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Effect of Process Parameters on Formation and Aggregation of Nanoparticles Prepared with a Shirasu Porous Glass Membrane

Jeong-Woong Seo, Kyung-Jin Kim, Su-Hyeon Kim, Kyu-Mok Hwang, Su Hyun Seok, and Eun-Seok Park*

School of Pharmacy, Sungkyunkwan University; Suwon 440–746, Republic of Korea.
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The objectives of this study were to prepare itraconazole (ITZ) nanoparticles using a Shirasu porous glass (SPG) membrane and to characterize the effects of diverse preparation parameters on the physical stability of nanoparticles. SPG membrane technology was used for the antisolvent precipitation method. The preparation of nanoparticles was carried out over a wide range of continuous-phase factors (type of surfactant, surfactant concentration), dispersed-phase factors (solvent type, solvent volume used to dissolve ITZ), and technical factors (pressure, membrane pore size, stirring speed in the continuous phase, temperature). Improved physical stability of nanoparticles was observed when surfactant with a lower molecular weight and higher hydrophilic segment ratio was used. The water miscibility of the solvent also had an effect on the physical stability. N,N-Dimethylacetamide contributed to creating a well-rounded shape and narrow size distribution due to high miscibility. Concentration of the surfactant and solvent volume used for dissolving ITZ were related to instability of nanoparticles, resulting from depletion attraction and Ostwald ripening. In addition to these factors, technical factors changed the environment surrounding ITZ nanoparticles, such as the physicochemical equilibrium between surfactant and ITZ nanoparticles. Therefore, the appropriate continuous-phase factors, dispersed-phase factors, and technical factors should be maintained for stabilizing ITZ nanoparticles.

Key words Shirasu porous glass membrane; antisolvent precipitation; nanoparticle; itraconazole; physical stability

Oral administration of poorly water-soluble drugs often leads to many problems in drug research and development. The aqueous solubility of a drug is an important factor for its dissolution rate. Poorly soluble compounds tend to be eliminated from the gastrointestinal (GI) tract before they have had opportunity to be absorbed into the circulation, and the low dissolution rate arising from low solubility results in low bioavailability. In such cases, dose escalation often causes topical toxicity in the GI tract and reduces patient compliance, which would be required for reaching therapeutic concentrations in the blood.1

There have been many attempts to increase bioavailability and dissolution rate of poorly water-soluble drugs. These include solid dispersions composed of solid state microfines or molecular dispersions, water-soluble cyclodextrin complexes and self-emulsifying drug delivery systems using diverse surfactants.2,3) The principle limitation of all these approaches is that the drug needs to have certain physicochemical properties. For example, sufficient solubility in oils or surfactants is needed for self-emulsifying drug delivery systems and the proper molecular size is essential for synthesizing a complex with cyclodextrin. In addition, the use of solubilizing excipients in drug solubilization is limited because of their toxicity or volume of formulation. Because of these limitations, there are the relatively low numbers of marketed products which use solubilizing techniques.3) For example, the commercial product, Sporanox®, an oral liquid of itraconazole (ITZ), has significantly greater oral bioavailability compared to the oral capsule already marketed. However, toxicity and side effects (e.g. Splenomegaly, ataxia) can occur when they are overdosed due to 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) used for complexation of ITZ and propylene glycol used as solubilizing agents.4,5)

Nanization could be a good alternative to improve solubility of poorly soluble drugs. The dissolution rate of nanosized drugs is enhanced by their increased surface area. A fast dissolution rate facilitates its use for Active Pharmaceutical Ingredients (API) with a narrow absorption window. Moreover, the saturation solubility of drugs in the nano-range is enhanced by a decrease in particle size.6,7) Numerous techniques for the preparation of spherical nanoparticles with a narrow size distribution have been developed. Methods for preparing solid drug nanoparticles can be divided into two ways; the top-down method and bottom-up method. The bottom-up method starts with dissolving the molecules and adding it to an antisolvent. The top-down method consists of milling, disintegrating and microfluidizing.7,8) In this report, antisolvent precipitation classified as the bottom-up method was used. The drug was dissolved in a solvent and this solution was added to a miscible antisolvent. Sudden supersaturation was caused by rapid addition of solvent to antisolvent and mixing, resulting in rapid nucleation and the formation of many small nuclei.3,5) The advantages of antisolvent precipitation method are that the equipment needed is relatively inexpensive, scaling up is simple, and high drug loading is possible. However, there is critical problem associated with physical instability of the nanoparticles which gives rise to crystal growth. The toxicity of non-aqueous solvents utilized in the precipitation process is another problem.3,6)

For precipitation method, Shirasu Porous Glass (SPG) membrane was used in the study. The dispersed phase is pressed through the pores of a microporous membrane, while the con-
tinuous phase flows along the membrane surface. The advantages of SPG membranes are the narrow pore size distribution and a wide range of available pore sizes. They facilitate control of droplet size with narrow size distribution. The potential for controlling droplet size is a distinguished feature of the SPG membrane. Additionally, the strengths of the technique lie in its simplicity, lower energy demands, and need for less surfactant.10,11)

A model drug used in this report is ITZ, an antimycotic drug administered for the treatment of systemic fungal infections. ITZ is practically insoluble in water at physiological pH conditions and soluble only under extremely acidic environments, leading to poor oral bioavailability with large individual deviations.12,13)

The purposes of this study were to prepare the ITZ nanoparticles using SPG membrane and to characterize the effect of diverse factors on the physical stability of nanoparticles. For these purposes, factors considered in this study were features of the dispersed phase, continuous phase, and technical points. The change of nanoparticle size distributions was also measured in this study.

Experimental

Materials ITZ was purchased from Nueland Laboratories (India), Poloxamer 188 (Lutrol® F68, Average molecular weight: 7680–9510), Poloxamer 237 (Lutrol® F87, Average molecular weight: 6840–8830), Poloxamer 338 (Lutrol® F108, Average molecular weight: 12700–17400), Poloxamer 407 (Lutrol® F127, Average molecular weight: 9840–14600), Polyoxyl 35 castor oil (Cremophor EL), and Polyoxyl 40 hydrogenated castor oil (Cremophor RH40) were purchased from BASF Co. (Ludwigshafen, Germany). Sodium n-dodecyl sulfate was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, U.S.A.). Polyoxyl hydrogenated castor oil 40, 50, and 60 (Nikkol HCO 40, 50, and 60) were purchased from Nikko Chemicals Co. (Tokyo, Japan). Polysorbate 20 and 60 were purchased from Samchun Chemicals Co. (Pyeongtaek, Korea). All other chemicals were reagent grade and used without further purification.

Methods Preparation of ITZ Nanoparticles In this experiment, SPG membrane technology was used for anti-solvent precipitation. The SPG membrane tubes were supplied from SPG Technology Co. (Miyazaki, Japan). ITZ nanoparticles were prepared using the cross-flow membrane system. The preparation of nanoparticles was carried out over a wide range of continuous phase factors, dispersed phase factors, and technical factors. Type of surfactants (non-ionic, anionic), and surfactant concentration (1–10%) were factors that influenced the characteristics of the continuous phase. Solvent types (benzyl alcohol, N-methylpyrrolidone and N,N-dimethylacetamide), and solvent volumes used to dissolve ITZ (1–10mL) were controlled factors for the dispersed phase. Pressure (30–100kPa), membrane pore size (300–1000nm), stirring speed of continuous phase (100–1100rpm), and temperature (0–60°C) technically influence the characteristic of nanoparticles. The SPG membrane was soaked into solvent and sonicated for 1 min on a wet membrane surface. ITZ was dissolved in the diverse dispersed phase in which ITZ was highly soluble. The continuous phase contained surfactants in 20mL of distilled water, which was precooled in an ice bath at 0°C with magnetic stirring. The dispersed phase was placed into the reservoir and pressurized using nitrogen gas that flowed through the pores of SPG membrane, while the continuous phase flowed along the membrane surface by stirring. The resultant samples were stored in 4°C.

 Determination of Particle Size and Size Distribution The mean particle size and polydisperse index (PI) of itraconazole nanoparticles for all samples were determined by a zetasizer (Zetasizer 3000HSA, Malvern Instruments Ltd., U.K.) using the Photon Correlation Spectroscopy (PCS) method, which enabled the detection of nanoparticles in the range of 1–3000nm. All samples were filtered and diluted with 10nM NaCl solution for analysis. The analysis was performed at a scattering angle of 90° and a temperature of 25°C. For each samples, the mean diameter of ten determinations was calculated using automatic analysis. Each measurement was per-
formed in triplicate.

**Scanning Electronic Microscopy (SEM)** To investigate the morphology of ITZ nanoparticles, the photomicrographs were obtained by field emission (FE)-SEM (JSM 6700F, JEOL, Japan). ITZ nanoparticles prepared by SPG membrane were ultra-centrifuged with protectants (10000 rpm, 10 min). The supernatant was decanted to remove the unbound stabilizer and remaining solvent. The solution containing ITZ nanoparticles was frozen at −40°C for overnight and subsequently freeze-dried. Resultant powders were scattered onto a carbon sticky tape and the coated with a platinum thin layer under vacuum for 1 min of sputtering time by a high resolution precision etching coating system.

**Results and Discussion**

**The Effect of Kinds of Surfactants** Four types of surfactants were screened; poloxamer, polyoxyethylene castor oil, polyoxyethylene sorbitan fatty acid esters, and sodium lauryl sulfate. As shown in Fig. 1, the size distribution of the initial particles was below 1 µm except particles using Polyoxy hydrogenated castor oil 50 and 60 as a stabilizer. The initial mean size of typical particles containing sodium lauryl sulfate (SLS) was 165 nm with a deviation of 17 nm. Suspension containing ITZ nanoparticles with SLS was clear, unlike those with other surfactants that were milky. However, the particles aggregated quickly and were large (>1 µm). Samples made with various surfactants also aggregated after one day, except for those prepared with poloxamer 188 or poloxamer 407 (Fig. 1). In the antisolvent precipitation method, nanoparticles are obtained by growth and nucleation. If surfactants used as stabilizers for nucleating have a good affinity for the drug, nano-suspension can be stabilized through steric or electrostatic mechanisms.14) In this study, based on the result that poloxamers were more effective surfactants for preparation of ITZ nanoparticles as compared with other surfactants, poloxamers 188, 237, 338, and 407 were further examined.

As shown in Fig. 2, the stability of particles was improved by orders of poloxamers 188, 237, 338 and 407. There are two explanations for this result. First, the molecular weight of the surfactants influenced the physical stability. Any increase in the molecular weight of surfactant decreases the exchange rate from micelle to monomer. Poloxamers 188 and 237 have much lower molecular weights than poloxamers 338 and 407. The unimer which had a low molecular weight escaped easily from micelle than those with high molecular weights, and stabilized the nanoparticles by adhering to their surface. Therefore, there was a difference in the stability between each classes of
poloxamer. Second, the ratio of the hydrophilic segment, poly-
ethylene oxide (PEO), could affect the stability. In the aqueous
phase, hydrophobic blocks of poloxamer adhere to the hydro-
phobic drug, ITZ. Hydrophilic layers consisting of PEO were
produced at the surface of ITZ nanoparticles. It could prevent
aggregation which was attributed to van der Waals forces be-
tween nanoparticles. In this study, poloxamers 188 and 338
were composed of 81–83% hydrophilic blocks and poloxamers
237 and 407 were composed of 72–73% hydrophilic blocks.
Therefore, poloxamers 188 and 338 have higher surface PEO
density and thickness. As a result, there were differences in
stability as seen in Fig. 2. We found that poloxamers 188 and
338 which have more hydrophilic blocks had more stable char-
acteristic than 237 and 407, respectively. In other words, if
two kinds of poloxamer had similar molecular weight, polox-
amer which had longer hydrophilic segment was more stable
than the other. Syntheticly, poloxamer 188 was selected as
the most suitable surfactant due to its low molecular weight
and higher ratio of hydrophilic blocks. The experiments were
set up to use poloxamer 188 as a surfactant.

The Effect of the Solvent Types To find an appropriate
solvent for preparing nanoparticles containing ITZ using the
antisolvent method, three types of solvents were screened.
ITZ is freely soluble in three types of solvents; benzyl alco-
hol, N-methylpyrrolidone, and N,N-dimethylacetamide. The
solubility of ITZ is 213 mg/mL in benzyl alcohol, 182 mg/mL
in N-methylpyrrolidone and 65 mg/mL in N,N-dimethylacet-
amide. All three types of solvents have a difference in degree
of water miscibility. The water miscibility of the solvent used
for anti-solvent precipitation method is the determining fac-
tor on nanoparticle formation. High water miscibility leads
to fast diffusion of solvent and drug precipitation. The water
miscibility of the solvents used in this study was 47 mg/mL
for benzyl alcohol, 82 mg/mL for N-methylpyrrolidone, and
517 mg/mL for N,N-dimethylacetamide. Benzyl alcohol and
N-methylpyrrolidone were therefore slowly diffused in contact
with the aqueous phase. Precipitated particles have no typi-
cal shape and the size was far over the nano-range (Fig. 3).
In contrast, nanoparticles made by N,N-dimethylacetamide
were well-rounded in shape and have a diameter of 247.9 nm
with polydispersity index of 0.49 which meant narrow size dis-
bution (data not shown). Therefore, N,N-dimethylacetamide
was selected as a suitable solvent for the preparation of ITZ
nanoparticles made by SPG membrane system.

The Effect of Surfactant Concentrations As shown in
Fig. 4, surfactant concentration is an important parameter for
size and physical stability of nanoparticles. Surfactants which
contain both hydrophilic groups and hydrophobic groups
could make a stable barrier at the interface, acting as a sta-
bilizer. The barrier enables the surfactants to prevent crystal
growth by providing steric repulsion between nanoparticles.
Therefore, the enough amount of surfactant is needed for cov-
erage on the nanoparticles. However, surfactant concentra-
tion was insufficient to cover the surface of nanoparticles at
a concentration of 0.1%. The initial particle size produced at
a surfactant concentration of 0.1% was about two times larger
than that of any other concentrations. The particles were also
unstable, aggregating within a day. At a surfactant concentra-
tion of 1%, nanoparticles preserve their stability for more than
13 d, which might be due to the fact that increased surfactant
concentration is sufficiently adsorbed onto the particle surface.

Specifically, most of surfactant was located at the air–water
interface. Concentration of amphiphilic surfactant at the
air–water interface was saturated as the total concentration
of surfactant was raised below the critical micelle concentra-
tion (CMC). As a result, the amount of surfactant monomer in
continuous phase was also raised. The surfactants adsorbed
onto the newly formed particle surfaces and functioned as
stabilizers through their ability to decrease the surface energy
and provide steric repulsion between particles. In conclusion,
nanoparticle system was stabilized by amphiphilic surfac-
tants present in particle surface. However, there is a point
where the more concentrated a solution, the lower the stabil-
ity. The particles formed in 10% (w/v %) surfactant solution
aggregated within 6 d, which showed that higher surfactant
concentration also caused instability. The phenomenon of in-
stability could be explained as depletion attraction. As the ITZ
particles come closer together, there is no surfactant between
the nanoparticles. The aqueous phase between nanoparticles
then tends to diffuse out to reduce the concentration gradient
carried by surplus poloxamer in the continuous phase. As a
result, ITZ nanoparticles are aggregated faster when the sur-
factant concentration is high.

The Effect of Volume of Disperse Phase As mentioned
above, fixed amounts of ITZ were dissolved in different vol-
umes of disperse phase, N,N-dimethylacetamide. As shown in
Fig. 5, the ITZ nanoparticles had a tendency to be unstable
when ITZ was dissolved in a higher volume of N,N-dimeth-
ylacetamide. This instability is attributed to the high N,N-
dimethylacetamide/water ratio after mixing the disperse phase
and continuous phase. In this environment, ITZ, which is
hydrophobic triazole derivates, is easy to dissolve in medium.
Diffusion and growth kinetics of ITZ nanoparticles become
active at the particle boundary layer interface. As a result,
the rate of Ostwald ripening increases, inducing the aggrega-
tion of nanoparticles. As a result, it is best to use minimum
amount of antisolvent for stabilizing nanoparticle system.
The Effect of Pressure

As shown in Fig. 6, there were no significant differences in the initial particle size of nanoparticles prepared under different pressures. Thirty kilo-Pascals was the minimum pressure at which the dispersed phase was forced to permeate through the membrane into the continuous phase, and 100 kPa was the highest pressure at which nitrogen did not bubble out. After 9 d, the particle size of the formulation made at 100 kPa began to increase, and after 10 d, the particles were no longer included in nano-range. However, the particle size of the formulation made at 30 kPa remained the same for more than 10 d, indicating that this formulation was more stable. This difference depends on the time needed for nanoparticle formation. When nanoparticles are made at high pressure, which induces fast flux, the polydispersity of nanoparticles size distribution increases. In this situation, smaller particles with a high solubility dissolve in the continuous phase and precipitate on the larger particles. It is ‘Ostwald ripening’ and reason for instability of the nanoparticles after 9 d.10,21)

The Effect of the Stirring Speed of Continuous Phase

As shown in Fig. 7, the stirring speed of continuous phase had a significant influence on initial particle size and physical stability of nanoparticles. The initial particle size made at 100 rpm was around 550 nm and was larger than that at 1100 rpm, which was around 330 nm. The diffusion rate of the solvent at 1100 rpm was faster than that at 100 rpm. Therefore, nanoparticles that were rapidly produced at 1100 rpm were smaller in size than that produced at 100 rpm. Nanoparticles made at 100 rpm were also comparatively unstable, and completely aggregated within 9 d. The initial particle size of nanoparticles lasted for 5 d, and nanoparticles began to aggregate over the next 4 d. The particle size changed progressively from 700 nm to micro-size, along with an increased polydispersity. In the precipitation method using the SPG membrane, the droplets are formed at the membrane surface. As soon as it detaches, the water-miscible N,N-dimethylacetamide dissolves into the continuous phase and nucleation of supersaturated ITZ occurs. To arrest particle growth, the stabilizer adsorption on the drug surface is needed. Once the ITZ nuclei are formed, a higher water phase flow can help disperse the nanoparticles through the mixing vessel and reduce the amount of coagulation in the environment near the membrane surface. However, under low stirring speed of continuous phase, generation of supersaturation and the subsequent nucleation rates may be relatively slow, leading to large polydisperse particles.

The Effect of Temperature Control

As shown in Figs. 8 (A) and (B), the initial particle size was nano-range at 0
The solubility of ITZ in water at different temperatures, the level of supersaturation is reduced because the process occurs through several pathways. First, at elevated temperatures, the increase of mean particle size and polydispersity was evaluated only about particles in nano-range. The size of the particles made at 20°C remained constant for only 4 d, and then the samples began to aggregate, while the 0°C samples were stable for more than 8 d. Several reports explain how temperature influences the precipitation process through several pathways. First, at elevated temperatures, the level of supersaturation is reduced because the solubility of ITZ in the water/N,N-dimethylacetamide mixture is increased. Second, precipitation can be explained by the properties of the poloxamer. Ethylene oxide (EO) blocks play an important role in preventing precipitation, and have a tendency to be dehydrated at an elevated temperature. Dehydration of the EO blocks leads to precipitation of nonionic EO-based surfactants. Finally, steric stabilization of drug particles is reduced resulting in greater particle growth.20,22

**Conclusion**

In this study, ITZ nanoparticles were prepared by anti-solvent precipitation using SPG membrane technology. According to continuous phase factors, dispersed phase factors, and technical factors, there are substantial differences in the physical stability of nanoparticles dispersed in the continuous phase. Improved physical stability of nanoparticles was observed when surfactant with a lower molecular weight and higher hydrophilic segment ratio was used. This phenomenon was ascribed to the decreased exchange rate from micelle to monomer and the higher surface density and thickness of surfactant. The water miscibility of the solvent used for precipitation also had an effect on the physical stability. N,N-Dimethylacetamide with high miscibility led to rapid diffusion and precipitation of ITZ, creating nanoparticles with a well-rounded shape and narrow size distribution. Concentration of the surfactant and solvent volume used for dissolving ITZ were related with depletion attraction and Ostwald ripening leading to instability. In addition these factors, technical factors (pressure, membrane pore size, temperature and rpm) changed the environment surrounding ITZ nanoparticles. As a result, the physicochemical equilibrium among surfactant, the amount of ITZ, and technical factors should be attained for stabilizing ITZ nanoparticles with improved bioavailability.

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**Conflict of Interest**

The authors declare no conflict of interest.

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