Ketoprofen Nanoparticle Gels Formed by Evaporative Precipitation into Aqueous Solution

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DOI 10.1002/aic.10848
Published online April 25, 2006 in Wiley InterScience (www.interscience.wiley.com).

Aqueous nanoparticle gels of a poorly-water soluble drug, ketoprofen, were produced by evaporative precipitation into aqueous solution (EPAS). Liquid droplets of surfactant stabilized ketoprofen containing residual solvent were dispersed in water from 60 to 90°C below the melting point of pure ketoprofen. The carboxylic acid group in ketoprofen dissociates in pure water, providing electrostatic stabilization of the droplets to complement steric stabilization. Stable amorphous ketoprofen particles with a mean size of 135 nm, measured by dynamic light scattering, were formed with only 0.1% w/v poloxamer 407, resulting in an exceptionally high drug-to-surfactant ratio of 10:1. For 5% w/v poloxamer 407, interactions with ketoprofen produced a bluish, transparent gel composed of ~50 nm particles. In 2 min, 98% of the ketoprofen in the gel nanoparticles dissolved. The favorable interactions between the ketoprofen and poloxamer 407, along with the electrostatic and steric stabilization, lead to gelation, which further stabilizes the unusually small particles. The rapidly dissolving wet gels with extremely small particle sizes, one month stability, and relatively low viscosities, are of interest in transdermal and parenteral delivery; furthermore, the gels may be dried for oral delivery. © 2006 American Institute of Chemical Engineers *AIChE J,* 52: 2428–2435, 2006
Keywords: ketoprofen, nanoparticle, precipitation, suspension, gel

Introduction

The bioavailability of many class II poorly water-soluble drugs is dissolution-rate limited as their high lipophilicity leads to rapid permeation of biomembranes.[1] According to the Noyes-Whitney equation,[2] dissolution rates may be increased by reducing the particle size to increase the surface area. Coating drug particles with polymeric and low molar mass hydrophilic stabilizers enhances wetting of this interfacial surface by intestinal fluids, thus further enhancing dissolution. Recently a new technology, evaporative precipitation into aqueous solution (EPAS), was introduced to form rapidly dis-

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solving poorly water-soluble drugs coated with hydrophilic stabilizers.[3-7] The process involves dissolving a drug in an organic solvent and spraying through an atomizer into an aqueous solution to produce an aqueous dispersion. Amphiphilic stabilizers diffuse to the surface of the growing particles to inhibit particle growth and, in some cases, crystallization. Although nanoparticles have been produced for cyclosporine A,[3] particle aggregates several microns in diameter composed of smaller primary particles have been reported for other drugs, such as danazol and itraconazole.[4,5] The relatively high surface areas (on the order of 5 m^2/g) led to rapid dissolution, which is desirable for oral delivery formulations. However, for parenteral, pulmonary, and transdermal applications, it would be desirable to produce aqueous dispersions in which the particle size is well below 1 micron.[8-10]

The formation of an aqueous gel during nucleation of a drug may offer a means to arrest the growth of drug particles and achieve particle sizes below 500 nm. The influence of various hydrogen bonding organic solvents, for example, glycerol and propylene glycol, on the stability of liquid crystalline gels has been reported.[11] Based on these studies, it is possible that a drug such as ketoprofen will favor the gelation of surfactants such as Poloxamer 407. After formation of the gel, it is often desirable to remove part of the solvent. The kinetics of formation of various lyotropic liquid crystal phases of poloxamer-based hydrogels upon drying has been analyzed.[12] The controlled release of small molecular weight molecules from these gels at high concentrations has been examined in vitro and modeled mathematically to describe the diffusion mechanism.[13] The release from gels may be manipulated with smart polymers, such as pH sensitive materials.[14,15]

The primary objective of this study was to develop an understanding of how to produce stable drug nanoparticles by gelation with polyethylene oxide-*b*-polypropylene oxide-*b*-polyethylene oxide triblock copolymers by EPAS. EPAS typically produces micron-sized particles.[4-6] Ketoprofen, a nonsteroidal anti-inflammatory drug, was chosen as a model drug on the basis of its tendency to ionize in neutral water and its low melting point. The particle size was studied as a function of the drug concentration in the organic feed solution, to influence the supersaturation, the surfactant structure, the suspension concentration (typically 10 to 30 mg/ml), and the surfactant concentration in the aqueous suspension. Poloxamer 407, a polyethylene oxide-*b*-polypropylene oxide-*b*-polyethylene oxide triblock copolymer, was used to form viscous hydrogels in the aqueous phase with ketoprofen upon cooling to prevent particle growth. Ketoprofen is shown to interact with the polymer to lower substantially the polymer concentration needed for gelation. In this study, amorphous particles are reported at low poloxamer 407 concentrations. Typically EPAS produces crystalline drugs,[4–5,16] with some exceptions.[3] Electrostatic repulsion of the particles is characterized as a function of pH and added salt. The dissolution rate of the ketoprofen in the original hydrogels, dried hydrogels, and non-gelled suspensions is reported, along with the change in the particle size in the gel after one month.

Materials and Methods

Materials

Ketoprofen, poloxamer 407, Pluronic F68, Polyvinylpyrrolidone (PVP K-15, M_w = 10,000), and hydrolyzed Polyvinyl alcohol (PVA, M_w = 22,000) were purchased from Spectrum Chemicals & Laboratory Products Corporation (Gardena, CA). Myrj 52 (Sigma, St. Louis, MO) was used as received. Spectral grade dichloromethane was from Fisher Scientific Co. (Fairlawn, NJ). HPLC grade methanol was obtained from EM Industries Inc. (Gibbstown, NJ).

Determination of ketoprofen solubility in poloxamer 407 micelles

Excess ketoprofen was added into 50 ml 1%, 2%, and 5% w/v poloxamer 407 aqueous solutions, respectively, and stirred with a magnetic stir bar for 24 h to achieve equilibrium. 10 ml suspensions were filtered with a 0.45 μm Acrodisc GHP syringe filter (Pall Corporation, Ann Arbor, MI), and then 2 ml of the filtrates were mixed with 0.2 ml of methanol/water mixture (v:v = 57:40). All samples were then filtered through 0.45 μm filters, and ketoprofen concentrations were measured by HPLC (Shimadzu, LC-10AT *VP*, Japan) with an Alltech 150 mm $\times$ 4.6 mm Intersil 5 μm ODS-2 reverse-phase column (Alltech Associates, Inc., Deerfield, IL).

Evaporative Precipitation into Aqueous Solution (EPAS)

The EPAS apparatus was described elsewhere.[3-6] The organic ketoprofen solution with or without surfactant solution in dichloromethane was atomized through an elliptical conical nozzle into hot water at a flow rate of 1 ml/min and pressure drop of 23 to 34 MPa. 100 ml aqueous stabilizing solution was added to a graduated cylinder that was submerged in a temperature-controlled water bath. The nozzle was submerged approximately 4 cm under the surface of the aqueous solution. Unless indicated otherwise, the stabilizer was added in the aqueous phase and was not present in the organic feed solution. After spraying for a required time, typically less than 20 min, to produce the desired drug/surfactant ratio, the suspension was recovered and analyzed within 2 h to determine the particle size by dynamic light scattering (DLS).

Particle size and particle size distribution

Unless specified elsewhere, the particle size distribution was measured in the aqueous suspensions by dynamic light scanning (Brookhaven Zetaplus, Brookhaven Instruments Corporation, Holtsville, NY). The EPAS suspension was diluted with distilled water until the count rate was in the range of 5-250 kcps. For example, for a suspension concentration of 30 mg/ml, 0.2 ml of the EPAS suspension was added to 10 ml of water. The particle size distributions were based on intensity. The size distribution of the EPAS suspensions was measured within 2 h and did not change overnight. The particle size of the ketoprofen gel increased only slightly over one month, as discussed below.

Crystallinity and surface area measurement

The crystallinity of the dry powders was examined by X-ray diffraction with a Philips PW 1720 X-ray generator (Philips Analytical Inc., Natick, MA). The reflected intensity of EPAS samples was measured between 5° to 45° with a step size of 0.05° and a dwell time of 1 s. The surface area of the dry powders was measured with a high-speed surface area BET

analyzer (NOVA 2000, Quantachrome Instruments, Boynton Beach, FL) with nitrogen as adsorbate.

Viscosity measurement

The viscosity of the ketoprofen-poloxamer 407 gel was measured with a Brookfield digital viscometer (model DV-I+, Brookfield Engineering Laboratories, Inc. Middleboro, MA) at room temperature. A spindle (LV 2 with a LV guardleg) was rotated at various speeds in a 500 ml gel sample contained in a 600 ml beaker. The viscosity was measured at 6 min after rotation of the spindle.

Dissolution test

After freeze drying or vacuum drying, dry powder containing ~5 mg ketoprofen was placed into a USP Apparatus II (Vankel 7000, Vankel Technology Group, Cary, NC) and stirred at 50 rpm in purified water. 5 ml aliquots of the dissolution medium were sampled and filtered through 0.45 μm syringe filters (Pall Corporation, Ann Arbor, MI); 2 ml of each sample were diluted with 0.1ml methanol before analysis. Ketoprofen concentrations were measured using HPLC as described above (Shimadzu, LC-10AT VP, Japan).

Results and Discussion

Ketoprofen solubility in poloxamer 407 aqueous solutions

The solubility of ketoprofen in water containing various concentrations of poloxamer 407, the primary surfactant in this study, was measured by HPLC in triplicate. The solubilities of ketoprofen in 1, 2, and 5% w/v poloxamer 407 solutions were 0.34 ± 0.01, 0.81 ± 0.03, and 2.67 ± 0.02 mg/ml, respectively, much higher than its solubility in pure water, 0.05 mg/ml. The solubility of ketoprofen in the micelles increased linearly with the concentration of poloxamer 407. In each case, the poloxamer 407 concentration was well above the critical micelle concentration (CMC), which is 4×10^{-3}% w/v.[17] In EPAS experiments, the drug concentration in suspension was much higher than its micellar solubility in poloxamer 407 aqueous solutions, indicating the drug particles were stabilized by the surfactant and not merely dissolved in the cores of micelles.

Appearance of ketoprofen dispersions in the aqueous suspensions formed by EPAS

Ketoprofen, with a carboxylic acid group, has a pKa of 4.45. Relative to other commonly studied water insoluble drugs such as danazol and itraconazole, ketoprofen has a rather high water solubility, 51 mg/L, and a relatively low melting point of 94°C. In water at pH 7, the carboxylic acid group in ketoprofen dissociates.

To investigate ketoprofen dispersions produced in EPAS, 20% w/v ketoprofen in dichloromethane was sprayed into pH 1.5 and pH 2.0 KCl/HCl buffer solutions and into pure water without any surfactant at temperatures of 60, 70, 80, and 90°C for 10 min. The 20 mg/ml ketoprofen dispersions were cooled down after the spray while stirring at room temperature. Photographs of ketoprofen dispersions formed in pH 1.5 buffer solution and pure water at 80°C are shown in Figures 1a and 1b. The white object at the bottom of the container was a magnetic stir bar. At pH 1.5, individual liquid droplets were visible with the unaided eye. It was easy to tell that these were liquid droplets and not solid particles as they were transparent. When the dispersions were cooled down, ketoprofen changed from a liquid (at 72 and 55°C) to a semisolid (46.2°C) and finally to a solid (43.9°C) as the dispersion became progressively more opaque. The same behavior was observed with pH 2.0 buffer solutions (not shown). The freezing point depression was due to residual dichloromethane ($T_m = -96.7$°C)[18] and the surfactants in the suspension. When ketoprofen was sprayed into pure water at neutral pH, the liquid emulsion was much milkier and the droplet size appeared to be significantly smaller than in the pH 1.5 buffer solution. Single droplets of ketoprofen in this fine emulsion were not evident to the unaided eye, as shown in Figure 1b. In pH 1.5 or 2.0 buffer solution below the pKa of 4.45, ketoprofen does not dissociate. In neutral water (pH = 7), the improvement in emulsification suggests that the anionic carboxylate moiety balances the hydrophobic residue to lower the interfacial tension between ketoprofen droplets and water. For a given shear produced by the nozzle, much smaller droplets were produced in neutral water where ketoprofen ionized, in the form of a milky white emulsion, than in the low pH buffer solutions. The same change in emulsion morphology was observed at other temperatures, 60, 70, and 90°C.

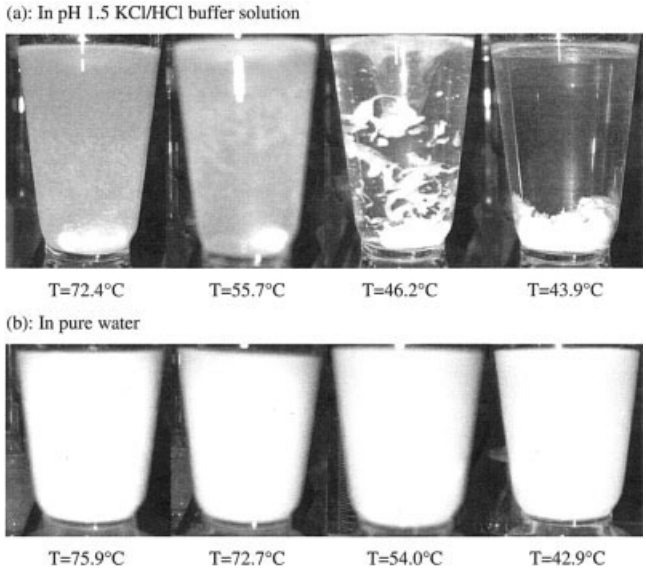

Figure 1. Liquid ketoprofen dispersed in aqueous solution.

20% w/v ketoprofen in dichloromethane was sprayed into a pH 1.5 KCl/HCl buffer solution and pure water without any surfactant at 80°C for 10 min. Ketoprofen concentration in the final dispersion was 20 mg/ml. The dispersion was cooled down at room temperature. (a): In pH 1.5 KCl/HCl buffer solution. (b): In pure water.

Effect of surfactant type and temperature on particle size of ketoprofen

A solution of 5% w/v ketoprofen in dichloromethane was sprayed into 1% w/v aqueous solutions containing various surfactants at 90°C for 20 min. The concentration of ketoprofen

Table 1. Effect of Surfactant Type and Temperature on Particle Size Measured at Room Temperature for Aqueous Suspensions that Did Not Gel

Aqueous Surfactant	T_{bath} (°C)	$T_{thermo\ couple}$ (°C)	D_{mean} (nm)	Particle Size Distribution (nm)
Poloxamer 407	90	81	230	16-25 (3%); 48-139 (50%); 214-626 (47%)
Pluronic F68	90	81	381	212-251 (51%); 485-597 (49%)
Myrj 52	90	81	383	224-264 (57%); 509-625 (43%)
PVA	90	81	2551	13-32 (14%); 56-237 (54%); >5 μm (32%)
PVP K-15				Did not form suspension
Poloxamer 407	80	75	184	17-22 (4%); 79-159 (67%); 278-487 (29%)
Poloxamer 407	70	64	114	27-39 (25%); 123-164 (75%)
Poloxamer 407 (a)	60	55	133	12-25 (5%); 53-135 (91%); 729-879 (1%); 1279-1860 (3%)
Poloxamer 407 (b)	60	54	126	17-24 (2%); 49-121 (95%); 1056-1266 (1%); 1816-2607 (2%)

Organic phase: 5% w/v ketoprofen in dichloromethane; aqueous phase: 1% w/v surfactant solution; t = 20 min; suspension concentration: 10 mg/ml.

in suspension was 10 mg/ml. The particle sizes of ketoprofen with various stabilizers, after cooling to room temperature, are shown in Table 1. The temperature of a thermocouple near the exit of the nozzle was cooler than the bath temperature due to the evaporation of the solvent. Some experiments were repeated to check experimental reproducibility and shown as (a), (b), and (c). Stable colloidal suspensions of ketoprofen with mean particle size smaller than 400 nm were formed with all three surfactants, poloxamer 407 ($HO(CH_2CH_2O)_{\sim 101}$-*b*-$(CH_2CH(CH_3)O)_{\sim 56}$-*b*-$(CH_2CH_2O)_{\sim 101}H$), Pluronic F68 ($HO(CH_2CH_2O)_{\sim 80}$-*b*-$(CH_2CH(CH_3)O)_{\sim 27}$-*b*-$(CH_2CH_2O)_{\sim 80}H$), and Myrj 52 ($CH_3(CH_2)_{\sim 16}(OCH_2CH_2)_{\sim 40}OH$).

For poloxamer 407, Pluronic F68, and Myrj 52, ketoprofen nanoparticles with mean particle size of 230, 383, and 381 nm, respectively, were formed in EPAS. Among these three copolymers, the hydrophilic EO moieties of poloxamer 407 were the longest. Greater steric stabilization with longer hydrophilic blocks would yield smaller particles if the other factors were equal, for example, the level of surfactant adsorption. Even though the lowest molecular weight surfactant Myrj 52 may be expected to diffuse the fastest, it produced larger particles than poloxamer 407. Perhaps the shorter stabilizing PEO chain provided weaker steric stabilization.[19] Another possibility is that a single PEO chain provides less stabilization than dual PEO end segments. The adsorption of poloxamer 407 on the particles may be expected to be higher than for Pluronic F68, since it has higher weight fraction of hydrophobic moieties. Differences in solubilities of ketoprofen in the three different types of micelles could also influence the particle size. Both surfactant micelles and free surfactant diffuse to the growing particle surface. The micelle diffusion and the deposition of surfactant from the micellar surface will depend upon the micelle size and surfactant mobility in the micelles.

The particle size was much larger for the homopolymer PVA than with the various copolymers. A dispersion was not formed with PVP K-15 in the aqueous phase. An oily ketoprofen liquid layer floated on the top of the aqueous phase. It is well known that a homopolymer is often a less effective stabilizer than a properly designed copolymer, since the copolymer has both hydrophilic and hydrophobic moieties. The hydrophobic moieties attach to the particle/droplet surface and hydrophilic moieties extend to water, stabilizing the particles/droplet from agglomeration, flocculation, and coalescence.

Experiments were conducted at various temperatures from 60 to 90°C with 1% w/v poloxamer 407 in aqueous solution. As the temperature was decreased from 80 to 70°C, the particle size distribution shifted significantly to smaller particles. This decrease can be influenced by several factors. Decreasing temperature may increase the steric repulsion between the EO blocks due to better solvation by water.[20] At the lower temperature, particle growth by diffusion of ketoprofen molecules in the organic phase is slower. In addition, the lower evaporation rate will slow down nucleation of the ketoprofen droplets. This slower production of nuclei may give the surfactant more time to diffuse from the water phase and coat the particles to lower growth. However, the evaporation was fast enough to prevent the buildup of large organic droplets in the water phase. As the temperature was lowered further, the average particle size increased slightly and some much larger particles were formed. The large particles may reflect too much time for growth in large slowly evaporating organic droplets.

Effect of drug feed concentration and concentration of the ketoprofen aqueous suspension on the particle size

To investigate the effect of the concentration of the drug in the aqueous suspension, 20% w/v ketoprofen solutions were sprayed into 1% w/v poloxamer 407 at a temperature of 80°C. As shown in Table 2, increasing the ketoprofen concentration in the aqueous suspension increases the particle size. At the given drug feed concentration, 20% w/v, the particle size increased from 136 nm to 401 nm when the suspension concentration increased from 10 to 30 mg/ml. The higher particle concentration in the suspension increases the collision rate and increases the drug-to-surfactant ratio. The higher drug-to-surfactant ratio may lead to reduced surfactant adsorption and, thus, weaker steric stabilization. Both of these factors would be consistent with the observed larger particles.

At a given ketoprofen aqueous suspension concentration of 10 mg/ml, the ketoprofen particle size decreased with increasing drug feed concentration. When the drug feed concentration increased, the mean particle size of ketoprofen decreased slightly, from 183 nm to 152 nm, and the percentage of the largest particles also decreased. At 1% w/v drug in the feed, 2% of the particles were larger than 2 μm, but at 20% w/v drug in the feed, all the particles were smaller than 300 nm. At a suspension concentration of 30 mg/ml, when the feed concentration increased from 20% w/v to 70% w/v, the mean particle size of ketoprofen decreased by a factor of more than three, with all particles smaller than 200 nm. With an increase in the drug feed concentration, the supersaturation increases during evaporation, leading to faster nucleation, resulting in smaller

Table 2. Effect of Feed Drug Concentration and Suspension Concentration on Particle Size at 80°C

C_{drug} in org. phase (% w/v)	$C_{susp.}$ (mg/ml)	t (min)	D/S ratio	D_{mean} (nm)	Particle Size Distribution (nm)
1	10	100	1	183	25-51 (15%); 104-253 (83%); 2129-3037 (2%)
5	10	20	1	184	17-22 (4%); 79-159 (67%); 278-487 (29%)
20[a]	10	5	1	136	10-13 (2%); 47-94 (56%); 163-327 (42%)
20[b]	10	5	1	147	35-46 (21%); 144-206 (79%)
20[c]	10	5	1	152	13-18 (3%); 46-73 (29%); 144-255 (68%)
20	20	10	2	169	27-51 (12%); 95-210 (80%); 395-635 (8%)
20	30	15	3	401	27-41 (6%); 95-222 (88%); 3474-5303 (6%)
70[a]	30	4.3	3	122	29-37 (16%); 118-163 (84%)
70[b]	30	4.3	3	122	30-40 (19%); 122-174 (81%)

These suspensions did not gel. Aqueous phase: 1% w/v poloxamer 407 solution.
[a,b,c]Experiment reproducibility for separate sprays.

particles. Furthermore, the shorter spray time for the higher feed drug concentration provided less time for particle growth. The formation of stable colloidal suspensions of ketoprofen, despite the very high drug-to-surfactant ratio of 3, is unique relative to other drugs, such as cyclosporine A, danazol, and itraconazole.[3-6]

Effect of surfactant concentration, pH, and ionic strength on ketoprofen particle size

The negative charge due to dissociation of the carboxylic acid groups was shown in Figure 1 to enhance the stability of the ketoprofen suspension in the aqueous phase by adding electrostatic stabilization to complement the steric stabilization from the nonionic surfactant. To study the influence of ketoprofen dissociation on the stabilization, 20% w/v ketoprofen solution in dichloromethane was sprayed into various concentrations of poloxamer 407 at two different values of pH at 80°C. The effect of drug-to-surfactant ratio has been characterized for the steric stabilization of drug particles by Young et al.[21] in a related process, rapid expansion from supercritical to aqueous solution (RESAS).

As shown in Table 3, with no surfactant in the aqueous phase, ketoprofen did not float to the top of the suspension, unlike the case for other drugs tested in EPAS, including danazol, itraconazole, and carbamazepine.[3-6] A milky suspension with a mean particle size of 4.0 μm formed. At 0.1% w/v poloxamer 407, the mean particle size of ketoprofen was 135 nm, and only 5% of the particles were larger than 500 nm. When the poloxamer 407 concentration was increased to 1% w/v, the mean particle size changed only slightly, but all particles were smaller than 210 nm.

To examine the effect of pH, the same ketoprofen organic solution was sprayed into 1% w/v poloxamer 407 dissolved in pH 2.0 KCl/HCl buffer solution to fully protonate the carboxylate groups. At a suspension concentration of 10 mg/ml, stable ketoprofen suspensions were still formed, with a mean particle size of 151 nm. But after 5 min where the suspension concentration reached 10 mg/ml, further spray would cause phase separation of liquid ketoprofen and water. The milky dispersion would suddenly become much clearer due to coalescence of ketoprofen droplets. In contrast, stable colloidal suspensions of ketoprofen were formed in pH neutral pure water at suspension concentrations of even 30 mg/ml, as shown in Table 3. It is apparent that electrostatic stabilization due to the negative charge on the ketoprofen droplets retarded emulsion flocculation and coalescence. Steric stabilization requires time for the surfactant to be adsorbed, whereas electrostatic stabilization is extremely rapid once the particles are surrounded by a sufficient amount of water. At low pH below the pK_a, 1% poloxamer 407 was not sufficient to stabilize concentrated ketoprofen particles/droplets without the electrostatic stabilization, as the droplets coalesced and phase separated.

To further characterize the electrostatic stabilization, the effect of ionic strength on the ketoprofen suspension was also investigated. A 20% w/v ketoprofen solution in dichloromethane was sprayed into 1% w/v poloxamer 407 dissolved in 1% w/v NaCl aqueous solution, which had an ionic strength of 0.17 M. At a suspension concentration of 10 mg/ml, a milky but unstable ketoprofen suspension was formed that coalesced at 80°C after stirring for only 1.3 min after the spray. This experiment further confirmed that electrostatic stabilization due to the negative charge on the ketoprofen droplets retarded emulsion flocculation and coalescence. The high ionic strength screened the electrostatic stabilization, and the emulsion coalesced, whereas it was very stable without added salt.

Ketoprofen-poloxamer 407 gel formation at high polymer concentration in the aqueous phase

For certain PPO-PEO-PPO block polymers (poloxamers) in water, a gel region in the phase diagram appears at high

Table 3. Effect of Aqueous Surfactant Concentration and pH on Ketoprofen Particle Size at 80°C

$C_{surf.}$ in aq. phase (% w/v)	pH	$C_{susp.}$ (mg/ml)	D/S ratio	D_{mean} (nm)	Particle Size Distribution (nm)
0	7	10	∞	3986	192-332 (38%); 861-1296 (20%); >5 μm (42%)
0.01	7	10	100:1	1378	42-178 (50%); 422-1778 (38%); >5 μm (12%)
0.1	7	10	10:1	135	89-136 (95%); 578-746 (5%)
1[a]	7	10	1:1	147	35-46 (21%); 144-206 (79%)
1[b]	7	10	1:1	163	20-30 (8%); 78-133 (50%); 203-345 (42%)
1	2	10	1:1	151	19-27 (3%); 57-85 (27%); 154-252 (70%)
1	2	14	1.4:1		Phase separation

These ketoprofen suspensions did not gel. Organic phase: 20% w/v ketoprofen solution in dichloromethane; suspension concentration: 10 mg/ml.

Figure 2. Thermoreversible gel of ketoprofen-poloxamer 407 formed at room temperature.

[Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

concentration (typically >20 wt%) and intermediate temperatures.[22-25] Both water-soluble and water-insoluble solutes may be incorporated into these gels, and they are of great interest in drug delivery.[25-27] For the poloxamer 407-water system,[22] the viscous gel region occurs at polymer concentration above approximately 18 wt%, but only at intermediate temperatures, such as between 20 and 85°C for a concentration of 25 wt%. The viscosity becomes low, even in concentrated solutions, at temperatures above or below the gelation temperature. According to Wanka et al.[17] and Mortensen et al.,[28] gels of poloxamers consist of a close-packed array of block copolymer micelles.

Gel formation was investigated in EPAS experiments. Either 20 or 70% w/v ketoprofen, with or without 5% poloxamer 407 in the organic phase, was sprayed into poloxamer 407 aqueous solution. The temperatures ranged from 60 to 90°C. Figure 2 shows a photograph of ketoprofen-poloxamer 407 gel formed at room temperature after cooling from the spray temperature of 90°C. When the gel was heated in hot water (80°C) for 3 min, the gel was converted to a turbid suspension. Upon cooling back to room temperature, the translucent gel reformed. Thus, the gelation was thermally reversible. The particle size of ketoprofen did not change during the heating and cooling. It was found that if the stirring was too strong and a lot of air was trapped in the gel, ketoprofen would crystallize at the air-water interface. In the air-water interface, the unprotected interfacially active ketoprofen that was not complexed with the gel began to crystallize and form large particles.

The conditions where the gel formed and the resulting particle sizes are shown in Table 4. Either with or without poloxamer 407 in the organic feed, when the poloxamer 407 concentration in the aqueous phase was 5% w/v, the sprayed suspension was as milky as in the above non-gelled suspensions in Tables 1-3, which had a smaller concentration of poloxamer 407, except more viscous. When the suspension was cooled down at room temperature with gentle magnetic stirring, a bluish, transparent ketoprofen-poloxamer gel formed. The ketoprofen particles in the gel were much smaller than those formed in the more dilute non-gelled suspensions in Tables 1-3 with lower poloxamer 407 concentrations. At least 90% of the particles were smaller than 50 nm in the gel, while the non-gelled suspensions contained particles larger than 150 nm. For surfactant concentrations lower than 5% w/v, no gel was formed upon cooling, as shown in Table 4.

Compared to the poloxamer 407-water binary system, a gel was formed at a much lower poloxamer concentration (5.75-6.61 wt% including the water evaporation during EPAS spray) with ketoprofen than without ketoprofen (>18 wt%). In the presence of 1 M NaCl, the gel concentration of poloxamer 407 only shifted from 18 wt% at 50°C to 17 wt% at 30°C.[22] Since the solubility of ketoprofen is only 0.05 mg/ml or 1.97×10^{-4} M at room temperature, it would not be expected to shift the poloxamer 407 gel concentration due to a general effect based on ionic strength. Ketoprofen particles may be distributed both inside the PPO core of poloxamer 407 micelles and in the PEO corona. The polar and ionic carboxylate groups will interact favorably with the EO moieties of poloxamer 407 to provide order and enhance gel formation. Also, since the size of the coated drug particles (50 nm) is larger than that of the poloxamer micelles (15-20 nm),[29] the Hamaker interaction between ketoprofen particles may also be expected to aid gelation. At high temperature, the lattice of the poloxamer 407 gel was disrupted by contraction of the micelles as the average polyethylene oxide hydrodynamic radius (R_H) decreased with temperature.[22]

The gel exhibited non-Newtonian rheology; the viscosity decreased from 1185 cp at 2 rpm shear rate to 294 cp at 10 rpm and leveled off to 85 cp at 100 rpm. Gels in this viscosity range are appropriate for parenteral injection.[30] Because of high viscosity of the gel, the ketoprofen diffused slowly in the gel, and particle aggregation and Ostwald ripening were very slow.

Table 4. Formation of Gels by EPAS as a Function of Poloxamer 407 Concentration in Aqueous Phase

C_{F127} in org. phase (% w/v)	C_{F127} in aqu. phase (% w/v)	C_{drug} in organic phase (% w/v)	t (min)	T_{bath} (°C)	Gel formation	D_{mean} (nm)	Particle Size Distribution (nm)
0	1	70	4.3	80	N	122	29-37 (16%); 118-163 (84%)
0	2	70	4.3	80	N	136	18-27 (14%); 45-76 (29%); 157-264 (57%)
0[a]	5	70	4.3	80	Y	95	18-32 (28%); 59-96 (58%); 251-408 (14%)
0[b]	5	70	4.3	80	Y	81	26-37 (50%); 104-165 (50%)
5	1	20	15	90	N	139	37-49 (21%); 140-198 (79%)
5	2	20	15	90	N	120	18-39 (23%); 85-185 (73%)
5	5	20	15	90	Y	71	21-39 (90%); 353-576 (10%)
5	5	20	15	80	Y	76	18-45 (98%); 2570 (2%)
5	5	20	15	70	Y	108	29-47 (41%); 123-222 (59%)
5	5	20	15	60	Y	50	24-32 (78%); 104-151 (22%)

Organic phase: 20% or 70% w/v ketoprofen solution in dichloromethane; suspension concentration: 30 mg/ml.

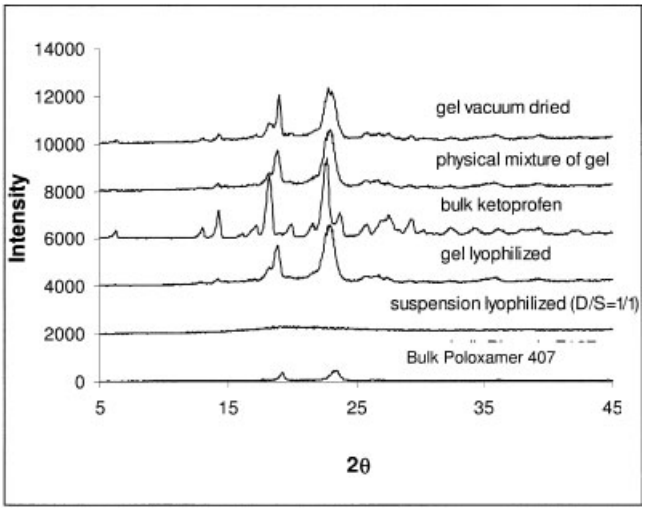

Figure 3. X-ray diffraction profile of dried ketoprofen gels and non-gelled suspensions by EPAS.

The high concentrations of surfactant in the aqueous solutions stabilized very small particles, and the gelation upon cooling preserved the small particles. A gel formed at 80°C and stored at 25°C was stable for 1 month, with a slight increase in mean particle size from 78 nm to 115 nm.

The crystallinity, surface area, and dissolution rate of dried ketoprofen-poloxamer 407 gel

A ketoprofen gel was formed by spraying 20% w/v ketoprofen + 5% w/v poloxamer 407 in dichloromethane into 100 ml of 5% poloxamer 407 for 15 min at 80°C, and cooling the suspension to room temperature with continuous stirring. The ketoprofen concentration in the gel was 30 mg/ml. The gel was dried by two different methods. One sample was vacuum dried at room temperature and −30 in. Hg. Here 100 ml of the gel was poured into three 500 ml beakers to form thin gel layers. The drying took more than 3 days due to slow diffusion of water. The other sample was dried by slowly injecting the gel into liquid nitrogen with a syringe and then lyophilizing the frozen mixture for 3 days.

A ketoprofen suspension, which did not gel, was formed by spraying 20% w/v ketoprofen in dichloromethane into 100 ml 1% poloxamer 407 for 5 min at 80°C. The ketoprofen concentration in the suspension was 10 mg/ml, and the drug-to-surfactant ratio was 1:1. The suspension was dried by flash freezing in liquid nitrogen and was lyophilized for 3 days.

The surface area of the ketoprofen gel dried by lyophilization was 9.7 m^2/g, while that of the gel dried with vacuum was 8.3 m^2/g, based on the BET technique. The surface area for discrete 50 nm particles, which were present in the gel before drying, would be much larger if the particles did not agglomerate upon drying. The dried gel was redispersed into water by stirring and the particle size was measured. The mean particle size of ketoprofen gel after drying by vacuum or lyophilization was 12.7 and 12.2 μm, respectively, as measured with a Mastersizer-S (Malvern Instruments Ltd., U. K.) since the particle size was too large for measurement by DLS. The surface areas are more consistent with these particle sizes than for the original 50 nm particles.

The X-ray diffraction patterns for the dried gel and dried non-gelled suspension are shown in Figure 3. The ketoprofen peaks were much smaller and broader in EPAS samples, as well as in the physical mixture, relative to pure ketoprofen. The highest ketoprofen peaks coincided with those of bulk poloxamer 407. However, ketoprofen shoulders at $2\theta = 18.4°$ to the left of the poloxamer peak were evident for each of the samples, except for the non-gelled suspension. Whereas ketoprofen in the non-gelled suspension remained amorphous after lyophilization, the ketoprofen in the gel dried by vacuum or by lyophilization was crystalline. One possibility is that the very small ketoprofen nanoparticles crystallized during drying due to air-water interfaces in vapor bubbles. Another possibility is that the ketoprofen at the surface of the thin film of the gel crystallized. Also, as the gels dried, the dehydrated PEO groups would provide less effective steric stabilization. Furthermore, the ketoprofen concentration in the gel increases upon drying, enhancing the contact between particles and potential for coalescence. Finally, as the gel dried, the residual water may have added mobility to the ketoprofen and caused crystallization.

The dissolution rates of the wet ketoprofen gel, the gels dried by vacuum and by lyophilization, the non-gelled suspension dried by lyophilization (formed with dilute poloxamer 407), and a physical mixture of ketoprofen and poloxamer 407 (with the same composition as the gel) are shown in Figure 4. The ketoprofen release from the wet gel or lyophilized non-gelled suspension, 98% and 94%, respectively, in 2 min was extremely rapid. However, when the gel was dried by vacuum or lyophilization, the dissolution rate of ketoprofen was much slower than for the wet gel. There are various reasons for the slower dissolution rates. One is the large particle size and smaller surface area after drying. Another is the slow hydration and swelling of the ketoprofen-poloxamer 407 complex that was observed during the dissolution test. In all cases, the release rate of ketoprofen gel was much higher than that of bulk ketoprofen and a physical mixture with poloxamer 407.

Conclusions

Upon contacting water, ionization of ketoprofen and gelation with poloxamer 407 produces stable nanoparticle gels, with smaller particle sizes than observed typically with the EPAS process. The favorable stabilization of ketoprofen by adsorbed

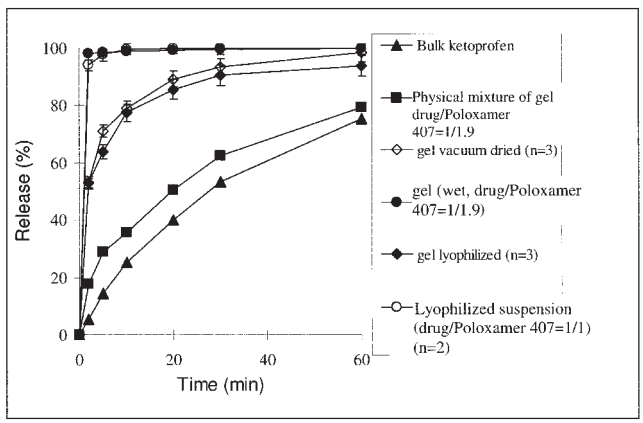

Figure 4. Dissolution profiles of the wet and dried ketoprofen gels and dried non-gelled suspension.

poloxamer 407, as well as by electrostatic stabilization, led to 135 nm amorphous particles, for an exceptionally high drug-to-surfactant ratio of 10:1. Typically crystalline particles are formed by EPAS, when only steric stabilization is present, although exceptions have been observed.[3] It is likely that this type of electrostatic stabilization and gelation would also be beneficial in other solution based particle formation processes. After drying, 94% of the ketoprofen dissolved in 2 min. For the higher poloxamer concentrations, interactions with ketoprofen induced gelation and produced particles as small as 50 nm, which were much smaller than reported previously in EPAS. The rapidly dissolving wet gels, with extremely small particle sizes, one month stability, and relatively low viscosities, are of interest in transdermal and parenteral delivery; furthermore, the gels may be dried for oral delivery.

Acknowledgments

The authors would like to gratefully acknowledge financial support from The Dow Chemical Company (Midland, MI) and the National Science Foundation STC Program of the National Science Foundation under Agreement No. CHE-9876674.

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Manuscript received Aug. 31, 2005, and revision received Mar. 2, 2006.