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# Evaluation of new propofol aqueous solutions for intravenous anesthesia

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**Abstract**

The aim of this study was to evaluate the potential of using three new aqueous formulations of propofol for intravenous (i.v.) anesthesia. The first formulation can be prepared by using hydroxypropyl-γ-cyclodextrin (HP-γ-CD) as a solubilizer. Phase-solubility analysis showed a linear increase in the solubility of propofol to a maximum of 16.6 mg/ml in 30% (w/v) HP-γ-CD. Moreover, phase-solubility studies demonstrated that 18% (w/v) HP-β-CD or SBE-β-CD and 24% HP-γ-CD solutions, respectively, are required to dissolve 10 mg of propofol in 1 ml of the vehicle; the corresponding solutions, however, are slightly hypertonic. Autoclaving the 10 mg/ml CD-based formulations for 15 min at 121 °C caused a change in pH which was more evident for the HP-β-CD-based formulation while, in any case, no detectable fall in propofol concentration was observed. The second formulation herein evaluated is a co-solvent mixture (i.e., propylene glycol:water (1:1), v/v) which is able to dissolve 10 mg/ml of the anesthetic agent. However, although it is simple to prepare, the stability of this formulation is limited. The third aqueous formulation can be prepared by using the prolinate ester of propofol and its water-soluble derivative dissolved in water at equimolar concentration. The efficacy of all these formulations as i.v. anesthetic agents was assessed using a pharmacodynamic measure (onset and duration of loss of the righting reflex, LORR), and compared with that of the commercial propofol formulation (Diprivan®, 10 mg/ml) in rats. It was found that minimizing the amount of cyclodextrin in all CD-based formulations, anesthetic effects comparable to those of propofol in Diprivan® were still observed. Moreover, the prolinate ester constituted an effective i.v. anesthetic formulation with the same duration of action but with a longer induction time than Diprivan®.


*Keywords:* Propofol; Aqueous solutions; Intravenous anesthesia; Cyclodextrins; Prodrug

## 1. Introduction

Propofol, 2,6-diisopropylphenol, (Fig. 1, **1**) is a short-acting hypnotic agent effective for short surgical procedures, and for the induction and maintenance of general anesthesia when administered by intravenous (i.v.) infusion. Propofol has the pharmacokinetic advantages of rapid onset and recovery even after long periods of anesthesia, and produces a low incidence of post-operative nausea and vomiting (Trapani et al., 2000; Langley and Heel, 1988). Presently, propofol

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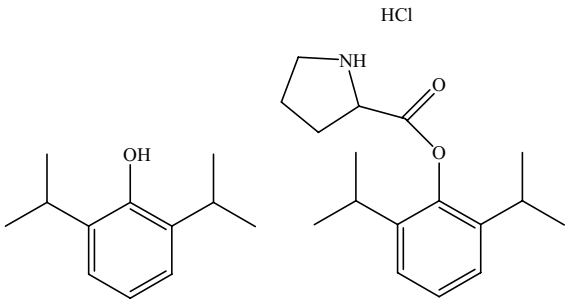


1 2

Fig. 1. Chemical strucuture of propofol (**1**) and its prolinate ester (**2**).

is formulated in an oil-in-water emulsion (1%, w/v) of soya bean oil, glycerol, and purified egg phosphatide (Diprivan®, Zeneca, UK). The i.v. injection of Diprivan® produces hypnosis rapidly with minimal excitation, but several adverse clinical effects are associated with propofol infusion such as bradycardia and reduction in blood pressure (Dollery, 1991). Some of these side-effects have been shown to be associated with the emulsion-based formulation of propofol. Moreover, this formulation suffers from further limitations, including pain at the site of injection, poor physical stability, rapid growth of microorganisms, and potential for embolism (Prankerd and Stella, 1990; Bennett et al., 1995). These drawbacks stimulated an active search aimed at developing safe alternative aqueous formulations of propofol.

A survey of the literature reveals that several approaches have been exploited, including complexation with hydroxypropyl- and sulfobutyl-β-cyclodextrin (Brewster et al., 1990; Trapani et al., 1996, 1998; Mosher and Thompson, 2002), chemical delivery systems (Pop et al., 1992), the main goals being increased hydrosolubility, improved patient acceptance (e.g., reduced pain at injection site), decreased side-effects and prolonged duration of action. Water-soluble prodrugs have also been prepared as suitable formulations for parenteral administration (Banaszczyk et al., 2002; Hendler et al., 1999; Sagara et al., 1999; Stella et al., 2000). However, in spite of this increasing interest in new aqueous propofol formulations, clinical application has been limited, with Aquavan (TM) injection being the notable exception. This formulation is currently under Phase II clinical trial in patients undergoing coronary artery surgery (Guilford Pharmaceuticals Inc., 2002). Thus, there still remains a need for the development of efficient propofol delivery systems that combine the advantages of aqueous formulations (sterilization, reduced pain at the injection site) and overcome the drawbacks associated with the marketed emulsion formulation.

As part of our program aimed at identifying new aqueous formulations of propofol (Fig. 1, **1**), we investigated the properties of some aqueous preparations containing either a CD together with the anesthetic agent or only its prolinate ester (Fig. 1, **2**), a water-soluble prodrug candidate previously studied by us (Altomare et al., 2003). The efficacy of these new formulations was assessed using a pharmacodynamic measure such as the onset and duration of loss of the righting reflex (LORR).

## 2. Materials and methods

### 2.1. Materials

Propylene glycol of pharmaceutical grade was purchased from ACEF (Azienda chimica e farmaceutica, Fiorenzuola d'Adda, Italy) and used as received. Hydroxypropyl-β-cyclodextrin was obtained as a gift from Roquette (Cassano Spinola, Italy) and its substitution degree (5.88) was calculated by means of $^{1}$H NMR (Pitha et al., 1986). Sulfobutylether-β-cyclodextrin sodium salt $(SBE)_{7m}$-β-CD (Captisol®, degree of substitution 6.4, determined by the supplier, referred to in the following text as SBE-β-CD) was purchased from CyDex Inc., Overland Park, KS. 2-Hydroxypropyl-γ-cyclodextrin, having a substitution degree of 0.6, was kindly donated from Waker Chemie (Peschiera Borromeo, Italy). This substitution degree was determined from the supplier by NMR analysis.

Propofol **1** was purchased from Aldrich (Milan, Italy) as extra pure reagent, and its freshly distilled samples were stored under nitrogen. Reagents used for the preparation of buffers were all of analytical grade. The buffers were prepared as reported in the European Pharmacopeia III Ed. Fresh deionized water from all glass apparatus was used in the preparation of all the solutions.

### 2.2. High-performance liquid chromatography (HPLC) analysis

High-performance liquid chromatography (HPLC) analyses were performed with a Waters Associates (Waters Corp., Milford, MA) Model 1515 isocratic HPLC pump equipped with a Waters 2487 dual λ absorbance UV detector and a Waters 717 plus autosampler. For analyses, a revesed phase symmetry $C_{18}$ (25 cm × 4.6 mm; 5 μm particles) column in conjunction with a Security Guard Cartridge was eluted with a mobile phase composed by methanol and deionized water (8:2). The flow rate of 0.8 ml/min was maintained. Quantification of propofol was carried out by measuring areas under peaks in relation to those of the standards chromatographed under the same conditions. Calibration curves were prepared using methanol as the solvent and were linear ($r^2 > 0.998$) over the range of concentrations of interest at the selective wavelength of 270 nm. Standard curves were prepared at a wavelength of 270 nm using methanol as the solvent and were linear ($r^2 > 0.998$) over the range of concentrations of interest.

### 2.3. Phase-solubility analysis

The phase-solubility study was performed according to the method of Higuchi and Connors (1965). Solubility measurements of propofol were carried out at a constant temperature (25 °C) using different amount of CD in 0.05 M potassium phosphate buffer (pH 6.5). A large excess of the anesthetic agent was added to 2 ml of the appropriate CD solution in screw-capped test tubes. The resulting mixtures were vortexed for about 5 min and shaken in a thermostatically controlled water bath at 25 ± 0.5 °C for 5 days. Then, an aliquot of aqueous phase of each mixture was withdrawn with a 10 ml glass syringe pre-heated at the above mentioned temperature, and after filtration through a 0.45 μm membrane filter (Millipore®, cellulose acetate), it was transferred in thermostated test tubes. Next, about 0.5 ml of the clear filtrate were subjected to appropriate dilution with 0.05 M potassium phosphate buffer (pH 6.5) and the test tubes were allowed to stand at 25 ± 0.5 °C until analyzed by HPLC. The injection volume was 20 μl. All these manipulations were made unless the removal of the test tubes from the water bath, using thermostated pipettes, syringes, and buffer solution. The apparent 1:1 stability constant ($K_c$) was estimated from the slope of the straight line of the phase-solubility diagram according to the following equation: $K_c = \text{slope}/S_0(1 - \text{slope})$ (Higuchi and Connors, 1965). The intrinsic solubility value ($S_0$) of propofol was determined directly in 0.05 M potassium phosphate buffer (pH 6.5) at the temperature of experiment (25 ± 0.5 °C).

### 2.4. Dosage form preparation

The aqueous HP-β-CD and SBE-β-CD dosage forms for i.v. administration were prepared by dissolving propofol (100 mg) in 10 ml of 18% (w/v) solutions of the corresponding CD in distilled water. The resulting mixtures were vortexed for 10 min and sonicated for 20 min at 25 °C. The aqueous HP-γ-CD dosage form was prepared as above reported for the β-CDs except for the fact that 100 mg of propofol were dissolved in 10 ml of a 24% (w/v) solution of HP-γ-CD. All aqueous CD-based formulations were allowed to stand at 25 °C to attain the equilibrium solubility. The propofol/co-solvent mixture dosage form was prepared by dissolving propofol (100 mg) in 10 ml of a mixture propylene glycol/water (1:1). The resulting mixture was vortexed for 10 min and sonicated for 20 min at 25 °C. This co-solvent cocktail and methodology was needed to maintain the propofol for i.v. administration. The aqueous solution of the prolinate ester of the propofol, a prodrug prepared in our laboratory, was freshly prepared by dissolving the 17 mg of prodrug in 1 ml of distilled deionized water. This solution is equivalent to 10 mg/ml of propofol.

### 2.5. Osmolarity measurements

All osmolarity measurements were carried out by the freezing point depression method using a Micro-Osmometer automatic type 13 RS, (Hermann Roebling Messtechnik, Berlin, Deutchland). At first, the appropriate CDs-based aqueous solutions (i.e., HP-β-CD, SBE-β-CD (18%, w/v) and HP-γ-CD (24%, w/v)) were prepared and in each of them propofol was added to ensure a 10 mg/ml concentration. Then, the CDs solutions were vortexed for 5 min, kept in an ultrasonic bath for 20 min and shaken (150 rpm) in a thermostatically controlled water bath

at 25 ± 0.5 °C until analyzed. To calibrate the instrument, an isotonic solution of NaCl and pure water were used to give 300 and 0 mOsm, respectively. The osmometer vials were filled by an automatic pipet taking up 100 μl of each solution of propofol. In this study, the resulting tonicity of the CDs-based aqueous solutions was not adjusted.

### 2.6. Sterilization and stability

Samples of the CD-based dosage forms and the prolinate ester were prepared as above reported. Aliquots (4 ml) were transferred into glass ampoules and sealed. The ampoules were then sterilized by heating at 121 °C for 15 min in an autoclave. A solution of the corresponding CD at the same concentration and distilled water was similarly treated as control. The stability of the formulation and control solutions before and after heating were examined by HPLC and pH measurements.

### 2.7. Pharmacological studies

Adult male Sprague–Dawley CD rats (Charles River, Como, Italy), with body mass of 200–250 g at the beginning of the experiments, were maintained under an artificial 12 h light–dark cycle (lights on from 08:00 to 20:00 h) at a constant temperature of 23 ± 2 °C and 65% humidity. Food and water were freely available, and the animals were acclimatized for >7 days before use. Experiments were performed between 08:00 and 14:00 h. Animal care and handling throughout the experimental procedure were performed in accordance to the European Communities Council Directive of 24 November 1986 (6/609/EEC). The experimental protocol was approved by the Animal Ethical Committee of the University of Cagliari.

Rats were injected intravenously (single bolus in the lateral tail vein) with the different formulations of propofol, each containing equimolar concentration of propofol (10 mg/(ml kg)). For i.v. administration, animals were restrained in an appropriate plexiglass cage and a tail vein was used. Following the drug administration, rats (five per treatment group) were observed for the following 60 min, and the onset and duration of loss of the righting reflex were recorded.

### 2.8. Statistical analysis

Solubility data are given as mean ± standard deviation. The statistical significance of differences in behavioral data were analyzed utilizing analysis of variance (ANOVA) followed by Scheffè's test (GraphPad Prism version 3 for Windows, GraphPad software, San Diego, CA). Differences were considered statistically significant at $P < 0.05$.

## 3. Results and discussion

In the developing safer parenteral dosage forms of **1**, an attractive possibility could be the use of cyclodextrins. Chemically modified cyclodextrins have been widely used to increase the solubility of poorly water-soluble drugs as well as to improve the stability of drugs (Loftsson and Brewster, 1966). Among them, HP-β-CD in particular has received attention due to its favorable physicochemical and biological properties. Recently, an injectable formulation employing a sulfoalkyl ether cyclodextrin such as Captisol® (SBE-β-CD) and forming a true aqueous solution has been patented (Mosher and Thompson, 2002). These cyclodextrins, in fact, have the distinct advantage of being more soluble and safer than β-CD due to their minimal toxicity profiles (Rajewski and Stella, 1996). However, all the cyclodextrin-based propofol formulations studied so far contained relative large amount of the complexing agent. In this context, Bielen et al. (1996) in a study on the cardiovascular profile of propofol dissolved in HP-β-CD 20% (w/v) in rats pointed out that propofol/HP-β-CD administration resulted in immediate bradycardia of variable duration, and these authors ruled out a direct dependence of these effects on HP-β-CD, since a solution of HP-β-CD 20% (w/v) produced no significant change in blood pressure. It may be suggested that an improved clinical efficacy and safety of the propofol/HP-β-CD system as well as of other CD-based formulations could occur by minimizing the amount of cyclodextrin needed to solubilize the anesthetic drug. Therefore, we have at first thoroughly evaluated the solubility profiles both in 0.05 M potassium phosphate buffer (pH 6.5) (Table 1) and in distilled water (data not shown) of the propofol/HP-β-CD and propofol/SBE-β-CD systems as well as that of

Table 1
Aqueous solubility of propofol (mg/ml) in 0.05 M potassium phosphate buffer (pH 6.5) at 25 °C in the presence of SBE-β-CD or HP-γ-CD

| SBE-β-CD (M)[a] | Propofol solubility (mg/ml) | HP-γ-CD (M)[a] | Propofol solubility (mg/ml) |
|---|---|---|---|
| 0 | 0.146 ± 0.02 | 0 | 0.146 ± 0.02 |
| 0.0071 (1.55%) | 1.16 ± 0.03 | 0.0095 (1.5%) | 0.97 ± 0.057 |
| 0.0143 (3.11%) | 1.98 ± 0.07 | 0.04745 (7.5%) | 3.72 ± 0.16 |
| 0.0286 (6.2%) | 4.20 ± 0.39 | 0.0949 (15%) | 7.64 ± 0.56 |
| 0.0573 (12.4%) | 8.10 ± 0.01 | 0.114 (18%) | 9.46 ± 0.42 |
| 0.0716 (15.5%) | 9.89 ± 0.007 | 0.152 (24%) | 12.69 ± 0.07 |
| 0.0832 (18%) | 15.32 | 0.19 (30%) | 16.60 ± 1.10 |

[a] Data are means ± standard deviation of three determinations. In parentheses, the corresponding percent w/v concentrations are reported.

propofol dissolved in the presence of HP-γ-CD. This last solution constitutes a new aqueous CD-based formulation of propofol. HP-γ-CD, indeed, is also useful to improve the aqueous solubility of many hydrophobic drugs and possesses low toxicity on i.v. administration (Waker Chemie, 1997).

From the phase-solubility studies, we deduced the concentrations of HP-β-CD, SBE-β-CD, and HP-γ-CD capable of dissolving propofol at a 10 mg/ml concentration in each case examined (Table 1). Thus, these phase-solubility diagrams showed that 18% (w/v) HP-β-CD or SBE-β-CD and 24% HP-γ-CD are respectively required to dissolve 10 mg of propofol in 1 ml of the vehicle. Furthermore, these concentrations are just sufficient to solubilize the drug and ensure that no precipitation can occur during storage. The HP-β-CD used in this study is characterized by a different degree of substitution from that previously investigated (Trapani et al., 1998); however, no significant changes in the corresponding phase-solubility diagram were observed. In all cases, a linear relationship was noted between propofol solubility and CD concentration, most likely due to the formation of a 1:1 inclusion complex. Because these profiles are characterized by slopes of less than 1 (i.e., 0.762 and 0.471 for SBE-β-CD and HP-γ-CD, respectively), the apparent stability constant values ($K_{1:1}$) (Table 1) could be estimated from the slope of the straight line of the phase-solubility diagrams according to the following equation: $K_c = \text{slope}/S_0(1 - \text{slope})$ (Higuchi and Connors, 1965) where $S_0$ is the solubility value of propofol in 0.05 M phosphate buffer (pH 6.5). The stability constant values with the β-CDs were higher than that observed with γ-CD (3439 and 3909 $M^{-1}$ for HP-β-CD and SBE-β-CD respectively versus 1085 $M^{-1}$ for HP-γ-CD) indicating that the stability of the complex depends on cavity size. The osmolarities of these solutions were determined and, as shown in Table 2, were slightly hypertonic; the rank order of osmolarity being: SBE-β-CD > HP-γ-CD > HP-β-CD.

Autoclaving the 10 mg/ml CD-based propofol formulations for 15 min at 121 °C caused a change in pH more evident in the case of the HP-β-CD (>1.7 pH units) containing solution, while in any case, no detectable fall in propofol concentration was observed by HPLC. The pH value of the 18% (w/v) SBE-β-CD solution and of the 24% (w/v) HP-γ-CD solution showed no significant change after autoclaving. The pH of the 18% (w/v) HP-β-CD solution changed from pH 3.90 to pH 5.60 after autoclaving. No significant change in pH or fall in propofol concentration was observed for CD-based formulations stored at room temperature for a week.

Another injectable propofol formulation described in literature is based on a mixture of propofol (10%, w/v), *N*-methyl pyrrolidone (30%, w/v), propylene glycol (40%, w/v), and water for injection q.s. to 100% (w/v). *N*-methyl pyrrolidone can be replaced

Table 2
Stability constants and osmolarity of CD-base formulations in 0.05 M potassium phosphate buffer (pH 6.5) at 25 °C

| CD | Apparent stability constant $K_{1:1}(M^{-1})$[a] | Osmolarity (mOsm/kg)[b] |
|---|---|---|
| SBE-β-CD | 3909 (8.8) | 639 ± 14 |
| HP-β-CD | 3439 (2)[c] | 443 ± 5 |
| HP-γ-CD | 1085 (9.6) | 569 ± 2 |

[a] Mean of three determinations, relative standard deviation (CV) values are reported in parentheses.
[b] Each value is the mean of two experiments ± standard deviation.
[c] From reference Trapani et al. (1998).

by 2-pyrrolidone (Komer, 1999). This is one of the very few examples utilizing co-solvents to provide an aqueous formulation of propofol. However, the *N*-methyl pyrrolidone toxicity profile is well-documented (Saillenfait et al., 2002). As a novel co-solvent based formulation, we evaluated the mixture propylene glycol:water (1:1, v/v) which is able to dissolve 10 mg/ml of the anesthetic agent. However, although simple to prepare, the stability of this formulation is limited. Laboratory experience with this mixture suggests that it should be freshly prepared when used, because droplets of propofol were detected after one day of storage at 4 °C. The osmolarity of the co-solvent mixture was not considered because it is well-known that the presence of organic co-solvents in the solution can disturb the freezing point of the aqueous solution (Ma et al., 1999). The third formulation herein presented is constituted by a propofol water-soluble derivative, the prolinate ester (Fig. 1, **2**) dissolved in water at equimolar concentration. We recently demonstrated that **2** is a derivative having the properties of a water-soluble prodrug of propofol (Altomare et al., 2003). The aqueous solution of the **2** was freshly prepared by dissolving 17 mg of prodrug in 1 ml of distilled water, the final pH of the solution being 3.20 pH units. Autoclaving a 17 mg/ml solution of **2** brought about no significant fall in prodrug concentration while the pH rose to 3.94.

The next aspect of these studies was to examine some pharmacodynamic properties of these CD-based formulations of propofol containing an amount of complexing agent just sufficient to solubilize the drug. We considered it to be of great interest to investigate whether there are any differences in terms of onset and duration of action in rat between the propofol-containing solutions herein presented. Moreover, two emulsion formulations were also included, that is, Diprivan® and propofol dissolved in saline with five drops of Tween 80 per 10 ml of emulsion, each containing equimolar concentrations of anesthetic agent (10 mg/(ml kg)). The obtained results are shown in Table 3. For all the formulations, except for that containing the prolinate ester, the onset time was immediate. In the case of the prodrug, the onset occurred after 22.75 ± 3.4 s. The delayed pharmacological effect observed for the prolinate ester was expected, as the prodrug approach is a well-known strategy for obtaining sustained release (Wermuth et al., 1996). The differences observed in the duration of loss of the righting reflex are also interesting. Data from this pharmacological paradigm, in fact, suggested that the two emulsion formulations produces a duration of loss of the righting reflex comparable with that of prolinate ester prodrug but longer than the CD-containing ones or the co-solvent mixture. Although the differences are not statistically significant, a clear trend in the duration of loss of the righting reflex was noted, that is, propofol/propylene glycol < propofol/SBE-β-CD < propofol/HP-γ-CD < propofol/HP-β-CD. It should be stressed that the efficacy of these new propofol formulations was assessed using a pharmacodynamic measure, and hence, pharmacokinetic data are necessary prior to definitive conclusions on their performance.

Table 3
Loss of the righting reflex (LORR) following intravenous administration of the examined propofol formulations to rats

| Formulation | LORR (second ± E.S.) | *N* of animals |
|---|---|---|
| Propofol/HP-β-CD | 454.8 ± 19.7 | 5 |
| Propofol/HP-γ-CD | 351.6 ± 47.8 | 5 |
| Propofol/SBE-β-CD | 342.4 ± 64.8 | 5 |
| Propofol/propylene glycol | 303.8 ± 72.3 | 5 |
| Prolinate ester | 527.7 ± 69.2 | 5 |
| Propofol/Tween 80 | 882.0 ± 130.4* | 4** |
| Diprivan® | 528.0 ± 79.3 | 5 |

* $P < 0.05$ vs. propofol/HP-β-CD, vs. propofol/HP-γ-CD, vs. propofol/SBE-β-CD, and vs. propylene glycol, but not vs. prolinate ester or Diprivan® (ANOVA followed by Scheffè's test).

** One rat died due to respiratory depression.

In conclusion, this study has demonstrated that a propofol 10 mg/ml aqueous solution in 24% (w/v) HP-γ-CD or in a propylene glycol:water (1:1) mixture or the prolinate ester (**2**) dissolved in water at equimolar concentration are new effective and simple-to-prepare anesthetic formulations. Except for the prolinate (**2**)-containing solution, the induction time and duration of action were comparable with that of Diprivan®. Furthermore, it has been found that minimizing the amount of cyclodextrin in all CD-based solutions, anesthetic effects similar to those of Diprivan® were still observed. Even though, we identified some subtle differences between the CD- and lipid-based formulations; overall, the pharmacodynamic properties of the

two propofol formulations appear to be very similar. This is consistent with the study on pigs by Egan et al. (2003) with a SBE-β-CD-based propofol formulation. On the other hand, an amount of 17 mg/ml aqueous solution of the prolinate (**2**) produced a longer induction time than a chemically equivalent dose of Diprivan®, although the duration of action was unchanged. In contrast to the formulation using a co-solvent mixture, the CD-based formulations were stable at 121 °C and on storage for at least a week. All these data may provide some impetus for the evaluation of these new solutions in clinical practice as efficient propofol delivery systems lacking the drawbacks associated with emulsion formulations.

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