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Amphiphilic amino acid copolymers as stabilizers for the preparation of nanocrystal dispersion

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Abstract

The recent advance of particle size engineering in nanometer ranges has widened the formulation opportunities of relatively water-insoluble drugs. However, the 'nanoformulation' suffers from a lack of systematic understanding about the requirements of polymeric stabilizers. Furthermore, the polymers that can be used for the preparation of nanocrystals are so limited that finding a proper stabilizer for a given formulation is often difficult. In this study, amino acid copolymers whose properties can systematically be tailored are developed, and their morphological and compositional effects are investigated. Copolymers containing lysine (K) as their hydrophilic segments, and phenylalanine (F) or leucine (L) as their hydrophobic segments successfully produce stable nanocrystals (200–300 nm) in water, while copolymers of K and alanine (A) could not generate nanosized particles. Not the morphology but the hydrophobicity of copolymers seems to be a critical parameter in the preparation of drug nanocrystals by wet comminution. The effective stabilization performance of copolymers requires the hydrophobic moiety content to be higher than 15 mol%. Comminution for only 5 min is long enough for nanocrystal preparation, and the crystallinity of drug is found intact after the processing.

Keywords: Nanoparticles; Nanocrystals; Particle size; Insoluble drug; Dispersion; Particle engineering

1. Introduction

Reducing the particle size of an active pharmaceutical ingredient (API) has opened new formulation opportunities in various dosage forms. Particularly, the bioavailability of relatively insoluble drugs, which is often limited by poor dissolution rates, benefits from the development of the 'nanoformulation' techniques (Lee et al., 2000; Serajuddin, 1999). Based on the Noyes–Whitney equation, which supports the linear dependence of the dissolution rate on the surface area, increasing surface area by reducing particle sizes from microns to nanometers leads to one or more order(s) of magnitude increase in the dissolution rate (Lee et al., 2000).

The technology of particle size reduction to nanometers has significantly advanced over the last several decades. Comminution, high pressure homogenization, impinging jet, electrospraying, liquid-based methods, and supercritical fluid processes are the technologies that are currently available or being actively developed for nanoparticle preparation (Lee et al., 2000; Serajuddin, 1999; Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Merisko-Liversidge et al., 1996; Zheng and Bosch, 1997; Yamada et al., 1999; Grau et al., 2000; Lee, 2003). Methods to prepare drug nanoparticles can be categorized into two approaches, i.e., thermodynamic and kinetic approach. In the approaches, energy inputs or surface stabilization compensate the dramatic increase in surface energy (extra Gibbs free energy) accompanying with particle size reduction (Lee et al., 2000; Serajuddin, 1999; Liversidge and Conzentino, 1995; Lee, 2004). In most processing, both the approaches are employed together.

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Stabilizers usually cover the surface of nanoparticles producing ionic or steric stabilization (Ploehn and Russel, 1990; Evertsson and Nilsson, 1997; Berglund et al., 2003a, b). Nanocrystal preparation by wet comminution uses polymeric stabilizers, which adsorb onto the surfaces of drug nanocrystals and provide steric stabilization effect (Evertsson and Nilsson, 1997; Berglund et al., 2003a, b). The adsorbed chain molecules on surface have ceaseless thermal motion, resulting in dynamically rough surface preventing coalescence by repulsive entropic force. Polymer steric stabilization is good for the stability of drugs during processing since it does not usually destroy the crystal structure of drug particles unlike the conventional surfactants of small molecular weights such as sodium lauryl sulfate. The surfactants tend to form micelles containing a small number of dissolved drug molecules (Adamson and Gast, 1997). For establishing successful stabilization within a reasonable processing time, strong and fast adsorption at full coverage and a long time scale for desorption are necessary (Evertsson and Nilsson, 1997; Berglund et al., 2003a, b) in addition to steric repulsion.

When a polymeric stabilizer is used in a wet comminution process, its physical adsorption onto the surface of drug particles and subsequent steric stabilization are important processes. However, they are not simple processes. Polymer diffusion, convection, adsorption, and desorption can occur simultaneously with the mechanical fracturing of crystal (Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Merisko-Liversidge et al., 1996; Zheng and Bosch, 1997; Yamada et al., 1999; Grau et al., 2000; Lee, 2003; Evertsson and Nilsson, 1997; Berglund et al., 2003a). Crystal surfaces freshly generated by fracturing will attract polymer chains if the entropy loss by physical adsorption is smaller than the related enthalpy gain. Thus, the physical and chemical properties of the crystal surfaces and the solution behaviors of polymers are important. The mechanism of fracturing and subsequent stabilization of particles in the wet comminution process remains largely unknown. To the best of our knowledge, the critical physical properties of polymers for the effective wet comminution have never been systematically examined yet, while the micelle forming properties of polymers have intensively been investigated (Ploehn and Russel, 1990; Evertsson and Nilsson, 1997; Berglund et al., 2003a, b; Torchilin, 2000). In industry, a proper polymeric stabilizer is chosen for a specific formulation by a simple trial-and error approach (Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Merisko-Liversidge et al., 1996; Zheng and Bosch, 1997; Yamada et al., 1999; Grau et al., 2000; Lee, 2003).

It is unrealistic to find a single polymer that properly works for all different wet comminution processes. Different formulation imposes different requirements on polymeric stabilizers. Furthermore, differences in the surface characteristics of drug crystals would require different properties of polymers. However, the properties of polymers critical in nanocrystal processing cannot easily be tuned for a specific formulation, due to the limited number of available polymers. Only a few polymers generally recognized as safe and effective (GRAS) can be employed (Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Merisko-Liversidge et al., 1996; Zheng and Bosch, 1997; Yamada et al., 1999; Grau et al., 2000; Lee, 2003). Finding a proper stabilizer among the currently available polymers is the leading key in the successful nanoformulation of a drug.

Although numerous nanocrystal formulations are under active development worldwide, efforts for developing a new class of materials for the stabilization of nanocrystals are still insufficient. To resolve the current limitations of nanocrystal processing, novel polymeric stabilizers should have flexibility in engineering their properties from synthetic point of view. Considering biocompatibility and diversity in the selection of monomers, amino acid copolymers are excellent candidates for this purpose (Szabo et al., 2002; Santosoa et al., 2002; Nowak et al., 2002; Hernández and Klok, 2003; Deming, 1997a, b, 2002; Chiang and Yeh, 2003; Iizuka et al., 1993). Their biocompatibility has been proved as can be found elsewhere (Szabo et al., 2002; Santosoa et al., 2002; Nowak et al., 2002; Hernández and Klok, 2003; Deming, 1997a, b, 2002; Chiang and Yeh, 2003; Iizuka et al., 1993). More than 20 amino acids and their derivatives are available for polymer construction. Depending on the kind of amino acids and their compositions, the chemical and physical properties of the copolymers can conveniently be adjusted for a specific nanoformulation. The wide range of hydrophobicity of amino acids imparts control over the systematic variation of hydrophobicity to resulting copolymers (Szabo et al., 2002; Santosoa et al., 2002; Nowak et al., 2002; Hernández and Klok, 2003; Deming, 1997a, b, 2002; Chiang and Yeh, 2003; Iizuka et al., 1993).

More importantly, tailoring amino acid copolymers will provide systematic understanding of the effect of their chain composition and morphology. This will be our initial step to reveal what are the critical physical properties of polymers for wet comminution processes. In this study, random and block copolymers based on lysine (K), phenylalanine (F), leucine (L), and alanine (A) were prepared via ring opening polymerization and were used for nanocrystal preparation (Kricheldorf, 1987; Deming, 1997a, b, 2000; Van Dijk-Wolthuis et al., 1997; Daly and Poche, 1988). The effects of their composition and morphology were systematically investigated. The amino acids, K, F, L, and A, were chosen because they have quite different hydrophobicities, and so the effects of hydrophobicity on nanocrystal processing can easily be compared. Naproxen was chosen as a typical water insoluble drug for easy comparison with the previous works on it (Liversidge and Conzentino, 1995; Lee, 2003).

2. Materials and methods

2.1. Materials

Triphosgene (98.0%), *n*-hexylamine (99.0%), hydrogen bromide (30 wt.% solution in acetic acid), and L-alanine

were purchased from Aldrich. N^{ε}-carbobenzyloxy-L-lysine (N^{ε}-CBZ-L-lys) was commercially available from Bachem AG. L-Phenylalanine and L-leucine were purchased from TCI America. Diethylamine (98.0%) was obtained from Yakuri Pure Chemical Co. and *N*,*N*′-dimethylformamide (99.5%) from Junsei. Tetrahydrofuran (THF, 99.0%), *n*-hexane (95.0%), and diethyl ether (97.0%) were purchased from Daejung Chemicals and Metals Co. THF, *n*-hexane and diethyl ether were refluxed over Na and freshly distilled before use. Diethylamine and *n*-hexylamine were distilled from CaH_2 and stored over molecular sieves. All other chemicals were used as received.

As an API for wet comminution, Naproxen (GMP) produced by Merck & Co. was used. The physical properties of crystalline Naproxen particles (purity > 95%) listed in Table 1 are the basic material parameters for this study. The particle morphology is also shown in a SEM micrograph, which is equant (cubic), plate-like or columnar. Hydroxypropyl cellulose (HPC, JP) was obtained from Nisso. Its average molecular weight was 60 kg/mol, and surface energy was 45 mN/m. Yittria-stabilized zirconium beads (performance ceramics, 0.8 mm diameter) and HPLC-grade water from Aldrich were used without any further purification.

2.2. Synthesis of amino acid N-carboxy anhydrides (NCAs)

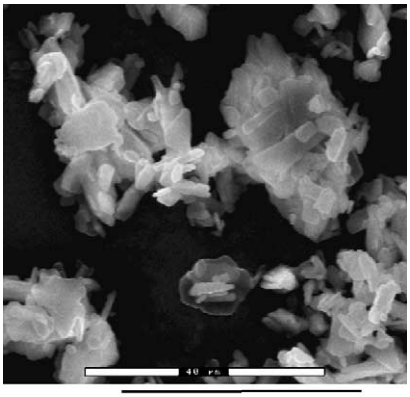
N^{ε}-CBZ-L-lysine, L-phenylalanine, L-leucine, and L-alanine were converted to corresponding *N*-carboxy anhydrides (NCAs). The ring opening polymerization of α-amino acid *N*-carboxy anhydrides (NCAs) is a fast and efficient route for the synthesis of polypeptides (Kricheldorf, 1987; Deming, 1997a, b, 2000; Van Dijk-Wolthuis et al., 1997; Daly and Poche, 1988). The lysine-based copolymers were synthesized by ring opening polymerization via the amino acid NCA using amino acids and triphosgene (Van Dijk-Wolthuis et al., 1997; Daly and Poche, 1988).

N^{ε}-CBZ-L-lysine, L-phenylalanine, L-leucine, and L-alanine were suspended in anhydrous THF (10 wt.%), and the reaction temperature was increased to 55 °C. A calculated amount of triphosgene, dissolved in anhydrous THF, was added dropwise into the reaction mixture and the suspended mixture became a clear solution as NCA was formed after a certain reaction time. To ensure the ring formation, the reaction mixture was vigorously stirred for an additional 1 h. The reaction mixture was condensed and poured to a 10-fold excess amount of anhydrous *n*-hexane to precipitate amino acid NCAs. The amino acid NCAs were obtained by filtration and dried in vacuum for 48 h. The amino acid NCAs were characterized by ^{1}H NMR.

2.3. Synthesis of amino acid copolymers

Calculated amounts of amino acid NCAs were placed in an 100 ml two-neck round bottom flask and dissolved in anhydrous DMF (10 wt.%) in nitrogen atmosphere (Van Dijk-Wolthuis et al., 1997). Freshly distilled *n*-hexylamine, previ-

Table 1
Properties of active pharmaceutical ingredient (API), Naproxen particles

API	Naproxen				
Molecular weight	230.266 g/mol ($C_{14}H_{14}O_3$)				
Particle size distribution	Bimodal				
Particle size (μm)					
Sonication	0s	30s	60s	120s	300s
Mean	37	40	46	38	15
D10	3.9	0.9	0.8	0.8	0.9
D50	13.7	13.3	16.3	8.1	0.9
D90	109.1	117.5	129.4	111.6	60.0
Contact angle	62° with water, 13° with ethanol				
True densities (g/cm^3)	1.243				
Surface area (m^2/g)	2.02 ± 0.13				
Particle morphology: SEM micrograph	 40 μm				

ously diluted in anhydrous DMF, was added into the solution to initiate ring-opening polymerization. In case of L-lysine and L-leucine copolymerization, diethylamine was used as an initiator for the measurement of copolymer molecular weight by ^{1}H NMR.

The polymerization was continued for 72 h at room temperature to make sure of the consumption of all the NCA monomers. The resulting slightly viscous solution was precipitated in 20-fold excess water. The precipitate was filtered and dried in vacuum overnight. The molecular weights of copolymers before deprotection were measured by ^{1}H NMR.

The protection groups of copolymers (CBZ groups in L-lysine side chains) were removed using HBr (Deming, 2000). Copolymers with protection groups were dissolved in 30% HBr/glacial acetic acid solution (20 ml/g). As deprotection reaction went on, carbon dioxide evolved out and the copolymer rapidly precipitated from the solution. After 30 min, a 10-fold excess amount of anhydrous diethyl ether was added to the reaction mixture. The precipitate was filtered and washed with diethyl ether. After drying, the deprotected polymer was dissolved again in deionized water, transferred to a pre-swollen dialysis membrane (MWCO = 500) and dialyzed against deionized water for 2 days. Poly(amino acid) copolymers for wet comminution were obtained by freeze drying for 3 days. The compositions of comonomers and the degree of polymerization were characterized by ^{1}H NMR.

2.4. Wet comminution

API particles were mixed in a 1.3 ml bottle with distilled water, polymeric stabilizer (1.33 wt.%) and polystyrene beads (500 μm, 0.354 g). Then, high speed shaking of 4800 rpm at room temperature produced a nanosuspension with a Mini-Beadbeater^{TM} (Biospec Products). The weight of the slurry (API + HPC + water) was 3.75 g, and the concentration of API in water was 8 wt.%. The concentrations of HPC mother liquid were 0.38, 0.77, 1.54, and 3.08 wt.%. Unless otherwise specified, the comminution time was 30 min.

2.5. Characterizations after comminution

The particle size distribution was measured in water using an Electrophoretic Light Scattering Spectrophotometer, ELS-8000 (Otsuka, 632.8 nm He–Ne laser (10 mW), 25 °C, relative refractive index = 1.3313, Marquadt analysis method, 100 repeated tests). The sonication power of the particle size analyzer was 70 W (39 kHz). For the differential scanning calorimetry (DSC) experiments, solid powders were prepared by centrifuging wet-milled suspensions at 17,000 rpm for 1 h using a Micro 17R centrifuge (Hanil).

DSC experiments were performed using a DSC-7 (Perkin Elmer) in nitrogen atmosphere. The samples were scanned from 50 to 200 °C at a heating rate of 5 °C/min, and the first heating scans were reported to measure the melting points and heat of fusion of nanocrystals.

For the atomic force microscopy (AFM), a PSIA XE-100 with TESP or TESP7 etched single crystal silicone probes was used (20–100 N/m force constant and 200–400 kHz resonance frequency). The surfaces of the dry compacts were scanned in a tapping mode at ambient conditions.

3. Results and discussion

3.1. Preparation of amino acid copolymers

Amino acids were successfully copolymerized into block and random copolymers (Fig. 1). Their designations and basic properties are given in Table 2. Random copolymers of K and F, K and L, and K and A were designated as K-r-F, K-r-L, and K-r-A, respectively. For block copolymers, the letter 'b' was used in the place of the letter 'r', e.g., K-b-F.

The molecular weights, degrees of polymerization (DP), and compositions of copolymers were measured by ^{1}H NMR. The degree of polymerization controlled by the initial concentration ratio of the NCA monomers to that of initiator, was calculated based on the integration ratio of a characteristic peak from the initiator to a peak from the amino acids in the repeating units. In the cases of polymerization initiated by *n*-hexylamine (DMSO-D_6), the peak from initiator at 0.83 ppm (t, 3H, $-RNCH_2(CH_2)_5C\mathbf{H}_3$) was compared to the peak at 4.99 ppm in the L-lysine side groups with a CBZ protection group (s, 2H, $ArC\mathbf{H}_2O$), 7.17 ppm in L-phenylalanine (m, 5H, Ar**H**), and 1.36 ppm in L-alanine (m, 3H, $CHC\mathbf{H}_3$). In the diethylamine-initiated polymerization (DMSO-D_6), the peak at 1.11 ppm (s, 6H, $-RN(CH_2C\mathbf{H}_3)_2$) was compared to that at 4.99 ppm in L-lysine (s, 2H, $ArC\mathbf{H}_2O$) and 0.80–0.90 ppm in L-leucine (m, 6H, $CH(C\mathbf{H}_3)_2$). The results are summarized in Table 2. In the *n*-hexylamine-initiated polymerization, the DP was almost equal to initial monomer-to-initiator ratio. However, the DP of diethylamine-initiated copolymers was about twice as high as that expected from initial concentration ratio, which follows the same trend reported previously (Van Dijk-Wolthuis et al., 1997). The amino acid compositions of copolymers were successfully controlled by the initial concentration ratio of amino acid NCAs.

3.2. Types of amino acids

Wet comminution in the presence of polymeric stabilizers can produce effective size reduction (Lee et al., 2000; Serajuddin, 1999; Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Merisko-Liversidge et al., 1996; Zheng and Bosch, 1997; Yamada et al., 1999; Grau et al., 2000; Lee, 2003). Polymer stabilizers tend to adsorb onto the surface of hydrophobic drug, since they have relatively hydrophobic moieties. Polymer chains anchored onto the surface provide steric or ionic stabilization depending on the characteristics of polymers (Ploehn and Russel, 1990). Without polymer adsorption, stabilization cannot occur, and so no dispersed nanoparticles result from wet comminution. The mechanical

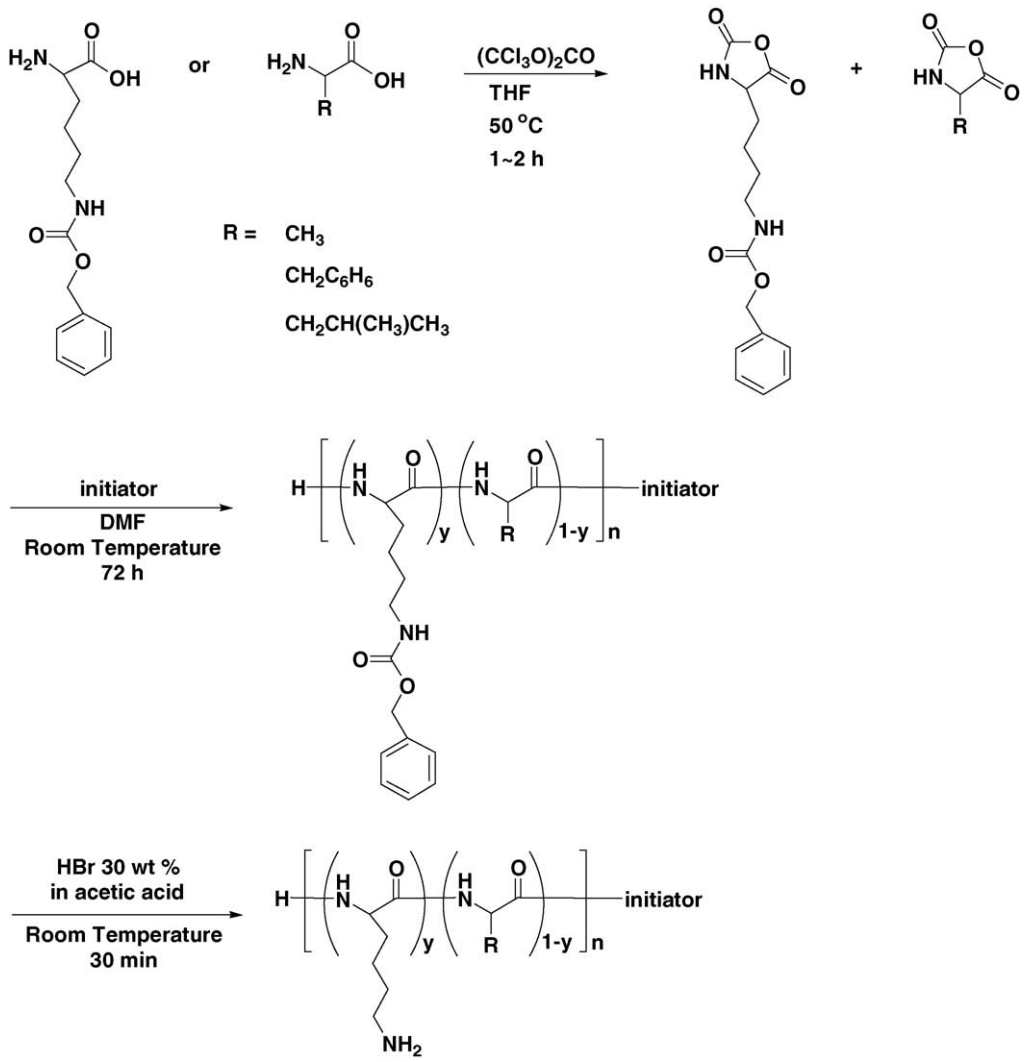


Fig. 1. Reaction schemes of amino acid copolymers.

Table 2
Designations and properties of amino acid copolymers

Amino acid copolymers: designations	Architecture (random or block copolymer)	K content (mol%)	Molecular weight, Mw	Degree of polymerisation, DP
K-r-F-1	Random	100	4800	38
K-r-F-2	Random	100	8400	66
K-r-F-3	Random	89	9200	70
K-r-F-4	Random	88	13300	102
K-r-F-5	Random	84	12600	96
K-r-F-6	Random	83	26800	204
K-r-F-7	Random	81	10100	77
K-r-F-8	Random	77	6300	48
K-r-F-9	Random	73	13300	100
K-r-F-10	Random	68	14000	105
K-r-F-11	Random	60	15200	112
K-r-F-12	Random	60	17500	129
K-r-F-13	Random	50	14700	107
K-b-F-1	Block	66	7500	56
K-b-F-2	Block	62	25200	186
K-b-F-3	Block	60	4800	35
K-r-L-1	Random	78	13100	105
K-r-A-1	Random	79	12900	110

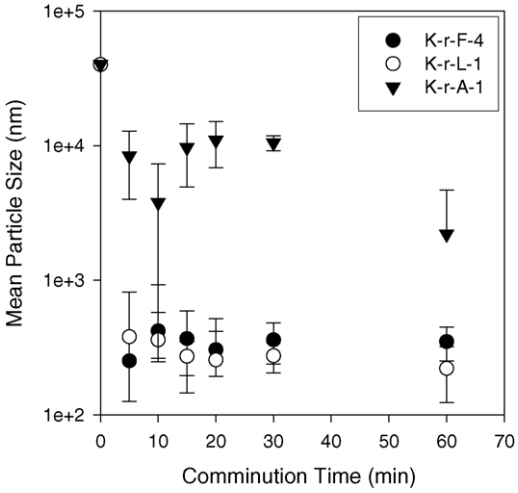


Fig. 2. Mean particle size changes of various copolymer systems as a function of comminution time.

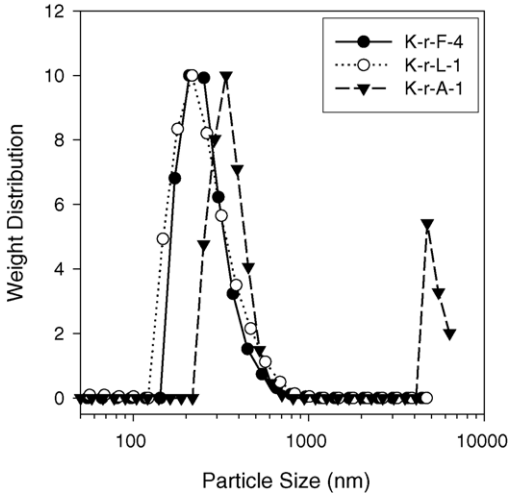


Fig. 3. Size distribution curves of drug particles measured by quasi-elastic light scattering method.

fracture energy input of comminution itself cannot produce stable nanoparticles since it cannot overcome and compensate the surface energy of nanoparticles.

The hydrophilic moiety of copolymers is K segment. Because of its hydrophilic nature, the segment will tend to locate itself toward water instead of hydrophobic drug surface. Thus, it can provide effective steric or ionic stabilization for drug nanoparticles. However, like the conventional surfactants of small molecular weights, it may excessively help the dissolution of hydrophobic drugs, resulting in the destruction of crystalline structures. The destruction produces micelle-like structures in liquid formulations. This possible series of events will be examined in the stability study of nanocrystals below.

While K was used as the hydrophilic moiety of copolymers, three other amino acids, F, L, and A, were used as hydrophobic moieties. The use of different amino acids imparts a different hydrophobicity to amino acid copolymers. If all other factors such as the K content, polymer morphology, etc., remain the same, the hydrophobicity of copolymers increases in the order of K-r-A, K-r-L, and K-r-F (Black and Mould, 1991). The hydrophobicity of hydrophobic moiety varies with 0.616 (A), 0.943 (L), and 1.000 (F) (according to the hydrophobicity scale of Black and Mould (1991)). The difference in the hydrophobicity of copolymers is the net changes in the driving force of the absorption of the copolymers onto hydrophobic drug surfaces. Desorption is also related with the hydrophobicity of copolymers.

Fig. 2 shows the effect of the hydrophobicity of constituting amino acids. The effect of hydrophobicity is distinct. K-r-A systems show significantly higher mean particle sizes (usually above micron) than the other K-r-L and K-r-F systems (near 200–300 nm). The differences between K-r-L and K-r-F systems are not statistically significant, although only K-r-F systems can have specific π–π interactions between the benzene rings of F and Naproxen (Adamson and Gast, 1997).

A longer comminution time than 5 min was not necessary to further decrease mean particle size (Fig. 2). The particle sizes seem to reach their steady state values within 5 min [particle size before processing = 15–46 μm (Table 1)]. This efficient processing time indicates that the mechanical fracturing of drug particles into several hundred nanometers and their stabilization by nearby copolymers can be completed in a short time period. The results support that the rate of polymer diffusion (under convection) and adsorption onto the surface of the hydrophobic drug is fast enough to stabilize particles within 5 min. Fig. 2 shows that K-r-F and K-r-L are likely to adsorb onto the surfaces within 5 min, but K-r-A does not significantly adsorb even in a processing time longer than 5 min. This result is in contrast to our previous study using common cellulose type polymers such as hydroxyl propyl cellulose (HPC) (Lee, 2003). Due to the viscose nature of cellulose polymers, processing using HPC seems to take a longer time. However, a direct comparison between the two sets of data is difficult because of differences in processing equipment, preparation scale, etc.

Fig. 3 shows the typical particle size distributions of three suspension systems, K-r-F, K-r-L, and K-r-A. The distribution curves show broad ranges of particle size produced by wet comminution. K-r-A system shows distinct bimodal distribution in bigger size ranges than the other two systems. Its distribution curve above micron is similar to that of drug particles as received (Table 1), and the particles should be unstable. Therefore, the K-r-A system seems to have physical adsorption process not enough to produce stable dispersions of nanocrystals.

3.3. Content of hydrophobic moiety

As the content of hydrophobic moieties increases, the adsorption of copolymers is expected to be enhanced. It is an interesting and valuable subject to figure out the minimum content of hydrophobic amino acids required to trigger

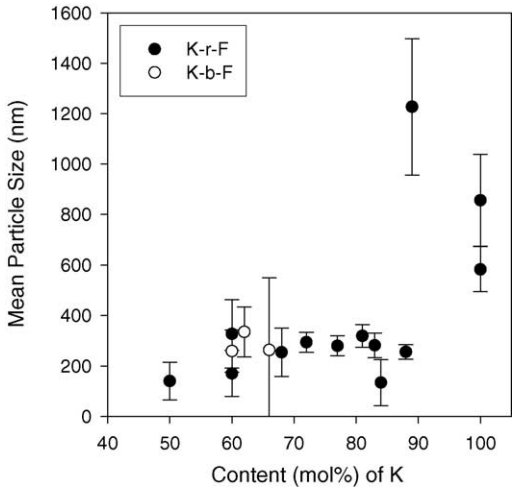


Fig. 4. Mean particle sizes of drug nanocrystals vs. K content in copolymers. The nanocrystals were prepared in the presence of amino acid copolymers of various K contents.

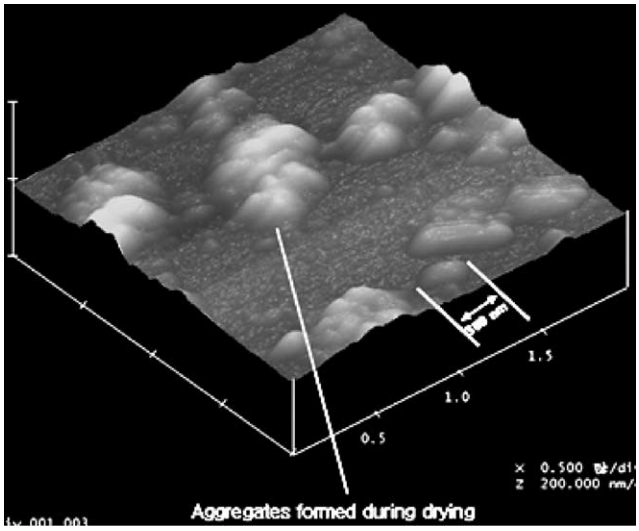


Fig. 5. Atomic force micrograph of drug nanoparticles prepared with using amino acid copolymers (K-b-F-1).

the adsorption of copolymers. Additionally, the morphology of copolymers, i.e., the distribution of hydrophobic moiety along a polymer chain, could be an important factor. To estimate the effect of the morphology, block and random copolymers having the same mole fraction of hydrophobic amino acids are compared.

Fig. 4 shows the effect of hydrophobic amino acid content on nanoparticle formation. A statistically meaningful result is that when the hydrophobic content is above 15 mol% (K content < 85 mol% in Fig. 4), the mean particle size can be reduced to below 400 nm. Below 15 mol% (K content > 85 mol%), it spans a wide size range of 500–1500 nm. Above 15 mol%, a significant change in mean particle sizes is not observed. Therefore, hydrophobic moiety of 15 mol% is enough for copolymer chains to attach onto the surface of drug particles and maintain stable physical adsorption (Ploehn and Russel, 1990; Evertsson and Nilsson, 1997; Berglund et al., 2003a, b). (They were sable up to at least 30 days as will be seen in Fig. 7.) Their desorption kinetics should be long enough for successful nanosuspension preparation.

In random copolymers, the hydrophobic amino acids are randomly distributed, but in diblock copolymers, they are localized in one segment. As a consequence, multiple attachments of polymer chains onto hydrophobic surface could be easier in random copolymer adsorptions than in diblock copolymer cases. However, the mean particle size results of Fig. 4 do not reflect the possible differences in the chain conformations of adsorbed copolymers. The morphology of copolymers, diblock or random copolymer chains, is not a determining factor in controlling particle size reduction.

The effect of molecular weight was not critical either (the details are not given in Fig. 4). The variation in molecular weight (Table 2) was intentionally generated to investigate its effect, but a systematic increase or decrease in particle size with an increase in molecular weight was not found. Since steric repulsion is known to depend on molecular weight, the variation in molecular weight may not be large enough to identify its effect. Yet, it is clear from the successful preparation of nanoparticles shown in Fig. 4 that the polymer chains of 5000–25,000 g/mol are long enough to provide steric repulsion (Adamson and Gast, 1997).

It should be mentioned that the differences among the three data points above 500 nm (Fig. 4) may not be significant, particularly due to the effect of sonication. Since these suspensions were not found thermodynamically and kinetically stable, their mean particle size strongly depended on the history of stirring and sonication. However, the wide size range might be related to a possible physical ion pairing between K and the acid functional groups of Naproxen. If any, the ion pairing can lead the K chain segments to partially adsorb onto the hydrophobic surfaces of Naproxen.

The nanoparticles prepared in the presence of amino acid copolymers were visualized using atomic force microscopy (AFM) and a typical micrograph is provided in Fig. 5. Particles were dried on a silicone wafer for microscopy study, and particle aggregation during the drying process was inevitable. In aqueous conditions, the surface energy of particles is counterbalanced by steric or ionic stabilization. However, in dried conditions, polymer chains solidify themselves and their stabilization can be no longer effective, resulting in significant aggregation as can be found in the previous study (Lee, 2003). Indeed, both single particles and aggregates can be found in the micrographs. They are not typically spherical, indicating their crystalline nature that can be confirmed in the thermal analyses discussed below.

3.4. Stability of nanoparticles

Retaining the crystallinity of drug particles during processing is beneficial to the stability of drugs. Wet comminution could sometimes significantly damage the crystallinity

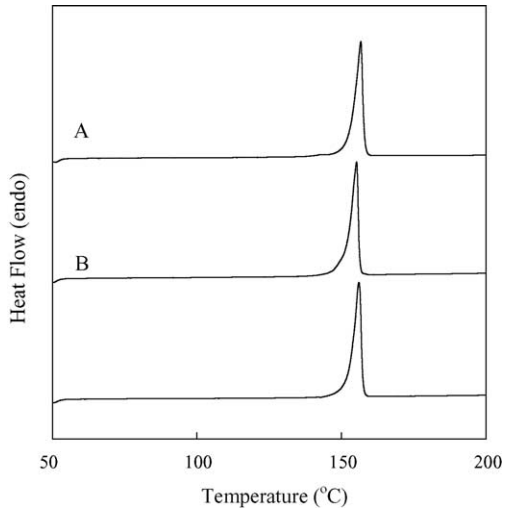


Fig. 6. DSC thermograms of drug nanocrystals stabilized by amino acid copolymers show the crystallinity of drug: (A) drugs as received, ΔH_f = 128 J/g; (B) nanoparticles with K-b-F-1, ΔH_f = 117 J/g; (C) nanoparticles with K-r-F-13, ΔH_f = 127 J/g.

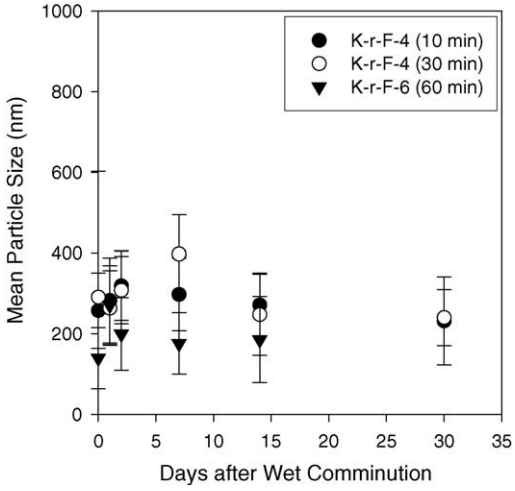


Fig. 7. Plot of mean particle size vs. time shows the physical stability of drug nanosuspensions at room temperature after wet comminution. Wet comminution times are given in parentheses.

of particles, and so possible changes in the crystallinity of the drugs by wet comminution were checked by DSC. Fig. 6 shows that the crystallinity of the drugs was not significantly changed by wet comminution. In the figure, changes in heat of fusion and melting temperature were almost negligible. Caution needs to be taken in the generalization of this result to other pharmaceutical active ingredients, since each drug crystal has a different physical stability.

After the preparation of nanoparticles by wet comminution with the aid of polymers, nanoparticles could slowly aggregate back into microparticles with a desorption process of polymers. Also, crystal growth via the Osward ripening (Adamson and Gast, 1997) due to the dissolution of drug particles can trigger significant changes in particle size. To investigate the physical stability of prepared nanoparticles in water, particle size was measured at an appropriate time interval up to 30 days at room temperature. Fig. 7 provides the typical data showing that there is no significant change in the mean particle sizes of nanosuspensions during the examined period of time. They seem to be stable, which opens the possibility in the application of the presented formulation process to an actual pharmaceutical unit operation.

3.5. Chain architecture

In this study, both the type of amino acids and their composition are the major factors determining particle size reduction. The hydrophobicity of polymers depends on the two factors. Thus, it is the case that the hydrophobicity of polymers is more important than the chain morphologies of polymers such as block and random copolymers. Interestingly, the particle size results of Figs. 2 and 4 show that the relationship between hydrophobicity and mean particle size may not be linear.

Hydrophobicity mainly influences the physical adsorption of polymers onto hydrophobic drug surfaces, while chain morphology mainly affects the adsorption conformation and resulting steric repulsion of polymers. As a result, this study indicates that the physical adsorption of polymers is the primary determining factor in the effective wet comminution and steric stabilization of drug nanocrystals. In fact, wet comminution continually provides mechanical fracture energy to drug crystals and creates fresh fracture surfaces (or cleavages), which will tend to fuse together unless quickly stabilized. Actual size reduction by fracturing could occur only with the *fast* adsorption of enough polymeric stabilizers onto the surfaces. The fast physical adsorption primarily requires a certain degree of hydrophobicity. This understanding can guide us to the future development of polymeric stabilizers for wet comminution processes, although it still needs further verification.

The amphiphilic copolymers in solid dispersion systems behave differently from those in emulsion systems (Ploehn and Russel, 1990; Adamson and Gast, 1997). In emulsions, they can form unique structures depending on the concentration of amphiphilic copolymers and their thermodynamic driving forces (Ploehn and Russel, 1990; Adamson and Gast, 1997). For example, critical micelle concentration depends on the concentration and hydrophobicity of copolymers. However, the preparation of drug nanocrystal dispersion depends on the fracture process of drug crystals and subsequent stabilization.

4. Conclusion

Amphiphilic amino acid copolymers having molecular weights of 5000–25,000 g/mol were found to be able to stabilize drug nanocrystals during wet comminution. K was used as the hydrophilic moiety of the copolymers, and A, L, and F

were employed as the hydrophobic moieties. In the case of A as the hydrophobic moiety, successful size reduction down to few hundred nanometers was not observed. Copolymers of K and the other two hydrophobic amino acids, i.e., F and L, were found to be efficient in nanoparticle formation by triggering stable polymer adsorption onto the hydrophobic surfaces of drugs for dispersion stabilization. For successful polymer adsorption and particle size reduction, the mole fraction of the hydrophobic moieties needed to be at least 15 mol%. The morphology of copolymers was not an important factor in determining particle size reduction. Once wet comminution in the presence of a proper amino acid copolymer produced drug nanocrystals, their particle size were found to be stable up to 30 days without significant aggregation. From these results, the hydrophobicity of polymer is a critical physical property, and amphiphilic amino acid copolymers seem to be an effective class of materials for pharmaceutical formulation of drug nanocrystals.

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