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Effect of PVP on the Physical Stability of O/W Emulsion

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O/W 유제의 물리적 안정성에 대한 PVP의 영향

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To make a stable o/w emulsion, the effects of egg lecithin as an emulsifier and polyvinylpyrrolidone (PVP) as an auxiliary emulsifier on the physical stability of emulsion were investigated. The oil-in-water emulsion system was manufactured by microfluidizer and evaluated the physical stability. Average particle size and size distribution of emulsion was measured by dynamic light scattering analyzer and interfacial tension was measured. From the interfacial tension tested, critical micelle concentration of the egg lecithin was 0.1 %w/v and optimal concentration for the preparation of emulsion was 1.0 %w/v. The mean particle size was about 0.2 μm which was suitable for injections. The short-term accelerated stability studies were conducted by centrifugation, freeze-thaw method and shaking of the emulsion samples. The addition of PVP was caused the reduction in the particle size and improved the physical stability of emulsion. These results suggested that a mixed interfacial film comprising the egg lecithin and PVP was formed at the o/w interface and it was effective in preventing phase separation under thermic or mechanical stress. We used antineoplaston A10 (A10) as a model drug which is peptide and amino acid derivative having a action to the living organism against the development of neoplastic growth by a nonimmunological progress. It has a poor solubility in water and there may be a difficulty in formulation of A10. Emulsion formulation study about A10 was performed. Solubility of A10 in emulsion was about five times as high as that in water. From the results of solubility and partition coefficient, almost A10 molecules in o/w emulsion exist in the interface between oil and water.

Keywords—Antineoplaston A10, O/W emulsions, Egg lecithin, Polyvinylpyrrolidone, Particle size, Stability

Emulsions as a drug carrier have the ability to incorporate hydrophilic or hydrophobic drugs within their innermost phase, thus a drug could be sequestered from direct contact with body fluids and tissues while being slowly delivered over a prolonged periods of time. Emulsion formulation may also be used as a means of delivering drugs to phagocytic cell of reticuloendotherial system for treatment of a variety of parasitic and infectious disease.[1] Lipid emulsion (o/w) are commonly made by mixing an oil phase with an excess of aqueous solution in the presence of emulsifying agents. Although emulsion formulations have the advantages of biode-

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gradable, large scale productive, shelf life stability and clinically acceptable characteristics, low physical stability of emulsion system is one of problems to be solved. Moreover, average particle size and size distribution of parenteral emulsion is very important in stability of emulsion, in vivo fate of emulsion droplets and clinical acceptance. For example, sedimentation and creaming tendencies of the emulsions during long-term storage increase the size to a mean diameter over 5 μm, thus promoting the formation of pulmonary emboli.[2] For this reason measurement of particle size becomes the most important parameter for emulsion studies, and is normally tested using accelerated testing methods including heating, freezing-thaw cycles and centrifugation. The properties of the emulsions, including particle size, stability and in vivo distribution, are primarily determined by the properties of emulsifier and the ratio of oil to emulsifying agents.[3] Synthetic and natural polymers have been used as auxiliary emulsifiers to improve the physical stability of emulsions.[4,5] Polymers are usually water soluble and are thought to stabilize emulsions by either modification of the rheological properties of the bulk phase or adsorption at the oil-water interface, thereby providing a steric or an electrostatic barrier.[6]

3-Phenylacetyl-amino-2,6-piperidinedione which is called antineoplaston A10 (A10) is a peptide and amino acid derivative produced by the living organism. The size and shape of A10 is similar to a DNA base pair and may intercalate stereospecifically between base pairs in double helical DNA.[7] Its major action is to protect the living organism against the development of neoplastic growth by a non-immunological progress which does not significantly inhibit the growth of normal cells. But it is poorly soluble in water and fairly resistant to acid hydrolysis at room temperature. By formulating A10 into emulsion, therapeutic efficacy and physicochemical stability of the drug will be improved and side effect of A10 will be reduced by targeted delivery.

In this paper, we studied the manufacturing procedure for the o/w emulsion, the effect of egg lecithin or polyvinylpyrrolidone (PVP) on the formation and physical stability of emulsion and the emulsion formulation of A10.

Experimental

Materials

Antineoplaston A10, egg lecithin, PVP and soybean oil were purchased from Sigma. Acetonitrile was high performance liquid chromatography (HPLC) grade, and all other reagents were analytical reagent grade.

Determination of interfacial tension between oil and water

Soybean oil solution with various concentrations of egg lecithin (0.001-20% w/v) was prepared. Soybean oil (20 m*l*) containing surfactant was poured carefully to 20 m*l* of water layer. The interfacial tension between oil and water was determined by du Noüy tensiometer (Krüss K8, Germany).

Preparation of emulsion

Into 100 m*l* round flask 10g of soybean oil was added and heated to 70°C. Egg lecithin (2g) in hot ethanol solution was slowly mixed with the oil. And then, ethanol was removed with rotary vacuum evaporator (Büchi R114, Swiss). To make o/w emulsion 70°C distilled water and glycerin or PVP were added to the oil. This solution was homogenized at 10,000 rpm for 20 min to make a crude emulsion. With 0.1 N NaOH, the pH of the crude emulsion was adjusted to neutral and finally the volume was adjusted to 100 m*l*. The crude emulsion was changed to fine emulsion by microfluidizer (Microfluidics M110Y, USA). The emulsion was cooled to room temperature and stored at 4°C in a refrigerator.

Determination of particle size distribution.

The particle size distribution of emulsion was measured by dynamic light scattering analyzer (Malvern Autosizer Lo-C, England). Sample was diluted to 100 fold for droplet size measurements. Emulsion drops were blended with demineralized water under gentle agitation to avoid breaking up oil droplets and immediately measured.

Effect of egg lecithin and PVP

Emulsion was prepared with 0.01-2 %w/v egg lecithin in order to evaluate the effect of concentration of emulsifier on the formation of o/w emulsion. The effect of concentration of PVP on the stability emulsion was also investigated.

Physical stability of emulsion

Emulsion samples were stored in a stoppered vessel at 25°C for up to 5 days and the degrees of creaming and separation at proper intervals were determined by the particle size analyzer. Higher coalescence rates at elevated temperatures were measured for o/w emulsion immersing the vessel into constant-temperature baths, and recorded the particle size as function of time. The short-term accelerated stability studies were conducted by centrifugation, freeze-thaw method and shaking of the emulsion samples. For the evaluation of physical stability by centrifugation, the centrifuge (Vision Sci. VS 4000, Korea) was operated at 4500 x g, and samples were examined for percent creaming after 10, 20, 40, 60, 80, 100 and 120 minutes. The height of the separated phase appearing in the lower part of the tube was determined as a index of the degree of separation. The emulsions were also subjected to freeze in a refrigerator maintained at -20°C and thaw at 26°C. At determined intervals, the particle size of samples were measured. To test the physical stability of emulsion under excessive shaking, the samples were placed in the oscillator (IKA Vibrax, Germany) shaking with 200 rpm. Sample was withdrawn at proper intervals and measured particle size.

Determination of drug solubility and partition coefficient

An excess amount of A10 was equilibrated with 5 m*l* of distilled water or oil at 37°C for 24 hours with constant shaking in a shaking incubator. The saturated A10 solution was then filtered through a membrane filter (0.45 μm). After proper dilution, the concentration was determined by HPLC.[8] The partition coefficient of A10 between organic phase and aqueous layer was determined. An adequate amount of A10 was dissolved with 50 m*l* of distilled water at 50°C for 4 hours. This solution was then filtered through a membrane filter. The solution of A10 was assayed by HPLC method. And then, the solution of A10 (20 m*l*) was mixed with equal amount of n-octanol or soybean oil in separating funnel with vigorous shaking. After 24 hours, the water layer was assayed by HPLC method. The HPLC system was consisted of a pump (Waters 501, USA), UV detector (Waters 484, USA), a 3.9×300 mm stainless-steel column packed with μ-Bondapak C18 and an integrator (Youngin Sci. D520A, Korea). The mobile phase was a combination of acetonitrile and water (50 : 50 %v/v) and column temperature was maintained at ambient. A flow rate of 1.0 m*l*/min yielded an operating pressure of about 1000 psi. The signal was detected at 254 nm of UV detector with a sensitivity of 0.005 AUFS. Under these conditions, A10 peak was appeared at the retention time of 3.6 min.

Results and Discussion

Interfacial tension of egg lecithin

Egg lecithin was used as a nonionic surfactant. The relationship of interfacial tension and concentrations of egg lecithin are shown in Figure 1. The interfacial tension was not changed largely from 0 to 0.05 %w/v of egg lecithin but declined rapidly from 0.05 to 0.1 %w/v and became comparatively constant above 1.0 %w/v. From these results, critical micelle concentration of egg lec-

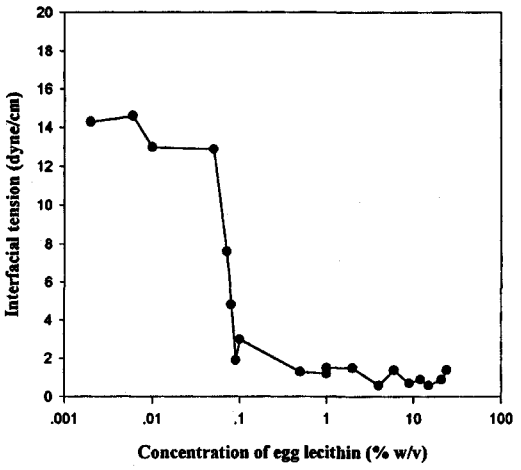

Figure 1—Interfacial tension of egg lecithin between soybean oil and water system.

ithin in the present study was 0.1 %w/v and the optimal concentration of egg lecithin for preparing a emulsion may be 1.0 %w/v. Afterwards, 1.0 % w/v of egg lecithin was used in the preparation of emulsion.

Particle size of emulsion

Particle size and distribution is very important in the stability of emulsion, tissue distribution and clinical acceptance.[9] Because greater shear forces are apparently required to achieve a stable emulsion having small particle size[10], we used microfluidizer as an emulsifier. The average particle size of emulsion depending on the number of cycles through microfluidizer was studied. As expected, the average particle size decreased as the pass number increased as shown in Figure 2. With increased cycling the higher shearing forces disrupt the oil droplets exposing the surfactant chains to the water.[11] After 6 passes through microfludizer, the emulsion comprised only submicron particles with a mean population diameter of approximately 210 nm. Particles above 5 μm are clinically unacceptable in injections because of the formation of pulmonary emboli, so the emulsion produced in the present study is suitable for injections.

Effect of concentration of egg lecithin

Mean droplet size of o/w emulsion decreased with increasing egg lecithin concentration (Figure 3). These finding indicated the formation of a better closed-packed interfacial film with improved mechanical and electrostatic properties which led to the formation of stabilized emulsified droplets of progressively diminishing particle size.[12,13] This interfacial film acted as a stabilizer at the stage of the emulsification process by forming a high energy barrier which caused repulsion of adjacent droplets and led to the formation of stabilized emulsified droplets of progressively decreasing size. This was confirmed by the influence of egg lecithin on the interfacial tension between oil and aqueous phase (Figure 1). Small particle size in an emulsion is associated with good stability during storage

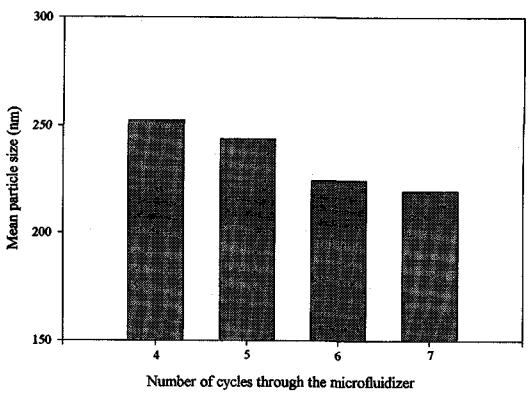

Figure 2—Average particle size of o/w emulsion depending on the cycle through microfluidizer.

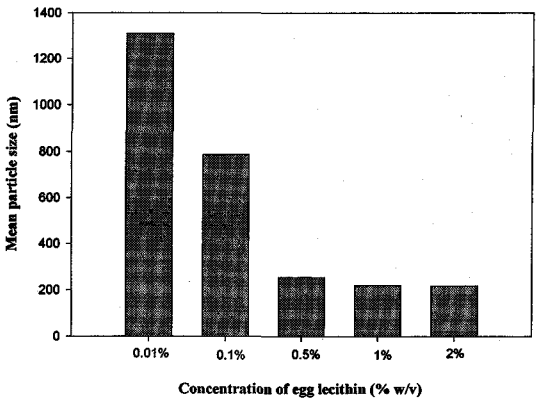

Figure 3—Average particle size of o/w emulsion depending on the concentration of egg lecithin.

and low toxicity in the body. By Ishii et. al.[14] at lower concentrations of 0.6-1.2 %w/w egg lecithin, the absorbed layer around the emulsion droplets formed, decreasing the particle size. At higher concentration of egg lecithin (above 1.6 % w/w), the surplus egg lecithin molecules associated to form micelles or vesicles, increasing the volume ratio between the dispersed phase and the continuous phase.

Effect of polyvinylpyrrolidone

Figure 4 shows the average particle size of o/w emulsion depending on the concentration of egg lecithin and PVP. The combination of egg lecithin and PVP in the formation of o/w emulsion yielded a fine emulsion with small particles and improved stability properties. Figure 5 shows that the mean droplet size of emulsion was a minimum at 5% PVP system. By Gullapalli and Sheth[15], the addition of methylcellulose caused a significant reduction and narrowing of the particle size distributions, but further increase in the polymer concentration exerted only slight changes in the distribution. This gradual decrease behavior reflects the formation of better close-packed mixed film of both emulsifying agents at the oil-water interface of the emulsified droplets. This interfacial film acted as a stabilizer at the earlier stage of emulsification process by forming a high-energy barrier which caused repulsion of adjacent droplets and led to the formation of stabilized emulsified droplets of progressively decreasing size. In this result the average size of 10% PVP system was higher than that of 5% system. The fact implies that hydrophilic-lipophilic balance of emulsion system may lean to hydrophilic side by the addition of excess quantity of hydrophilic auxiliary emulsifier.

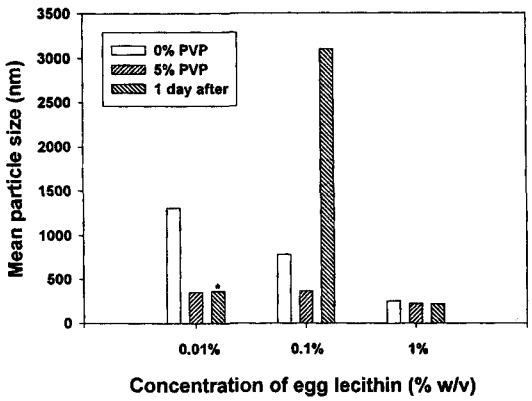

Figure 4—Effect of egg lecithin and PVP on the formation and stability of o/w emulsion. *Partial separation of emulsion.

Physical stability of emulsion

Temperature stress testing has been employed to accelerate the creaming of the investigated emulsion. The stability during storage with respect to particle size was monitored using submicron particle size analyzer. The change in size of the particles during a storage period of 5 days

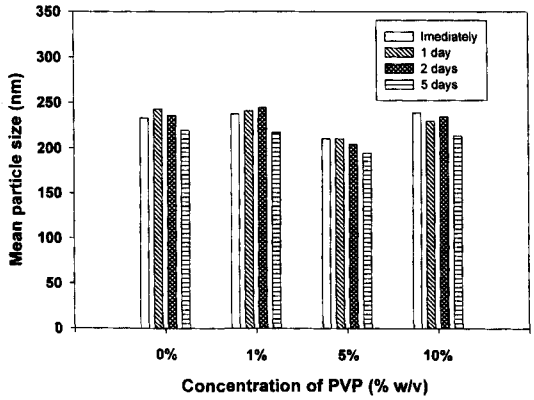

Figure 5—Physical stability of o/w emulsion at 25°C depending on the concentration of PVP.

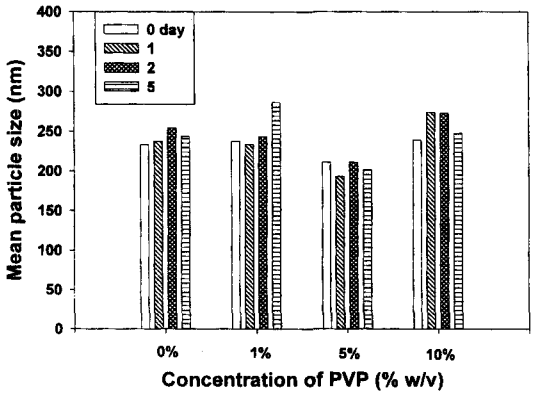

Figure 6—Average particle size of o/w emulsion depending on the concentration of PVP at 60°C.

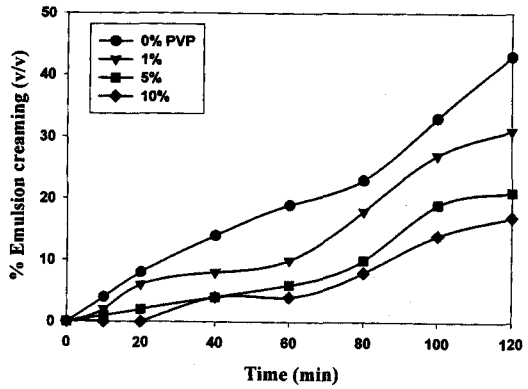

Figure 7—Emulsion creaming as a function of concentration of PVP and time of centrifugation at 4500×g.

at room temperature appears to be insignificant irrespective of the PVP composition. The particle size was found to remain constant at PVP ratio of 1, 5 and 10% (Figure 6). The stability was found to be best at 5%. The degree of acceleration of creaming will obviously depend upon the speed of centrifugation.[16] With regard to the centrifugation stress test, the employed centrifugal field may lead to overstressing an emulsion system. Therefore, if under centrifugation no creaming can be observed, it is normally correct to assume that it will be stable under normal gravitational field. The result (Figure 7) that the creaming of emulsion decreased as the concentration of PVP increased was due to increase

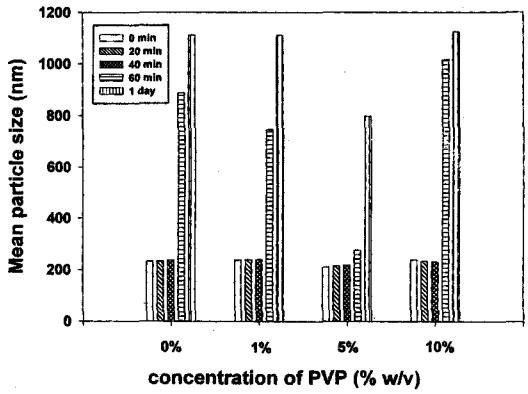

Figure 8—Physical stability of o/w emulsion on a freeze-thaw cycle.

of viscosity. Figure 8 shows the physical stability of o/w emulsion on a freeze-thaw experiment. It could be observed that the 5% PVP system is resistant to various mechanical and thermal stresses. These results clearly suggested that a mixed interfacial film comprising the phospholipid and PVP was formed at the o/w interface. The physico-mechanical properties of the mixed emulsifying interfacial film were strong enough to prevent any droplet coalescence upon random collision over prolonged periods of storage under thermic or mechanical stress.[17] The adsorbed layers on the droplets, evidently act by forming a high-energy barrier with the macromolecular chains oriented toward the continuous phase, an arrangement leading to steric stabilization by repulsion of surfactant chains adsorbed on adjacent dispersed droplets as they approach each other.[12]

Solubility and partition coefficient of antineoplaston A10

Solubility affects the dissolution rate, absorption and ultimately bioavailability of a drug. A solubility having less than 1 mg/m*l* indicates the need for special formulation to increase solubility or dissolution rate. The solubility of A10 in water was 670 μg/m*l* and that in emulsion was 3.53 mg/m*l*. The solubility of A10 was increased about five times in emulsion formulation. Partition coefficient is important in formulation, drug absorption and ultimately bioavailability. So the partition coefficient information is needed prior to the formulation of a drug. The partition coefficient of A10 between n-octanol and water was 0.921. This result implied that the partition of A10 in n-octanol is nearly equal to that in water. The partition coefficient of A10 between soybean oil and water was 0.098. By Yamamura et.al.[18] although the drug intrinsically has a lipophilic nature, but has a greater affinity to the oil/water interface with the aid of egg phosphatidylcholine. This results also implied that almost A10 molecules in emulsion are existed in the interface between oil and

water, so surfactant played the important role in emulsion formulation of A10.

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