Exceptionally Stable Fluorous Emulsions for the Intravenous Delivery of Volatile General Anesthetics

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Abstract

Background—Intravenous delivery of volatile fluorinated anesthetics has a number of potential advantages when compared to the current inhalation method of administration. We reported previously that the IV delivery of sevoflurane can be achieved through an emulsion composed of a linear fluorinated diblock copolymer, a stabilizer, and the anesthetic. However, this original emulsion was subject to particle size growth that would limit its potential clinical utility. We hypothesized that the use of bulkier fluorous groups and smaller poly(ethylene glycol) moieties in the polymer design would result in improved emulsion stability while maintaining anesthetic functionality.

Methods—The authors prepared emulsions incorporating sevoflurane, perfluorooctyl bromide as a stabilizing agent, and combinations of linear fluorinated diblock copolymer and a novel dibranched fluorinated diblock copolymer. Emulsion stability was assessed using dynamic light scattering. The ability of the emulsions to induce anesthesia was tested *in vivo* by administering them intravenously to fifteen male Sprague-Dawley rats and measuring loss of the forepaw righting reflex.

Results—20% (volume/volume) sevoflurane emulsions incorporating mixtures of dibranched- and linear diblock copolymers had improved stability, with those containing an excess of the dibranched polymers displaying stability of particle size for over one year. The ED50s for loss of forepaw righting reflex were all similar, and ranged between 0.55 and 0.60 ml/kg body weight.

Conclusions—Hemifluorinated dibranched polymers can be used to generate exceptionally stable sevoflurane nanoemulsions, as required of formulations intended for clinical use. Intravenous delivery of the emulsion in rats resulted in induction of anesthesia with rapid onset and smooth and rapid recovery.

Introduction

Altering the way with which drugs are administered has the potential to change their pharmacokinetic and even pharmacodynamic properties.[1–3] Fluorinated anesthetics are

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currently administered through inhalation. However, intravenous delivery of these anesthetics has a number of potential advantages when compared with inhalation.[4–6] In principle, IV delivery could eliminate the need for a vaporizer [4–6] and allow more rapid changes in anesthetic depth.[7] Indeed, by eliminating the equilibration steps between the vaporizer outflow and the breathing circuit and between the alveoli and the blood, anesthetic induction and recovery have been shown to be extremely rapid – as rapid for induction with intravenous emulsified isoflurane as for intravenous propofol, with even more rapid recovery than from propofol.[8]

Because of these potential benefits, the intravenous administration of fluorinated anesthetics has been investigated for more than 40 years. Early efforts of direct IV delivery of methoxyflurane and halothane led to lethal damage for humans and animals.[9–13] Lipid emulsions such as Intralipid have been tested for IV delivery of volatile anesthetics without adverse effects in animals.[8,14–18] However, the fluorophilic nature of these anesthetics,[19] with limited solubility in aqueous or lipid phases, has prevented the use of classic lipids and surfactants in the preparation of stable emulsions. Furthermore, only small concentrations of emulsified fluorinated anesthetic (3–8% volume/volume) can be achieved by using Intralipid[8,17,18,20–22] or fluorocarbons such as Oxygent, a second-generation blood substitute.[23]

In an effort to develop a formulation sufficiently concentrated for possible clinical use, we previously created a nanoemulsion composed of sevoflurane, perfluorooctyl bromide serving as a stabilizing agent, and a semifluorinated diblock copolymer serving as an interfacial agent, and reported that it could be used to dissolve sevoflurane in water with concentrations up to 20% volume/volume.[24] This formulation was tested *in vivo* in rats and shown to induce general anesthesia with very rapid onset and recovery. However, as we report here, our follow-up studies revealed insufficient long-term stability. The main mechanism of destabilization in these emulsions is that of Ostwald ripening, in which smaller particles dissolve as larger particles grow. This mechanism eventually leads to emulsions with particles too big to be used safely. The emulsions we originally prepared and studied used semifluorinated linear diblock copolymers similar to M_1F_{13} (Figure 1, molecule **1**).[24] In an attempt to reduce particle growth as much as possible, we designed a new hemifluorinated dibranched polymer $M_1diH_3F_8$ (Figure 1, molecule **2**). The main feature of these polymers is the doubling in size and volume of the perfluorinated moiety. We hypothesized that this change in design would allow us to prepare nanoemulsions in which the individual nanodroplets composed of a mixture of sevoflurane and perfluorooctyl bromide would be surrounded by a more dense and compact shell of polymer (Figure 2). The synthesis and physicochemical characterization of these new polymers have been described elsewhere.[25] Here, we report on the ability of these novel polymers to provide exceptionally stable concentrated emulsions of sevoflurane, in which no significant ripening is observed over one year, and on their ability to induce anesthesia *in vivo*.

Materials and methods

Materials

Sevoflurane was purchased from Abbott Labs (N. Chicago, IL) and perfluorooctyl bromide was obtained from SynQuest Laboratories, Inc (Alachua, FL). Sodium chloride (NaCl) was purchased from Fisher Scientific (Fair Lawn, NJ). High purity water (MilliQ, Millipore, Billerica, MA) was used during the studies.

Preparation of 20% (volume/volume) sevoflurane emulsion

Polymer aqueous solutions were prepared by first dissolving the fluoropolymers (500 mg) in saline (0.9% sodium chloride solution, 11.9 ml). Sevoflurane (3.4 ml) and perfluorooctyl bromide (1.7 ml) were added to the aqueous solution, for a total volume of 17 ml. The mixture was initially homogeneized with a high speed homogenizer (Power Gen 500, Fisher Scientific, Hampton, NH) for 1 min at 21,000 rpm at room temperature. The crude emulsion was then microfluidized with a Microfluidizer (model 110 S, Microfluidics Corp., Newton, MA) for 1 min under 5,000 psi at 15°C maintained through a cooling bath. The resulting nanoemulsion was filtered through a 0.45 µm nylon syringe filter. Nanoemulsions were stored in 15 ml sterile centrifuge tubes (Corning Inc., Corning, NY) at 4°C. We prepared several sevoflurane emulsions with various composition ratios of the two fluorous surfactants: $M_1diH_3F_8$/ M_1F_{13} (0/100, 28/72, 54/46, 77/23 and 100/0, weight/weight) and evaluated their physicochemical stability by analyzing particle sizes over one year.

Emulsion particle size determination

Dynamic light scattering was used to analyze the emulsion particle size. Measurements were performed with a NICOMP 380 ZLS (Particle Sizing Systems, Santa Barbara, CA) equipped with a 639 nm laser positioned at a scattering angle of 90°. The nanoemulsions were diluted at the dynamic light scattering intensity factor of 300 by mixing 20 µl of the emulsion and 3.0 ml of Millipore water. Each particle size measurement was run for 5 min at room temperature and repeated three times. The raw data were analyzed using a Guassian fit with the ZW380 software (v. 1.61A, Particle Sizing Systems). Results were reported as volume weighted average diameters.

Emulsion physical stability

The nanoemulsions were stored at 4°C for periods up to 357 days. The emulsion particle size was analyzed at 1, 2, 7, 14, 21, 28 days and then every two weeks up to 357 days.

Animal studies

All animal studies were approved by the University of Wisconsin Animals Care and Use Committee, Madison, Wisconsin, and were performed in accordance with the guidelines laid out in the Guide for the Care and Use of Laboratory Animals published by the National Research Council.

Dose-response data (ED50) for the intravenous delivery of sevoflurane emulsions were measured on male Spraque-Dawley rats (Harlan Spraque-Dawley, Inc., Indianapolis, IN) weighing approximately 280 g. The rats were received with a jugular catheter surgically implanted by the supplier. The rats were weighed and the emulsion dose was adjusted according to the body weight. Fifteen rats were divided into three groups of five rats and each group was used to test one specific emulsion. The same five rats were also used to study the effect of multiple doses of the emulsions. In all cases, the rats received only one dose of anesthetic per day.

The sevoflurane emulsions were administered by first restraining the rat with a towel. The plug placed at the end of the catheter was then removed and replaced with a 22-gauge needle connected to an insulin-type syringe. To remove the heparin-based fill solution and check that no blockage was obstructing in the catheter, the syringe plunger was slowly withdrawn until blood filled the catheter. The rat was then placed in a transparent cage for observation. The 22-gauge needle was then connected to the syringe containing the sevoflurane emulsions. Forty µl of the sevoflurane emulsion, corresponding to the volume of the catheter, was injected to prime the catheter and then the administration of the emulsion was started. The emulsion injection rate was controlled through an infusion pump (11 plus;

Harvard Apparatus, Holliston, MA). A bolus dose was delivered within 20 s regardless of the volume. Immediately after injection, the rat was rolled onto its back and loss of righting reflex (LORR) was evaluated. The time to achieve and to recover from LORR and non-coordination were recorded. When the rat completely recovered from LORR, non-coordination and disorientation (within 3 min in all cases), the catheter was flushed with 0.08 ml of a normal saline solution to remove the residual emulsion and then refilled with 0.08 ml of a heparin-based fill solution. The end of the catheter was sealed with a sterile plug.

In experiments to determine the effective dose for anesthetizing 50% of the population (ED50), doses of the emulsions were administered to the grouped five rats in irregular order. The emulsion starting dose was derived from our previous study in which the ED50 of sevoflurane emulsified with the linear semifluorinated polymer **1** was found to be 0.41 ml / kg.[20]

Statistical analysis

To calculate ED50 for LORR, we determined the number of rats that lost the righting reflex out of the total number that received intravenous delivery of the emulsions. ED50 values were calculated through nonlinear regression, data were fitted to a sigmoidal dose-response relation using the program Graphpad Prism (ver. 5.01; GraphPad Software, Inc., San Diego, CA), according to the equation:

$$Y=Y_{min}+\frac{Y_{max}-Y_{min}}{1+10^{(logED50-X)\times Hillslope}}$$

where X is the logarithm of concentration and Y is the response. 95% confidence intervals of ED50 were also calculated using the program Graphpad Prism (ver. 5.01; GraphPad Software, Inc., San Diego, CA).

Statistical analyses were performed using Student's unpaired *t*-test and one-way analysis of variance (ANOVA), as indicated in the text. A *p*-value of less than 0.05 was considered significant.

Values are presented as mean ± standard deviation.

Results

Emulsion stability

The dibranched polymers of the general molecular formula $M_zdiH_xF_y$ are made through a multistep synthesis and are relatively expensive compounds. Thus, we tested various mixtures of the two most promising polymers, M_1F_{13} and $M_1diH_3F_8$, to establish whether stable sevoflurane emulsions could be formed by a combination of the relatively inexpensive surfactant M_1F_{13} and the more expensive $M_1diH_3F_8$. Immediately after preparation, the emulsion particle size did not show significant differences between the various surfactant ratios, and ranged from 126 to 138 nm in diameter. In all cases the emulsion particles grew in size during the first week. This initial increase was followed by slower ripening. The ripening was maximum in the case of M_1F_{13}, and minimum in the case of pure $M_1diH_3F_8$ (Figure 3A). As shown in Figure 3B, the emulsion containing pure M_1F_{13} (0/100) increased to 284 ± 1 nm during the first 14 days, and then the increasing ratio was slower. The emulsions with 28% of $M_1diH_3F_8$, (28/72), 54% of $M_1diH_3F_8$ (54/46), and 77% of $M_1diH_3F_8$ (77/23) increased to 236 ± 2 nm, 240 ± 5 nm and 253 ± 7 nm respectively, during the first seven days. Particle size ripening slowed significantly after the seventh day, and the

particle growth rate was greater with increasing M_1F_{13} concentration. Specifically, the particles in the emulsion made by using only M_1F_{13}, increased to 912 ± 24 nm during 161 days and sedimentation was then observed. After one year the particle size of the emulsions containing $M_1diH_3F_8/M_1F_{13}$ ratios of 28/72 and 54/46 were 663 ± 20 nm, and 460 ± 2 nm respectively. Sedimentation was observed in both emulsions starting on day 301. The particle size of the emulsion containing an $M_1diH_3F_8/M_1F_{13}$ ratio of 77/23 increased slightly faster than the particles in the emulsion with pure $M_1diH_3F_8$ during the first seven days. After this time, the rates of ripening of this emulsion and that with pure $M_1diH_3F_8$ were similar. After one year the 77/23 emulsion contained particles of the average size 365 ± 11, still within a safe therapeutic range according to United States Pharmacopeia Chapter 729 guidelines.[26] Remarkably, after one year, the particle size of the emulsion containing pure $M_1diH_3F_8$ (100/0) was only 285 ± 5 nm. Figures 3A, B show complete growth plots for the five emulsions investigated in this study.

Dose-response studies of sevoflurane emulsions

Three different formulations ($M_1diH_3F_8/M_1F_{13}$ ratios of 100/0, 77/23 and 0/100) were chosen for dose-response studies in rats. The dose-response curves for LORR of the three different emulsions are shown in Figure 4. Figure 4 indicates the doses given to the rats and the corresponding ED50 values, which were all similar, ranging from 0.55 to 0.60 ml/kg. In all cases, the lowest administered dose that induced LORR in all rats (i.e. 0.69 ml/kg for $M_1diH_3F_8/M_1F_{13}$ ratios of 100/0 and 0/100, and 0.57 ml/kg for 77/23) produced this effect after 18 ± 1 sec from the start of injection. The duration of anesthesia, as measured from the start of LORR (induction) to recovery from LORR (emergence from anesthesia) ranged from 10 to 30 sec, in a dose-dependent manner (ANOVA) for all three formulations (Figure 5).

Discussion

The original formulation we developed for the intravenous delivery of sevoflurane allowed us to dissolve up to 20% sevoflurane as an emulsion in saline solution.[24] This formulation was tested *in vivo* and proved to be able to induce and maintain general anesthesia, with a therapeutic Index of 2.6. However, emulsions are not inherently thermodynamically stable. Various physicochemical mechanisms may contribute to particle growth, which may be followed by phase separation. Therefore, as potential pharmaceutical development requires emulsions to be stable for an extended period of time, we were confronted with the need to stabilize our emulsions. As shown in Figure 3, our original formulations exemplified by the emulsion containing only the M_1F_{13} surfactant showed a relatively fast rate of particle growth, not compatible with clinical use.

In our attempts to create an emulsion with improved stability, we hypothesized that changes in the size and shape of the polymer would be able to induce the formation of a more dense and compact polymeric shell around the emulsion particles. This effect, in turn, would stabilize the emulsions by reducing particle growth. The additional protection provided by the new dibranched fluoropolymer did indeed strongly enhance the emulsion stability for up to one year --the longest duration tested (Figure 3).

We performed dose-response (ED50) studies with emulsions with a ratio $M_1diH_3F_8/M_1F_{13}$ of 0/100, 77/23, and 100/0. The three emulsions had similar ED50s, ranging from 0.55 to 0.60 ml/kg. Loss of righting reflex was rapid – within the twenty second injection period – and recovery of righting reflex was also rapid in comparison with other intravenous induction agents such as propofol or isoflurane in lipid emulsion.[7, 8] The rapid induction of anesthesia indicates that despite its stability in water, the emulsion releases sevoflurane quickly; the recovery profile is presumably due to both redistribution and elimination of the

anesthetic through the lungs.[27] The underlying reasons for these ultrarapid kinetics are not known, but we speculate that it relates to the rapid disaggregation of the nanoemulsion into components or structures that have limited carrying capacity, such as micelles.

This rapid pharmacokinetic profile may prove advantageous under certain conditions, such as increasing anesthetic depth immediately before skin incision, or matching the duration of anesthesia to a brief but intense stimulus such as laryngoscopy or insertion of cranial pins. IV delivery of fluorinated ethers also offers a host of other advantages.[4,5] Recent reports have shown that protection against ischemia and reperfusion injury can be achieved with concentrations of anesthetic insufficient to cause hypnotic and anesthetic effects.[28] The IV delivery of small amounts of concentrated emulsified sevoflurane would be well suited to such applications. However, it must also be acknowledged that the potential benefits of IV administration remain unproven, and that important questions remain regarding the practical utility of IV administration, the optimal approach to measuring and maintaining consistent depth of anesthesia using IV delivery (possibly in combination with inhalational administration), the pharmacokinetic profile and elimination characteristics when administered as a bolus or infusion, the potential to utilize this method for other agents such as desflurane, which boils at room temperature and pressure, and the safety of administration in situations were the airway remains unprotected, such as in a "preconditioning clinic" or Magnetic Resonance Imaging scanner. Similarly, the disposition of the copolymer surfactant and the perfluorinated stabilizing agent also must be addressed by future studies. When administered as an Magnetic Resonance Imaging contrast agent, perfluorooctyl bromide is removed from the circulation primarily by the parenchyma and macrophages of the liver and reticuloendothelial system and ultimately excreted by the lungs through breathing.[29] The surfactant may remain assembled as micelles with limited carrying capacity, or dissociate into monomers, but its eventual disposition remains unknown. The present study, demonstrating that exceptional stability of emulsions under storage conditions is nevertheless compatible with rapid onset of action and recovery, is an important step in continuing to develop such formulations that can be used for such studies, and ultimately for possible clinical use.

In conclusion, sevoflurane nanoemulsions containing ratios of dibranched to linear polymer higher than 50% are characterized by a high drug content and an exceptional physicochemical stability. When these formulations were administered intravenously, a predictable and rapidly reversible anesthesia with rapid onset and smooth recovery was induced in rats. These results suggest a potential clinical use for the described sevoflurane emulsions, particularly in situations where an extremely rapid pharmacokinetic profile might improve physiological stability or extremely rapid recovery is desirable.

Final Box Summary Statement

What we already know about this topic

- Intravenous delivery of volatile anesthetics has potential advantages such as more rapid induction and titration of anesthesia compared to inhalation

- Previous emulsion formulations of fluorinated anesthetics have met with limited success

What this article tells us that is new

- An improved nanoemulsion of sevoflurane in a fluoropolymer shell was shown to induce general anesthesia with rapid onset and recovery in rats

> • This preparation is a promising lead for the intravenous delivery of volatile anesthetics

Acknowledgments

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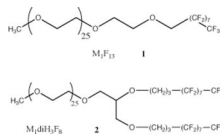
Figure 1.
Chemical structures of fluoropolymers. Molecule 1: M_1F_{13}. The number following the letter M indicates the molecular weight of the poly(ethylene glycol). Thus, M_1 indicates a methyl-capped poly(ethylene glycol) of average molecular weight 1,000. The number after the F indicates the number of carbon atoms in the fluorocarbon chains. Molecule 2: $M_1diH_3F_8$. The number after the H indicates the number of carbon atoms composing the intermediate hydrocarbon segment
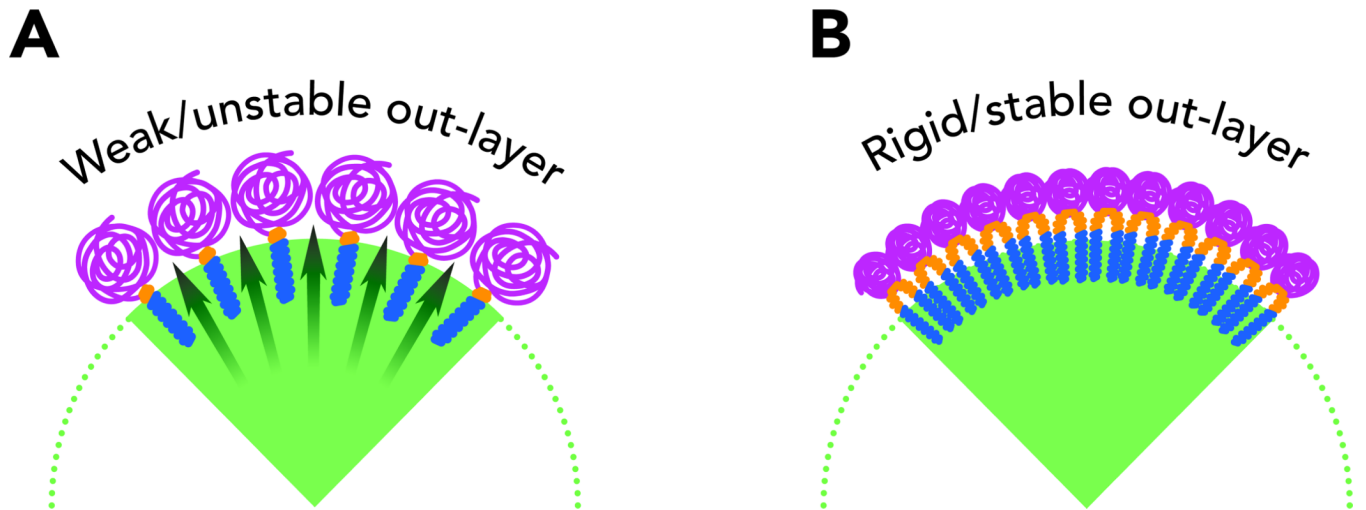
A Weak/unstable out-layer

B Rigid/stable out-layer

Figure 2.
Schematic structure of a section of a fluorous emulsion nanoparticle. (**A**) The original lineal diblock copolymer forms a polymer shell around the fluorous particle that allows easy diffusion of anesthetic molecule in solution. This phenomenon leads to the growth of the nanoparticles volume and destabilizes the emulsion. (**B**) The use of a smaller poly(ethylene glycol) and a much bulkier fluorous tail leads to a more compact polymer shell around the fluorous nanoparticle. The rate of diffusion of anesthetic molecules in solution is reduced and the emulsion is stabilized.
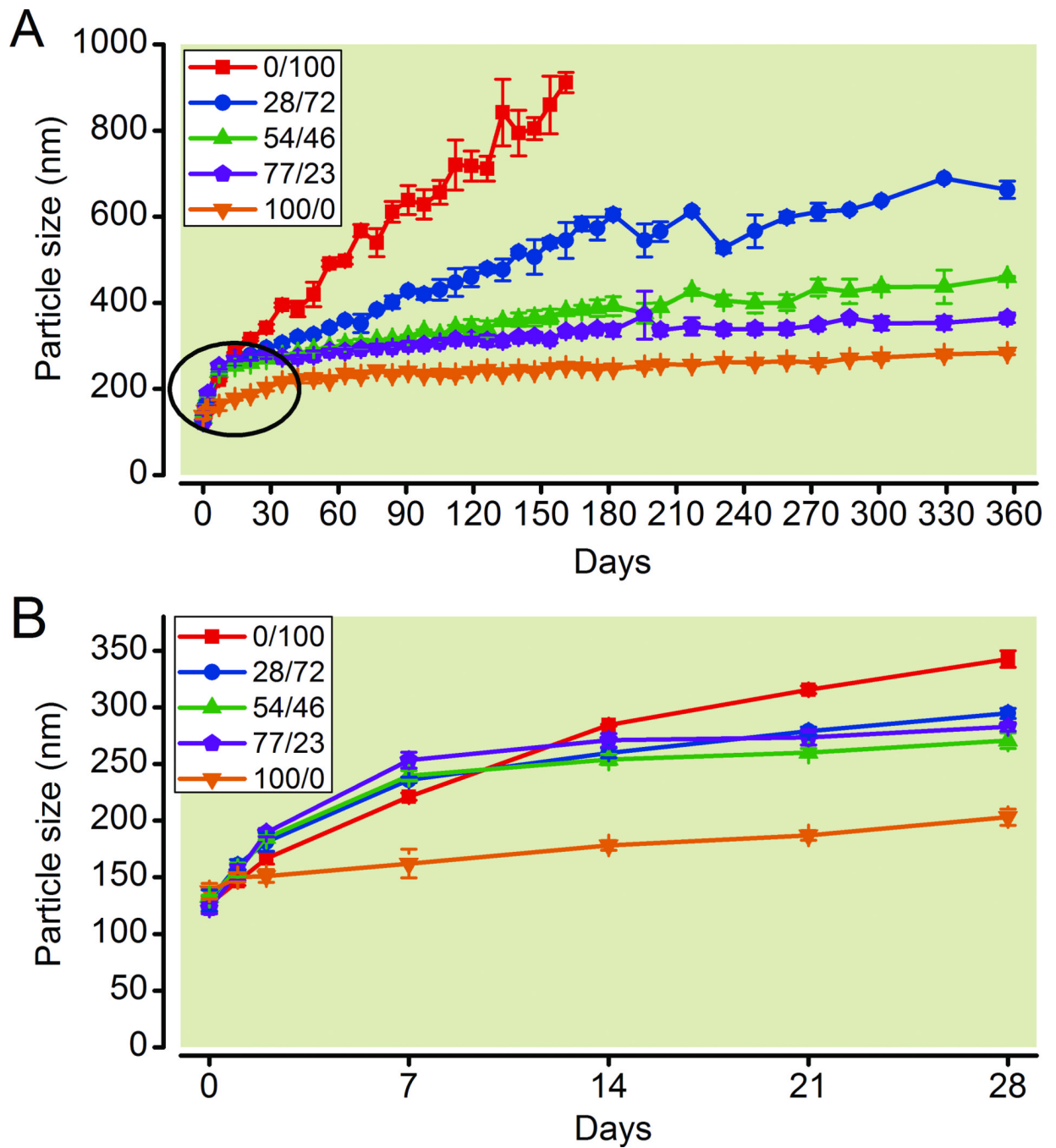
Figure 3.
Particle size of emulsions with varying $M_1diH_3F_8$ / M_1F_{13} composition ratios. (A) Particle size over 1 yr. (B) Expansion to show details of particle size increase during the first 28 days (encircled in A). Values are mean ± standard deviation (n = 3).
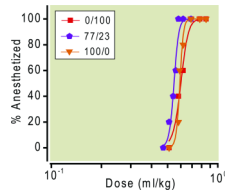
Figure 4.
ED50 Dose-response curves for the emulsions with the $M_1diH_3F_8 / M_1F_{13}$ composition ratios of (⬤) 0/100, ED50 = 0.6; (▲) 77/23, ED50 = 0.55; (◼) 100/0, ED50 = 0.59.
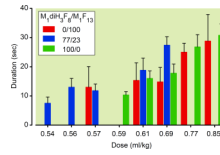
Figure 5.
Average duration of loss of righting reflex for rats. Values are mean ± standard deviation (n = 3).