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Preparation and Characterization of a Propofol-loaded Polymeric Micellar System: Nanoparticular Stability

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ABSTRACT – A propofol delivery system was prepared using two biocompatible polymeric surfactants, poloxamer 407 and PEG 400. The nanoparticular stability of the micellar system was evaluated in terms of temperature change, storage time and composition. The particle size of the system was slightly increased with elevating temperature from 4°C to 25°C, but its distribution was unimodal. At 40°C, the system presented a bimodal particle size distribution and the increase in the fraction of particles larger than 15 nm. This result might be due to the expansion of the nanoparticles through micellar swelling at the high temperature. It was found that propofol was gradually come out of the system, stored for a month at three different temperatures (4, 25 and 40°C). The drug loss was apparently dependent on temperature and the system composition. Increasing temperature induced the acceleration of the drug loss of 7~10% at 4°C and 14~16 % at 40°C. This may be owing to the high diffusivity resulting from the swelling of the hydrophilic surface of the nanoparticle at high temperature. However, the addition of PEG 400 to the system led to the reduction of the drug loss. This result is associated with the previous investigation that PEG coverage decreased diffusion coefficient because of the formation of the denser structure on the surface of nanoparticulate. Nevertheless, the limited amount of PEG, less than 2% (w/v), should be used to prevent the precipitation and discoloration of the system.

Key words – Propofol, Poloxamer, Nanoparticular Stability, Drug Delivery System, Micelle

2,6-diisopropylphenol (propofol) has been extensively used as an anesthetic agent. Propofol is practically insoluble in water due to its higher hydrophobic characteristics (log octanol-water partition coefficient log K_{ow}=4.33).[1] Propofol delivery systems have been developed by solubilizing the drug with surfactants and lipids. The commercialized propofol product was prepared with a polyoxyethylene caster oil, (Cremophore EL), which can be intravenously administrated unlike other gaseous anesthetics. The Cremphore EL is considered as an unacceptable material because of the occurrence of anaphylactoid reaction to some patients.[2] Another propofol system was developed using the composition of several lipids. The system was composed of propofol of 1% (w/v), soybean oil of 10% (v/v) and egg phophatide of 1.25% (w/v).[2,3] This system has been commercialized as a brand name of diprivan®. The use of lipids may cause the acceleration of microorganism growth[4] and hyperlipidemia.[5] The lipid-induced growth of microorganisms was solved by the use of the small quantity of a preservative ethylene diamine tetraacetic acid (EDTA).[6] Nevertheless, it would be difficult to state that diprivan® is free from risks of hyperlipidemia and low physical stability. It is, therefore, needed to develop an advanced propofol system to overcome the possible disadvantages exerted by diprivan®.

In this study, a new propofol delivery system prepared using two polymeric surfactants was investigated with respect to its physicochemical properties and stability. Its stability was evaluated by monitoring various properties such as pH, particle size, turbidity and drug loading efficiency in terms of temperature change, storage time and system composition. The results could provide the important information in developing a more acceptable propofol delivery system.

Materials and Methods

Materials

2,6-diisoproylphenol (purity >97%: propofol), ethyl alcohol, acetonitrile, methyl alcohol and sodium chloride were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). A triblock copolymer, poloxamer 407 (polyethylene oxide-polypropylene oxide-polyethylene oxide), was purchased from BASF Co. Ltd. (Ludwigshafen, Germany). The molecular weight of the copolymer was between 9,840 and 14,600 g/mol. Polyethylene glycol 400 (PEG 400: M.W.: 400 g/mol) was provided from Duksan Chemicals Co. Ltd (Ansan,

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Table I–*Chemical Composition of Individual Nanoparticular System*

Sample	Propofol	Poloxamer 407	PEG 400	Ethanol	Sodium chloride
P07N	1	4	-	-	0.71
20P05N	1	4	2	-	0.56
P20E	1	4	-	2	-
5P15E	1	4	0.5	1.5	-
10P10E	1	4	1	1	-

Unit: w/v %

Korea). All chemicals were reagent grade and used without any further treatment.

Preparation of propofol-based system

Poloxamer 407 was dissolved in deionzed water at room temperature and mechanically mixed using a magnetic stirrer (Corning Co. Ltd., MA, USA) with a speed of 150 rpm for 1 hour. Propofol was added to the poloxamer 407 aqueous solution and followed the strong agitation (500 rpm) for 3 hours. PEG 400, ethanol and sodium chloride were added to the propofol/poloxamer 407 aqueous solution. The composition of each sample is shown in Table I. The volume of the final product was 100 mL. The filtration was performed using a membrane (Pore size: 0.22 μm: Millipore, MA, USA) in order to remove microorganisms and impurities.

Evaluating physicochemical properties of propofol-based system

Particle size, pH and turbidity measurements were performed in order to evaluate the stability of individual propofol system. Its mean particle size and particle size distribution was characterized using a zeta-potential/particle size analyzer ELS-Z2 (Photal Otsuka Electronics Co. Ltd., Osaka, Japan). The pH and turbidity value of each propofol system were measured with a pH meter (Orion 720A+ pH/ISE meter, Thermo Electron Co. Ltd, MA, USA) and a turbidity meter (Hanna Instruments Ltd., Seoul, Korea), respectively. All physicochemical properties had been investigated for a month at the range of temperature (4~40°C) in terms of the system composition.

Determination of drug loading efficiency

High performance liquid chromatography analysis (HPLC) was employed to characterize the drug loading efficiency in the nanoparticles. For HPLC analysis, a mobile phase was composed of 70% (v/v) acetonitrile, 20% (v/v) methyl alcohol and 10% (v/v) deionized water. A flow rate of 0.7 mL/min and an injection volume of 10 μL were applied during an isocratic time of 30 minutes. A wavenumber of 270 nm was used to characterize the propofol concentration.

Prior to estimating the drug loading efficiency, a calibration curve was plotted as a function of a propofol concentration ($C_{Pol.}$) using the following equation:

$$A = 5.7 \times 10^6 C_{pol.} + 6 \times 10^4 \quad (R^2 = 0.999) \tag{1}$$

where A is the peak area of a retention time of 5 minutes. In order to estimate the drug loading efficiency, the propofol-loaded nanoparticle aqueous solution of 2 mL was centrifuged with a rapid of 13,000 rpm (HANIL Science Industrial Co., Ltd., Incheon, Korea) for 15 minutes. The supernatant was characterized by HPLC analysis at 25°C. The drug loading efficiency (E_{DL}) was calculated as follows:

$$E_{DL} = \frac{D_i(mg) - D_s(mg)}{D_i(mg)} \times 100(\%) \tag{2}$$

where D_i is the initial weight of propofol added to the propofol formulation and D_s is the drug weight in the supernatant.

Results and Discussion

Preparation of propofol basic system

The nanoparticular system was formulated when propofol was completely dissolved in the poloxamer 407 aqueous solution. The poloxamer is a three block copolymer composed of 30% propylene oxide (PPO) and 70% ethylene oxide (PEO).[7] The block copolymer forms micelles above a critical micelle concentration (CMC). It is known that the micelle is composed of a PPO core and a PEO shell.[7] The hydrophobic drug propofol could be dissolved in the PPO core of the nanoparticular system. The final products were transferred in a sterilized glass vial, but lots of small bubbles were found on the top of the bottle. The bubbles were removed by inserting nitrogen gas in the bottom. Precipitation and discoloration were investigated for 20P05E during the preparation process, indicating low physical stability. Thus, the stability of 20P05E was not investigated in this study. Figure 1(a) shows the photo of 5P05E as an example of all samples except for 20P05E. The system was

Figure 1–Photographs of (a) a new propofol formulation, 5P15E and (b) a commercialized product, DIPRIVAN®. P07N, P20E and 10P10E were clear and light blue like 5P15E. (Photographs not shown.)

transparent and light blue, but a commercial product diprivan® was opaque (Figure 1(b)).

Effect of composition on the propofol basic system

Each propofol system was evaluated in terms of mean particle size, drug loading efficiency, pH, and turbidity at 25°C. The results were shown in Table II. The mean particle size of each sample was mainly dependent on its system composition. The addition of ethanol decreased it particle size; for example, the mean particle size was 11.7±2.9 for P07N and 9.0±2.6 for P20E (Table II). By HPLC analysis, the propofol of over 97.5% was initially loaded into the nanoparticle regardless of the composition of each system. The pH value of each system was between 6.3 and 6.8. It slightly increased in the presence of various chemicals such as PEG 400 and ethanol. The turbidity measurements confirmed the result of the particle size analysis. The particle size was directly proportional to turbidity. The large particles scattered light more so that it increased turbidity value.

Effect of temperature on the propofol basic system

Physicochemical properties of a nanoparticular system were investigated as a function of temperature (4~40°C) to monitor its stability. Figure 2 shows the temperature-dependence of the particle size for 5P15E. As shown in the figure, the distribution was unimodal at 4 and 25°C, but bimodal at 40°C. In addition, all particles were less than 50 nm regardless of temperature and chemical composition. Table III shows the mean particle size. The size was increased at high temperature. Increasing temperature may decrease the number of the intramoleuclar hydrogen bonds.[8,9] Thus, the polyethylene oxide moieties on the surface of the nanoparticular system may be unfolded freely[8] and swell in solution, resulting in the increase of the particle size.[10] However, the effect of temperature was insignificant to physicochemical properties such as pH, turbidity and drug loading efficiency (Table III).

Effect of storage time on the propofol basic system

All nanoparticle systems were stored for a month at various temperatures (4~40°C). The nanoparticular stability was evaluated by investigating the change in several parameters such as particle size, pH, turbidity, and drug loading efficiency. Table IV shows that the effect of the storage time was negligible on the physicochemical properties of the nanoparticles measured at 40°C. Figure 3 shows the change in the drug loading efficiency after the storage for a month at a variety of temperatures (4~40°C). The mean drug loading efficiency of each system was initially over 97.5%. However, it was significantly decreased when the particle was stored for a month in a sealed-glass vial. The lower drug loading efficiency was presented at higher temperatures. The reduction of drug loading efficiency may be related to the occurrence of propofol release from the nanoparticles during the storage time.

Table II–*Physicochemical Properties of Each Nanoparticular System at 25°C*

Sample	Particle size (nm)	Drug loading efficiency (%)	pH	Turbidity (FTU)
P07N	11.7±2.9	98.3±2.1	6.38±0.03	17.9±0.9
P20E	9.0±2.6	97.5±1.3	6.84±0.02	13.4±0.3
5P15E	10.7±2.1	99.8±0.3	6.82±0.01	13.7±0.3
10P10E	11.0±1.1	98.4±1.6	6.78±0.02	15.5±1.1

Table III–*Effect of Temperature on Physicochemical Properties for 5P15E*

Sample	Particle size (nm)	Drug loading efficiency (%)	pH	Turbidity (FTU)
4°C	9.3±0.6	97.9±1.8	-	13.6±0.5
25°C	10.7±2.1	97.5±1.3	6.82±0.01	13.7±0.3
40°C	5.0±1.1 19.9±0.8	96.7±1.9	-	13.4±0.3

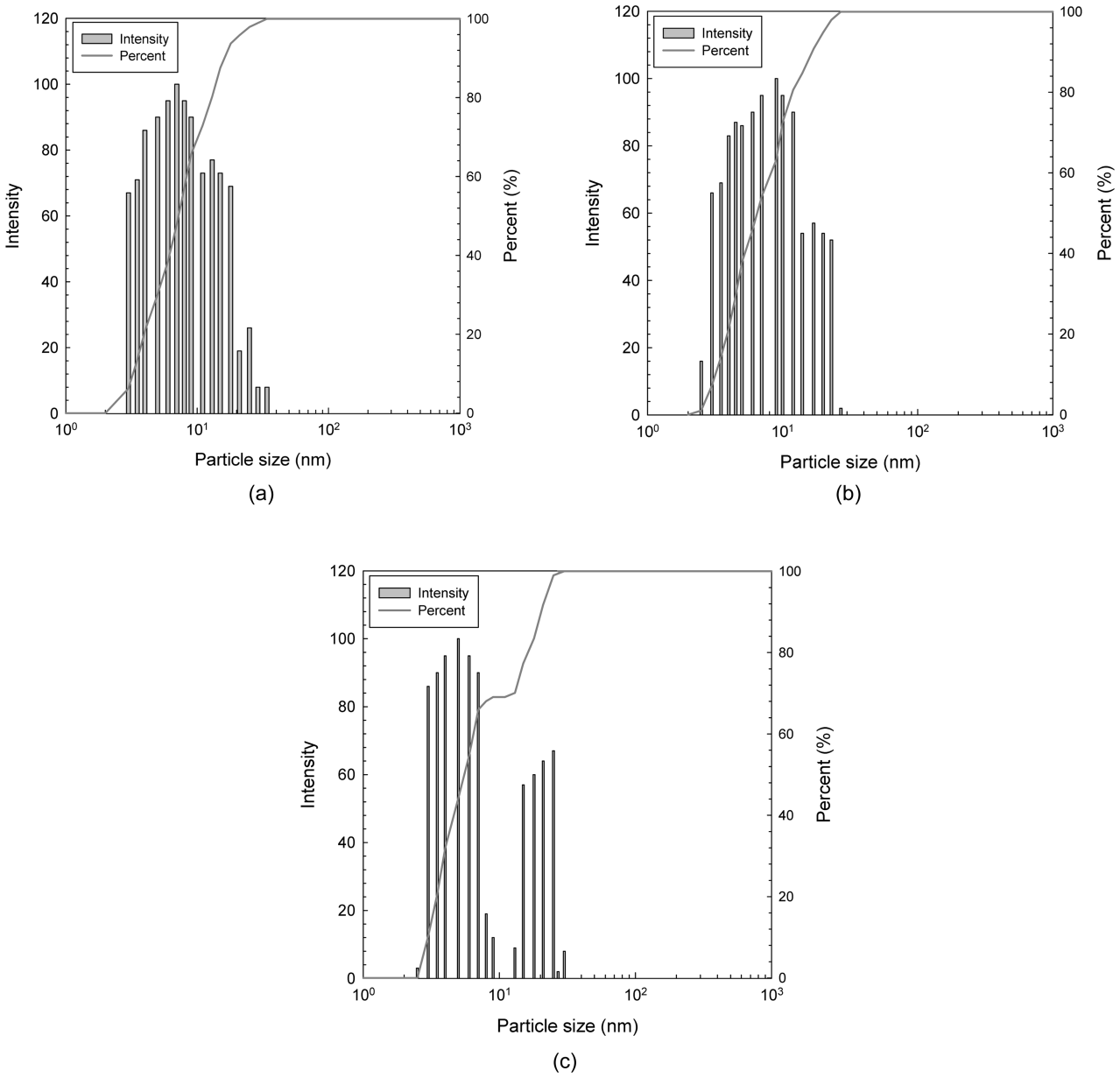

Figure 2–Temperature dependence of the particle size for 5P15E. (a) 4°C, (b) 25°C and (c) 40°C.

Koizumi and Panomsuk (KP)[11,12] proposed a drug release from polymer-based delivery systems by modifying Higuchi model[13,14] and KP model is described as follows:

$$R_D(t) = 4\pi r^2\left[\sqrt{2(C_0-C_s)C_sDt} + \frac{4C_sDt}{9r}\cdot\left(\frac{C_s}{2C_0-C_s}-3\right)\right] \quad (3)$$

where $R_D(t)$ is a cumulative amount of drug released a certain time, t. The r represents the radius of spherical delivery system. The C_0 and C_S are the initial drug concentration and the solubility of the drug in the systems, respectively. The D is the diffusion coefficient of the drug and can be described by the Stokes-Einstein equation:[15,16]

$$D = \frac{kT}{6\pi\eta r_s} \quad (4)$$

where k is the Boltzmann constant, 1.3805×10^{-23} J/K, T represents the absolute temperature; η is the viscosity of the solvent and r_s is the radius of the drug. The viscosity of water is temperature-dependent and it can be described as follows:[17]

$$\eta = a10^{\frac{b}{T-c}} \quad (5)$$

where the constants a, b and c are 2.414×10^{-5} Pa·s, 247.8 K

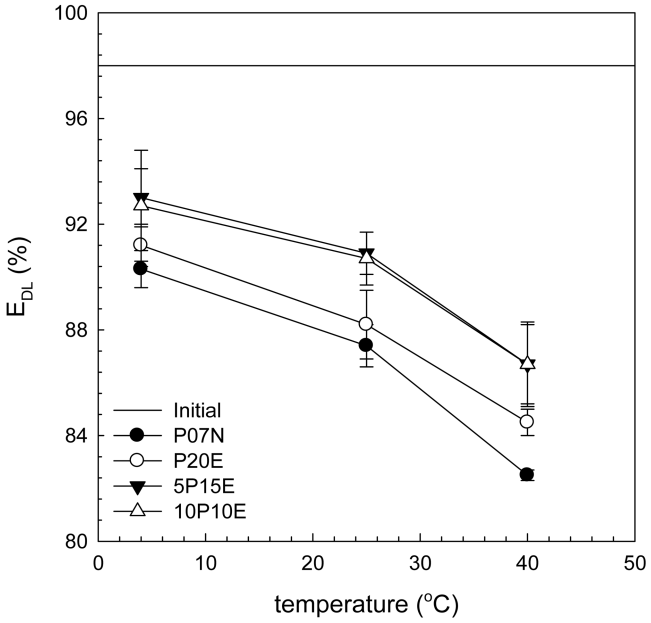

Figure 3–The change in drug loading efficiency (E_{DL}) of each composition after storing one month under various temperatures.

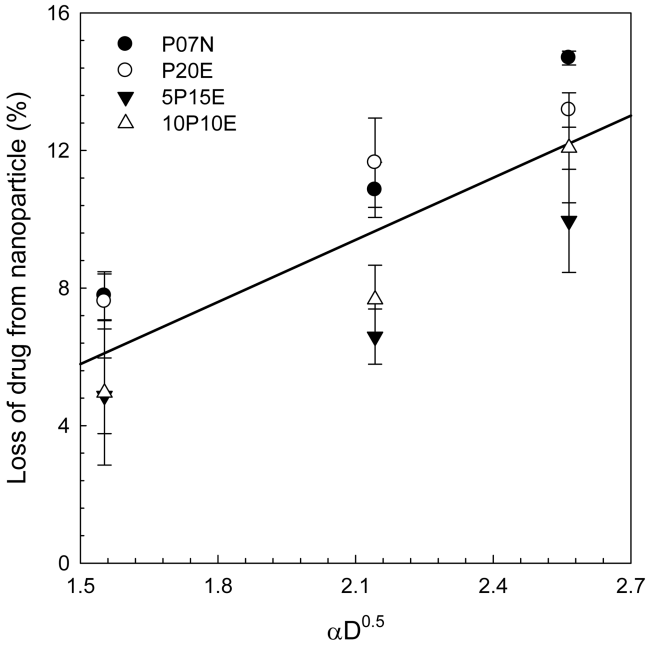

Figure 4–The relationship between drug loss and diffusion coefficient.

and 140 K, respectively. As shown in Table V, the viscosity of the water was getting lower at high temperature. In addition, the radius of propofol was calculated from its molecular volume, 0.641 nm^3,[18] and it was 5.35 Å. Finally, diffusion coefficient (D) was determined at each temperature (Table V). The D value was increased at high temperature, indicating the acceleration of drug release.[19] The percentage of drug loss for

Table IV–*Effect of Storage Time on the Physicochemical Properties of 5P15E*

Sample	Initial	1 Month
Particle size (nm)	10.3	10.0
Turbidity (FTU)	13.6	13.5
pH	6.8	6.8

Table V–*The Estimated Solvent Viscosity (η) and Diffusion Coefficient (D) at Each Temperature*

T (°C)	η (mPa·s)	D ($\times 10^{-10}$ m^2/s)
4	1.58	2.41
25	0.89	4.59
40	0.65	6.58

a month (100 (%)−E_{DL} (%)) at each temperature was linearly proportional to the magnitude of diffusion coefficient ($\alpha D^{0.5}$) as shown in Figure 4. Increasing temperature induced the strengthened molecular mobility of the polymer composed of the surface of the nanoparticles.[8,20] Enhanced mobility leads to the rearrangement of the segments of the macromolecules to allow drug to diffuse, resulting in the higher diffusion rate.[19] In addition, the portion of the particle size larger than 15 nm was found at 40°C (15% at 4 and 25°C, but 25% at 40°C), indicating the increase of the free volume to diffuse.[21,22] This expanded free volume accelerated the diffusion of the drug from the nanoparticles. In Figure 3, the loss of drug was smaller (or lower D) when PEG 400 was used to formulate the nanoparticles. Liu et al. reported the reduction of the diffusion coefficient in the presence of polyethylene glycol (PEG).[23] The denser hydrophilic PEG on the surface of the nanoparticles may hinder the transport of the small molecules from polymer to solvent, resulting in the reduction of the diffusion coefficient. Even though the addition of PEG to the poloxamer 407-based nanoparticles retards the drug release during storing the samples (or increase stability of the nanoparitles), but it should be not be overused due to the occurrence of the precipitation and discoloration (low stability).

Conclusions

A nanoparticular system to deliver propofol was designed in this study. The stability of the nanopartilce was dependent on temperature and its composition. Increasing temperature induced the acceleration of drug loss during storing the samples due to high diffusivity. When dense hydrophilic PEG covered on the surface of the nanoparticle, the nanoparticular stability was strengthened, resulting in reducing propofol release. Nevertheless, the use of the limited PEG concentration

is recommended in order to prevent the precipitation and discoloration of the system. Further study in our laboratory will focus on evaluating the biocompatibility of the propofol-loaded nanoparticuar system and increasing its physicochemical stability at high temperature.

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