives. Freezing and thawing emulsions can exacerbate destabilization by altering oil, water, and surfactant interactions during crystallization and melting which often occurs at different temperatures for each phase present (Degner et al., 2014). For each freeze–thaw cycle, emulsions were placed in a standard freezer at −18 °C for 16 h, followed by 8 h of thawing at room temperature. Visual observations and droplet size measurements were taken after each thaw. Samples of emulsions were also left undisturbed on the laboratory bench top for extended durations and droplet size measurements were taken at various times. In some cases where emulsions had creamed, size distributions were measured for both serum and cream phases to investigate the mechanism of creaming.

Droplet size distributions of emulsions were obtained using dynamic light scattering (DLS, Malvern Zetasizer Nano-ZS). Emulsions must have a mean intensity-weighted diameter of 500 nm or less and a volume-weighted percentage of droplets over 5 μm of 0.05% or less to be suitable for parenteral injection (The United States Pharmacopeial Convention, 2013). Thus, the intensity mean diameter of the emulsion sample was measured and reported. Emulsions were often too concentrated to give reliable droplet size measurements, therefore samples were diluted 10:1 (v/v) with DI water to prevent multiple scattering effects. DLS also provides the emulsion polydispersity index (PDI) between 0 and 1, a measurement of the uniformity of emulsion droplets. Low polydispersity index values (PDI = 0.1 and below) indicate a very uniform droplet size range and are an indication of a stable emulsion. We were unable to use DLS to measure the volume percentage of droplets over 5 μm due to the upper size limits of the instrument. The most stable emulsions with reduced free drug concentrations were then investigated for their kinetic stability as explained below.

2.3. Investigating mechanism of kinetic stability

Emulsions which exhibited good kinetic stability were modified in several ways in attempt to determine the dominant stability mechanism. Parameters which were altered included nonionic surfactant type and concentration, electrolyte concentration, and ionic surfactant concentration. Emulsions were formed as described above and evaluated for stability according to the above criteria unless otherwise noted. A brief description of these experiments is included in Supplemental data.

2.3.1. Nonionic surfactant type and concentration

Several emulsions containing 1% propofol, 5% soybean oil, 5% ethyl butyrate, and 10^{-4}% sodium stearate were prepared with several biocompatible nonionic surfactants at concentrations between 1 and 5% to explore their effect on the kinetic stability of the proposed formulation. The surfactants used were Pluronic F68, Tween 80, and Brij 78.

2.3.2. Electrostatic repulsions and zeta potential

Changes to the solution conductivity and pH will alter the electrostatic interactions between droplets which may have profound effect on emulsion stability. To investigate this possibility, we prepared mixtures of 10% w/w soybean oil in aqueous solutions of various pH and electrolyte concentrations and sonicated the mixtures according to the above emulsion preparation protocol. Aqueous solutions used were DI water, PBS, 5% NaCl, 0.01 M HCl, and 0.01 M NaOH. These experiments were also repeated with 1% w/w Pluronic F68 added to the mixture to explore whether surfactant interactions or electrostatic repulsions are dominant in stabilizing emulsions.

Immediately after the mixtures were sonicated, the zeta potential of the mixture was evaluated using electrophoretic light scattering (Malvern Zetasizer Nano-ZS). Samples were diluted 20:1 (v/v) with identical aqueous phase solutions to minimize changes in sample conductivity. Smoluchowski fitting was used for these experiments since the Debye lengths are smaller than the droplet radii of sonicated mixtures.

Additionally, stability of the sonicated mixtures was evaluated visually by photographing the mixtures at different durations of extended shelf storage. The zeta potential measurements and stability observations were then compared.

2.3.3. Addition of electrolytes to stable emulsions

To further explore the importance of ionic effects on emulsion stability, we added electrolytes to a shelf-stable emulsion. Several concentrations of NaCl (0.45%, 0.9%, 1.8%, and 5% all w/w) were added to a kinetically stable emulsion containing 1% propofol, 5% soybean oil, 5% ethyl butyrate, 1% Pluronic F68, 10^{-4}% sodium stearate (all w/w). These emulsions were evaluated for stability as described in section 2.2.2.

2.3.4. Ionic surfactants

Next, we explored the effect of modified ionic surfactant concentration on the resulting emulsion stability. We prepared emulsions containing 1% propofol, 10% soybean oil, and 2% Pluronic F68 with increased concentration of sodium stearate (0.01% and 0.05% all w/w). The upper limit of sodium stearate concentration was limited to 0.05% due to the low solubility limit of sodium stearate at room temperature. Formulations with sodium stearate concentrations approaching the solubility limit tend to partially solidify or gel when finely dispersed under sonication and cooled to room temperature.

Finally, two emulsions containing 1% propofol, 10% soybean oil and 2% nonionic surfactant (Pluronic F68 or Tween 80 all w/w) were made with no ionic surfactant. The stability of these formulations was also evaluated with freeze–thaw cycling and extended shelf life.

3. Results and discussion

An optimal formulation for intravenous injection of propofol should have high partitioning of the drug in the oil phase resulting in a low aqueous phase drug concentration. The optimal formulation should also be as shelf-stable as possible. To achieve the dual goals of low aqueous drug concentration and high kinetic stability, suitable excipient oils and surfactants must be selected. Below we present the results drug partitioning in various oils, followed by emulsion stability, and finally our results from investigating the mechanism of emulsion kinetic stability.

3.1. Reducing free drug concentration in emulsion

3.1.1. Equilibrium drug partitioning in pure excipient oils

Table 1 lists the molecular weight and densities of various excipient oils considered, along with measured aqueous phase drug concentrations and calculated logarithmic oil–water partition coefficients. The drug concentration in the aqueous phase decreases with increasing oil loading as a higher fraction is retained in the oil phase. Due to dilute drug conditions, the partition coefficient is independent of the oil loading. However, the partition coefficients vary significantly with different excipient oils with a clear dependence on the molecular weight of the oil. Among the excipient oils studied, ethyl butyrate with the lowest molecular weight and soybean oil with the highest molecular weight had log(K) values of 4.3 and 3.6, respectively. It is clear excipient oil type and concentration are both important when considering the aqueous phase concentration of drug in emulsion formulations.

3.1.2. Direct measurement of free propofol concentration in emulsion

The aqueous phase drug concentrations obtained from partition coefficient (19.1 ± 3.8 mg/L) and dialysis experiments (16.2 ± 1.8 mg/L) are in good agreement. These results indicate that the presence of emulsion droplets and surfactant have little effect on the equilibrium partitioning of drug between the excipient oils and the aqueous phase. Thus, the data for the aqueous phase concentrations obtained with bulk oil and water phases can be considered as equal to the free drug concentration in an emulsion formulation with the corresponding ratio of oil to water.

3.1.3. Equilibrium drug partitioning in mixtures of excipient oils

The partition coefficient of propofol varies considerably with oil type, and as we show later, emulsion stability also depends on oil type. Fig. 1 shows the aqueous phase drug concentrations of bulk mixtures of drug, water, and varying ratios of two excipient oils. As the portion of ethyl butyrate increases, the aqueous phase drug concentration reduces significantly. By assuming ideal mixing of ethyl butyrate, soybean oil, and the drug in the oil phase, a drug mass balance gives the following equation:

$$M_{\text{drug}} = V_{\text{aq}} c_f + (K_{\text{EB}} V_{\text{EB}} + K_{\text{SO}} V_{\text{SO}}) c_f \quad (3)$$

where EB and SO represent ethyl butyrate and soybean oil, respectively. The dashed line in Fig. 1 is the best fit curve to the above equation with values of 19,900 and 5200 for K_{EB} and K_{SO}, respectively. The model fits are in good agreement with the experimental data suggesting that the oil mixtures can be considered ideal. A 50:50 mixture of ethyl butyrate and either soybean or olive oil reduces the aqueous drug concentration by over 50% when compared with the pure vegetable oil excipient.

We were unable to accurately measure the free drug concentration in emulsions containing ethyl butyrate due to its volatility. In the time required for equilibrium to be reached with the dialysis method (approximately 24–48 h), most of the ethyl butyrate had evaporated which resulted in erroneously high free drug concentration values. This would not be a serious issue in commercial formulations since the vial would likely be sealed under nitrogen or other inert gas until its use. However, equilibrium partitioning data was in very good agreement with dialysis data for soybean oil formulations indicating the free drug concentration of emulsions containing binary mixtures of oil match the equilibrium partitioning data.

This emulsion containing a 50:50 mixture of ethyl butyrate and soybean oil reduces free drug concentration to 6.7 μg/mL, an over 50% reduction from Diprivan® and 30% reduction from 1% Propofol Lipuro®. Patients have reported less severe and a 73% reduction in incidence of pain when injected with Propofol Lipuro® over Diprivan® (Larsen et al., 2001), therefore this formulation likely will reduce the incidence of pain on injection even further.

Table 1
Aqueous phase drug concentrations and calculated partition coefficients of systems with 1% drug loading and either 5, 10 or 15% excipient oil loading of various oils. The water phase was pure deionized water. All percentages shown are w/w.

Excipient oil type	M_W excipient oil (g/mol)	Oil density (g/mL)	5% excipient oil		10% excipient oil		15% excipient oil	
			C_{free} (mg/L)	$\text{Log}_{10} K$	C_{free} (mg/L)	$\text{Log}_{10} K$	C_{free} (mg/L)	$\text{Log}_{10} K$
Ethyl butyrate (EB)	116.16	0.869	6.93	4.33	4.60 ± 1.83	4.32	2.48	4.35
Octanoic acid (OA)	144.21	0.898	33.5	3.65	–	–	10.0	3.76
Isopropyl myristate (IM)	270.45	0.843	16.9	3.92	–	–	4.39	4.08
Isopropyl palmitate (IP)	298.50	0.841	28.1	3.71	–	–	5.17	4.01
Soybean oil (SO)	874	0.909	51.2	3.48	19.12 ± 3.80	3.65	12.6	3.66

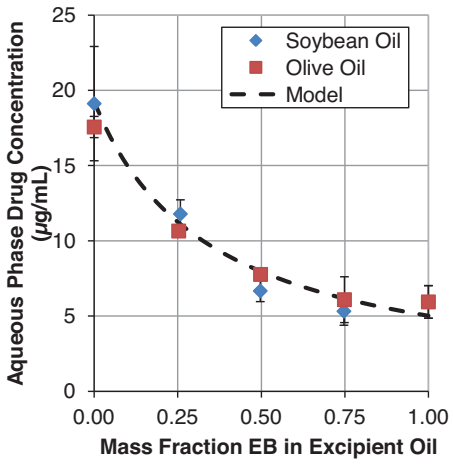


Fig. 1. Free drug concentration from systems with binary excipient oil mixtures of soybean oil and ethyl butyrate or olive oil and ethyl butyrate. Best fit model also shown.

3.2. Emulsion stability

3.2.1. Excipient oils and emulsion creaming

We have demonstrated how the excipient oil affects drug partitioning, but its drastic effect on emulsion stability is best observed visually with creaming experiments. Fig. 2 shows the initial and final images of several formulations and notes the time which they have creamed. Initial images of two formulations for each of the five excipient oils are included in the top row of Fig. 2. Two different concentrations (10^{-3}% and 10^{-4}%) of sodium stearate (SS) were used for each of the excipient oils studied.

Ethyl butyrate emulsions showed very rapid phase separation even before images could be acquired showing that these emulsions are highly unstable. While ethyl butyrate exhibits the highest drug partitioning, it demonstrates poor emulsion stability. Thus, emulsions with ethyl butyrate alone as excipient oil are not suitable for intravenous delivery of propofol. Since it was immediately realized that ethyl butyrate emulsions are highly unstable, a 50:50 mix of ethyl butyrate and soybean oil as the excipient (5% w/w each) was also included in these creaming experiments. All the emulsions systems except pure ethyl butyrate appear homogeneous, opaque, and white just after formation. Visual observations of the emulsions showed that within a year, all emulsions except soybean oil and the 50:50 mix of soybean oil and ethyl butyrate separated into two layers comprising of a clear lower serum layer and an upper opaque cream layer. The serum phase is mostly water, and the cream phase appears to be an emulsion with higher oil loadings compared to the starting value. After longer times, the cream layer can completely destabilize leaving a non-emulsified “free” oil layer at the top.

Images of the emulsions were digitally measured to determine the serum height as a function of time for all the emulsion systems (Fig. 3). Plots of serum heights over time show that lower molecular weight excipient oils suffered from poor emulsion stability despite reducing aqueous phase drug concentrations. Intermediate oils such as isopropyl myristate and isopropyl palmitate required several days or weeks to begin creaming, while the larger triglyceride oils tended to remain stable to creaming. The results also showed very little difference in creaming between 10^{-4}% and 10^{-3}% sodium stearate.

The plots of serum heights as a function of time (Fig. 3) are non-linear with an initial stable phase during which the emulsions appear to be homogeneous with no observable separation followed by rapid creaming. Once a serum layer appears, the rate of creaming accelerates. Complete creaming occurs in a fraction of the time that it took for the first onset of creaming. The increase in creaming rates could potentially be due to aggregation forming larger size droplets that have an increased rising velocity.

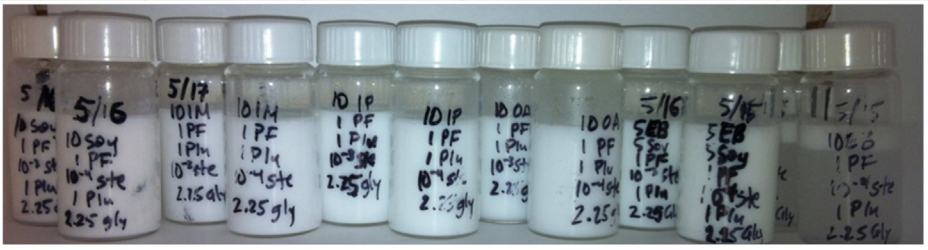
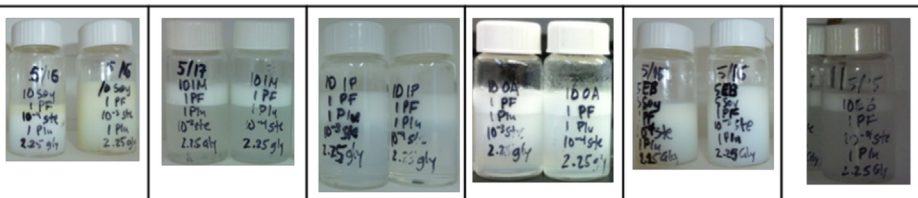
Formulation	10% Soybean oil	10% Isopropyl myristate	10% Isopropyl palmitate	10% Octanoic acid	5% Ethyl butyrate + 5% Soybean oil	10% Ethyl butyrate
Initial Photo						
Days Elapsed	Initial photo – 0 days elapsed					
Creamed Photo						
Days Elapsed	374 days	33 days	257 days	9 days	374+ days	0 days

Fig. 2. Initial and creamed images of emulsion formulations of various oils. The elapsed time at which the final creamed photo was taken is noted below the photo. The 5% ethyl butyrate and 5% soybean oil formulation has not fully creamed in over one year on the shelf. All percentages shown are w/w.

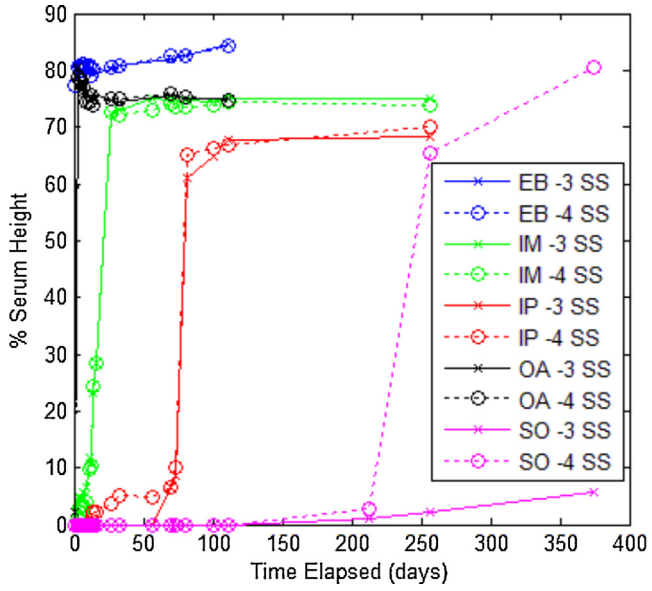


Fig. 3. Creaming heights over time of emulsions prepared with a single excipient oil type.

3.2.2. Destructive or reversible creaming

As seen in Fig. 3, even soybean oil emulsions exhibited some degree of creaming over one year of shelf storage. Whether this creaming was caused by gravimetric settling alone (reversible) or droplet coalescence (destructive) was unknown. To better understand creaming dynamics, size distributions were measured in the serum and cream layers for two of the emulsion systems, those formed with isopropyl myristate and soybean oil. Emulsions of isopropyl myristate reached serum heights of over 70% in less than two months, while those of soybean oil exhibited less than 10% serum height after thirteen months. Fig. 4a shows size distributions in the cream and serum for isopropyl myristate after two months of shelf life. The initial emulsion size distribution is included for comparison. The data shows that the droplet size in the cream layer is much larger than the original diameter, while the serum layer has droplets of similar or smaller size. Additionally, visual transparency of the serum phase suggests a very small volume fraction of droplets. After mixing the serum and cream phases, the droplet size distribution remains similar to the cream phase indicating an irreversible growth of droplet size has occurred. This data is consistent with the hypothesis that the cream layer is formed by droplet aggregation and coalescence which results in an increase in size and a consequent increase in the rising velocity. The size of the emulsions in the cream layer is nearly ten times that of the starting size, which would imply a nearly 100-fold larger rising velocity since settling velocity scales with the square of droplet size.

Conversely, size distributions of the serum and cream layers of a soybean oil formulation after 13 months of storage are shown in Fig. 4b. The cream size distribution is very similar to the starting distribution suggesting negligible aggregation has occurred during the 13 months of storage. Essentially, the cream layer is an emulsion with slightly higher oil loading compared to the original formulation but no change in the droplet size, and it formed due to density differences between the oil and the continuous phases. The droplet sizes in the serum phase are significantly smaller than in the cream and may be due to surfactant micelles. There was no phase separation (oiling off) occurring in the cream layer, which further suggests that the cream layer is still an emulsion. In this case, gentle shaking is sufficient to render the emulsion uniform with size distributions similar to the starting distribution. While soybean oil emulsions creamed about 10% after one year, the 50:50 mixture of soybean oil and ethyl butyrate did not exhibit any creaming even after 13 months in spite of the lower density of ethyl butyrate (0.869 g/mL) compared to soybean oil (0.909 g/mL) possibly due to smaller size of the oil droplets as discussed below.

3.2.3. Long-term stability of pure and binary excipient oil mixtures

The creaming studies described above clearly show that emulsions of pure soybean oil and 50:50 mixtures of soybean oil and ethyl butyrate are stable for a long period of time. The binary mixture appears to be more stable however as it did not exhibit any creaming in 13 months. Since creaming was attributed to rising of the larger oil droplets, we hypothesized that increasing the surfactant concentration will reduce oil droplet size and minimize creaming. Accordingly, the emulsions discussed below were prepared with higher concentration of nonionic surfactant (5% w/w) to reduce the droplet size. Each emulsion containing soybean oil was also replicated with olive oil in this experiment.

The mean droplet sizes of several emulsions and Diprivan® after freeze–thaw cycling and shelf life are shown in Fig. 5a and b. The mean sizes remain unchanged for the entire duration of about a year for all of the systems. Also, the emulsions remain stable to freeze–thaw cycles, which further suggest that these emulsion

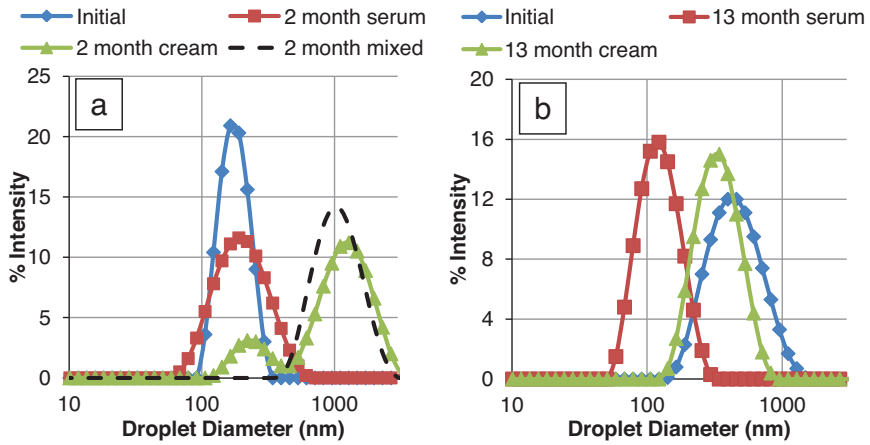


Fig. 4. Intensity droplet size distributions of serum and cream phases of 10% isopropyl myristate, 3% Pluronic F68, 0.1% sodium stearate emulsion (a) and 1% propofol, 10% soybean oil, 1% Pluronic F68, 10-3% sodium stearate emulsion formulation (b). All percentages are w/w.

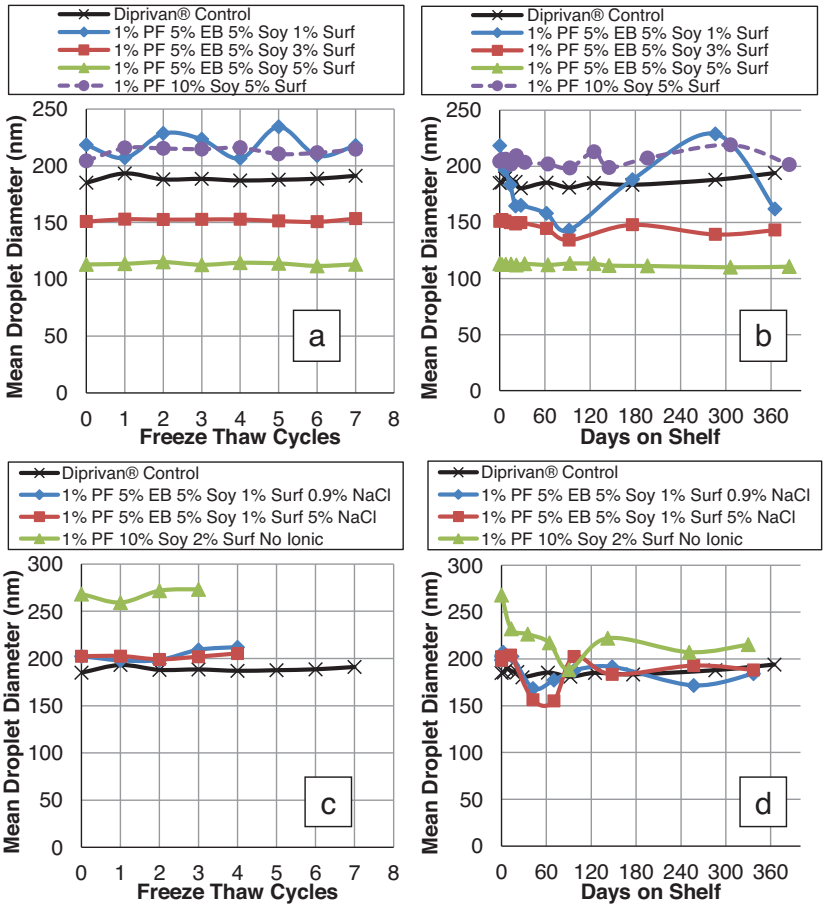


Fig. 5. Mean droplet size of several emulsions after freeze–thaw cycles (frames a and c) and various shelf life durations (frames b and d). All percentages are w/w.

systems are kinetically stable. Increasing the surfactant loading from 1% to 5% reduces the droplet size by 27% and 46% for pure soybean oil and 50:50 ethyl butyrate and soybean oil mixture formulations respectively. The mean sizes of the olive oil emulsions are comparable to soybean oil and Diprivan®, but introducing ethyl butyrate significantly reduces the size for both olive and soybean oils. Also, the polydispersity index (PDI) decreases with inclusion of ethyl butyrate from 0.180 for pure oils to about 0.130 for the binary mixtures.

Thus, the benefits of including ethyl butyrate are twofold. Improved kinetic stability, smaller droplet size, and reduced polydispersity were observed by mixing ethyl butyrate with other excipient oils over pure excipient oil formulations. Adding ethyl butyrate also greatly increases the partitioning of drug into the oil phase which reduces the aqueous phase concentration of the drug which is believed to be critically important to reduce patient pain and discomfort on injection. Based on these results, we consider ethyl butyrate an ideal additive to accomplish the design goals for an improved propofol formulation. Ethyl butyrate is recognized as safe by the FDA and is widely used as a food additive, so we expect this excipient to be highly biocompatible. However, ethyl butyrate has not yet been approved for parenteral use despite being suggested for several intravenous therapies (Baravkar et al., 2014; Varshney et al., 2004).

3.3. Investigating mechanism of emulsion kinetic stability

The most stable emulsion formulation containing 1% propofol, 5% ethyl butyrate, 5% soybean oil (all w/w) was observed to resist creaming for over 12 months without major changes in droplet size even after freeze–thaw cycling. These results suggested the formation of a kinetically stable emulsion, and this formulation was the basis of the investigations into kinetic stability presented below.

3.3.1. Nonionic surfactant concentration

Fig. 5a and b shows the changes in droplet size with freeze–thaw cycling and long shelf life for proposed emulsions with surfactant loadings of 1, 3, and 5% Pluronic F68. Larger amounts of nonionic surfactant yield smaller emulsion droplets with less variability in size and polydispersity after both freeze–thaw cycling and shelf life. PDI decreased from 0.146 to 0.109 for 1% and 3% Pluronic formulations of the 50:50 mixture of soybean oil and ethyl butyrate respectively. A sample lot of Diprivan® was also included for comparison (PDI value 0.066). The formulation with 1% and 3% Pluronic formed emulsions of larger and smaller droplets than Diprivan® respectively. In comparison, Diprivan® contains 1.2% egg lecithin as a surfactant and includes only one excipient oil, 10% soybean oil (w/v). Olive oil formulations seemed more variable in size and polydispersity, therefore this excipient oil was not studied further.

3.3.2. Nonionic surfactant type

There was very little variation in droplet size obtained from the three different nonionic surfactants for freeze–thaw cycling and shelf life studies (see Supplemental data). Pluronic formulations had the lowest amount of polydispersity. PDI values obtained were 0.127, 0.202, and 0.174 for 2% Pluronic, Tween, and Brij respectively. These results suggest there are no major differences in effectiveness between the three nonionic surfactants considered for the

Table 2
Zeta potentials and emulsion stability of several formulations of soybean oil prepared in aqueous solutions of varying ionic strength and pH. Formulations were studied with and without 1% w/w Pluronic F68 nonionic surfactant. All zeta potentials were performed at 20:1 volume dilution, and all percentages shown are by weight. No zeta potential was detectable at 5% NaCl and 1% Pluronic.

Oil	Aqueous phase	No surfactant		1% Pluronic F68	
		Zeta potential $\pm\sigma$ (mV)	Days of stability	Zeta potential $\pm\sigma$ (mV)	Days of stability
10% soybean oil	DI water	-91.1 ± 0.1	1–2	-24.6 ± 0.7	>365
	PBS	-30.0 ± 0.2	<1	-1.0 ± 1.3	>182
	5% wt NaCl	0.8 ± 1.1	<1	–	>365
	0.01 M HCl	-7.6 ± 0.5	<1	0.2 ± 1.0	<76
	0.01 M NaOH	-47.7 ± 1.5	<182	-2.7 ± 0.7	>182

proposed emulsion formulation. Additionally, 2% w/w of nonionic surfactant appears to have very similar droplet size to the Diprivan® control formulation. Shelf life stability is comparable to the Diprivan® control sample, thus 2–3% nonionic surfactant (in particular Pluronic F68) is recommended for the improved propofol formulation.

3.3.3. Zeta potential and electrostatic repulsions in emulsion stability

Up to this point, all emulsions considered contain a very small loading (0.0001–0.001% wt) of the ionic surfactant sodium stearate. Only a very small fraction of this amount is expected to be in the anionic form due to the very low pK_a of stearic acid (Kanicky and Shah, 2002). Thus, kinetic stability is likely not arising from ionic surfactants adsorbed at the interface. Several studies suggest that Pluronics and other nonionic surfactants adsorb hydroxide ions at the interface (Elworthy et al., 1971; Gotchev et al., 2011; Schick, 1987). Other investigations demonstrate the presence of surface charge in oil and water systems in absence of surfactant (Carruthers, 1938; Dickinson, 1941; Marinova et al., 1996; Taylor and Wood, 1957). It is thus feasible that electrostatic interactions are important in these systems even though the concentration of ionic surfactant is negligible.

Table 2 shows a comparison of measured zeta potential and observed days of stability for emulsions made with 10% soybean oil with or without 1% Pluronic F68 surfactant (all w/w) in various aqueous systems. Finely dispersed droplets of soybean oil in DI water have a highly negative zeta potential of −91.1 mV which reduces to −30 mV in PBS and to a negligibly small value in 5% w/w NaCl solution. This proves that ions are indeed adsorbing to the oil droplet surface even without surfactants. In acid, the zeta potential of the mixture becomes −7.6 mV, which further suggests that it is hydroxyl ions that are adsorbing to the oil–water interface. Soybean oil dispersions prepared in DI water remained stable for about one day, while those in PBS and acid destabilized very rapidly. Thus, stronger electrostatic repulsions demonstrated by the larger zeta potential magnitudes are likely responsible for the limited stability of soybean oil dispersions without surfactants. The correlation between zeta potential and emulsion stability was not observed when surfactant was included. Adding surfactant to each of the systems significantly decreases the magnitude of zeta potential despite greatly increasing the stability of the mixtures. The decrease in the magnitude of the zeta potential could be due to a lower affinity for charge adsorption at the interface with Pluronic or due to shifting of the slip plane further away from the surface where ions may adsorb. These results suggest electrostatic repulsions are not dominant in providing the long-term kinetic stability of emulsions as shown above.

3.3.4. Addition of electrolytes to stable emulsions

Fig. 6 shows photographs of emulsions with increased electrolyte concentration taken at long times after salt addition. The images and the size distributions clearly show that the emulsions remain stable for longer than a year even for salt concentrations of 5%. There was however some evidence of discoloration of the formulations starting at about 2 months which appears to be proportional to salt concentration. Discoloration may be due to dimerization of propofol which occurs spontaneously with exposure to oxygen. Elevated salt concentration may exacerbate the dimerization reaction, but this has no effect on the droplet size or stability of the emulsion (see Fig. 5c and d). These results suggest that elevated electrolyte concentration and solution conductivity are not critical for long term stability of emulsions.

3.3.5. Increased ionic surfactant concentration

Increasing the concentration of ionic surfactant did not affect the long-term stability of the proposed formulations. Photographs of emulsions with increased sodium stearate loading at long shelf storage are shown in Supplemental data. The visible stability of these formulations combined with stable droplet sizes (data not shown) suggest that increasing the concentration of sodium stearate up to 0.05% has little to no effect on emulsion stability. Again it is noted that due to stearic acid's low pK_a of about 4.9 (Kanicky and Shah, 2002), only a small fraction of stearate at the interface will be in its ionized form at biological pH.

3.3.6. No ionic surfactants

Removing ionic surfactant entirely from the proposed formulation had very little effect on the resulting emulsion stability as seen in Fig. 5c and d and Supplemental data. These results suggest ionic surfactants are not necessary to achieve long-term kinetic stability of emulsions.

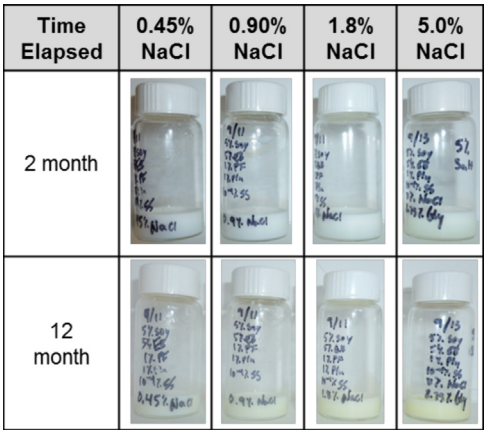


Fig. 6. Photos of proposed emulsion formulations with different amounts of salt added at different shelf life intervals. All percentages shown are w/w. At 2 months, only a small amount of discoloration is seen in the high salt concentration sample. At 12 months, discoloration is observed in all samples. Droplet size remains stable despite discoloration.

4. Conclusions

Incidence of pain on injection of propofol emulsions is correlated with aqueous phase drug concentration which is driven by the equilibrium partitioning of the drug into the emulsified excipient oil and water phases. Modifying the excipient oil used in emulsion formulation can significantly alter the free drug concentration. Ethyl butyrate exhibits high solubility of propofol with a demonstrated oil/water partition coefficient of 19,900, which is about 4-fold higher than soybean oil. An excipient oil mixture of ethyl butyrate and soybean oil reduces the free propofol concentration to 7 μg/mL, or over 50% reduction from Diprivan® and 30% reduction from 1% Propofol Lipuro®.

The excipient oils used to alter drug partitioning strongly affect the resulting emulsion stability. Several other oils explored here including ethyl butyrate, octanoic acid, isopropyl myristate, and isopropyl palmitate did not yield stable emulsions with a correlation observed between stability and molecular weight of the excipient oil. While pure ethyl butyrate emulsions were highly unstable, adding ethyl butyrate to soybean oil emulsions improved their stability. Mean droplet sizes of emulsions containing 1% propofol and 10% soybean oil, or 10% mixtures of soybean oil with ethyl butyrate remained unchanged for over a year with concentrations of 1–5% Pluronic F68, Tween 80 or Brij 78 surfactants (all w/w). A low concentration of sodium stearate was used in most of the formulations, but it was eventually concluded that ionic effects play a negligible role in stability which allowed sodium stearate to be removed from the formulation without affecting stability. We hypothesize the kinetic stability of these emulsions is likely due to increased rigidity of the oil–water interface caused by strong interactions of the surfactant with phases at the interface. This strong interface rigidity prevents droplets from deforming and coalescing during collisions leading to enhanced kinetic stability.

As an excipient, ethyl butyrate reduces the aqueous concentration of the drug while improving emulsion stability, making it an ideal addition to the soybean oil based formulations of propofol. Although it is safely and widely used as a food additive, ethyl butyrate has not yet been approved for parenteral use. Based on all the results from this study, we propose an emulsion with 1% w/w propofol, 5% ethyl butyrate, 5% soybean oil and 2–3% Pluronic F68 as an improved formulation with excellent kinetic stability and greater than two-fold reduced aqueous concentration compared to Diprivan®. With the exception of ethyl butyrate, all components of these formulations are considered as safe and have been tested in various animal and human studies at comparable or larger loadings. While the results from these *in vitro* results are very encouraging, *in vivo* studies are the necessary next step to prove efficacy and possibly reduced pain compared to Diprivan® and Propofol Lipuro®. Also, *in vitro* size measurements clarifying the volume percentage of droplet size larger than 5 μm are necessary to establish the suitability of these formulations for intravenous administration.

Acknowledgements

We would like to thank the University of Florida, Graduate School Fellowship providing partial funding and support for this project.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2015.03.057.

References

Altomare, C., Trapani, G., Latrofa, A., Serra, M., Sanna, E., Biggio, G., Liso, G., 2003. Highly water-soluble derivatives of the anesthetic agent propofol: in vitro and in vivo evaluation of cyclic amino acid esters. Eur. J. Pharm. Sci. 20, 17–26. doi:http://dx.doi.org/10.1016/S0928-0987(03)00161-1.

Bagwe, R.P., Kanicky, J.R., Palla, B.J., Patanjali, P.K., Shah, D.O., 2001. Improved drug delivery using microemulsions: rationale, recent progress, and new horizons. Crit. Rev. Ther. Drug Carrier Syst. 18, 77–140.

Baker, M.T., Naguib, M., 2005. Propofol: the challenges of formulation. Anesthesiology 103, 860–876.

Baravkar, V., Jirage, A., Kate, V., Payghan, S., D'souza, J., 2014. Potential application of non aqueous emulsion for drug delivery. Asian J. Biomed. Pharm. Sci. 4, 10–18.

Carruthers, J.C., 1938. The electrophoresis of certain hydrocarbons and their simple derivatives as a function of pH. Trans. Faraday Soc. 34, 300. doi:http://dx.doi.org/10.1039/tf9383400300.

Date, A.A., Nagarsenker, M.S., 2008. Parenteral microemulsions: an overview. Int. J. Pharm. 355, 19–30. doi:http://dx.doi.org/10.1016/j.ijpharm.2008.01.004.

Degner, B.M., Chung, C., Schlegel, V., Hutkins, R., McClements, D.J., 2014. Factors influencing the freeze–thaw stability of emulsion-based foods: freeze–thaw stability of food emulsions. Compr. Rev. Food Sci Food Saf. 13, 98–113. doi:http://dx.doi.org/10.1111/1541-4337.12050.

Derjaguin, B., Landau, L., 1941. Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. Acta Physicochim. 633.

Dickinson, W., 1941. The effect of pH upon the electrophoretic mobility of emulsions of certain hydrocarbons and aliphatic halides. Trans. Faraday Soc. 37, 140. doi:http://dx.doi.org/10.1039/tf9413700140.

Driscoll, D.F., Nehne, J., Peterss, H., Franke, R., Bistrian, B.R., Niemann, W., 2002. The influence of medium-chain triglycerides on the stability of all-in-one formulations. Int. J. Pharm. 240, 1–10.

Elworthy, P., Florence, A., Rogers, J., 1971. Stabilization of oil-in-water emulsions by nonionic detergents. J. Colloid Interface Sci. 35, 23–33. doi:http://dx.doi.org/10.1016/0021-9797(71)90180-9.

Gotchev, G., Kolarov, T., Khristov, K., Exerowa, D., 2011. Electrostatic and steric interactions in oil-in-water emulsion films from Pluronic surfactants. Adv. Colloid Interface Sci. 168, 79–84. doi:http://dx.doi.org/10.1016/j.cis.2011.05.001.

Gregory, J., 1995. Polymers at interfaces, by G. J. Fleer, M. A. Cohen Stuart, J. M. H. M. Scheutjens, T. Cosgrove and B. Vincent. Chapman and Hall, London, 1993. Pp. xv + 502, price £65.00. ISBN 0-412-58160-4. Polym. Int. 36, 102–102. doi:10.1002/pi.1995.210360115

Hasani, A., Jashari, H., Gashi, V., Dervishi, A., 2012. Propofol and postoperative pain: systematic review and meta-analysis. In: Racz, G. (Ed.), Pain Management – Current Issues and Opinions. InTech.

Hayashi, M., Nakamura, Y., Higashi, K., Kato, H., Kishida, F., Kaneko, H., 1999. A quantitative structure–activity relationship study of the skin irritation potential of phenols. Toxicol. In Vitro 13, 915–922. doi:http://dx.doi.org/10.1016/S0887-2333(99)00077-6.

Hulman, G., 1995. The pathogenesis of fat embolism. J. Pathol. 176, 3–9. doi:http://dx.doi.org/10.1002/path.1711760103.

Kanicky, J.R., Shah, D.O., 2002. Effect of degree, type, and position of unsaturation on the pK_a of long-chain fatty acids. J. Colloid Interface Sci. 256, 201–207. doi:http://dx.doi.org/10.1006/jcis.2001.8009.

Knibbe, C.A.J., Melenhorst-de Jong, G., Mestrom, M., Rademaker, C.M.A., Reijnvaan, A.F.A., Zuideveld, K.P., Kuks, P.F.M., Van Vught, H., Danhof, M., 2002. Pharmacokinetics and effects of propofol 6% for short-term sedation in paediatric patients following cardiac surgery: propofol pharmacokinetics in children. Br. J. Clin. Pharmacol. 54, 415–422. doi:http://dx.doi.org/10.1046/j.1365-2125.2002.01652.x.

Larsen, B., Beerhalter, U., Biedler, A., Brandt, A., Doege, F., Brün, K., Erdkönig, R., Larsen, R., 2001. Less pain on injection by a new formulation of propofol? A comparison with propofol LCT. Anaesthesist 50, 842–845.

Lee, H.-Y., Kim, S.-H., So, K.-Y., 2011. Prevention of microemulsion propofol injection pain: a comparison of a combination of lidocaine and ramosetron with lidocaine or ramosetron alone. Korean J. Anesthesiol. 61, 30. doi:http://dx.doi.org/10.4097/kjae.2011.61.1.30.

Lee, S.K., 2010. Pain on injection with propofol. Korean J. Anesthesiol. 59, 297. doi:http://dx.doi.org/10.4097/kjae.2010.59.5.297.

Li, G., Fan, Y., Li, X., Wang, X., Li, Y., Liu, Y., Li, M., 2012. In vitro and in vivo evaluation of a simple microemulsion formulation for propofol. Int. J. Pharm. 425, 53–61. doi:http://dx.doi.org/10.1016/j.ijpharm.2012.01.011.

Marinova, K.G., Alargova, R.G., Denkov, N.D., Velev, O.D., Petsev, D.N., Ivanov, I.B., Borwankar, R.P., 1996. Charging of oil–water interfaces due to spontaneous adsorption of hydroxyl ions. Langmuir 12, 2045–2051. doi:http://dx.doi.org/10.1021/la950928i.

McClements, D.J., 2007. Critical review of techniques and methodologies for characterization of emulsion stability. Crit. Rev. Food Sci. Nutr. 47, 611–649. doi:http://dx.doi.org/10.1080/10408390701289292.

Morey, T.E., Modell, J.H., Shekhawat, D., Grand, T., Shah, D.O., Gravenstein, N., McGorray, S.P., Dennis, D.M., 2006. Preparation and anesthetic properties of propofol microemulsions in rats. Anesthesiology 104, 1184–1190.

Ozawa, A., Isono, M., Ueno, T., Kosaka, Y., Nakano, T., Inamura, M., Kanai, A., Okamoto, H., Hoka, S., Nomiyama, S., 2005. Comparison of propofol LCT and propofol MCT/LCT regarding the injection pain at different sites and the memory. Masui 54, 1241–1246.

Ryoo, H.-K., Park, C.-W., Chi, S.-C., Park, E.-S., 2005. Development of propofol-loaded microemulsion systems for parenteral delivery. Arch. Pharm. Res. 28, 1400–1404.
Schick, M.J., 1987. Nonionic Surfactants: Physical Chemistry. M. Dekker, New York.
Sim, J.-Y., Lee, S.-H., Park, D.-Y., Jung, J.-A., Ki, K.-H., Lee, D.-H., Noh, G.-J., 2009. Pain on injection with microemulsion propofol. Br. J. Clin. Pharmacol. 67, 316–325. doi:http://dx.doi.org/10.1111/j.1365-2125.2008.03358.x.
Spernath, A., Aserin, A., 2006. Microemulsions as carriers for drugs and nutraceuticals. Adv. Colloid Interface Sci. 128–130, 47–64. doi:http://dx.doi.org/10.1016/j.cis.2006.11.016.
Sundarathiti, P., Boonthom, N., Chalacheewa, T., Jommaroeng, P., Rungsithiwan, W., 2007. A comparison of propofol-LCT with propofol-LCT/MCT on pain of injection. J. Med. Assoc. Thai. 90, 2683–2688.
Tadros, T., 2006. Principles of emulsion stabilization with special reference to polymeric surfactants. J. Cosmet. Sci. 57, 153–169.
Taylor, A.J., Wood, F.W., 1957. The electrophoresis of hydrocarbon droplets in dilute solutions of electrolytes. Trans. Faraday Soc. 53, 523. doi:http://dx.doi.org/10.1039/tf9575300523.
The United States Pharmacopeial Convention, 2013. Globule Size Distribution in Lipid Injectable Emulsions, USP 36 NF 31.
Thompson, K.A., Goodale, D.B., 2000. The recent development of propofol (DIPRIVAN). Intensive Care Med. 26 (Suppl. 4), S400–S404.
Trapani, G., Lopedota, A., Franco, M., Latrofa, A., Liso, G., 1996. Effect of 2-hydroxypropyl-β-cyclodextrin on the aqueous solubility of the anaesthetic agent propofol (2,6-diisopropylphenol). Int. J. Pharm. 139, 215–218. doi:http://dx.doi.org/10.1016/0378-5173(96)04595-4.
Varshney, M., Morey, T.E., Shah, D.O., Flint, J.A., Moudgil, B.M., Seubert, C.N., Dennis, D.M., 2004. Pluronic microemulsions as nanoreservoirs for extraction of bupivacaine from normal saline. J. Am. Chem. Soc. 126, 5108–5112. doi:http://dx.doi.org/10.1021/ja0394479.
Verwey, E., Overbeek, T., 1948. Theory of the Stability of Lyophobic Colloids. Elsevier.
Yamakage, M., Iwasaki, S., Satoh, J.-I., Namiki, A., 2005. Changes in concentrations of free propofol by modification of the solution. Anesth. Analg. 101, 385–388. doi:http://dx.doi.org/10.1213/01.ANE.0000154191.86608.AC table of contents.