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REGULAR ARTICLE

A liposomal dispersion formulation of propofol: formulation, pharmacokinetics, stability, and identification of an oxidative degradant

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Abstract Propofol is a common and highly effective anesthetic. Commercially available formulations of propofol, which is only sparingly soluble in aqueous systems, utilize emulsion technology. A liposome based dispersion formulation of propofol has been produced which exhibits good long term colloidal and chemical stability and pharmacokinetcs indistinguishable from the emulsion systems. Inclusion of 0.01% ascorbic acid in the final formulation arrests the growth of an oxidative degradant of propofol both during production and on stability. This degradant is identified as being the dimer 4,4′-dihydroxy-3,3′, 5,5′-tetraisopropylbiphenyl by mass spectroscopy and comparison of the infrared spectrum of the purified degradant to predicted spectra generated using density functional theory.

1 Introduction

Propofol (2,6-diisopropylphenol, Fig. 1), is a highly effective and commonly used injectable anesthetic [1] used for the creation and maintenance of sedation. Propofol is a non-barbiturate and is short-acting. Since propofol has minimal solubility in water, injectable formulations exist as emulsions, which emulsions are based on technology developed in the paint industry. Commercial formulations of propofol include the emulsions Diprivan® (AstraZeneca) and 1% Propofol Injectable Emulsion (Baxter).

As an alternative to an emulsion, we sought to formulate propofol as a liposome based dispersion. Liposomes are microscopic vesicles made, in part, from phospholipids which form closed, fluid filled spheres when mixed with water. Phospholipid molecules are polar, having a hydrophilic ionizable head, and a hydrophobic tail consisting of long fatty acid chains. When sufficient phospholipid molecules are present in water, the tails spontaneously associate to exclude water. The result is a bilayer membrane in which fatty acid tails converge in the membrane interior and the polar heads point outward toward the aqueous medium. As the liposomes form, hydrophobic and reasonably lipophilic molecules tend to be incorporated into the lipid bilayer. Liposomes may be either multilamellar, onion-like structures, with liquid separating multiple lipid bilayers, or unilamellar, with a single bilayer surrounding an entirely liquid center. For our purposes, liposomes would be small (<100 nm in diameter) and unilamellar in character. Liposomes have long been utilized as drug delivery systems designed to provide therapeutic index improvement through passive tissue targeting and enhancement of biodistribution and pharmacokinetics profile [[2–6] and references therein]. A newer technology application of liposomes provides for simple solubilization of insoluble drug substances in a lipid dispersion [[7], see example in Fig. 2]. The goal of the formulation is to provide a low-cost, non-toxic, stable colloidal dispersion for injection. Ideally, the system has the following features: drug loaded into the lipid phase of the liposome, ability to perform sterilizing 0.2 μm filtration, multi-year shelf life, colloidal stability, is non-hemolytic, can be used either as an intravenous bolus injection or for infusion, provide for quick release of the drug, exhibit pharmacokinetics equivalent to other carrier systems, and would afford low cost of materials.

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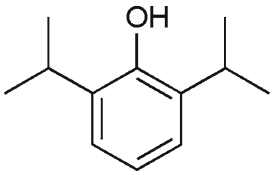


$C_{12}H_{18}O$
Mol. Wt.: 178.271

Fig. 1 The structure of propofol

Fig. 2 A Soy-PC:DSPG liposome dispersion

Here we will examine a liposomal dispersion formulation of propofol ("L-propofol"), and will look at pharmacokinetics, along with chemical and colloidal stability. In the context of chemical stability, an oxidative degradant is identified and a stabilized formulation is derived to prevent such degradation.

2 Methods

2.1 Liposome production

Soy-phosphatidylcholine (Soy-PC, phospholipon 90 from Phospholipid GmbH, Köln, Germany), distearoylphosphatidylglycerol (DSPG from Nippon Fine Chemical) and propofol (from Research Biochemicals International, Natick, MA, USA) were dissolved in a 1:1 (v:v) mixture of methanol and chloroform at a molar ratio of Soy-PC:DSPG of 1:0.2 and a molar ratio of (Soy-PC+DSPG):propofol of 5:1. Once all components were dissolved, solvents were removed by evaporation under continuous nitrogen flow. Residual solvent was removed by storing the container containing the material in a desiccator under vacuum for not less than 48 hours. The dried lipid was then hydrated in 9% sucrose at desired drug concentrations and processed through a high shear homogenizer to form liposomes [5]. The final buffer was established at 5 mM phosphate, pH $\sim$ 7.5, containing 0.01% by weight of ascorbic acid. The resulting solution was filtered through a 0.2 μm filter (cellulose acetate filters worked well; polyethersulfone filters sponsored propofol degradation) and then evaluated.

2.2 Hemolysis testing

Hemolysis was evaluated against dog, rat, and human blood. Negative controls (0.85% saline) along with positive controls (purified water and 0.5% w/v saponin) were included. Liposomes were diluted 2-fold, 10-fold and 50-fold into whole blood containing an anticoagulant. Results are subjective (in the form of a numeric score) and objective (in the measurement of extra-cellular potassium or hemoglobin measurement). For objective measurements, the background from the negative control is subtracted from all observed measurements and then divided through by the positive control less background. This is then reported as a percentage of maximum hemolysis. Whole rat and dog blood was collected in sodium heparin and stored at 2–8°C until use (Lampire Biologicals, Pipersville, PA, USA). Human blood was collected from normal volunteers, also in sodium heparin. Saponin was from Calbiochem (La Jolla, CA, USA). Potassium was measured using an EL/ISE electrolyte analyzer (Beckman Diagnostics Systems Group, Brea CA, USA). Hemoglobin was determined using a Wako Pure Chemical non-cyanide hemoglobin assay (PN 271–73901).

2.3 Pharmacokinetics

Male Sprauge–Dawley rats were administered 8 mg/kg propofol in a single intravenous dose using L-propofol, or one of the two commercially available emulsions (Priority Healthcare Corporation, Altamont Springs, FL, USA). Animal work was performed at EPO (Berlin-Buch, Germany). Plasma samples were drawn prior to dosing and at 5, 30, 60 120, 240, 360, 480, 720, 1,440 and 2,880 min after dosing. Samples for 6 animals each were then analyzed for drug levels by HPLC. The HPLC method is as described below with the following modifications: the standard curve was lowered and made in rat plasma to accommodate the concentrations and processing seen in the samples, and the detection method was switched to fluorescence, with excitation at 271 nm and detection at 310 nm. Samples and standards were diluted in methanol and centrifuged, with the supernatant used for analysis. Concentration vs. time data were fit with WinNonlin

(Pharsight Corporation, Mountain View, CA, USA) to obtain pharmacokinetic parameters.

2.4 Analytical characterization

To assay L-propofol, samples were measured for drug and drug related impurities by HPLC. This assay of L-propofol (after dilution in methanol) included a Kromasil 100 C8 column, equipped with a guard column, isocratic elution with acetonitrile/water 40/60 v/v brought to an apparent pH of 3 with 85% phosphoric acid, a flow rate of 1 mL/min, 25 μL injection volume, detection by UV at 271 nm, a run time of 16 min, and a column temperature of 35°C. Under these conditions, propofol would elute at ∼5.5 min and a degradant would elute at ∼ 13.5 min. The degradant had a peak UV absorbance at 262 nm, but area percent values for the degradant were assumed to have a response factor of 1 at 271 nm. Quantitation was based on an external standard developed from propofol raw material. The dispersion was assayed for median particle size by dynamic light scattering and for aggregation/flocculation by turbidimetry, both as previously described [5]. L-propofol attributes were measured after initial production and during storage at 2–8°C for a period of 2 years.

LC-MS (both positive and negative mode) and GC-MS analysis of propofol and an oxidative degradant were carried out in conjunction with Mass Consortium (San Diego, CA, USA). The electrospray mass spectrometry experiments were performed on a Hewlett-Packard 1100 MSD electrospray mass spectrometer. Electrospray samples were introduced in the analyzer at 12 μL per minute. The positive and negative ions generated by the ion evaporation process entered into the analyzer through an interface plate with 100 μm orifice, while the declustering potential was maintained between 50 and 200 V to control the collision energy of the entering ions. The HPLC assay for LC-MS was the same as that noted above. The GC assay conditions for use with GC-MS were again based on samples diluted at least five fold in methanol, an inlet temperature of 290°C, 1 μL injection volume, a purged splitless injector mode 120 mL/min at 0.5 min, a column flow of 4 mL/min helium, an RTx-1 30 m × 0.53 mm ID column with 1 μm film, and FID detection at 300°C. Oven program was 175°C initial temperature, 1 min initial time, a 10°/min rate, and a 310°C final temperature and 5 min final time. For this assay propofol eluted at ∼ 3.3 min and the degradant at ∼ 14.4 min. In both LC-MS and GC-MS, to enhance analysis of the degradant, samples were thermally treated to enhance degradant content to about 30% relative to propofol. All GC and LC procedures were confirmed to exhibit acceptable linearity, accuracy, precision, limits of detection, and limits of quantitation for the given purpose.

IR analysis was performed on a Nicolet Nexus 470 FTIR coupled to a GC interface. Both GC-IR and neat sample IR (at 8 cm^{-1} resolution) by ATR probe were obtained. The latter were used to measure the IR spectrum of the degradant, which was purified by heat degrading L-propofol, separation by LC, and purification of the dark brown material out of methanol. This material was re-injected both into GC and HPLC assays to confirm identity as the degradant.

2.5 Computational methods

IR spectra for propofol and the candidate structure and plotted for the oxidative degradant (see below) were calculated and plotted using the GAUSSIAN 03 suite of programs [8] as previously described [9]. The geometries and harmonic vibrational frequencies were calculated using spin-restricted density functional theory with the hybrid B3LYP functional [10,11] and a 6–31G* basis set.

3 Results and discussion

Analysis of L-propofol produced as described above revealed a drug concentration of 12 mg/mL and a lipid to drug molar ratio of about 5–1. Thus, a substantial and commercially relevant concentration of propofol had been achieved. The pH was measured to be ∼ 7.2. Analysis by dynamic light scattering revealed a unimodal distribution with a median size of 22 nm. This relatively small size for a small unilamellar liposome is typical of some of the liposomes produced with this formulation family [7]. Turbidity as measured by the apparent absorption at 600 nm remained below 0.05 absorbance units, and thus no evidence of flocculation or aggregation was evidenced nor was there evidence of any significant presence of particles above 100 nm in diameter.

The formula was shown to be non-hemolytic (Grade 0 and 0% hemolysis), with behavior in the assays described herein identical to saline.

The concentration, pH, median size, and turbidity value did not change after 24 months storage at 2–8°C, indicative of excellent chemical and physical stability of the dispersion. These properties were also stable for 4 months at 25°C.

A chemical degradant, seen in both GC and HPLC assays, was present at about a 1% level in the propofol raw material used throughout these studies. This level was seen to increase during processing and during storage in a temperature dependent manner. In order to identify the degradant, samples were analyzed by LC-MS and GC-MS. The degradant was found to have a molecular weight of 354, indicating that dimerization was a possibility. Purified degradant was collected and an IR measurement was made. From the experimental spectra, IR spectral differences between propofol and the degradant are not significant at wavelengths above 1,600 cm^{-1}. Key

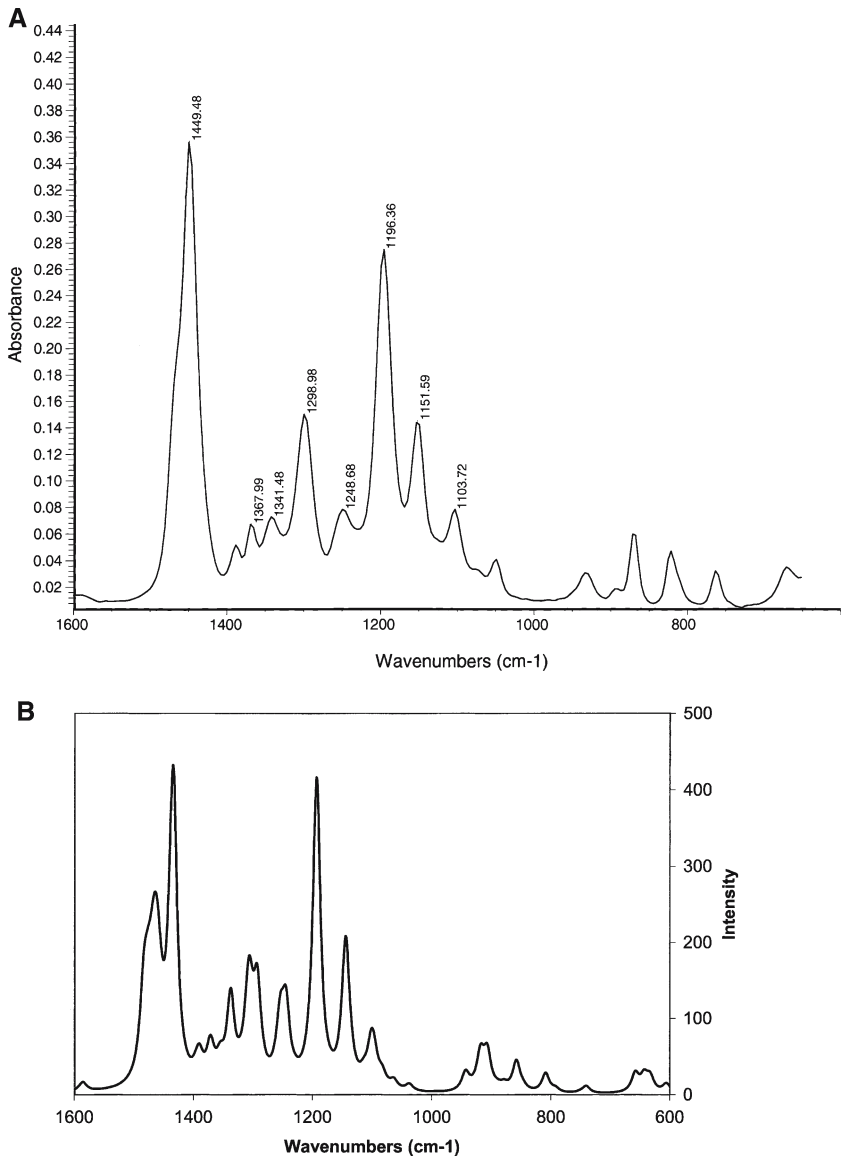
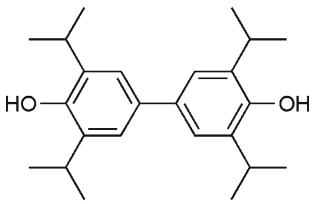


Fig. 3 **a** Experimental IR spectrum of the oxidative degradant of propofol. **b** Theoretical simulation of the IR spectrum of 4,4′dihydroxy-3,3′ 5,5′-tetraisopropylbiphenyl

features of this spectrum in the region 1,600–600 cm^{-1} do show the most significant changes, and the degradant spectrum is shown in Fig. 3a. A candidate structure with the correct mass involved an oxidative dimerization of propofol to 4,4′-dihydroxy-3,3′, 5,5′-tetraisopropylbiphenyl (Fig. 4). To compare this candidate with the experimentally determined IR spectrum, theoretical spectral simulations were generated. Features most altered going from propofol to the degradant are in the range 650–1,000 cm^{-1} and 1,300–1,600 cm^{-1} (data not shown). The theoretical calculations for both propofol and the degradant candidate noted above also exhibit these shifts (data not shown). The theoretical spectrum of the degradant candidate is shown in Fig. 3b. The spectra in Figure 3a and b are in excellent accord. A doublet at 1,450 cm^{-1} in the experiment compares to a pair of peaks in the calculation at 1,464 and 1,434 cm^{-1}. Bands at 1,299,



$C_{24}H_{34}O_2$
Mol. Wt.: 354.526

Fig. 4 The structure of the proposed oxidative propofol degradant 4,4′-dihydroxy-3,3′, 5,5′ tetraisopropylbiphenyl

1,249, 1,196, 1,142, and 1,103 cm^{-1} correspond to theoretical bands at 1,305/1,294, 1,246, 1,193, 1,144, and 1,100 cm^{-1}. Spectra in the region 650–1000 cm^{-1} are also in good correspondence. The results overall support the assignment of the

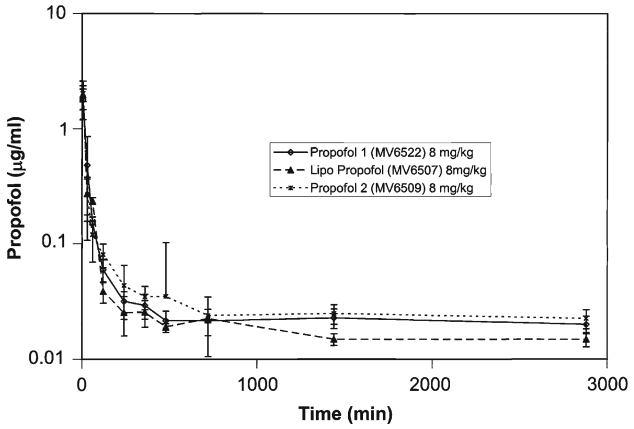


Fig. 5 Concentration of propofol in rat plasma followed by injection of 8 mg/kg propofol as L-propofol ("Lipo Propofol" MV6507), Diprivan ("Propofol 2" MV6509), and 1% Propofol Injection Emulsion ("Propofol 1" MV6522)

oxidative candidate degradant as 4,4′-dihydroxy-3,3′, 5,5′-tetraisopropylbiphenyl.

Prevention of the formation and growth of this degradant is key to a pharmaceutically acceptable preparation. Given an oxidative mechanism, formulations with alpha-tocopherol, butylated hydroxytoluene, and ascorbate were evaluated. The final formula, as noted above, contains 0.01% by weight ascorbate and the degradant level remained at the 1% level in the raw material through production and through storage for 24 months at 2–8°C. After 4 months stability at 25°C, the peak had only increased slightly. Based on the product package inserts, the 1% propofol injectable emulsion contains 0.25 mg/mL metabisulfite. Diprivan does not, but contains 0.005% disodium edetate. L-propofol utilized nitrogen sparged buffers, but was not filled into vials anaerobically. It is interesting to note that oxidation during IV infusion has been noted for metabisulfite containing propofol [12].

Data from analysis of plasma samples from the single dose rat pharmacokinetics study are shown in Fig. 5, comparing L-propofol to the two emulsions. For all three formulas, rapid release of drug is evidenced by the clear observation of rapid distribution in high volume. The results parallel those reported in the package inserts of the two emulsions. This high volume of distribution and lack of difference between the emulsions and L-propofol indicate that the drug is rapidly released from the liposome, as seen previously for this type of formulation [7]. AUC_∞ values for L-propofol were 235 ± 86, 207 ± 49 and 144 ± 72 min* µg/mL for L-propofol, Diprivan, and the 1% Propofol Injectable Emulsion, respectively. Overall, the pharmacokinetics of all three formulations are indistinguishable. During the pharmacokinetics study, the animals experienced sedation during the early time points, but evaluation of this aspect was not an element of the study design.

4 Conclusion

Evaluating the goals for the formulation, we conclude that L-propofol has been successfully formulated as a colloidally stable dispersion. The lipid based excipients are widely used and considered safe for parenteral formulation. The predominant lipid component is the inexpensive Soy-PC, with a relatively small amount of DSPG to afford anionic character and thence the observed colloidal stability. The formula is observed to be stable over two years during storage at 2–8°C. The drug concentration and lipid to drug ratio achieved are well within dosing practicability. The formulation was easily sterile filtered through cellulose acetate 0.2 µm membranes. (Emulsion formulations cannot be so filtered.) The formulation exhibited no hemolysis, and exhibited in the simple rat study performed pharmacokinetics equivalent to two commercially available emulsions. Though not demonstrated in this study, the formulation has the propensity to avoid the pain on injection associated with the emulsion formulations.

Acknowledgments This paper is dedicated to Professor Philip J. Stephens in celebration of his lasting contributions to science. Thanks are due to Don Hodgson, who first brought attention to propofol, to Charles Yuan and Steven Sladky for early efforts in study of the degradant, and to all in Pharmaceutical Development, Product and Process Characterization, and Development and Technical Services at Gilead. This work was supported in part by a grant of HPC time from the DoD HPC Center, US Army Research Laboratory.

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