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Preparation of stabilized lidocaine particles by a combination of supercritical CO_2 technique and particle surface control

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Abstract Lidocaine particles were prepared by the rapid expansion of the supercritical fluids into aqueous solution (RESAS). About 150–300 nm lidocaine particles could be temporarily observed when collected in water at concentration of 10 mg/cc, but the particles developed into 50 μm needle crystals just 30 min later. To prevent the fast aggregation of the particles as well as further crystal growth, modifications on particle surfaces by adding surfactants and introduction of electrostatic repulsion between particles were conducted. When a surfactant (sucrose stearic acid ester S-1570) was added to the collecting aqueous solution, the particle growth was alleviated and no large needle crystals were formed. However, the long-term stability needs to be improved because the lidocaine particles tend to grow from submicron to a few microns in a few days even stored in the 1% S-1570 solution. Electrostatic repulsion between particles is found effective to stabilize the submicron particles during the storage. When the pH value of the aqueous solution with 1% S-1570 was adjusted to 8.5 by adding KOH, the lidocaine particles suspending in this solution showed good stability that the particle size was able to be controlled in submicron level in 3 months.

Introduction

Manufacture of fine pharmaceutical particles by using supercritical CO_2 has been studied for two decades. Particles as small as submicrons were able to be produced by the rapid expansion of supercritical solutions (RESS). Nano particles of fluorinated porphyrin smaller than 100 nm were successfully generated by RESS without adding other solvents as reported by Sane et al. [1]. Generally, the minimum size of organic particles by RESS is in the range of 20–50 nm in theory [2, 3], but nano organic particles (<100 nm) are hardly obtained in practice. Particle collisions in RESS are often considered as one of the main reasons for the particle growth [2]. To control the particle growth, rapid expansion of supercritical solution to an aqueous solution (RESAS) [4] or rapid expansion of supercritical solution into a liquid solvent (RESOLV) [5] had been developed so that the stabilization of particles in aqueous solutions became possible. Addition of agents of low surface tension is a popular way to stabilize submicron particles in aqueous solutions. Usually, non-ionic surfactants are favorable candidates for stabilization of pharmaceuticals because of their low toxic. Young et al. showed that Tween-80 was capable of stabilizing 400–700 nm cyclosporine [4]. Türk et al. demonstrated that phytosterol particles less than 500 nm could be stabilized in four different surfactant solutions (Tween-80, SLS, poloxamer and polyethylene glycol-15-hydroxystearate). They reported modest particle growth and broadening of size distribution of phytosterol particles in one-year storage [6]. Attempts to stabilize nano particles in a long term is as important as producing nano particles itself. On the other hand, pH adjustment to the particle suspension is a common way to prevent the aggregations for various metal and ceramic powders. Although preparations of some pharmaceutical particles in specific pH values by RESS were reported [7], study of the pH effects on the long-term stability of pharmaceutical nanoparticles seems to be very scares according to our investigation. Therefore, a

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combination of supercritical CO_2 technique and particle surface control by pH adjustment is of significance.

In our RESAS experiments, fast aggregation of submicron lidocaine particles in water was observed when the concentration was about 10 mg/cc (about two times of its water solubility). Existence of 150–300 nm lidocaine particles was confirmed by the laser diffraction measurement as soon as they were collected in pure water, but the particles developed into 50 μm long needle crystals in 30 min. The fast crystal growth of the RESAS processed lidocaine particles does not occur for non-processed lidocaine powders (10–200 μm) no matter what the concentration is. It could be a disaster for the application to drug delivery system if the grow rate were not limited to a satisfied level. The atomization effect of RESS depends on materials properties and processing conditions. For low melting point drugs, nano particles (<100 nm) are hardly obtained by RESS [8]. Lidocaine is a water insoluble drug of low melting point (341 K) and its solubility in supercritical CO_2 is quite high (>26 wt%) [9]. In this study, lidocaine was employed as the model drug to investigate the particles behaviors in RESAS. Effects of adding surfactants as well as introduction of electrostatic repulsion between particles on the particle growth of lidocaine were investigated.

Experimental

CO_2 (99.99%) was used in the study. Volume of the solutions employed in RESAS to collect the particles was 50–100 cc. Distilled water passed through 0.1 μm filter (Millex-VV, Millipore) was utilized to prepare the aqueous solutions. The lidocaine was provided by Teikoku Seiyaku Co., Ltd. The solubility in supercritical CO_2 at 313 K and 20 MPa is roughly estimated as about 37 wt% according to the results measured by Frank and Ye [9]. Lidocaine has both lipophilic and hydrophilic characteristics at opposite ends in the molecule structure as shown in Fig. 1. The lipophilic end is attracted to lipids, and the hydrophilic end is attracted to water. The surfactant was selected from a kind of sucrose fatty acid ester made by Mitsubishi Kagaku. It is used as emulsifiers in food industry because its hydrophilic lipophilic balance (HLB) can be easily adjusted. In this study, solutions of 0.1–1% surfactant S-1570 (sucrose stearic acid ester, HLB15, monoester 70%) were used to collect and stabilize the lidocaine particles produced by RESAS.

A brief illustration of the RESAS equipments is shown in Fig. 2. A heating unit was connected to the collection chamber to avoid solution freeze due to the Joule–Thompson effect. The operation pressure and temperature of the supercritical CO_2 were 20 MPa and 313 K, respectively. Volume of the high-pressure dissolution tank was

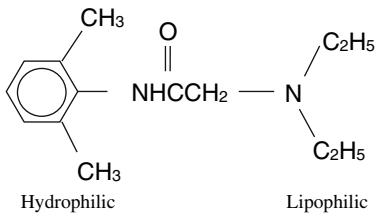

Fig. 1 Molecule structure of lidocaine

300 cc. To obtain nano particles as small as possible, low loading concentration of lidocaine as 0.1 wt% was employed. A 5 μm clearance nozzle was used to produce the lidocaine particles [10]. The concentration of lidocaine in the solution was determined by a high performance liquid chromatographer (HPLC) D-7000, Hitachi. The particle size in the aqueous solutions was measured by a laser diffraction analyzer, Microtrac UPA 9340, Honeywell Int., Inc. The analyzing volume for all solution samples was 8 cc. The particle morphology in the aqueous solution was studied by a scanning electron microscope (SEM) JSM 5310 after drying a few drops of sample solutions.

Results and discussion

Effect of adding S-1570

Solutions of 0.1–1% surfactant S-1570 were prepared as the aqueous solutions of RESAS to modify the surface of lidocaine particles. Powders of the S-1570 were stirred in hot water till completely dissolved. Filtration of the solution was conducted by a 0.1 μm membrane filter (Millex-VV, Millipore) before it was used as the aqueous solution in RESAS. The micelle size in water was measured as 9–13 nm.

As a comparison, the huge needle crystals formed in pure water just 30 min after the RESAS processes were

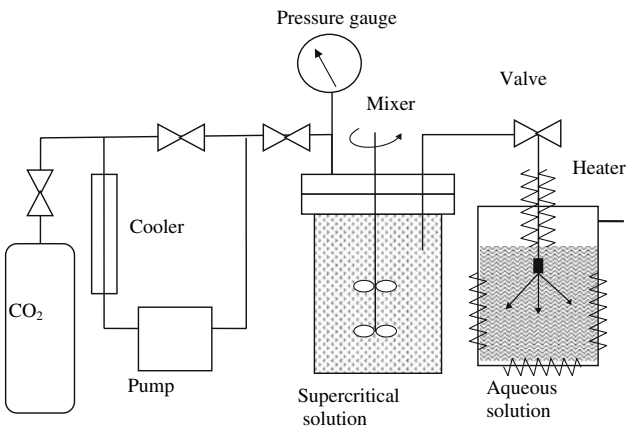

Fig. 2 RESAS equipments

shown in Fig. 3. When 0.1–1% S-1570 solutions were used, the fast particle growth of lidocaine was no longer found for lidocaine concentrations up to 10 mg/cc. For the case of 1% S-1570 solution, a time dependence of the size of lidocaine particles was shown in Fig. 4. The dispersed submicron particles were able to be observed even 1 day after produced. A SEM picture was given in Fig. 5. It indicates that good dispersion of lidocaine particles in above solution was achieved. The experiment results suggest that addition of the surfactant successfully decreased the probability of collisions between lidocaine particles so that the particle growth process slowed down compared to that in pure water.

It was also found that ultrasonic vibrations could temporally reduce the mean size of particles measured by UPA 9340. Taking the measurement to the sample stored for 3 h as an example, the mean particle size decreased from 441 ± 134 nm to 254 ± 55 nm after 10 s ultrasonic vibrations. However, these submicron particles still turn into powders in microns after one week's storage in the 1% S-1570 solution and they could not be turn back to submicron particles again by ultrasonic vibrations. Probably, the failure of controlling the particle growth in a long term can be attributed to the weak physical adsorption of the surfactant molecules to the surface of lidocaine particles. To prepare stabilized nano particles of lidocaine in a long term, other treatments are needed to prevent the lidocaine particle growth.

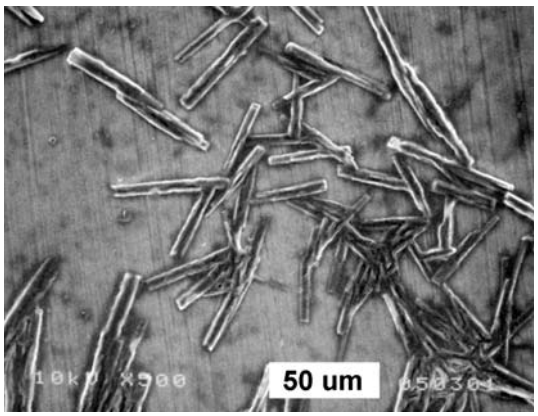

Fig. 3 Lidocaine needle crystals observed in water 30 min after preparation by RESAS

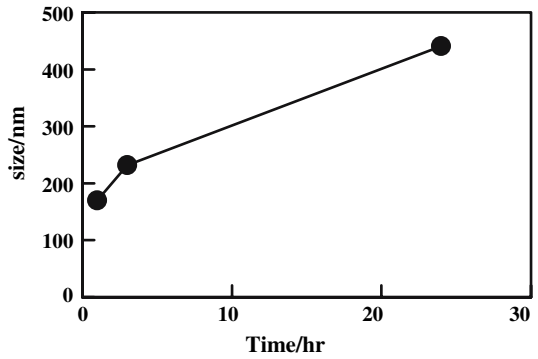

Fig. 4 Time dependence of particle size of lidocaine in 1% S-1570 solution after preparation by RESAS

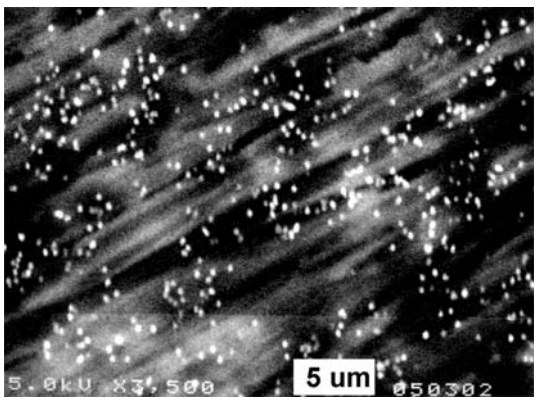

Fig. 5 Lidocaine particles dispersed in 1% S-1570 solution 24 h after preparation

Effect of the pH adjustment

It is well known that charged particles repel each other while uncharged particles are free to aggregate. Stabilized polymer nano particles less than 50 nm in NaOH solution (pH = 11) were reported by Meziani et al. [11]. To maximum the repulsive force between particles, zeta-potential analysis is very helpful. Thus, the zeta-potentials of the lidocaine particles in the 1% S-1570 solution produced in the previous experiments were measured by the Malvern zetasizer 3000HS. The zeta-potentials as a function of pH values shown in Fig. 6 suggested that a strong electrostatic effect can be achieved if pH > 8. So, a weak alkaline solution of pH = 8.5 was prepared to test the anti-aggregation effect of electrostatic repulsion.

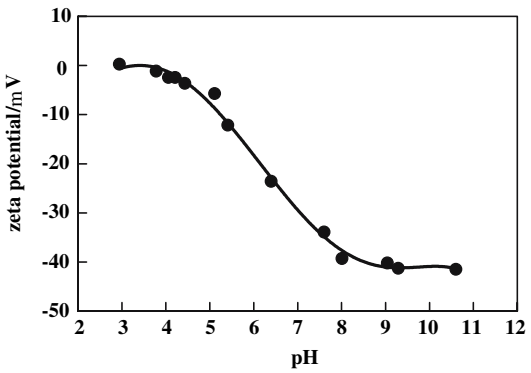

Fig. 6 Zeta-potential of lidocaine particles as a function of pH in 1% S-1570 solution

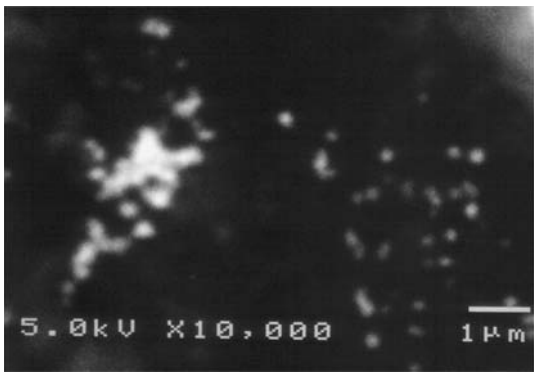

Fig. 7 Lidocaine particles stored for 3 months after preparation by RESAS

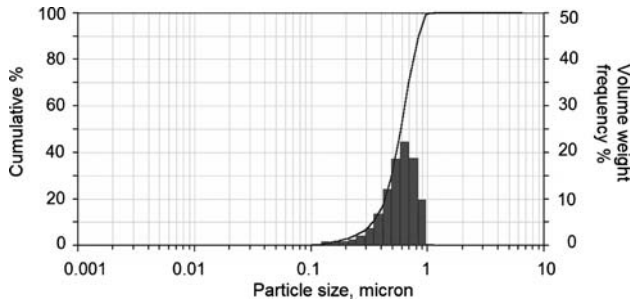

Fig. 8 Size distribution of the lidocaine particles stored for 3 months after preparation by RESAS

In the RESAS process, lidocaine particles were first collected in 1% S-1570 solution as the previous experiment. The solution was then evacuated for 1 h to remove CO_2 dissolved in the aqueous solution during the spray. Then, KOH solution was added into the above 1% S-1570 solution till pH = 8.5. The sample lidocaine particles stored in the pH adjusted S-1570 solution was examined after 3 months storage without any other ultrasonic vibrations. The dispersed lidocaine particles in size of about 200–300 nm together with aggregates less than 1 μm were observed by SEM as shown in Fig. 7. The size measurement by the laser diffraction particle analyzer gave no evidence of large aggregations over 1 μm as shown in Fig. 8. Generally, the experimental results indicate that better anti-aggregation effect was obtained compared to using 1% S-1570 solutions along.

Discussion

It seems that particle growth of other organics produced by RESS or RESAS up to date were not as fast as that of lidocaine found in the present study. Even in Frank's dissolution measurements, fast particle growth of lidocaine particles (~100 nm) was not reported either [9]. Therefore, it is very interesting to discuss why lidocaine particles generated in the present study aggregate so fast in pure water. Sane et al. investigated the influences of materials properties and operation conditions on the particle size obtained by RESS. According to their research, nano particles of organic substance with high melting points are relatively easier to produce while coalescence of particles in the free jet is considered as the main reason for formation of large particles [8]. This is theoretically consistent with the findings in our laboratory because the fast aggregations occurred both in lidocaine (melting point 341 K) and phenanthrene (melting point 353 K) [10]. Secondly, particle size also has great influence on the aggregation process because of the difference in specific surface area. Generally, the smaller the particles, the faster they grow up. In a separate study in our laboratory, the 5 μm clearance nozzle showed dramatic atomization effect compared to using normal capillary nozzle [10]. Thus, it is reasonable to assume that the initial size of lidocaine particles generated by the clearance nozzle were smaller than 100 nm. If the assumption is true, the initially formed nano lidocaine particles aggregated very fast so that huge needle crystals were observed. However, it is very difficult to obtain solid evidence of the formation of lidocaine particles less than 100 nm even if the lidocaine particles were collected in the S-1570 solutions. The SEM pictures taken by drying a few drops of sample solutions only reflected particles larger than 100 nm. The size measurements on the lidocaine particles in the solutions gave unstable results within the initial 30 min after preparation by RESAS. Dynamic movements of lidocaine particles, surfactant molecules as well as micro bubbles of CO_2 might affect the particle size measurements. We tend to make an induction that nano lidocaine particles aggregate until enough surfactant molecules diffuse into the surfaces of lidocaine particles. So, the aggregation process is hardly prevented especially in the initial stage. Thirdly, the effect of particles size on the dissolution rate is another important factor of significance. The dissolution rates of RESS processed lidocaine particles and those without processing had been studied by Frank and Ye [9]. The fine powders in size of about 100 nm produced by RESS did not show significant improvement on the dissolution rate of lidocaine as pointed out by Perrut [7]. If lidocaine particles were dispersed in water without presence of surfactants, aggregations into large particles were unavoidable according to the present study. This would be a possible explanation to the modest increase of dissolution rate of lidocaine particles mentioned above.

Thus, proper surface treatments to the low melting drugs are essential as soon as the particles were produced. RESAS followed by surface treatments to the particles is a potential way to prepare stabilized pharmaceutical nano particles in a long term for low melting point drugs.

Conclusions

Preparation of fine lidocaine particles by RESAS was conducted. Fast particle growth of the low melting point lidocaine in water was observed. The effects of addition of surfactant and pH adjustment to the collecting solutions were investigated. It was found that adding S-1570 to the collecting water effectively alleviated the particle aggregation process and prevented the formation of large crystals over a few microns. To stabilize the submicron particles of lidocaine for months, the influence of electrostatic repulsion between particles were studied. The experimental results showed that submicron lidocaine particles could be stored for 3 months when the pH value of the 1% S-1570 solution is adjusted to 8.5.

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References

1. Sane A et al (2003) Chem Commun 21:2720
2. Debenedetti PG (1994) In: Supercritical fluids: fundamentals for application. Kluwer Academic Publications, pp 719–729
3. Helfgen B, Turk M, Schaber K (2003) J Supercrit Fluid 26:225
4. Young TJ et al (2000) Biotechnol Prog 16:402
5. Sun YP et al (2001) Langmuir 17:5707
6. Türk M, Lietzow R (2004) AAPS Pharma Sci Tech 5(4) Article 56
7. Perrut M et al (2005) J Supercrit Fluid 288:3
8. Sane A, Thies MC (2007) J Supercrit Fluids 40:134
9. Frank SG, Ye C (2000) In: Eckert C, Teja A (eds) Proceedings (CD-ROM) of the fifth international symposium on supercritical fluids (ISSF2000), Atlanda, GA, April 2000
10. Huang J, Moriyoshi T (2006) J Supercrit Fluid 37:292
11. Meziani MJ et al (2004) Angew Chem Int Ed 43:704