Preparation and Anesthetic Properties of Propofol Microemulsions in Rats

Timothy E. Morey, M.D.,* Jerome H. Modell, M.D., D.Sc. (Hon.),† Dushyant Shekhawat, B.S.,‡ Todd Grand, B.S.,§ Dinesh O. Shah, Ph.D.,‖ Nikolaus Gravenstein, M.D.,# Susan P. McGorray, Ph.D.,** Donn M. Dennis, M.D., F.A.H.A.††

Background: The lipophilicity of propofol has required dispersion in a soybean macroemulsion. The authors hypothesized that the anesthetic properties of propofol are preserved when reformulated as a transparent microemulsion rather than as a turbid macroemulsion and that the dose–response relation can be selectively modified by altering the microemulsion's surfactant type and concentration.

Methods: Microemulsions of propofol were formulated using purified poloxamer 188 (3%, 5%, 7%), and sodium salt of fatty acids (C_8, C_{10}, C_{12}) in saline and characterized using ternary/binary diagrams, particle sizing, and stability upon dilution. Rats received propofol ($10 \, mg \cdot kg^{-1} \cdot min^{-1}$) as either a microemulsion or a conventional macroemulsion to determine these end points: induction (dose; stunned; loss of lash reflex, righting reflex, withdrawal to toe pinch) and recovery (recovery of lash, righting, withdrawal reflexes). After a 14-day recovery period, rats were crossed over into the opposite experimental limb.

Results: Forty-eight microemulsions (diameter, 11.9–47.7 nm) were formulated. Longer carbon chain length led to a marked increase in the volume of diluent necessary to break these microemulsions. All rats experienced anesthetic induction with successful recovery, although significantly greater doses of propofol were required to induce anesthesia with microemulsions irrespective of surfactant concentration or type than with macroemulsions. The sodium salt of C_{10} fatty acid microemulsion required the greatest dose and longest time for anesthetic induction.

Conclusion: Propofol microemulsions cause induction in rats similar to that from macroemulsions. The surfactant concentration and type markedly affect the spontaneous destabilization and anesthetic properties of microemulsions, a phenomenon suggesting a mechanism whereby dose–response relation can be selectively modified.

* Associate Professor, Department of Anesthesiology, University of Florida. † Professor Emeritus, Department of Anesthesiology, University of Florida. Chief Scientific Officer, NanoMedex, Inc. ‡ Graduate Assistant, Department of Chemical Engineering, University of Florida. § Biological Scientist, NanoMedex, Inc. ‖ Professor Emeritus, Departments of Chemical Engineering and Anesthesiology, University of Florida. Vice President for Research, NanoMedex, Inc. # The Jerome H. Modell, M.D. Professor and Chairman, Department of Anesthesiology, and Professor of Neurosurgery, University of Florida. ** Research Assistant Professor, Division of Biostatistics, Department of Epidemiology and Health Policy Research, University of Florida. †† The J.S. Gravenstein, M.D. Professor of Anesthesiology, Department of Anesthesiology, and Professor of Pharmacology and Experimental Therapeutics and of Psychiatry, University of Florida.

Address correspondence to Dr. Dennis: Department of Anesthesiology, University of Florida, P. O. Box 100254, Gainesville, Florida 32610-0254. ddennis@ufl.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

PROPOFOL possesses several favorable characteristics, including an antiemetic effect and rapid emergence from unconsciousness with minimal residual drowsiness.[1-4] However, a primary drawback of propofol (2,6-diisopropylphenol) is this drug's extreme lipophilicity, which necessitates dispersion in soybean macroemulsions to produce white, opaque formulations. This solvent requirement potentially causes several adverse drug outcomes, including bacterial growth leading to postoperative infection, pain in a significant number of patients with peripheral intravenous injection, inclusion of egg products, and others.[5-8] Alternative formulations yielding similar pharmacodynamic characteristics as the conventional formulations, but without the associated liabilities, would be useful to enhance patient safety and comfort. Recently, efforts have been made to achieve these goals using other lipid solvents and concentrations, cyclodextrin formulations, microemulsion technology, and prodrug techniques that depend on native enzymes such as alkaline phosphatase to metabolize a parent compound (*i.e.,* phosphono-2,6-diisopropylphenol) to the active drug molecule (*i.e.,* propofol).[9-14]

To address these drawbacks of conventional formulations, we hypothesized that propofol could be associated with biocompatible surfactants to form transparent, colorless, thermodynamically stable, low viscosity, oil-in-water microemulsions with droplets having a 10- to 50-nm diameter (fig. 1). Thus, instead of regarding the extreme lipophilicity of propofol as a hindrance to be overcome, the lipophilicity of propofol (which exists as an oil at room and physiologic temperatures) was leveraged to construct the physical core of the microemulsions. Therefore, propofol served dual roles as both the lipid oil core of the microemulsion and the pharmacologically active agent to induce unconsciousness. In addition, we hypothesized that the spontaneous destabilization of the microemulsion nanodroplets to release propofol could be selectively altered by modifying the concentration and nature of the associated surfactants with resultant changes in latency to anesthetic induction. That is, we hypothesized that the dose–response relation of propofol microemulsions could be modified by varying the nature and concentration of surfactants. To examine these hypotheses, we first determined the physical limitations of possible propofol microemulsions using a variety of propofol and surfactant concentrations to construct pseudoternary phase diagrams of these systems. Pseudoternary diagrams are equilateral triangles that describe the compositional phase behavior of microemulsions wherein the apices represent pure compo-
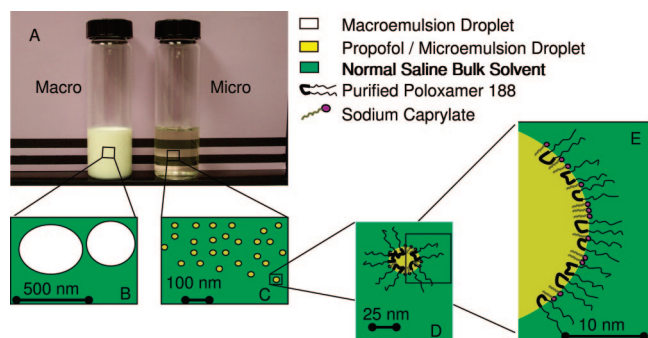
Fig. 1. Photograph and cartoons of a macroemulsion and microemulsion of propofol. (*A*) Shown in the photograph is an opaque, white macroemulsion (Macro) and a clear, colorless microemulsion (Micro) of propofol. (*B* and *C*) Cartoon demonstrating the relative particle sizes of the macroemulsion (*B*) and microemulsion (*C*). (*D*) Cartoon of a microemulsion in cross section. (*E*) Magnified view of cross section of a microemulsion particle demonstrating propofol surrounded by a corona of purified poloxamer 188 and sodium caprylate surfactant in a bulk media of normal saline. *Calibration bars* apply only to their respective panels. *Color legend* applies to all panels.

nents, the boundaries denote two component systems, and the interior demonstrates all three components in the microemulsion system.[15] These diagrams can be used to note the physical boundary conditions of oils and surfactants necessary to formulate thermodynamically stable microemulsions. The diagram is defined as pseudoternary, *vis-à-vis* ternary, because the fourth component of the microemulsion system, 0.9% NaCl in water, is not plotted but did not change throughout the studies. Second, we measured several parameters of anesthetic induction and emergence in rats receiving the experimental and conventional formulations of propofol using a randomized, crossover design.

Methods and Materials

Synthesis of Propofol Microemulsions

Propofol was obtained from Albemarle Corporation (Baton Rouge, LA). Purified poloxamer 188, a nonionic coblock polymer consisting of polyethylene and polypropylene monomers, was purchased from the BASF Corporation (Florham Park, NJ). Sodium salts of fatty acid (C_8, C_{10}, and C_{12}) were supplied by Sigma Chemical Co. (St. Louis, MO). Microemulsions were prepared by combining propofol (0–100 mg/ml) with purified poloxamer 188 (0–70 mg/ml), and a fatty acid salt (0–12.5 mg/ml) in normal saline (0.90 mg/ml NaCl) bulk media. Water was ultrapurified using a water purification system (Nanopure; Barnstead/Thermolyne, Dubuque, IA) to provide a minimal electrical resistance of 18.2 MΩ. After agitation with a magnetic stirrer, these components combined to form clear, colorless microemulsions with adjustment of pH to 7.40 using either HCl or NaOH. All experimental formulations were stored under a nitrogen headspace. To characterize the dimension of the

individual droplets, the effective droplet size of the nanoparticles was measured by the dynamic light-scattering method using a submicron particle sizer analyzer (90Plus; Brookhaven Instruments Corporation, Holtsville, NY) as previously described.[16]

Animal Preparation

All experimental protocols involving the use of animals were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee, Gainesville, Florida. Sprague-Dawley rats (350–500 g; either sex) were purchased from Charles Rivers Laboratories (Wilmington, MA). Before delivery of animals to the investigators by the vendor, rats underwent catheterization of the left femoral vein with subcutaneous tunneling to an exit site between the scapulae. Thus, rats were supplied by the vendor with preimplanted, heparinbonded, femoral vein catheters and did not require additional instrumentation at the time of the experiments that might require sedation or anesthesia that could confound interpretation of results. Rats were caged singly to avoid damage to the catheter by cage mates. They were allowed unlimited access to food and water with a 12:12-h light:dark cycle. On the day of experimentation, each rat was weighed. Thereafter, the central venous line of each rat was easily accessed in conscious, unrestrained rats. All catheters were aspirated until blood was observed and then flushed with 0.5 ml normal saline. Subsequently, rats entered the animal experimental protocol.

Animal Experimental Protocol

Rats were randomized using a random number generator to receive either (1) an experimental propofol microemulsion (n = 6/microemulsion) after filtration through a 200-nm pore filter to ensure sterility or (2) a conventional macroemulsion of propofol in a soybean-based solvent (Diprivan; AstraZeneca Pharmaceuticals, Wilmington, DE). In both cases, the formulation was infused at a rate of 10 mg · kg^{-1} · min^{-1} propofol *via* a microprocessor-controlled syringe pump (sp2000i; World Precision Instruments, Sarasota, FL) to avoid varying rates of infusion associated with manual injection that could potentially confound anesthetic induction time parameters. The end points of anesthetic induction were total drug dose, time to loss of exploratory behavior (*i.e.*, stunned), time to loss of righting reflex, time to loss of lash reflex to gentle stroking (*i.e.*, canthal reflex), and time to loss of reflexive withdrawal of the leg after a great toe pinch every 10 s by a metal clamp. In these experiments, the metal clamp was rubber shod to avoid tissue damage during the experiment. After loss of withdrawal to the toe pinch, the drug infusion was discontinued. End points of anesthetic recovery were return of the withdrawal response to a toe pinch, recovery of spontaneous eye blinking, recovery of a sustained head

lift, and recovery of the righting reflex. After the first anesthetic, each rat recovered for at least 14 days before enrollment in the experimental limb opposite the original assignment. Thus, each rat was anesthetized with both experimental and conventional propofol formulations with random initial assignment and with a 14-day recovery interval before crossover between the formulations. Following a 14-day period of observation after participation in the crossover limb, the experiment was ended.

Statistical Analysis

Measurements are reported as mean ± SD. Statistical analysis was performed with SAS 9.1 (SAS Institute, Inc., Cary, NC). Linear mixed models for incomplete randomized block design were used to analyze the data from this crossover study.[17] Outcome variables were evaluated for normality assumptions using the Box-Cox procedure, with transformations (*i.e.*, square root, inverse square root) if indicated.[18] Initial models included terms for order effect and crossover effect. These were not included in final models if not significant, based on the Akaike Information Criterion. Models were parameterized to include treatment *versus* control differences, purified poloxamer 188 concentration category, fatty acid salt carbon chain length category, and interactions between treatment difference and propofol formulation variables. Prespecified contrasts were used to compare treatment differences at each level of the formulation variables and between levels of the formulation variables. A *P* value of less than 0.05 was considered statistically significant.

Results

Microemulsion Synthesis and Characterization

Forty-eight microemulsions of propofol were formulated using varying concentrations of propofol, purified poloxamer 188, and C_8 fatty acid salt in a normal saline bulk media to construct a pseudoternary diagram of these systems (fig. 2). Depending on the concentrations of surfactants, propofol concentrations of 12–15 mg/ml or less produced microemulsions. In contrast, propofol concentrations exceeding 20 mg/ml with these concentrations of surfactants formed opaque, white macroemulsions similar to conventional propofol formulations. For purposes of testing in rats, the final concentration of propofol in the microemulsions was selected to be 1% (10 mg/ml), a concentration equal to that in the conventional commercially available macroemulsion formulation.

Subsequently, we more closely investigated the interaction of the concentration and types of surfactants and cosurfactants necessary to form microemulsions (or macroemulsions) while holding the concentration of
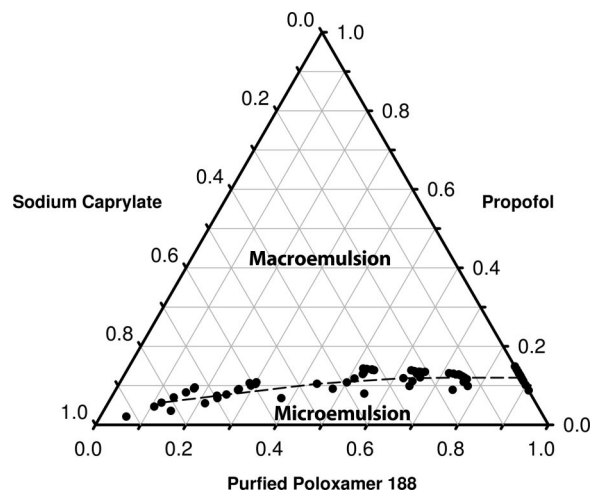
Fig. 2. Pseudoternary diagram of an oil-in-water propofol microemulsion of propofol (oil), purified poloxamer 188 (nonionic surfactant), and sodium caprylate (ionic cosurfactant; C_8 fatty acid salt) (the diagram is defined as pseudoternary, *vis-à-vis* ternary, because the fourth component of the microemulsion system is the bulk media, 0.9% wt/vol NaCl in water, and is not plotted, but did not change throughout the studies). The *regions of the phase diagram below and above the dashed line* represent microemulsion and macroemulsion regions, respectively. As is typical of these diagrams, the axes are normalized with values representing a fraction of the maximal concentration of components used (propofol: 100 mg/ml; purified poloxamer 188: 70 mg/ml; sodium caprylate: 12.5 mg/ml) and are, therefore, without units of measure.

propofol constant at 10 mg/ml. At the greatest concentration of purified poloxamer 188 (70 mg/ml), no fatty acid cosurfactant was required to create microemulsions of propofol as shown in the binary diagram (fig. 3). However, as the concentration of purified poloxamer 188 decreased, the concentration of the fatty acid surfactant necessary to form a microemulsion increased. In addition, the nature of the fatty acid significantly affected its concentration of fatty acid salt necessary to create a

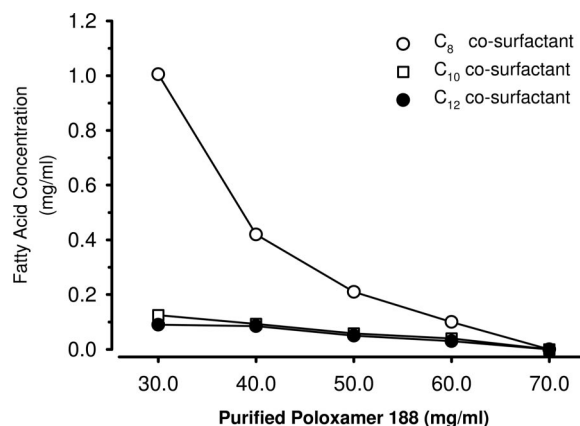Fig. 3. Binary diagram noting the concentrations of purified poloxamer 188 and the cosurfactant fatty acids necessary to form propofol microemulsions in a bulk media of normal saline. The concentration of propofol was constant at 10 mg/ml. For a given fatty acid cosurfactant, the *region below the line* is a microemulsion, whereas the *area above the line* represents a macroemulsion formulation.
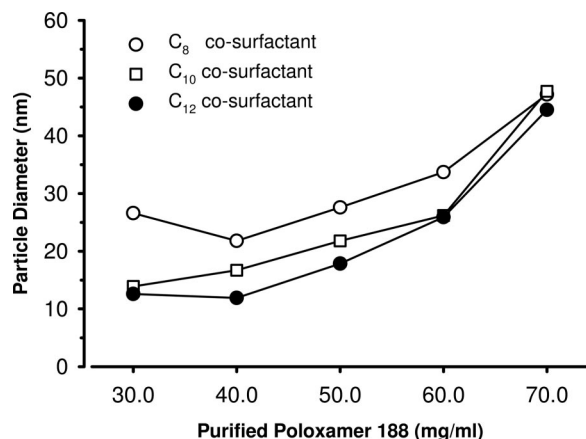
Fig. 4. Effects of purified poloxamer 188 concentration and fatty acid chain length on nanodroplet diameter for propofol microemulsions. In these systems, the concentration of propofol was held constant at 1% (10 mg/ml), and the minimum necessary amount of fatty acid salt was added to formulate propofol microemulsions.
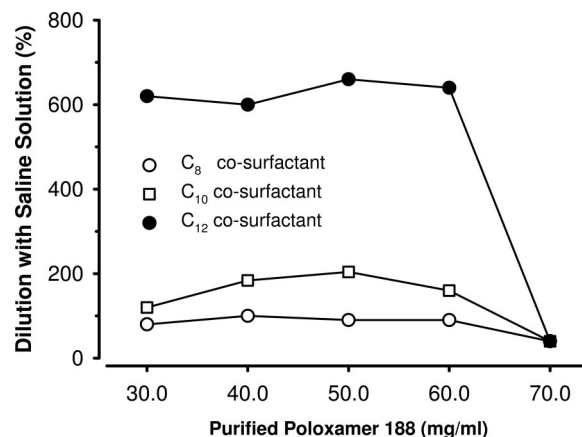

Fig. 5. Effect of dilution on propofol microemulsions formulated using various concentrations of purified poloxamer 188 (see *abscissa*) and several fatty acid salts with variable carbon chain length. Shown are the relative volumes of 0.9% NaCl diluents that caused the microemulsions to break into macroemulsions (*i.e.*, a transition from a transparent to a turbid state). The concentration of propofol in the undiluted microemulsions was constant at 1% (10 mg/ml).

microemulsion. Thus, the concentration of fatty acid salt required to formulate propofol as the oil core of a microemulsion was markedly diminished by lengthening the carbon chain of the cosurfactant fatty acid from 8 to 10 or 12 carbon atoms at any concentration of purified poloxamer 188.

The measured particle sizes for various formulations of the microemulsions varied from 11.9 to 47.7 nm (fig. 4). In general, as the concentration of purified poloxamer 188 was increased, the diameter of the particles also increased irrespective of the carbon length of the fatty acid cosurfactant. No change was noted in the dimension of the microemulsion droplets containing C_8 fatty acid salt after 4 months of storage at room temperature.

We also investigated the relative stability of these propofol microemulsions to dilution with normal saline. That is, we determined the volume of the saline diluent necessary to break the microemulsion into a macroemulsion (fig. 5). The volume of diluent required to cause these changes increased with lengthening of the carbon chain in the fatty acid salt. This increase in the diluent volume was most noticeable for the C_{12} propofol microemulsions that required approximately a sixfold dilution to break the microemulsion.

Anesthetic Properties in Rats

Thirty-eight rats were enrolled in this protocol, but eight rats completed only one limb of this protocol because of retraction of the catheter under the skin during the recovery interval from the first limb of the study. Data from these eight rats (n = 5 in microemulsion group, n = 3 in macroemulsion group) were removed from the study. The remaining rats (n = 30) completed the crossover study and were anesthetized with these microemulsions (n = 6/experimental group; five experimental groups) and with the macroemulsion

control (n = 30). All rats experienced rapid induction without apnea and recovered for at least 14 days without apparent injury. After the 14-day observation period after crossover, the experiment was terminated. The summary data from these experiments are presented in table 1.

Discussion

Propofol is an organic liquid that exists as an oil at room temperature with minimal aqueous solubility. This insolubility is both a fundamental property of propofol and a significant problem for purposes of drug delivery. To address this issue, propofol was formulated in a castor bean oil macroemulsion in the early 1980s, but this solvent was subsequently abandoned because of its propensity to cause anaphylaxis.[19,20] Instead, a soy bean oil macroemulsion was selected as an alternative emulsification agent to deliver propofol.[21] Currently, these white, opaque macroemulsions available from different pharmaceutical companies are formulated with the following components: propofol (10 mg/ml), soybean oil (100 mg/ml), glycerol (22.5 mg/ml), egg lecithin (12 mg/ml), and a preservative (0.05 mg/ml disodium edetate or 0.25 mg/ml sodium metabisulfite). The requirement that propofol be dissolved in 10% soybean oil with associated surfactant has caused a number of liabilities, including support of bacterial growth, addition of egg products that is objectionable to vegans, and the necessity of preservatives.[5,6,8]

The need for a suitable alternative formulation is evidenced by past and ongoing research into other types of propofol delivery systems. Several investigators have reported favorable results using formulations composed of medium- and long-chain triglyceride macroemulsions to

Table 1. Effects of Purified Poloxamer 188 and Fatty Acid Chain Length Concentrations on Anesthetic Induction and Emergence

Parameter	Macroemulsion	Microemulsions				
Purified poloxamer 188, %	Not applicable	3	5	7	5	5
Fatty acid salt length	Not applicable	C_8	C_8	C_8	C_{10}	C_{12}
n	30	6	6	6	6	6
Anesthetic induction						
Dose, mg/kg	20.5 ± 4.4	26.6 ± 4.0*	30.4 ± 4.5*	30.2 ± 4.5*	41.9 ± 3.4*†	33.9 ± 1.6*†§
Stunned, s	40 ± 9	48 ± 8*†	69 ± 13*	64 ± 12*	69 ± 10*	83 ± 12*§
Loss of righting reflex, s	55 ± 12	68 ± 11*	91 ± 12*	78 ± 15*	110 ± 13*	100 ± 13*
Loss of lash reflex, s	89 ± 17	93 ± 17	129 ± 24*	106 ± 16	156 ± 11*	142 ± 33*
Loss of leg withdrawal, s	123 ± 26	160 ± 23*	182 ± 26*	181 ± 28*	251 ± 20*†	203 ± 10*†§
Anesthetic emergence						
Return of leg withdrawal, s	312 ± 163	368 ± 77	405 ± 193	274 ± 105*†‡	318 ± 63	516 ± 176*†§
Return of lash reflex, s	341 ± 191	347 ± 84	362 ± 195	230 ± 107‡	334 ± 57	528 ± 240
Return of righting reflex, s	658 ± 119	460 ± 64*	629 ± 126	581 ± 155	712 ± 192	889 ± 175*†§
Return of sustained head lift, s	684 ± 113	495 ± 83*	644 ± 133	596 ± 143*	725 ± 197	850 ± 119*†§

Dose and latency intervals for anesthetic induction and emergence in rats after intravenous infusion of a propofol macroemulsion formulation and several propofol microemulsion formulations with differing fatty acid salt carbon chain length and differing purified poloxamer 188 concentrations. Data are expressed as mean ± SD of n number of experiments. Propofol was given as a macroemulsion or a microemulsion in a rat femoral vein catheter using a randomized, crossover design (see Methods and Materials) separated by 14 days of rest. The anesthetic emergence intervals started when the propofol infusion was discontinued.

$P < 0.05$: * microemulsion formulation compared with the macroemulsion formulation; † compared with microemulsion formulated with 5% purified poloxamer and C_8; ‡ compared with microemulsion formulation with 3% purified poloxamer and C_8; § compared with microemulsion formulation with 5% purified poloxamer and C_{10}.

deliver propofol at varying concentrations of 10–60 mg/ml.[11,22,23] In addition, propofol has been complexed with sulfobutylether 7-β-cyclodextrin molecules to form clear, colorless formulations that are stable at a wide range of temperatures (4°–50°C), a clear benefit compared with macroemulsion technology, and that have pharmacokinetic properties similar to the conventional drug delivery system.[12,24] Finally, prodrug methods have been exploited to develop phosphono-2,6-diisopropyl-phenol, an agent that is metabolized by native alkaline phosphatase to generate the active agent, propofol, and two by-products, formaldehyde and phosphate.[25] In this report, we demonstrate that microemulsion methods represent another suitable technology to deliver propofol intravenously with anesthetic parameters similar to the commercially available macroemulsions.

Microemulsion Technology for Drug Delivery

Microemulsions have been previously used to deliver several drugs by the oral and transcutaneous routes. Currently, an oral microemulsion of cyclosporine is available for prevention and treatment of transplant rejection of solid organs.[26] In addition, a number of other drugs have been formulated in microemulsions for oral (*e.g.*, paclitaxel, heparin) and transdermal (*e.g.*, apomorphine, estrogen) delivery.[27–30] Fewer drugs have been delivered using an intravenous route but include *trans*-retinoic acid and flurbiprofen.[31,32] Unlike other oil-in-water microemulsions wherein the active drug is dissolved in an oil excipient, propofol acted in two different, complementary roles in the current investigation. That is, the need for an excipients oil for propofol dispersal was obviated by the fact that propofol is itself an oil at room and physiologic temperatures. Therefore, propofol could serve not only as the active pharmacologic agent, but also could exist as the physical platform for the preparation of these microemulsions. By doing so, the need for additional excipients oils (*e.g.*, soybean or castor bean oil) is eliminated along with the potential for these excipients to nourish bacteria. Although the current authors also hypothesize that propofol microemulsions will not support bacterial growth to the same extent as macroemulsions, additional investigations are needed to test this thesis.

The onset of induction with the propofol microemulsion was similar to that caused by the commercially available propofol macroemulsion. The time to achieve induction (defined by the protocol as loss of leg withdrawal to a pinch) during macroemulsion injection was 123 s, whereas the latency periods during microemulsion treatment were 160–251 s. This delay is favorable when compared with prodrug technologies that rely on metabolism, a phenomenon that may vary within any patient population.[33] The differences in induction times between experimental groups reported herein may be caused by differential release of propofol from the individual droplets into the blood. That is, the different propofol nanoparticles have markedly different stabilities against dilution by blood based on the emulsifier structure and concentration selected for the formulation. In general, a microemulsion is thermodynamically stable at equilibrium. One can destabilize a microemulsion by significantly changing pressure, temperature, or chemical compositions.[15] The last variable can be

changed simply by diluting a microemulsion with saline or blood. It is well recognized that the formation of microemulsions requires an ultralow interfacial tension (*e.g.*, approximately 10^{-3} mN/m) at the oil–water interface.[15] Upon dilution with saline, the interfacial tension will increase substantially as the emulsifier molecules (*i.e.*, both poloxamer 188 and fatty acid salt molecules) desorb from the droplet surface. This event will markedly increase the interfacial tension at the droplet surface and ultimately destabilize the microemulsion, with release of the active pharmacologic core (*i.e.*, propofol). However, each emulsifier film around the microemulsion droplet has inherent molecular packing and, hence, stability. The extent of dilution required to destabilize a microemulsion represents its inherent stability. Thus, a microemulsion requiring greater dilution for destabilization indicates that its emulsifier film has a greater stability. The data in figure 5 suggests that the C_{12} fatty acid salt microemulsions are the most stable because they require the greatest dilution to become a turbid macroemulsion. It is likely, however, that microemulsion destabilization is affected by more than just dilution *in vivo* because the C_{10} fatty acid microemulsion required the longest time to cause anesthetic induction, whereas the C_{12} fatty acid microemulsion was most stable to dilution *in vitro*. Further understanding and selectively modifying these destabilization rates by adapting surfactant type and concentrations alludes to the possibility of controlling release times of active pharmaceuticals from nearly immediate (*e.g.*, propofol) to longer times (*e.g.*, chemotherapeutic or antifungal agents). Moreover, although an understanding of the parameters governing release rate allows further refinement of these microemulsions, only a fraction of the formulations presented herein could be considered for additional study with an aim toward clinical use. That is, the induction times for some microemulsions (*e.g.*, 5% purified poloxamer 188 with C_{10}) were more than double that caused by the macroemulsion. Whether this observation is species specific or a clinically significant result remains to be determined.

Selection of Surfactants

The nonionic and ionic surfactants used to formulate propofol microemulsions were carefully selected not only for their ability to combine with the drug to form stable microemulsions, but also because both surfactants have been previously injected intravenously into humans without reported adverse incidents. Justification for choosing these surfactants is presented subsequently.

Nonionic Surfactant. The selection of purified poloxamer 188, an ethylene oxide–propylene oxide block copolymer, was justified based on a number of characteristics related to chemical and medical requirements. First, this surfactant allowed rapid and reproducible preparation of propofol microemulsions that possessed good stability over time. In general, microemulsions are thermodynamically stable and hence exhibit infinite shelf life. These formulations are destabilized upon dilution with blood or plasma or saline beyond its tolerance limit. Second, purified poloxamer 188 has been previously administered intravenously in large doses to humans, without any apparent adverse effect. For example, this surfactant was infused in patients experiencing chest crisis due to sickle cell disease in an attempt to reduce the duration and severity of the crisis.[34,35] Although not efficacious to treat chest crisis at large doses of purified poloxamer 188 (*e.g.*, 88 g), no apparent untoward effects were noted in the subjects. Third, this surfactant is primarily ($> 95\%$) excreted by kidneys and does not stimulate or inhibit human cytochrome P-450 systems.[36] In fact, others have hailed this coblock surfactant as a model surfactant for pharmacologic use, although additional toxicologic evaluation may be needed before more widespread use in the clinical arena.[36] For example, the current investigators have also demonstrated that poloxamer surfactants may reduce platelet function *in vitro* as assessed by thromboelastography.[37] Additional work is needed to determine whether this effect on platelets translates into functional defects in thrombosis.

For the experiments examining microemulsions formulated with C_8, C_{10}, or C_{12}, the authors held the purified poloxamer 188 concentration constant at 5% (*vis-à-vis* 3% or 7%). The 5% concentration of purified poloxamer 188 was selected for further study because microemulsions formulated with 7% purified poloxamer 188 required minimal to no fatty acid surfactant (fig. 2), whereas microemulsions with 3% purified poloxamer 188 required concentrations of fatty acid necessary to form stable propofol microemulsion likely to be unacceptable for *in vivo* use in humans.

Ionic Surfactant. Sodium caprylate was chosen as the best ionic surfactant for the following reasons. First, the surfactant allows reliable synthesis of propofol microemulsions with particle diameters of less than 50 nm range. Second, sodium caprylate is already present in many blood products (*e.g.*, human albumin) currently in clinical use to stabilize proteins.[38,39] Third, the smaller length of the carbon backbone reduces the risks of cellular injury associated with fatty acids with longer carbon chains such as sodium oleate that is used to cause lung injury as a model of acute respiratory distress syndrome.[40] For these reasons, sodium caprylate is the best choice currently available for additional investigations using microemulsions of propofol, although the information from use of C_{10} and C_{12} allowed us to understand how surfactant selection can affect the dose–response of propofol microemulsions. In addition to the selection of surfactant type, however, the concentration of this ionic surfactant must be carefully considered. In the future, the current investigators hope to determine whether the

concentrations of sodium caprylate used in this study interact with erythrocyte membranes or cause hemolysis as can occur *in vitro* with higher concentrations (EC_{50} of 213 mM) of sodium caprylate.[37]

In conclusion, we report the successful preparation, characterization, and use of propofol microemulsions in rats to induce anesthesia similar to that caused by propofol macroemulsions. Formulation of propofol microemulsions was achieved although no excipient oil (*e.g.,* soybean or castor bean oil) was used because propofol exists as an oil and can serve as its own core of an oil-in-water microemulsion. Thus, propofol was used both to solubilize itself and as an active pharmaceutical. The differential diluent volumes *in vitro* and destabilization times *in vivo* based on changing the concentration and type of surfactant allude to the possibility of selectively modifying the dose–response properties of propofol and potentially other lipophilic drugs. Future work will be aimed toward exploring these differences for propofol and other lipophilic agents and toward determining whether these propofol microemulsions reduce the liabilities associated with soybean oil–based macroemulsions.

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