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Development of Propofol-loaded Microemulsion Systems for Parenteral Delivery

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The aim of the present study was to develop the aqueous parenteral formulation containing propofol using o/w microemulsion systems. Propofol itself was chosen as the oil phase and its content was fixed to 1%, w/w. Pseudoternary phase diagrams were constructed to obtain the concentration range of surfactant and cosurfacatnt and the optimum ratio between them for microemulsion formation. Consequently, the suitability of the chosen microemulsion system as a parenteral formulation was evaluated from the stability and hemolysis tests on that. Among the surfactants and cosurfactants screened, the mixture of Solutol HS 15-ethyl alcohol (5/1) showed the largest o/w mocroemulsion region in the phase diagram. When 1% (w/w) of propofol was solubilized with 8% (w/w) of Solutol HS® 15-ethyl alcohol (5/1), the average droplet size (150 nm) and the content of propofol in the systems were not significantly changed at 40°C for 8 weeks. The hemolysis test showed that this formulation was nontoxic to red blood cells. In conclusion, propofol was successfully solubilized with the o/w microemulsion systems.

Key words: Propofol, Microemulsion, Solubility, Parenteral delivery, Hemolysis, Anesthetics

INTRODUCTION

Propofol (2,6-diisopropylphenol), an intravenous sedative hypnotic agent, causes rapid and reliable loss of consciousness by acting at $GABA_A$ receptor (Concas *et al.*, 1990). It has additional desirable properties such as rapid and smooth recovery, even after a prolonged infusion, and the absence of emetic sequelae (Dollery, 1999). Propofol is a colorless liquid at room temperature (m.p. 19°C) and practically insoluble in water. Solubilities of propofol were reported as 146 ± 8.9 μg/mL, 154 ± 10 μg/mL in water and 0.05 M phosphate buffer of pH 7.4, respectively (Trapani *et al.*, 1996). The commercial intravenous preparation of propofol, therefore, is formulated as a lipid emulsion. This formulation, however, has shown some drawbacks such as the risk of hyperlipidemia in patients under long-term use, the risk of bacterial contamination after exposure to air, pain on injection, and the phase separation by concomitant use of local anesthetics, e.g. lignocaine (Tan *et al.*, 1998; Lilley *et al.*, 1996; Masaki *et al.*, 2000; Park *et al.*, 2003). Developing more stable aqueous parenteral formulation is desirable to overcome those drawbacks, and microemulsion systems can be a choice for those purposes.

Microemulsions offer several potential advantages as drug delivery systems arising from their solubilization capacity, transparencies, high stability, and simplicity of manufacture (Attwood, 1994; Tenjarla, 1999). Optimization of solubility of poorly water-soluble drugs in pharmaceutical dosage forms presents a challenge, due to the severe restrictions on the choice of solvents suitable for parenteral use. The finely dispersed oil droplets of oil-in-water microemulsions offer a potential solvent system for such drugs, although the toxicity of the microemulsion components still imposes limitations on their utilization. Moreover, the transparency of microemulsions enables them to be visually assessed for microorganism growth and also allows inspection for the presence of undissolved drug.

The aim of the present study was to develop the aqueous parenteral delivery system for poorly water-soluble propofol (1% w/w) using o/w microemulsions. The effect of formulation variables on the particle size of microemulsions was also investigated.

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MATERIALS AND METHODS

Materials

Propofol and egg york lecithin were obtained from Cilag AG (Switzerland) and Doo San Biotech (Korea), respectively, and the following reagents were used as received without further purification: PEG-40 hydrogenated castor oil (HCO-40), PEG-50 hydrogenated castor oil (HCO-50), PEG-60 hydrogenated castor oil (HCO-60) (Nikkon, Japan), HPLC grade acetonitrile, methanol, acetic acid, absolute ethanol (J.T. Baker Chemical Co., U.S.A.), sorbitan monolaurate (Span 20), sorbital monooleate (Span 80), polysorbate 20 (Tween 20), polysorbate 80 (Tween 80) (ICI Americas, U.S.A.), glycerin, polyethylene 400 (PEG 400), propylene glycol (PG) (Shinyo Pure Chemical Co., Japan), benzyl alcohol, glycofurol, sorbitol (Sigma chemical Co., U.S.A.), polyoxy 35 castor oil (Cremophor EL), polyoxy 60 hydrogenated castor oil (Cremophor RH 60), poloxamer 199, and polyethylene glycol-12-hydroxystearate (Solutol HS 15) (BASF Wyandotte Co., Germany). Purified deionized water was prepared by the Milli-Q Plus system (Millipore, Milford, U.S.A.). All other chemicals and solvents were of analytical grade.

Phase studies

To formulate parenteral aqueous systems containing 1% (w/w) propofol, various surfactants (S) and cosurfactants (CoS) were screened as a function of o/w microemulsion forming capacity. For each surfactant, pseudoternary phase diagram was constructed for S-CoS (ethanol)/propofol/water system in which the S/CoS ratio and the content of the propofol were fixed at 1/1 and 1% w/w, respectively. The boundaries of the oil-in-water microemulsion domains were determined by slowly titrating mixtures of surfactant and cosurfactant containing propofol with water at ambient temperature and each sample was assessed visually for clarity. The existence area of the o/w microemulsion in the pseudoternary phase diagrams was measured by Image Pro-Express software (Ver. 4.5, Media Cybernetics, Inc., U.S.A.).

The influence of cosurfactants on the extent of the microemulsion domain has been also determined. After surfactant decided, for each cosurfactant, same phase studies were conducted. The surfactants and cosurfactants screened are listed in Table I.

In addition, the influence of the S/CoS ratio on the area of existence of microemulsion phase diagrams were investigated. The S/CoS ratio was varied as 1/1, 3/1, 5/1, and 10/1 and phase studies were conducted.

Table I. List of surfactants and cosurfactants screened

Surfactant	Cosurfactant
Solutol HS® 15	Benzyl alcohol
Cremophor EL	Ethanol
Cremophor RH 60	Glycerin
Tween 80	Glycofurol
Tween 20	Polyethylene glycol 400
Lecithin	Propylene glycol
Myri 52	Sorbitol
Sod. deoxylate	Transcutol
HCO-60	
HCO-50	
HCO-40	

Stability test

The o/w microemulsion systems containing 1% (w/w) of propofol were prepared using the selected surfactant and cosurfactant for the stability study and the content of the mixture of S/CoS were varied from 4 to 8%. Vials containing 2 mL of the microemulsion were stored in a stability chamber (Fine Scientific Instrument, FLT-600D, Korea) at 40°C for 8 weeks. At 1, 2, 4, 6, and 8 weeks, samples were withdrawn and the physicochemical changes in the microemulsion system were observed. The droplet sizes were determined by light scattering techniques and the concentrations of propofol in the systems were measured by a HPLC method.

Determination of droplet size

The size of oil droplets in microemulsion was determined using a dynamic light scattering method employing He-Ne laser (Lexer Laser Inc., Model 127, U.S.A.).

HPLC Analysis

The concentration of propofol was determined with a slight modification of the HPLC method reported previously (Yeganeh *et al.*, 1997). The HPLC system consists of a controller (Waters 600, Milford, U.S.A.), an isocratic pump (Waters 600), a data module (Waters 746) and an UV/Vis detector (Jasco UV-976, Tokyo, Japan). A Cosmosil C_{18} column (4.6×150 mm, 5 mm particle size, Nacalai Tesque, Japan) was used. The mobile phase consisting of acetonitrile : deionized water (7:3, v/v, pH 4.0) was pumped at a flow rate of 1.0 mL/min. The UV/Vis detector was set at 276 nm and the injection volume was 50 μL. All operations were carried out at room temperature.

Hemolysis test

The hemolytic potential of the microemulsion systems developed was investigated using an *in vitro* method (Reed and Yalkowsky, 1984). In brief, 0.1 mL of the o/w microemulsion system was mixed with 1 mL of 4% red blood cell dispersions in normal saline. The remaining intact red blood cells were washed with normal saline, and then lysed with deionized water. The hemoglobin concentration was determined by the difference of absorbance at 540 nm (Shimadzu, UV mini 1240, Japan) between the control, 0.1 mL of normal saline, and the microemulsion systems (Fu *et al.*, 1987).

Statistics

All experiments were repeated three times and their mean values with standard error were presented. Student's *t*-test was performed to see any significant difference between the test formulation and the reference product.

RESULTS AND DISCUSSION

Screening of surfactant and cosurfactant for O/W microemulsions

The construction of phase diagram makes it easy to find out the concentration range of components for the existence range for microemulsions. To find out appropriate surfactant to form o/w microemulsions containing 1% propofol, phase diagrams were constructed (Fig. 1). Ethanol was used as a cosurfactant, propofol as an oil phase, and the S/CoS ratio was fixed to 1/1 (w/w). Monophasic, isotropic areas were seen to occur along the water-S/CoS axis in the o/w microemulsion systems. The existence area of the o/w microemulsion in the phase diagrams was measured and the results are tabulated in Table II. The o/w microemulsion existence area was largest with Solutol HS 15 followed by Cremophor RH 60, HCO 60, and Cremophor EL. Tween 80 resulted in a very small o/w microemulsion existence area. Solutol HS 15 was subsequently used as the surfactant for the screening of cosurfactant. The influence of cosurfactants on the extent of the microemulsion domain has been also investigated. For each cosurfactant, a phase diagram was constructed and the o/w microemulsion existence area was measured (Table III). Solutol HS 15 was used as a surfactant, and S/CoS was fixed to 1/1 (w/w). Ethanol resulted in the largest microemulsion existence area compared to other cosurfactants. Based on the phase studies, Solutol HS 15 and ethanol were chosen as a surfactant and a cosurfactant, respectively, for developing the o/w microemulsions containing propofol.

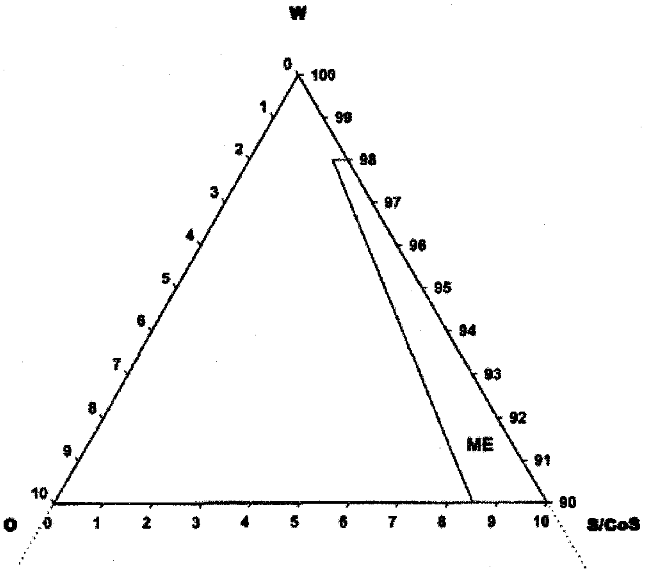

Fig. 1. Representative phase diagram of the systems containing water/Solutol HS 15(S)/ethanol(CoS)/propofol with the S/CoS ratio of 1/1. ME represents o/w microemulsion existence area.

Optimization of S/CoS

The extension of the microemulsion region was strongly dependent upon the nature of the surfactant and cosurfactant and the S/CoS ratio (Trotta *et al.*, 1996). To find out the effect of the S/CoS ratio on the area of existence

Table II. Effect of surfactant (S) on the o/w microemulsion existence area in the pseudoternary phase diagram. Ethanol was used as a cosurfactant (CoS). The ratio of S/CoS and the content of the propofol were fixed at 1/1 and 1% w/w, respectively.

Surfactant	Area (cm^2)*	Portion of o/w microemulsion area in the phase diagram (%)
Solutol HS 15	12.89	25.83
Cremophor EL	11.56	23.16
Cremophor RH 60	11.67	23.38
Tween 80	4.34	8.50
Tween 20	8.51	17.06
Lecithin	6.48	13.01
Myri 52	7.76	15.56
Sod. deoxylate	8.59	17.21
HCO-60	11.63	23.29
HCO-50	9.44	18.92
HCO-40	9.38	18.80

*The area was measured by Image Pro-Express software (Ver. 4.5, Media Cybernetics, Inc., U.S.A.).

Table III. Effect of cosurfactant (CoS) on the microemulsion existence area in the pseudoternary phase diagram. Solutol HS 15 was used as a surfactant (S). The ratio of S/CoS and the content of the propofol were fixed at 1/1 and 1% w/w, respectively.

Cosurfactant	Area (cm^2)*	Portion of o/w microemulsion area in the phase diagram (%)
Ethanol	10.89	21.82
Benzyl alcohol	1.61	3.22
Glycofural	9.27	18.58
Transcutol	4.55	9.12

*The area was measured by Image Pro-Express software (Ver. 4.5, Media Cybernetics, Inc., U.S.A.).

Table IV. Effects of the ratio of surfactant to cosurfactant (S/CoS) on the microemulsion existence area in the phase diagram. Solutol HS 15 and ethanol were used as a surfactant and a cosurfactant, respectively.

S/CoS	Area (cm^2)*	Portion of o/w microemulsion area in the phase diagram (%)
1/1	7.09	14.20
3/1	9.57	19.18
5/1	12.11	24.25
10/1	9.60	19.23

*The area was measured by Image Pro-Express software (Ver. 4.5, Media Cybernetics, Inc., U.S.A.).

of o/w microemulsion, S/CoS was varied as 1/1, 3/1, 5/1, and 10/1 for Solutol HS 15 (S)/ethanol (CoS)/propofol/ water systems and the o/w microemulsion existence area was measured (Table IV). As the S/CoS ratio was increased, the existence area of o/w microemulsion became enlarged, reaching a maximum at S/CoS of 5/1.

Stability study

In microemulsion systems, physical instability is indicated by droplet size change, flocculation, creaming, coalescence, and oil separation. Chemical instability is indicated by oxidation and hydrolysis of the oil or emulsifier, change in emulsion pH, and increase in free fatty acid component or acidity of the oil. Additionally, the chemical stability of any incorporated drugs must be monitored (Floyd, 1999). In this study, physical and chemical stability tests were carried out for formulations containing different amounts (4%, 6%, and 8%) of the mixture of surfactant and cosurfacatnt (S/CoS = 5/1) at 40°C for 8 weeks. All formulations showed no significant changes in propofol concentrations at 40°C for 8 weeks. The initial droplet sizes in all prepared systems were smaller than 150 nm and did not change when 1%(w/w) of propofol was solubilized with 8% (w/w) of Solutol HS 15/ethyl alcohol. However, the droplet size increased more than 200 nm in 8 weeks when the content of S/CoS mixture was less than 6% (Fig. 2).

Hemolysis test

There is a risk of lysis of blood cells with the subsequent release of hemoglobin in the plasma when surfactants and cosolvents are used in parenteral preparations. The increase of free hemoglobin in plasma is associated with undesirable medical conditions, including renal dysfunction, jaundice and kernicterus. Therefore, the potential of the microemulsions to induce intravascular hemolysis is needed to be evaluated (Kryzaniak *et al.*, 1997). All the formulations examined in this study did not cause hemolysis and appeared to be nontoxic to red blood cells.

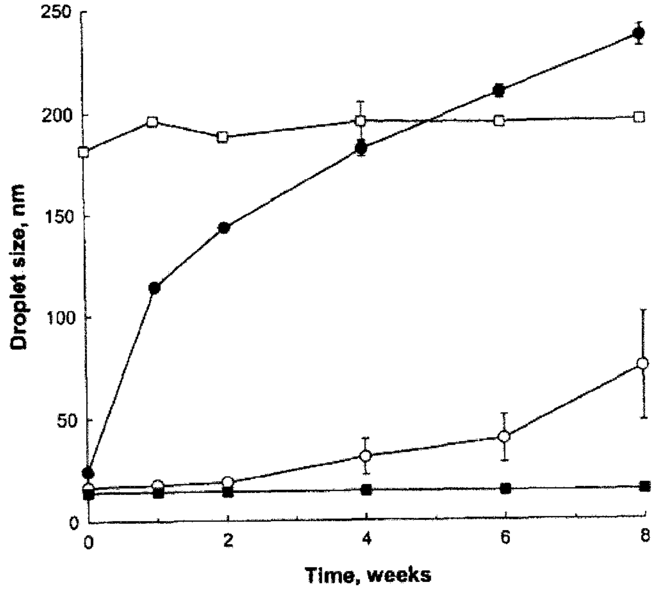

Fig. 2. The effect of the content (%) of S/CoS mixture on droplet size of the microemulsion at 40°C (Mean ± S.E., n=3). Key: ●; S/CoS = 4%, ○; S/CoS = 6%. ■; S/CoS=8%, □; Control (Diprivan®)

CONCLUSION

Propofol was successfully solubilized with the o/w microemulsion systems. Among the surfactants and cosolvents screened, the mixture of Solutol HS 15-ethyl alcohol (5/1), propofol and water system showed the largest o/w mocroemulsion region. When 1% (w/w) of propofol was solubilized with 8% (w/w) of Solutol HS 15/ ethanol (5/1), the droplet size and the amount of propofol in the systems did not significantly change at 40°C for 8 weeks. *In vitro* hemolysis test showed that this formulation was nontoxic to blood cells.

REFERENCES

Concas, A., Santoro, G., and Mascia, M. P., The general anaesthetic propofol enhances the function of gamma-amino-butyric acid-coupled chloride channel in the rat cerebral cortex. *J. Neurochem.*, 55, 2135-2138 (1990).

Dollery, C., Propofol In: Therapeutic Drugs, 2nd Ed., Churchill Livingstone, Edinburgh, P251-P256 (1999).

Floyd, A. G., Top ten considerations in the development of parenteral emulsions. *Pharm. Sci. Technol. Today*, 2, 134-143 (1999).

Fu, R. C., Lidgate, D. M., Whatley, J. L., and Mccullough, T., The biocompatibility of parenteral vehicles-*in vitro/in vivo* screening comparison and the effect of excipients on hemolysis. *J. Parenter. Sci. Technol.*, 41, 164-168 (1987).

Kryzaniak, J. F., Raymond, D. M., and Yalkowsky, S. H., Lysis of human red blood cells 2: effect of contact time on cosolvent induced hemolysis. *Int. J. Pharm.*, 152, 193-200 (1997).

Lilley, E. M. M., Isert, P. R., Carasso, M. L., and Kennedy, R. A., The effect of the addition of lignocaine on propofol emulsion stability. *Anaesthesia*, 51, 815-818 (1996).

Masaki, T., Tanaka, M., and Nishikawa, T., Changes in propofol concentration in a propofol-lidocaine 9:1 volume mixture. *Anesth. Analg.*, 90, 989-992 (2000).

Park, J. W., Park, E. S., Chi, S. C., Kil, H. Y., and Lee, K. H., The effect of lidocaine on the globule size distribution of propofol emulsions. *Anesth. Analg.*, 97, 769-771 (2003).

Reed, K. W. and Yalkowsky, S. H., Lysis of human red blood cells in the presence of various cosolvents. *J. Parenter. Sci. Technol.*, 39, 64-69 (1984).

Tan, C. H. and Onsiong, M. K., Pain on injection of propofol. *Anaesthesia*, 53, 468-476 (1998).

Tenjala, S., Microemulsions: An overview and pharmaceutical applications. *Crit. Rev. Ther. Drug Carrier Systems*, 16, 461-521 (1999).

Trapani, G., Lopedota A., Franco M., Latrofa A., and Liso G., Effect of 2-hydroxypropyl-β-cyclodextrin on the aqueous solubility of the anaesthetic agent propofol (2,6-diisopropylphenol*). Int. J. Pharm.*, 139, 215-218 (1996).

Trotta, M., Cavalli, R., Ugazio, E., and Gasco, M. R., Phase behaviour of microemulsion systems containing lecithin and lysolecithin as surfactant. *Int. J. Pharm.*, 143, 67-73 (1996).

Yeganeh, M. H. and Ramzan, I., Determination of propofol in rats whole blood and plasma by high performance liquid chromatohraph. *J. Chromatogr. B*, 691, 478-482 (1997).