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Flocculation of Polymer Stabilized Nanocrystal Suspensions to Produce Redispersible Powders

Xiaoxia Chen, Michal E. Matteucci, Connie Y. Lo, and Keith P. Johnston
Department of Chemical Engineering, University of Texas at Austin, Austin, TX, USA

Robert O. Williams III
College of Pharmacy, University of Texas at Austin, Austin, TX, USA

Aqueous suspensions of crystalline naproxen nanoparticles, formed by antisolvent precipitation, were flocculated with sodium sulfate, filtered, and dried to form redispersible powders for oral delivery. The particles were stabilized with polyvinylpyrrolidone (PVP K-15) and/or poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (poloxamer 407). The yield of the drug in the powder was typically 92–99%, and the drug loading was reproducible to within 1–2%. The filtration process increased the drug loading by up to 61% relative to the initial value, as unbound surfactant was removed with the filtrate. Upon redispersion of the dried powder, the average particle size measured by light scattering was comparable to the original value in the aqueous suspension prior to flocculation, and consistent with primary particle sizes observed by scanning electron microscopy (SEM). For 300-nm particles, up to 95% of the drug dissolved in 2 min. The dissolution rate was correlated linearly with the specific surface area calculated from the average particle diameter after redispersion. The redispersion of dried powders was examined as a function of the salt concentration used for flocculation and the surfactant composition and concentration. Flocculation followed by filtration and drying is an efficient and highly reproducible process for the rapid recovery of drug nanoparticles to produce wettable powders with high drug loading and rapid dissolution.

Keywords salt flocculation; nanoparticles; naproxen; antisolvent precipitation; dissolution

INTRODUCTION

It is estimated that more than one-third of the compounds discovered by the pharmaceutical industry are poorly water soluble (Lipinski, 2002; Radtke, 2001). The bioavailability of class II drugs is often dependent upon the dissolution rate of the drug in the gastrointestinal tract (Oh, Curl, & Gl, 1993). Dissolution rates may be increased by reducing the particle size to increase the surface area, and by coating drug particles with hydrophilic surfactants to enhance wetting and salvation (Chen, Vaughn, Yacaman, Williams, & Johnston, 2004; Rogers et al., 2003; Sarkari et al., 2002).

Poorly water-soluble drugs may be formulated with submicron features to enhance dissolution rates (Rabinow, 2004). However, the recovery of nanoparticles (<500 nm) from aqueous suspension is a formidable challenge, particularly if it is desired to achieve the original particle size upon redispersion in aqueous media, for example, in oral drug delivery. Budesonide particles with a size range from 1 to 10 μm were filtered with 0.8-μm pore size polycarbonate membranes (Ruch & Matijevic, 2000). Filtration becomes much more challenging, however, for particle sizes below 1 μm. Ketoconazole, itraconazole, and ibuprofen micronized particles have been recovered by spray drying (Rasenack & Muller, 2002). For particles produced by antisolvent precipitation, that is mixing of organic and aqueous solutions, the organic solvent may be stripped from the aqueous suspension prior to spray drying to minimize Ostwald ripening (Elder et al., 2003; Liu, Kathan, Saad, & Prud'homme, 2006).

Antisolvent precipitation is a widely used process to prepare inorganic and organic particles (Falk, Randolph, Meyer, Kelly, & Manning, 1997; Jarmer, Lengsfeld, & Randolph, 2004; Randolph, Randolph, Mebes, & Yeung, 1993) including nanoparticles of poorly water-soluble drugs (Elder et al., 2003; Johnson & Prud'homme, 2003; Rasenack & Muller, 2002; Ruch & Matijevic, 2000). In this process, a poorly water-soluble drug with or without a polymeric surfactant(s) is dissolved in a water-miscible organic solvent, such as methanol, ethanol, tetrahydrofuran (THF), and acetonitrile. The organic solution is then mixed with an "antisolvent," usually an aqueous solution containing a surfactant(s) by a confined impinging jets (CIJ) mixer (Johnson & Prud'homme, 2003), controlled precipitation (Elder et al., 2003; Rogers et al., 2004) sonication (Ruch & Matijevic, 2000), or direct addition (pouring) of the antisolvent into organic drug solution (Rasenack & Muller, 2002). Supercritical fluid antisolvents can be used to achieve a

Address correspondence to Keith P. Johnston, Department of Chemical Engineering, University of Texas at Austin, Austin, TX 78712, USA. E-mail: kpj@che.utexas.edu

great deal of control over particle morphology, as a result of rapid two-way diffusion (Dixon, Johnston, & Bodmeier, 1993; Palakodaty & York, 1999; Randolph et al., 1993; Yeo, Lim, Debenedetti, & Bernstein, 1993). Upon mixing, the supersaturation and concentration of polymeric steric stabilizers may be controlled to manipulate the nucleation and growth of drug particles (Jarmer et al., 2004; Matteucci, Hotze, Williams III, & Johnston, 2006; Rogers et al., 2004). With sufficient supersaturation, and arrested growth by surfactant stabilization, it becomes possible to form suspensions of submicron particles in the aqueous solution.

The objective of this study was to recover nanoparticles of poorly water-soluble drugs produced by antisolvent precipitation at low temperatures from 0 to 22°C by flocculation and filtration, and to examine their morpholgy and dissolution rates. The temperature for antisolvent precipitation is shown to have a large effect on the particle size distribution in the suspension. The particles were separated from the solvent by flocculation with a concentrated salt solution, to desolvate and collapse the polymeric steric stabilizers on the particle surface. The large flocculated particles were recovered from the aqueous solution by filtration and were vacuum dried. The surfactant composition and structure, and the type and concentration of salt in the drug suspension were optimized to produce large, loose flocs that could be redispersed into pure water readily after filtration and drying. The increase in drug loading (wt. drug/total weight excluding salt) upon filtration and the reproducibility in the composition and yield of the powder were examined. Another goal was to achieve particle sizes after redispersion similar to those in the original suspension prior to flocculation. The dissolution rate of naproxen powder produced by this technique was compared with that of identical aqueous drug suspensions dried by lyophilization. X-ray diffraction was used to investigate the crystallinity of naproxen. Optical microscopy and scanning electron microscopy (SEM) were used to characterize the morphologies of the naproxen flocs in the suspension and after drying. The concentration of residual salt in the dried samples was measured by conductivity and shown to be far below the toxic limit.

Compared to other solvent removal techniques, the use of flocculation and filtration offers several advantages. The flocs may be filtered much more rapidly and efficiently than the original nanoparticle suspension. Rapid filtration reduces the time for the growth of the primary particles in the concentrated precipitate. Particle growth is a potential problem in this step as the stabilizers on the particle become less solvated by water as the filtrate is removed, and the presence of organic solvent enhances Ostwald ripening (Liu et al., 2006). The filtration can be operated at low temperatures more easily than in the case of spray drying and other solvent evaporation techniques. For example, drying at high temperatures may lead to undesirable particle growth (Elder et al., 2003). Separation by filtration avoids challenges in evaporation, for example, for solvents such as ethanol that form azeotropes with water. Whereas it may take days for lyophilization, the time for flocculation and filtration will be shown to be on the order of minutes. Finally, the loading of the drug can be increased in the filtration step since the dispersed particle phase contains a higher fraction of drug than the continuous phase, as soluble surfactant stabilizers are removed with the filtrate.

EXPERIMENTAL SECTION

Materials

Naproxen, ketoconazole, polyvinylpyrrolidone (PVP K-15, M_w = 10,000), and poly(ethylene oxide-*b*-propylene oxide-*b*-ethylene oxide) (poloxamer 407) with a nominal molecular weight of 12,500 and a PEO/PPO ratio of 2:1 by weight were purchased from Spectrum Chemical Mfg. Corp. (Gardena, CA, USA). Sodium sulfate (anhydrous) and sodium carbonate (anhydrous) were obtained from Fisher Scientific Company (Fairlawn, NJ, USA). Sodium phosphate tribasic dodecahydrate was from EM Science (Gibbstown, NJ, USA). HPLC grade acetonitrile and methanol were obtained from EM Industrial Inc (Gibbstown, NJ, USA).

Antisolvent Precipitation

A schematic of the antisolvent process is shown in Figure 1. The naproxen solution in methanol or ethanol was fed by a HPLC pump through a 1-m-long 1/16 in. o.d. × 0.030 in. i.d. stainless-steel tube. The organic solution was sprayed to form a cylindrical jet roughly the inside diameter of the tube without atomization into precooled aqueous surfactant solution. The aqueous surfactant solution was contained in a 250-mL glass cylinder submerged in a water/ethylene glycol bath controlled to 3°C. The tip of the stainless-steel tube was submerged approximately 4 cm under the surface of the aqueous solution. To enhance the mixing between organic phase and aqueous phase, a magnetic stir bar was placed inside the glass cylinder and stirred at a fixed rate to form a vortex. Unless specified elsewhere, 7% (wt/vol) naproxen with or without surfactant was dissolved into methanol. The organic solution was then sprayed into 50 mL aqueous solution at 5 mL/min for 1.4 min to yield a suspension concentration of 10 mg/mL. After spraying for a required time to produce the desired suspension concentration, the suspension was analyzed within 5 min to determine the particle size by static light scattering with Malvern Mastersizer-S (Malvern Instruments Ltd., Malvern, UK). The suspension was sonicated in the Mastersizer to break up the aggregates until a stable particle size distribution was obtained. The particle size of the same suspension was also measured after 5 min sonication with a powerful sonicator (Branson Sonifer 450, Branson Ultrasonics Corp., Danbury, CT, USA) in an ice/water bath at an output control of 7 and 30% duty cycle.

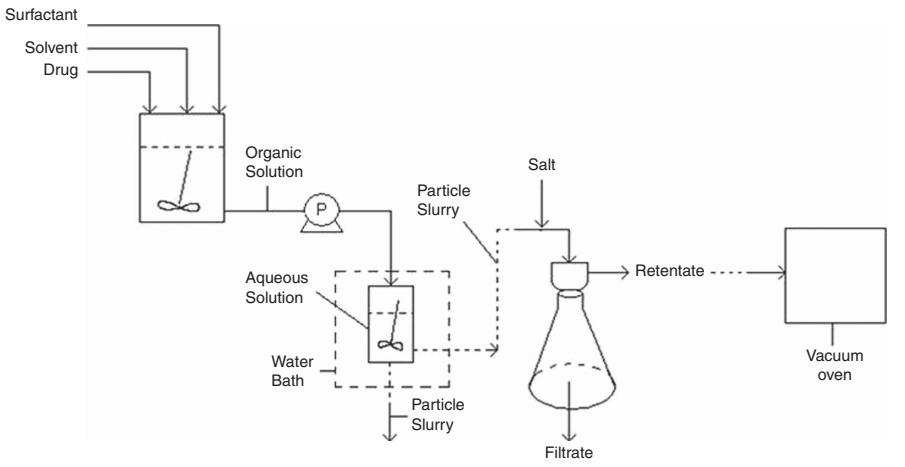

FIGURE 1. Process for producing pharmaceutical powder by antisolvent precipitation, flocculation with salt, filtration, and vacuum drying.

Recovery of Naproxen Nanoparticles by Salt Flocculation Followed by Filtration and Vacuum Drying

After particle size measurement, another suspension produced at exactly the same experimental conditions was sonicated in an ice/water bath at an output control of 7 and 30% duty cycle for 5 min. Then, a given volume of 20% (wt/vol) sodium sulfate was added into the suspension and mixed thoroughly with a spatula. The suspension was left at room temperature for 3 min to form large flocs. The flocs were filtered with P2 filter paper (Fisher Scientific, Fair lawn, NJ, USA) under vacuum (–27 in. Hg). Gentle stirring was necessary in the first 3 min of filtration to prevent forming a dense precipitate cake, which would increase resistance to filtration and result in a long filtration time. The filtration was continued for 10 s after no more water droplets were formed at the tip of the ceramic funnel. The precipitate was placed into a vacuum oven and dried overnight at room temperature and a vacuum of –30 in. Hg. To check the reproducibility of this process, for each formulation, triplicate samples were prepared.

The precipitate weight was determined after vacuum drying. A known amount of this dry powder, about 5 mg, was dissolved into 50 mL acetonitrile/water mixture, 50:49 (vol/vol). The naproxen loading was determined by measuring the naproxen concentration by HPLC (Shimadzu, LC-10AT *VP*, Kyoto, Japan). The salt concentration was determined from the electrical conductivity as described below. The surfactant composition in the final powder was calculated by difference given the total precipitate weight, the drug loading, and the salt concentration. The drug recovery was calculated from the HPLC measurement and the total amount of drug fed to the suspension in the antisolvent process. The surfactant recovery was calculated with the same method, given the surfactant composition in the dry powder. Wide-angle X-ray scattering was employed to detect the crystallinity of naproxen. Cu $K\alpha_1$ radiation with a wavelength of 1.54054 Å at 40 kV and 20 mA from a Philips PW 1720 X-ray generator (Philips Analytical Inc., Natick, MA, USA) was used. The samples were well-mixed to minimize the effects of preferred orientation. The reflected intensity was measured at a 2θ angle between 5° and 45° with a step size of 0.05° and a dwell time of 1 s.

Particle Size

Particle size distributions based on volume fraction were measured for the original antisolvent suspension prior to flocculation and for the dried powders after redispersion and sonication with laser light scattering (Mastersizer-S, Malvern Instruments Ltd., Malvern, UK). Approximately 5 mL of the suspension with a concentration of 10 mg drug/mL water was diluted with 500 mL distilled water, to produce a light obscuration in the desired range of 10–30%. The amount of drug in the water was several fold above the solubility limit. In control experiments, samples were stirred in the Mastersizer for up to 10 min, and there was very little change in the particle size distribution. To study the redispersibility of the dry powders, about 100 mg dry powder was suspended into 500 mL distilled water to produce an obscuration in the range 10–30%. After 1 min, the particle size distribution was measured. Ultrasound was used in the measurement to break up any aggregated particles. The uncertainty in mean particle size for different sprays at the same experimental conditions was about 10–15%.

Measurement of Residual Salt Concentration in Flocculated Samples by Conductivity

The residual concentration of sodium sulfate was measured by conductivity. The conductivities of a series of standard concentration of sodium sulfate solutions in acetonitrile/water mixture (vol/vol = 50:50) were measured with a conductivity

probe with cell constant of 1 cm^{-1} (Model 3252, YSI Inc., Yellow Springs, OH, USA). For salt concentrations ranging from 0.003 to 0.05 mg/mL, a linear standard line was obtained with a correlation coefficient of 0.9999. A small amount, about 5 mg, of dried naproxen powder was dissolved in the same acetonitrile/water mixture to yield a salt concentration in the linear range of the standard curve to determine the conductivity.

Morphology of the Flocs in the Suspension and After Drying

Optical microscopy and SEM were used to visualize the morphology of the naproxen flocs in the suspension and in the dried powder. A drop of antisolvent suspension flocculated with sodium sulfate solution was placed onto a microscope slide (25×75 mm^2, Erie Scientific Co. Portsmouth, NH, USA) and carefully covered with a cover glass (22×22, Fisher Scientific, Pittsburg, PA, USA). The morphology of naproxen flocs in the suspension was examined with an optical microscope (Axioskop 2 plus, Carl Zeiss Vision GmbH, Jena, Germany). Another suspension produced with the same experimental conditions was filtered with a P2 filter paper under vacuum for 11 min. The dried powders were mounted on an aluminum cylinder using double adhesive carbon conductive tabs (Ted Pella Inc., Redding, CA, USA) and coated with Au for 25 s using a Pelco Model 3 sputter-coater under an Ar atmosphere. A Hitachi S-4500 SEM (Hitachi Instruments Inc., Irvine, CA, USA) at an accelerating voltage of 10 kV with a secondary electron detector was used to obtain digital images of the samples.

Dissolution Test

The dissolution of naproxen powder was tested in pure water using a USP Apparatus II (Vankel 7000, Vankel Technology, Cary, NC, USA) at 50 rpm. All dissolution tests were conducted at sink conditions, in this case at 15% of its solubility in water. Antisolvent powders containing approximately 2 mg naproxen were added to 900 mL pure water at 37°C. Aliquots of the dissolution medium (5 mL) were sampled at 2, 5, 10, 20, and 30 min. The aliquots were filtered through 0.45-μm syringe filters and 2 mL of each sample was diluted with 0.1 mL acetonitrile before analysis. Naproxen concentrations were measured using HPLC (Shimadzu, LC-10AT *VP*, Japan). Dissolutions were repeated in duplicate or triplicate for the powder aliquots, and the average deviations are reported in the dissolution figures.

RESULTS AND DISCUSSION

Effect of Temperature on Drug Particle Size in Antisolvent Precipitation

The effect of temperature on the particle size distribution in the aqueous suspensions produced by antisolvent precipitation was determined. A mixture of 5% (wt/vol) naproxen and 2% (wt/vol) poloxamer 407 in ethanol solution was sprayed into 50 mL 3% (wt/vol) PVP K-15 at flow rate of 1 mL/min for 5 min. The final suspension concentration was 5 mg/mL. The organic solution was at room temperature. As shown in Figure 2, the mean particle size doubled from 270 nm at 0°C to 540 nm at 30°C. At temperatures higher than 30°C, the mean particle size increased markedly and reached 9.3 μm at 60°C. Several factors may lead to larger particles at high temperature, including degree of supersaturation, nucleation rate, crystal growth rate, interparticle attractive interactions between surfactant chains, and Ostwald ripening. The solubility of drug in the organic–water mixture increases with temperature. Thus, for a given drug concentration in the feed, the supersaturation decreases with temperature. The lower supersaturation lowers the nucleation rate. The smaller number of nuclei may be expected to produce larger particles for a given drug concentration in the final suspension. Also, the diffusion rate of drug molecules to the surface of the growing particles and the kinetics of addition of drug molecules to the surface increase with temperature. Furthermore, Ostwald ripening or diffusion of molecules from smaller crystals to larger crystals is also favored by higher solubility at higher temperature. The solvation of ethylene oxide groups in the poloxamer tails becomes stronger as temperature decreases due to hydrogen bonding between water and the ether oxygen (Blankschtein, Thurston, & Benedek, 1986; Chen, Young, Sarkari, Williams, & Johnston, 2002). Greater solvation will improve the repulsive forces between ethylene oxide blocks on approaching particles to improve steric stabilization. Based on these experiments, all other experiments in this paper were performed at or below room temperature.

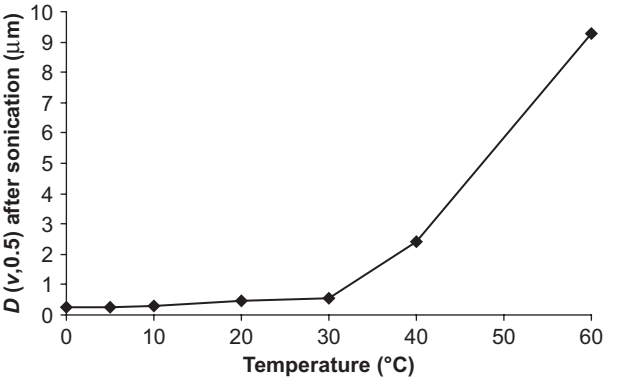

FIGURE 2. Temperature effect on particle size of naproxen suspensions produced by antisolvent precipitation. Organic phase: 5% (wt/vol) naproxen + 2% (wt/vol) poloxamer 407 in ethanol; aqueous phase: 50 mL 3% (wt/vol) PVP K-15; flow rate Q = 1 mL/min; t = 5 min; suspension concentration = 5 mg/mL.

Compositions of the Formulations

As shown in Table 1, four systems were studied. In system A, only 10% (wt/vol) PVP K-15 was used in the organic phase

TABLE 1
Systems Investigated

Systems	Organic Phase	Aqueous Phase	Drug/Poloxamer/PVP Ratio in the Suspension	Total Solid Weight (g)	Original Drug Loading (%)
A	10% PVP K-15	Pure water	1/0/1.4	1.22	41.2
B	10% poloxamer 407	3% PVP K-15	1/1.4/3	2.72	18.5
C	5% poloxamer 407	3% PVP K-15	1/0.7/3	2.36	21.3
D	5% poloxamer 407	1% PVP K-15	1/0.7/1	1.36	37.0

and there was no surfactant in the aqueous phase. This system was designed for long-term stability since PVP K-15 has a high glass transition temperature which enhances drug stability by decreasing the mobility of drug molecules. In system B, 10% (wt/vol) poloxamer 407 was used in the organic phase, as it has been shown to be an effective stabilizer (Chen, Lo, Sarkari, Williams, & Johnston, 2006; Chen et al., 2002; Matteucci et al., 2006) and 3% (wt/vol) PVP K-15 was included in the aqueous phase to raise the T_g of the final powder. To attempt to raise the loading of the drug, in system C the poloxamer 407 concentration was reduced to 5% (wt/vol) and in system D, the PVP K-15 concentration was reduced to 1% (wt/vol). The drug/poloxamer 407/PVP K-15 ratios in the final suspension based on the amounts fed from both organic and aqueous phase during spray time are also given. The total solid weight and total drug loading (weight of drug/total weight excluding salt) are listed for each system.

Particle Size of Naproxen in Aqueous Suspensions

As shown in Tables 2 and 3, aggregated naproxen nanoparticles were produced during antisolvent precipitation at both 22 and 3°C. With only PVP K-15 in the organic phase and pure water in the aqueous phase (system A, drug/poloxamer 407/PVP K-15 ratio = 1:0:1.4), the particle sizes were large in the original suspensions after spraying at either 22 or 3°C. Even after sonication in the laser light scattering chamber, the mean particle size was still undesirably large, as shown in Table 2. After the original suspensions were stirred with a magnetic stir bar for 20 h at room temperature and then sonicated, the mean particle sizes became extremely small, well below 0.4 μm at both temperatures. More surfactant may adsorb to the particles during stirring and aid breakup of the aggregates.

In order to attempt to reduce the aggregation of the particles at 3°C, 5% (wt/vol) poloxamer 407 was added to the aqueous phase to modify system A. The mean particle size of the original suspension was 15.9 μm. After 6 min sonication in the Mastersizer, the mean particle size decreased to 0.29 μm. In this case, the aggregates broke up into small particles without the need to stir the solution for 20 h. Because the copolymer poloxamer 407 has hydrophobic moieties that adsorb onto hydrophobic drug particle surfaces and two separate hydrophilic blocks, the adsorption of poloxamer 407 with PVP K-15 may be expected to provide greater steric repulsion and looser aggregates. The looser aggregates may be broken up more easily by sonication on the basis of the smaller particle sizes.

The particle sizes for the formulations in addition to system A are presented in Table 3. The results for the suspensions prior to flocculation are shown in the second and third columns. To avoid the need for stirring for long periods of time to break up aggregates, the organic phase stabilizer was changed to poloxamer 407 for all of the systems in Table 1 except system A. In addition, PVP K-15 was utilized in the aqueous phase to achieve a sufficiently high T_g for the final powder. For systems B and C, the mean particle sizes were extremely small, below 0.5 μm, in the original suspension even without sonication. After sonication, the particle size decreased only a small amount. However, when the PVP K-15 concentration in the aqueous phase was lowered to 1% (wt/vol) in system D, the mean particle size of the original suspension increased to 9.8 μm. These aggregates readily broke up upon sonication. After sonication the mean particle size was 0.29 μm for systems B–D, although the D (v, 0.9) was 7.0 μm for system D. Without sonication, a higher concentration of 3% (wt/vol) PVP K-15 in the aqueous phase was helpful in forming the nanosuspension. In systems B and C, the overall concentration of PVP K-15 was much higher than that of poloxamer 407, unlike the case for system D. The higher overall stabilizer concentration may have been required to provide enough steric stabilization to prevent aggregation of naproxen nanoparticles.

Polymer-Salt Cloud Point Concentrations and Flocculation of Naproxen Nanoparticles with Various Salts

Sterically stabilized dispersions may be flocculated by reducing the solvency of the dispersion medium for the stabilizing moieties to induce the onset of instability. Pelton (1988) reported that the critical flocculation temperature (CFT) corresponds to the cloud point temperature of the stabilizing polymer. A few reports have described the influence of inorganic salts on the cloud point behavior of poloxamers (Bahadur, Li, Almgren, & Brown, 1992; Bahadur, Pandya, Almgren, Li, & Stilbs, 1993; Pandit, Trygstad, Croy, Bohorquez, & Koch, 2000) and PVP (Güner & Ataman, 1994; Salamova & Rzaev, 1996; Sekikawa, Hori, Arita, Ito, & Nakano, 1978). As shown in Figure 3, the cloud point temperatures of PVP 44,000 and

TABLE 2
Particle Size of Naproxen Suspensions Produced by Antisolvent Precipitation Versus Time for System a Without any Added Salt

Temperature	T = 22°C			T = 3°C		
Suspension	$D(v, 0.1/0.5/0.9)$ w/o Sonication (μm)	$D(v, 0.1/0.5/0.9)$ After Sonication with Mastersizer (μm)	Sonication Time (min)	$D(v, 0.1/0.5/0.9)$ w/o Sonication (μm)	$D(v, 0.1/0.5/0.9)$ After Sonication with Mastersizer (μm)	Sonication Time (min)
Original suspension in DI water	7.5/15.9/28.4	2.4/5.3/7.2	7	6.2/12.6/153.1	2.3/4.5/6.3	15
After 20 h stirring at room temperature	4.5/6.4/11.0	0.15/0.36/2.1	3	6.1/12.1/19.7	0.11/0.29/0.88	5
Original suspension in 5% (wt/vol) poloxamer 407				6.9/15.9/42.4	0.10/0.29/3.3	6

Organic phase: 7% (wt/vol) naproxen + 10% (wt/vol) PVP K-15 in methanol.

TABLE 3
Particle Size of Naproxen in the Original Suspension Before and After Sonication, and After Flocculation, Filtration, Vacuum Drying, and Redispersion into Pure Water

Systems	$D(v, 0.1/0.5/0.9)$ in the Original Suspension (μm)	$D(v, 0.1/0.5/0.9)$ After 5 min Sonication (μm)	V of Salt (mL)	C_{salt} in Suspension (M)	$D(v, 0.1/0.5/0.9)$ After Redispersion into Water (μm)	$D(v, 0.1/0.5/0.9)$ After Redispersion and Sonication (μm)	$t_{son.}$ (min)	Release in 2 min (%)
A	4.5/6.4/11.0	0.15/0.36/2.1	175	1.10	16.5/91.6/243.0	0.12/0.32/3.24	3	27.3
A			200	1.13	29.4/155.7/447.1	0.22/2.02/11.6	6	25.5
B	0.14/0.48/95.2	0.11/0.29/1.01	100	0.94	0.10/0.30/6.8	0.10/0.27/3.07	1	95.4
B			125	1.01	0.11/0.31/1.87	0.11/0.28 /1.13	1	96.4
B			150	1.06	0.23/0.48/19.3	0.11/0.32/7.72	1	74.8
C	0.11/0.30/3.8	0.12/0.29/0.68	125	1.01	0.11/0.31/2.57	0.10/0.25/0.79	1	94.9
D	0.14/9.8/173.9	0.10/0.29/6.95	125	1.01	0.28/2.65/22.3	0.19/0.38/4.10	2	49.5

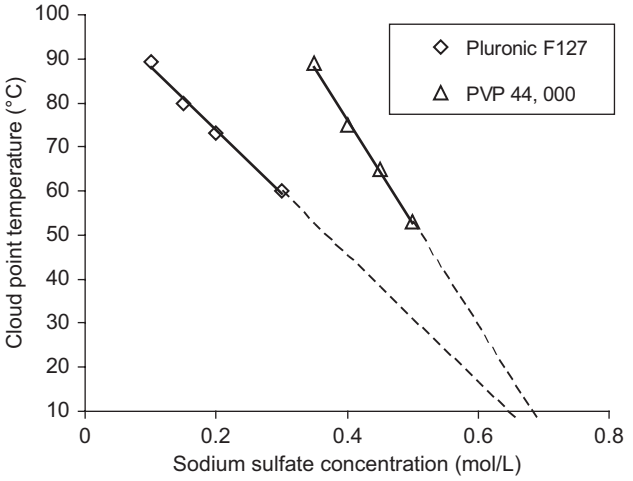

FIGURE 3. Cloud point temperature of PVP (Salamova & Rzaev, 1996) and poloxamer 407 (Pluronic F127) (Pandit et al., 2000) at various sodium sulfate concentrations in water.

poloxamer 407 decrease linearly with increasing concentrations of sodium sulfate. At a given temperature, the polymer precipitates as the salt concentration increases. In Figure 3, the concentration of PVP was 0.74% (wt/vol) on the basis of a partial specific volume of 0.952 cm^3/g for PVP (Pleštil & Al, 2001). The concentration of poloxamer 407 was 5 mg/mL or 0.5% (wt/vol). PVP contains N–C = O units on the lactam rings that hydrogen bond with water as observed with viscometric measurements (Guven & Eltan, 1981) and spectrophotometric studies (Turker, Guner, Yigit, & Guven, 1990). The decrease of the Huggins constant with the addition of inorganic salt into PVP aqueous solution indicates a loss in water–PVP H-bonding, and thus in PVP hydration, leading to precipitation (Salamova & Rzaev, 1996). For the same reasons, salt also precipitates PEO homopolymers (Khoultchaev, Kerekes, & Englezos, 1997a; Khoultchaev, Kerekes, & Englezos, 1997b; Pang & Englezos, 2002a; Pang & Englezos, 2002b) and of PEO-containing nonionic surfactants such as poloxamers (Bahadur et al., 1992; Bahadur et al., 1993; Pandit et al., 2000).

The reciprocal of cloud point temperatures, $1/T_{cp}$ of two fractionated and two unfractionated PVP samples have been shown to be linear in $1/\overline{M}_w^{1/2}$ (Güner & Ataman, 1994). With a lower molecular weight of 10,000 PVP, more sodium sulfate is required to precipitate the PVP K-15 used in this study than for the PVP 44,000 shown in Figure 3 (Sekikawa et al., 1978).

The stabilizing moieties collapse at the onset of the cloud point where the solvent is worse than a θ-solvent, as a result of the large number of interactions of the hydrophilic groups (Napper, 1983). Steric repulsion becomes weak and the stabilizing chains interact with each other leading to sticky Brownian collisions and flocculation. Some free surfactant might also precipitate out of solution and interact with flocculating particles. Because the particle size of coated naproxen was typically larger than 200 nm and the molecular weight of stabilizing polymers was not more than 12,500, the steric stabilization of the polymer chains does not completely screen the van der Waals attraction between the particles (Napper, 1983).

To more fully understand the effect of salinity on flocculation, the minimum salinity to desolvate polymer was measured for a 1.26% (wt/vol) concentration of either PVP K-15 or poloxamer 407. The solvent was a mixture of methanol (12.6%, vol/vol) and water (87.4%, vol/vol) with the same polymer composition as the system A suspension. A concentration of 1.06 M sodium sulfate was needed to precipitate PVP from solution at room temperature in the methanol/water mixture while only 0.98 M was needed for the case of methanol-free water. For pure poloxamer 407, 0.71 M sodium sulfate concentration was needed for the methanol/water mixture, while 0.58 M was needed without methanol. Apparently, at the same concentration and temperature, poloxamer 407 was precipitated much more easily with sodium sulfate than was PVP K-15, which is consistent with Figure 3. Less salt is needed to desolvate poloxamer 407 than PVP (M_w = 44,000) at 25°C from pure water. This difference would be expected to be even larger for PVP K-15 as a greater salt concentration would be required to collapse the lower molecular weight (Sekikawa et al., 1978). The salinities required to precipitate the polymers, despite the presence of methanol, are in the same order expected from the cloud points. Methanol also raises the cloud point temperature for poloxamine 908, a copolymer containing polyethylene oxide moieties (Na, Yuan, Stevens, Weekley, & Rajagopalan, 1999).

A much lower salt concentration is required for a divalent sulfate to precipitate these polymers relative to a monovalent anion (Pandit et al., 2000; Salamova & Rzaev, 1996). Three multivalent inorganic salts which are known to produce a large reduction in the cloud point temperature of PVP (Salamova & Rzaev, 1996) and poloxamer 407 (Pandit et al., 2000), sodium carbonate, sodium sulfate, and sodium phosphate, were used to attempt to flocculate polymer-stabilized naproxen nanoparticles. Large flocs were formed with sodium sulfate, and they were readily filterable. Solutions of 20% (wt/vol) sodium carbonate and 10% (wt/vol) sodium phosphate were also tested for flocculation. In each case, when a small volume of the salt solution was added to the suspension to produce a molarity less than 0.04 M, a clear solution was formed as the naproxen particles dissolved in the basic media. When 0.4 mL sodium carbonate or 1 mL sodium phosphate solution was added to 25 mL of the suspension, the pH of the solution increased to 11.3 for CO_3^{2-} and 11.9 for PO_4^{3-} due to the acid–base hydrolysis. The solubility of naproxen increases from 0.0159 mg/mL in pure water to 196.7 mg/mL at a pH higher than 8 (Chowhan, 1978), which is well above the pK_a of naproxen. This solubility is much higher than the naproxen concentration in the suspension, consistent with our observation of dissolution. Based on these results all of the flocculation experiments were performed with sodium sulfate.

Dissolution Rate

The dissolution rates of the dried naproxen powders are shown in Figure 4A for System B and for various formulations in Figure 4B. The dissolution rates were reproducible as shown by the error bars that represent the average deviation, defined by $(1/n)\sum|x-\bar{x}|$. The dissolution rates were very high for systems B and C, and significantly slower for systems A and D. As shown in Figure 4A, approximately 100% naproxen was dissolved in 2 min for the suspension dried by lyophilization. For samples flocculated with 100 or 125 mL salt solution, the dissolution rates of naproxen were only slightly slower than for the lyophilized ones. The flocculated sample of system B was stored with desiccant under vacuum at room temperature. After 1 month, the dissolution rate of this sample did not change, as shown in Figure 4B. The differences in these dissolution rates will now be analyzed in terms of the particle size distributions.

Particle Redispersibility into Water after Flocculation Followed by Filtration and Vacuum Drying

The dry powder was redispersed into pure water, and the particle sizes are shown in the sixth and seventh columns of Table 3. For system A flocculated with a salt concentration of 1.10 or 1.13 M, the filtration time was approximately 8 min and the dried naproxen particles redispersed to form a very coarse suspension upon stirring without sonication. The mean particle size of the dried particles was 91.6 μm at 1.10 M salt concentration, but decreased to 0.32 μm after 3 min sonication. With a higher salt concentration of 1.13 M, the mean particle size of naproxen decreased from 155 μm without sonication to 2.02 μm after 6 min sonication. Because the mean particle size was only 0.29 μm after flocculation and filtration, but before drying, the particle growth must have taken place during the vacuum drying step. The slow dissolution rates in system A are consistent with the large particle sizes upon redispersion without sonication.

To study the reversibility of flocculation, a suspension flocculated at 1.13 M salt concentration was filtered with a 0.45-μm Whatman® nylon membrane filter paper (Whatman International Ltd., Maidstone, England) for 43 min. The particle size of redispersed naproxen after drying was 17.1 μm after sonication. Apparently, if the particles are allowed to remain flocculated for more than 40 min, redispersion is no longer complete. Perhaps slow diffusion of the stabilizer away from the high-energy zones that are created by the close approach of the particles leads to irreversible aggregation (Napper, 1983). In contrast, the filtration times were on the order of 10 min for the other experiments.

For system B, with either 0.94 or 1.01 M salt, the dried particles could be redispersed into water readily with particle sizes similar to the original suspensions. Consequently, the dissolution rates were extremely rapid with 96% release in 2 min. As the salt concentration was increased to 1.06 M, the mean size of redispersed particles increased modestly to 0.48 μm with a

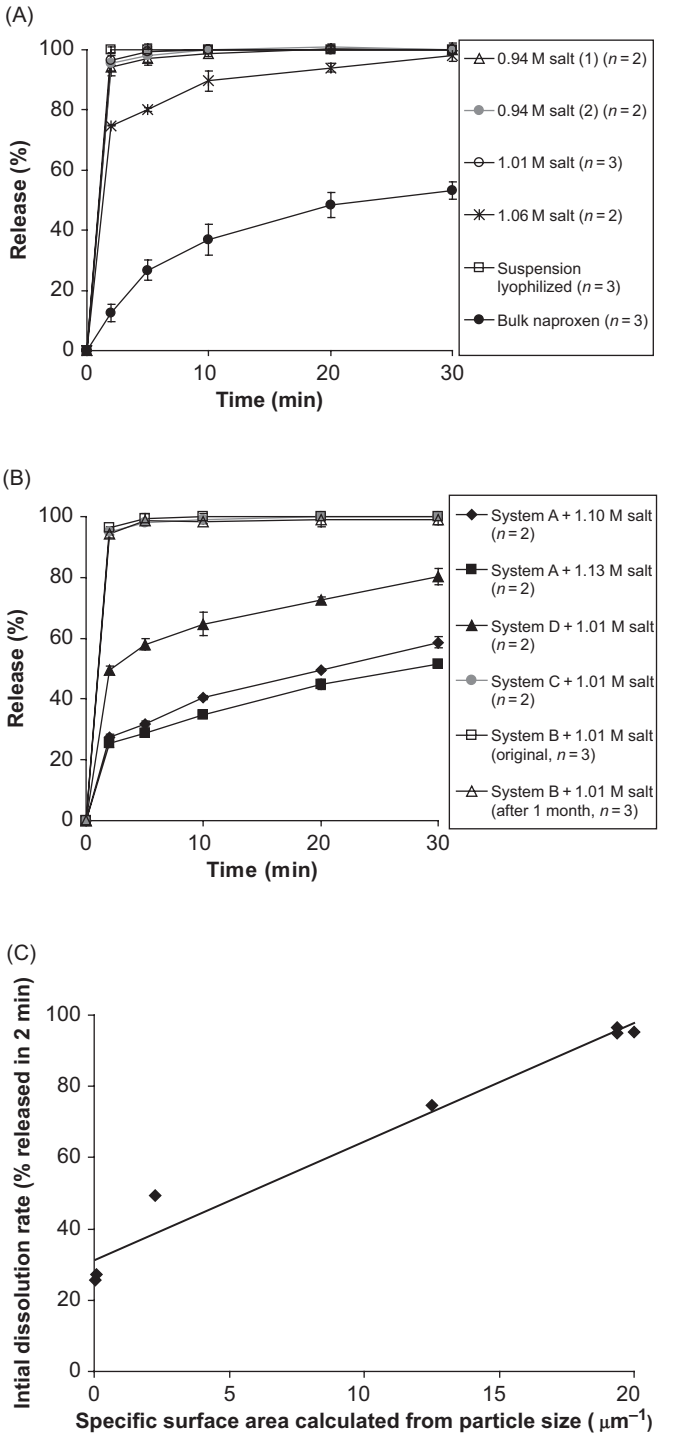

FIGURE 4. (A) Effect of salt concentration on dissolution rate of flocculated, filtered and vacuum-dried naproxen nanoparticles produced by antisolvent precipitation (system B). Conditions were the same as in Tables 3 and 4. As indicated, the suspension was lyophilized in one case. (B) Effect of stabilizers on dissolution rate of flocculated, filtered, and vacuum-dried naproxen nanoparticles produced by antisolvent precipitation. Conditions were the same as in Tables 3 and 4. (C) Correlation between dissolution rate and specific surface area of flocculated, filtered, and vacuum-dried naproxen particles (R^2 = .97).

high D (v, 0.9) of 19.3 μm. Likewise, the dissolution rate decreased to 75% in 2 min. Here, 1 min sonication was needed to reduce the particle size to the original value prior to flocculation. Higher salt concentrations appeared to produce tighter flocs after drying, requiring sonication to fully break them up, whereas sonication was not needed for the lower salt concentrations.

For system C, particles flocculated at 1.01 M salt concentration could be redispersed easily after drying to achieve a similar particle size as in the original dispersion. Consequently, the dissolution rate was extremely fast as in the case for the two lowest salinities in system B. However, for system D, with a low PVP K-15 concentration in the aqueous phase, the mean particle size of the redispersed dried powders without sonication was extremely large, 2.7 μm. The size decreased most of the way to the original value prior to flocculation after 2 min sonication. The dissolution rate was much slower than in systems B and C. Therefore, the dissolution rate is more closely related to the aggregate size than the size of the particles after sonication.

For all of the examples in Table 3, the dissolution rate was correlated closely with the particle size of the redispersed powder without sonication. Assuming spherical particles, the specific surface area of naproxen particles was calculated by, $S = 6/D$, where D is the average diameter of the particles from light scattering shown in the sixth column of Table 3. As shown in Figure 4C, a straight line with a correlation coefficient of 0.97 was obtained between initial dissolution rate (% released in 2 min) and specific surface area of naproxen particles after redispersion. This correlation is consistent with Noyes–Whitney equation, where the dissolution rate is proportional to the specific surface area of drug particles.

Reproducibility of Salt Flocculation Process on Precipitate Weight, Drug Loading, Salt Concentration, Surfactant Concentration, Drug, and Surfactant Yield

The filtration time and properties of the precipitate including naproxen loading (drug/total solids, wt/wt), drug yield (fraction in precipitate vs. total amount fed), surfactant yield (fraction in precipitate), and filtration selectivity ([g precipitate drug/g filtrate drug]/[g precipitate surfactant/g filtrate surfactant]) for four systems flocculated with various concentrations of sodium sulfate are shown in Table 4. Compared to the original naproxen loadings in Table 1 for the suspensions, the naproxen loadings in the precipitate were higher, since some free or nonadsorbed surfactant was removed with the filtrate. They were higher despite the presence of salt in Figure 4, relative to no salt for the drug loadings in Table 1. For system A, even at a salt concentration of 1.10 M, the resulting flocs were not stable. Stirring or pouring of the suspension broke up the fragile flocs. Thus, the precipitate weight was not reproducible, relative to the other systems. The poor flocculation was due to a salinity too close to the critical flocculation salinity, 1.06 M with 12.6% (vol/vol) methanol in the suspension. When salt concentration was increased to 1.13 M, larger flocs formed and consequently the drug and surfactant yields increased.

For system B, a control was performed in which the suspension was filtered without adding any salt. Without flocculation by salt, only 0.050 g precipitate was recovered as the particles were too small for the filter. At salt concentration of 0.47 M, only a small amount of flocculation occurred, and the drug particles plugged up the filter paper in 5 min. As the salt concentration increased from 0.94 to 1.01 to 1.06 M, the drug and surfactant yields increased significantly. A drug yield higher than 92% was obtained at a salt concentration higher than 1.4 times the critical flocculation salinity of poloxamer 407. The naproxen loading decreased a small amount with salinity and was about double the original loading. The relative deviation (average deviation/mean value) of the precipitate weight decreased from 5.5% with 0.94 M salt to 0.9% with 1.01 or 1.06 M salt. The increase of reproducibility of precipitate weight and drug yield with salt concentration likely indicates more stable, stronger and larger aggregates that could be filtered more effectively. An increase in aggregate size with an increase in the distance above the cloud point temperature has also been observed for clay particles stabilized with PEO formed at higher temperature above the cloud point (Pang & Englezos, 2002a). The increase in the flocculation efficiency was attributed to an increase in the tendency for the PEO to phase separate and adsorb onto the clay particles (Pang & Englezos, 2002a).

As the poloxamer 407 concentration was lowered twofold in system C relative to system B, for a given salinity, the drug loading increased from 36.7 to 54.1%, yet the dissolution rate remained extremely high. The drug loading was over 2.5 times that of the original loading in the suspension. The drug yield increased to 97.6% while the surfactant yield decreased significantly. Furthermore, the particle size changed very little. Given these beneficial results, the PVP K-15 concentration was reduced by a factor 3 in system D relative to system C and the drug yield increased to nearly 100%. The concentrations of drug and surfactant were similar, while the salt concentration decreased to 6.4%.

The selectivity for drug particles over surfactants ([g precipitate drug/g filtrate drug]/[g precipitate surfactant/g filtrate surfactant]) in the filtration process was very high and usually quite reproducible as shown is the last column of Table 4. The high deviation for system C was due to high drug yield, and thus the very small amount of drug in the filtrate. The excellent reproducibility in nearly all of the properties in Table 4 for most systems makes the flocculation/filtration concept relevant for practical application.

Based on dissolution rate and drug recovery, systems B and C, flocculated at 1.01 M salt concentration, may be expected to produce the highest drug bioavailability. The recommended daily dose of naproxen in adults for rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis is 500–1,000 mg

TABLE 4
Properties of the Precipitate After Vacuum Drying Including Selectivity for Drug Versus Surfactant

Systems	C_{salt} in Suspension (M)	Filtration Time (min)	Precipation Weight (g)	Drug Loading (%)	Salt Concentration (%, wt/wt)	Surfactant Conc. (%, wt/wt)	Drug Yield (%)	Surfactant Yield (%)	Selectivity for Filtration
A	1.10	8.6	0.5083 ± 0.0994 (n = 3)	69.0 ± 0.2 (n = 3)	25.2 ± 0.3 (n = 3)	5.8 ± 0.4 (n = 3)	70.2 ± 14.0 (n = 3)	4.4 ± 0.2 (n = 3)	94.5 ± 73.9
A	1.13	7.7	0.6552 ± 0.0030 (n = 2)	66.3 ± 0.7 (n = 2)	19.6 ± 0.7 (n = 2)	14.1 ± 1.4 (n = 3)	86.9 ± 1.3 (n = 3)	12.9 ± 1.2 (n = 3)	45.5 ± 6.8
B	0.94	16.5	1.0768 ± 0.0596 (n = 3)	38.2 ± 0.8 (n = 3)	10.6 ± 1.4 (n = 3)	51.2 ± 1.1 (n = 3)	79.7 ± 1.4 (n = 3)	24.1 ± 0.9 (n = 3)	12.4 ± 0.5
B	1.01	11	1.2523 ± 0.0113 (n = 4)	36.7 ± 0.7 (n = 4)	10.5 ± 0.5 (n = 4)	52.9 ± 0.5 (n = 3)	91.8 ± 1.4 (n = 3)	29.9 ± 0.3 (n = 3)	28.8 ± 8.5
B	1.06	11.5	1.4825 ± 0.0139 (n = 3)	31.8 ± 0.7 (n = 3)	9.3 ± 0.5 (n = 3)	58.9 ± 1.0 (n = 3)	94.1 ± 1.7 (n = 3)	39.4 ± 1.0 (n = 3)	28.9 ±12.3
C	1.01	12	0.9021 ± 0.0017 (n = 2)	54.1 ± 0.5 5 (n = 2)	8.8 ± 0.0 (n = 2)	37.1 ± 0.7 (n = 2)	97.6 ± 1.5 (n = 2)	18.0 ± 0.3 (n = 2)	316.1 ± 206.5 5
D	1.01	14	0.8930	55.7 ± 0.5 (n = 2)	6.4 ± 0.2 (n = 2)	37.9 (n = 2)	99.4 (n = 2)	39.6 (n = 2)	256.8

(Fuller & Sajatovic, 1999). According to the ratio of sodium sulfate to naproxen in systems B or C, the daily dose of sodium sulfate from these powders would be 81–286 mg. This amount is much less than the commercial daily dose from OCL®, a saline laxative (Abbott), in which 1.29 g sodium sulfate was used, indicating that the sodium sulfate would not present toxicity limitations.

Morphology of Flocculated Naproxen Nanoparticles by Optical Microscope and SEM

The morphology of naproxen nanoparticle flocs of system B flocculated at 1.01 M salt concentration in the suspension and after drying is shown in Figure 5A and B. As shown in Figure 5A, naproxen flocs with sizes ranging from 5 to 150 μm were formed in the suspension. These flocs were larger than the 2 μm limit for P2 filter paper and thus more than 90% of the naproxen was recovered. As shown in Figure 5B (left panel), a floc with size larger than 30 μm was formed during flocculation and drying. This particle, however, was composed of primary nanoparticles of approximately 300 nm, shown on the right, consistent with the size measured by light scattering.

X-Ray Diffraction

X-ray diffraction was used to analyze the crystallinity of the dry powders. As shown in Figure 6, the largest naproxen peaks coincided with those of bulk poloxamer 407. The naproxen peaks were much smaller and broader in the physical mixtures of the various formulations as well in the dried powders produced by antisolvent precipitation. However, the naproxen peaks at 2θ = 22.6° and 23.9°, especially in the samples with

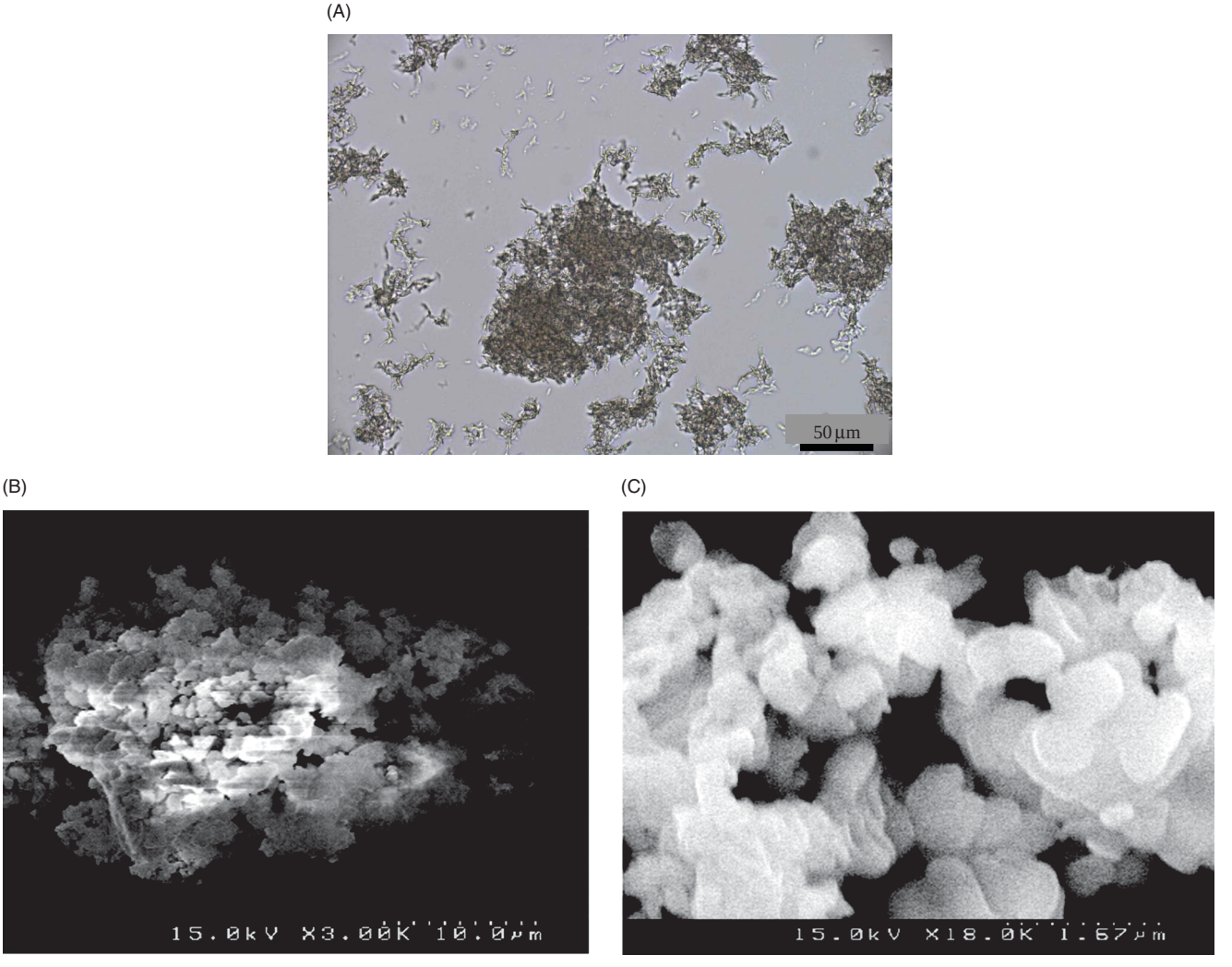

FIGURE 5. (A) Microscopic picture of naproxen flocs of system B in suspension at salt concentration of 1.01 M. (B) and (C) SEM pictures of naproxen flocs after filtration and vacuum drying. Same sample as in A.

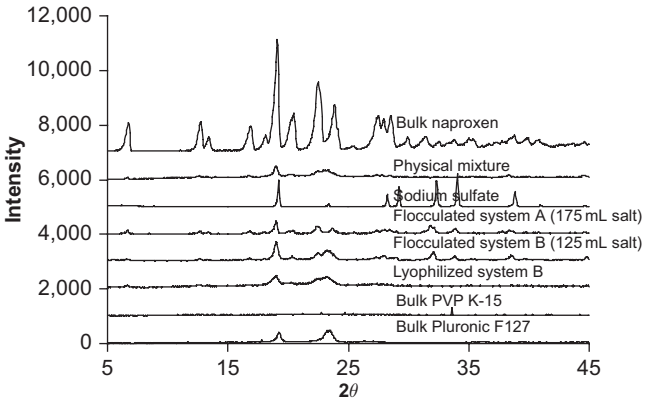

FIGURE 6. X-ray diffraction of flocculated, filtered, and vacuum-dried or lyophilized naproxen particles produced by antisolvent precipitation. Conditions were the same as in Tables 3 and 4.

high drug loading produced by flocculation, were evident. Thus, crystalline naproxen was formed whether the suspension was dried by lyophilization or flocculation followed by filtration and vacuum drying. The presence of sodium sulfate in the final powder is also apparent in the diffraction pattern.

CONCLUSIONS

Crystalline naproxen submicron particles were formed by antisolvent precipitation in the presence of polymeric steric stabilizers. The nanocrystals were successfully recovered from aqueous suspensions by flocculation with sodium sulfate followed by filtration and vacuum drying. The flocculation was often reversible as the particle size upon redispersion in water, without sonication, was on the order of only 300 nm and comparable to the original particle size in the aqueous suspension prior to flocculation. The size measured by light scattering was consistent with the primary particle size in the powders measured by SEM. Less salt was needed for flocculation when the stabilizer was poloxamer 407, with hydrophilic ethylene oxide groups, than for PVP K-15, with N–C=O units on lactam rings, consistent with the trends in the cloud point phase equilibria curves. The dissolution rate was linearly correlated with the specific surface area calculated from the average particle diameter after redispersion without sonication. Extremely rapid dissolution, up to 95% of the powder in 2 min, was achieved for 300-nm particles. The salt concentration could be optimized to control the flocculation to balance the drug yield versus loading with excellent reproducibility in both properties (average deviation ranged in 1–2%). The drug loading of flocculated samples was enhanced in the filtration step by up to 61% relative to the initial value, due to removal of surfactant with the filtrate. The yield of the drug in the powder was typically 92–99% and the drug loading varied by only 1–2%. The residual sodium sulfate concentration in the dried powders, typically 10% or less was far below toxic limits based on the recommended dose of naproxen. The flocculation/filtration process simplifies the drying step relative to lyophilization and spray drying. Flocculation followed by filtration and drying is an efficient and highly reproducible process for the rapid recovery of drug nanoparticles to produce wettable powders with high drug loading and high dissolution rates.

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