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Hydrophilic and hydrophobic amino acid copolymers for nano-comminution of poorly soluble drugs

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ABSTRACT

Nano-comminution has successfully brought nanoparticle formulations of poorly soluble drugs to our daily life. The key for the successful nano-comminution of a drug is the choice of a proper polymeric steric stabilizer. To systematically elucidate the rationale of stabilizer selection, two types of helical amino acid copolymers, relatively hydrophilic and hydrophobic copolymers, were used in nano-comminution. The hydrophilic copolymers had lysine as their major component. The addition of relatively hydrophobic leucine and phenylalanine to them could not make significant changes in particle size. However, when a small amount of hydrophilic glutamic acid or lysine was added into elastin-like hydrophobic copolymers of valine, glycine, and proline, significant composition dependence was found. Therefore, specific interactions between the functional groups of polymers and drug surfaces seem to be important for successful nano-comminution. The stimuli responsive behavior of the hydrophobic copolymer induced the temperature dependence of particle size.

1. Introduction

The use of drug nanoparticles has opened novel oral solid formulation opportunities for poorly water-soluble drugs (Lee et al., 2000; Torchilin, 2000). The drugs can have improved bioavailability and adsorption rate by increasing their dissolution rate (Amidon et al., 1995; Godwin et al., 2006; Elkharraz et al., 2006; Frick et al., 1998; Liversidge and Cundy, 1995; Kesisoglou et al., 2007; Rasenack and Muller, 2002). The dissolution rate depends on the surface area of drugs which can be increased by particle size reduction to nanometers (Craig, 2002; Leuner and Dressman, 2000; Takano et al., 2006).

A decrease in particle size resulted in an increase in Gibbs free energy by increasing the extra 'subdivision potential' term, which is mainly related to the increase in surface energy (Adamson and Gast, 1997; Hill, 2001). How to compensate for this term is the main issue to both the preparation and subsequent unit operations of nanoparticles (drying, granulation, compaction, etc.). Various methods to prepare drug nanoparticles such as liquid-based methods (e.g. nano-emulsions, nano-precipitation), nano-comminution, impinging jetting, and supercritical fluid methods use surface stabilizing materials and an external energy source to compensate for the extra Gibbs energy related to particle size reduction (Lee et al., 2006; Kesisoglou et al., 2007; Six et al., 2004; Verreck et al., 2005). For example, nano-comminution uses mechanical energy with the aid of one or more stabilizers (Grau et al., 2000; Liversidge and Conzentino, 1995; Merisko-Liversidge et al., 1996; Schonert, 1989, 1995). It is a commercially successful method used to produce Rapamune®, Emend®, Tricor®, and Megace® ES (Serajuddin, 1999; Yamada et al., 1999; Yin et al., 2005; Zheng and Bosch, 1997).

Ionic or steric stabilizations are the common mechanisms to stabilize the surface of hydrophobic drugs (Abdelwahed et al., 2006; Berglund et al., 2003a,b; Evertsson and Nilsson, 1997; Morrison and Ross, 2002; Ploehn and Russell, 1990). In the nanocrystal preparation, wet comminution uses polymeric stabilizers that adsorb onto the surfaces of drug particles and provide steric repulsion. For successful nano-comminution, the use of a proper stabilizer is critical since mechanical energy only cannot produce nanoparticles. Successful stabilization requires repulsive entropic force from the ceaseless thermal motion of polymeric chains, fast adsorption at full coverage, relatively long time scale for desorption, the absence of micelle forming or surface destructive behavior, etc. (Ploehn and Russell, 1990).

The selection of a proper stabilizer is not straightforward. The selection process has been developed only by trial and error. The previous results show that a drug requires a specific stabilizer (Choi et al., 2005; Lee, 2003; Lee et al., 2005, 2008a,b; Sepassi et al., 2007). The limited combinations of polymers and drugs restrict the freedom of formulation. Furthermore, the limited number of available pharmaceutical polymers further restricts the freedom.

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In the previous report, we developed a type of amino acid copolymer as a new class of materials for the stabilization of nanocrystals (Lee et al., 2005), which could have flexibility in engineering the chain architecture and biocompatibility (Yang et al., 2009). By controlling the hydrophobicity of amino acid copolymers using lysine (K), phenylalanine (F), leucine (L), and alanine (A), the effect of polymer properties on the mean particle size obtained from nano-comminution could be systematically elucidated. The chain architecture regarding random or block distribution of monomers along molecular chains was not a dominant factor, but the hydrophobicity of whole polymer was important. To have a significant particle size reduction, the content of hydrophobic amino acids should be more than 15 wt%.

The previous study used only one type of drug and four amino acids (Lee et al., 2005). In this study, the nano-comminution processes of amino acid copolymer are further studied using various drugs, and hydrophilic and hydrophobic amino acid copolymers, i.e., fully synthetic hydrophilic copolymer obtained by NCA-ring-opening polymerization and hydrophobic elastin-like polymer obtained by recombinant DNA technique. Both the hydrophilic and hydrophobic polymers are known to have helical structures instead of random chain structures. Unexpectedly, not just hydrophobic interactions but specific interactions of functional groups between polymers and drugs are found to be important.

2. Materials and methods

2.1. Materials

Various poorly soluble drugs (Fig. 1), i.e., ibuprofen (Dr. Reddy's, India), digitoxin (HPLC, >99%, Fisher, U.S.A.), naproxen (Tokyo Kansei Kogyo, Japan), predinisolone acetate (M-030402, Shaanxi Rainbow Pharm., China), nifedipin (DY-Mach, India), hydrocortisone acetate (HAC030304, Tianjin Tianyao Pharm., China), and itraconazole (Choongwae Pharm, GMP0004, South Korea) were used without preparation. Their particle sizes were all above 1 μm (ibuprofen, digitoxin, naproxen, predinisolone acetate, nifedipin, hydrocortisone acetate, itraconazole = 48, 2.8, 67, 20, 24, 4.1, 9.3 μm, respectively). The original particle sizes were checked following the method described below. Triphosgene (98.0%), n-hexylamine (99.0%), hydrogen bromide (30 wt% solution in acetic acid), and L-alanine from Aldrich were used. N^{ε}-carbobenzyloxy-L-lysine (N^{ε}-CBZ-L-lys) was commercially available from Bachem AG. L-Phenylalanine and L-leucine from TCI America, diethylamine (98.0%) from Yakuri Pure Chemical Co., N,N′-dimethylformamide (99.5%) from Junsei, and tetrahydrofuran (THF, 99.0%), n-hexane (95.0%), and diethyl ether (97.0%) from Daejung Chemicals and Metals Co., were used. 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. Hydroxypropyl cellulose (HPC, JP) was obtained from Nisso. Its average molecular weight was 60 kg/mol (surface energy 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 copolymers

The amino acid copolymers were synthesized following the method described in our previous report (Lee et al., 2005). 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). In short, N^{ε}-CBZ-L-lysine, L-phenylalanine, and L-leucine were converted to corresponding N-carboxy anhydrides (NCAs). The lysine-based copolymers were synthesized by ring opening polymerization with n-hexylamine as an initiator via the amino acid NCA (Van Dijk-Wolthuis et al., 1997; Daly and Poche, 1988). The compositions of comonomers and the degree of polymerization were characterized by ^{1}H NMR (Lee et al., 2005).

The elastin-like polymers (ELPs) were $(GVGVP)_{251}$ [designations: ELP-V], $(GVGIP)_{260}$ [ELP-I], (GVGVP GVGVP GEGVP GVGVP GVGVP GVGVP)$_{35}$(GVGVP) [ELP-E] and (GVGVP GVGVP GKGVP GVGVP GVGVP GVGVP)$_{35}$(GVGVP) [ELP-K], and their molecular weights were 102, 109, 88, and 88 kg/mol. The experimental details on the biosynthesis and purification of protein-based polymers could be found elsewhere in details (Guda et al., 1995; McPherson et al., 1996; Urry et al., 1998). Following the same technique, protein-based polymers were expressed from recombinant DNA in *Escherichia coli* (Bioelastics Research, Ltd.). In the subsequent purification after biosynthesis, the phase separation property of the Tt transition was used (Lee et al., 2001a,b,c). After purification, polymers were lyophilized. MALDI-TOF (matrix-assisted laser desorption ionization time-of-flight) mass spectrometry and ^{1}H NMR were used to confirm the synthesis following the same experimental methods previously reported (Urry et al., 1998).

2.3. Wet comminution

Drug particles were mixed in a 30 mL bottle with distilled water, polymeric stabilizer (1.33 wt% in slurry) and yttria-stabilized zirconia beads (50%, v/v). Then, the nano-comminution process was performed by ball milling for 4 days at 100 rpm at room temperature. The weight of the slurry (drug + stabilizer + water) was 7.5 g, and the concentration of drug in water was 8 wt%. Temperature increase due to ball milling was +4 °C, which was measured by inserting a thermocouple into the slurry. The 4-day comminution was enough to make the volume average size of drug particles to reach a steady state value. After filtering out zirconia beads, the suspension was stored at 5 °C for further characterizations.

2.4. Characterizations after comminution

A Horiba Laser Light Scattering Particle Size Analyzer LA-910 was used to measure the particle size of drug (refractive index = 1.06, ultrasonic chamber power = 40 W 39 kHz, 340 mL/min stirring flow (level 3), 95–100 mL water medium). Drug concentration for the measurement was ca. 0.02 wt%, and the repeated measurements of at least 3 times produced the error ranges of volume-averaged sizes. Particle morphology was investigated by a Hitachi (Japan) scanning electron microscope (SEM) S-4700 at 4 kV and 0.5 Hz. Samples were prepared by drying suspension drops on SEM sample stages previously cleaned, and they were coated with Pt-Pd at a coating speed of 6.7 nm/min for 2 min. Thermal characterization was obtained from differential scanning calorimeter (DSC, TA2010, TA Instrument, U.S.A.) measurements. Samples of 5 mg were scanned from 25 to 250 °C at 10 °C/min heating ramp.

3. Results and discussion

3.1. Hydrophilic lysine copolymers

The amino acid copolymers used in this study are composed of phenylalanine (F), leucine (L), lysine (K), glutamic acid (E), glycine (G), valine (V), and proline (P). The polymers can be categorized into two types, i.e., one relatively hydrophilic polymers (mainly K with a small mol. fraction of F or L (<22%)), and the other relatively hydrophobic copolymers (mainly G, V, and P). Although the typical polymers used for nano-comminution had been relatively

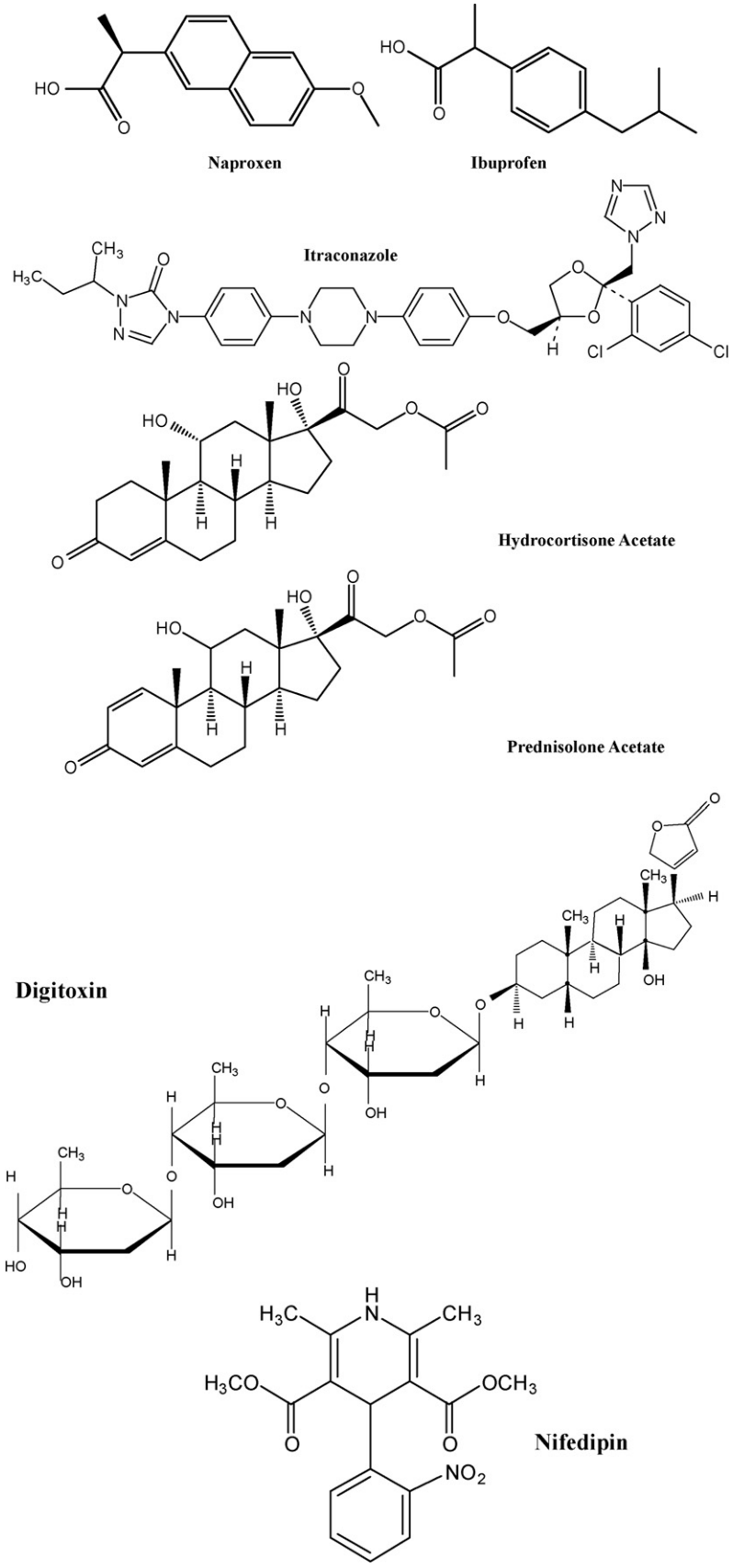
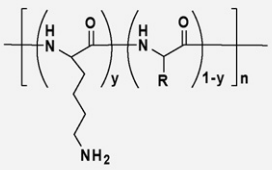


Fig. 1. Chemical structures of drugs used in nano-comminution.

Table 1
Designations and properties of amino acid copolymers.

Amino Acid Copolymers: Designations	K content (mol%)	Molecular Weight (Mw)	Degree of Polymerization (DP)	Chemical structure (R = F or L)
K10	100	8400	66	
K9F1	89	9200	70	
K7F3	68	14000	105	
K8L2	78	13100	105	
ELP-V	$(GVGVP)_{251}$			
ELP-I	$(GVGIP)_{260}$			
ELP-E	$(GVGVP\ GVGVP\ GEGVP\ GVGVP\ GVGVP\ GVGVP)_{35}(GVGVP)$			
ELP-K	$(GVGVP\ GVGVP\ GKGVP\ GVGVP\ GVGVP\ GVGVP)_{35}(GVGVP)$			

hydrophobic or amphiphilic, the relatively hydrophilic copolymers of K and F (or L) were able to stabilize naproxen nanoparticles in our previous study (Lee et al., 2005). The hydrophilic polymers have primary amines at their side chains. The hydrophobic polymers, often called ELP (elastin-like polymers), have a pentapeptide sequence that is commonly found in the tropoelastin structures (Lee et al., 2001a,b,c). The hydrophobic polymers have no polar functional groups (ELP-V and ELP-I) or they have a carboxylic acid or a primary amine at every 30 amino acid residues (ELP-E and ELP-K). Table 1 shows the designations of polymers.

Table 2 shows the particle sizes of drugs achieved in presence of lysine copolymers at the steady state of nano-comminution. All the particle sizes above 1 μm are written as 'm' to clearly identify the successful cases of nano-comminution with considering the error ranges. The drugs are listed in the order of their surface energies measured by the contact angle measurement (Lee et al., 2005, 2008a). The mean particle sizes were mostly between 300 and 700 nm. This is relatively large compared to the other nano-comminution cases (Lee et al., 2005, 2008a; Kesisoglou et al., 2007). Significant particle size reduction is obtained in the 18 cases, while the other 10 cases (denoted as 'm') do not show significant size reduction. Except the cases of digitoxin and nifedipin, all the other cases of insufficient particle size reduction are found in the two drug systems, i.e., itraconazole and ibuprofen.

The change in the content of hydrophilic amino acid (change in lysine content) does not seem to produce significant change in the volume-averaged particle size. Our previous report on the nano-comminution of naproxen using amino acid copolymers showed that an increase in lysine content slightly reduced the particle size reduction ability (Lee et al., 2005). The same effect is not significant in the other 6 drug cases in Table 2 using amino acid copolymers of 70–100 mol% K contents. In the cases of nifedipin, hydrocortisone acetate, prednisolone acetate, and digitoxin, no significant changes are observed as a function of lysine content. Therefore, the incorporation of hydrophobic amino acids such as L and F into polylysine (K10) backbone does not significantly change steric stabilization ability.

The presence of L instead of F reduces the effectiveness of nano-comminution in the cases of digitoxin and nifedipin, but it is hard to decide whether the trend is meaningful. The phenyl group in F might be more helpful in stabilizing the surface of digitoxin and nifedipin.

The overall results of Table 2 show us that the amino acid copolymers having high lysine contents can act as proper steric stabilizers in the nano-comminution of poorly soluble drugs. Even polylysine can be a successful steric stabilizer. Among amino acid polymers, polylysine is a relatively hydrophilic polymer having strong hydrogen bonding capability. Therefore, it is a rather unexpected result that the hydrophobic surface of various poorly soluble drugs can be stabilized by the relatively hydrophilic polymer. The typical hydrophilic polymers such as polyethylene glycol, polyacrylic acid, and polyvinyl alcohol have never been reported as good steric stabilizers (Grau et al., 2000; Lee et al., 2008a; Liversidge and Conzentino, 1995; Merisko-Liversidge et al., 1996; Schonert, 1989, 1995).

3.2. Hydrophobic elastin-like copolymers (ELP)

If the use of a hydrophilic amino acid polymer is successful for nano-comminution, the use of the other extreme polymer such as ELP might not be successful. Fig. 2 shows the results of nano-comminution of naproxen using ELPs. HPC is used for comparison, which has been known as a good stabilizer for naproxen (Evertsson and Nilsson, 1997; Lee et al., 2008a). At 25 °C, the polymer of GVGVP (ELP-V) sequence is unable to produce significant particle size reduction. Only ELP-E and ELP-K produced particle sizes comparable to the cases of HPC.

The nano-comminution experiments using ELPs were performed at different temperatures. This is because the polymers are known to be temperature responsive. They have critical temperatures (inverse transition temperature, Tt) above which their chain

Table 2
Particle sizes (μm, standard deviations in the parenthesis) of drugs after 1 h nano-comminution in the presence of four different copolymers.

Drugs	Polymers			
	K8L2 (37.0)	K7F3 (36.6)	K9F1 (38.4)	K10 (36.7)
Ibuprofen (52)	m	m	m	m
Naproxen (43)	0.36 (±0.11)	0.59 (±1.60)	0.77 (±2.02)	0.52 (±1.16)
Digitoxin (43)	m	0.36 (±0.10)	0.36 (±0.10)	0.35 (±0.10)
Prednisolone acetate (40)	0.54 (±0.29)	0.36 (±0.10)	0.44 (±0.16)	0.38 (±0.11)
Nifedipin (39)	m	0.57 (±0.27)	0.61 (±0.29)	0.54 (±0.25)
Hydrocortison acetate (38)	0.48 (±0.40)	0.41 (±0.12)	0.45 (±0.16)	0.43 (±0.14)
Itraconazole (36)	m	m	m	m

Surface energies of polymers and drugs obtained by the static contact angle measurement are given in the parentheses (dyn/cm^2). The 'm' indicates the systems of mean particle sizes above 1 μm.

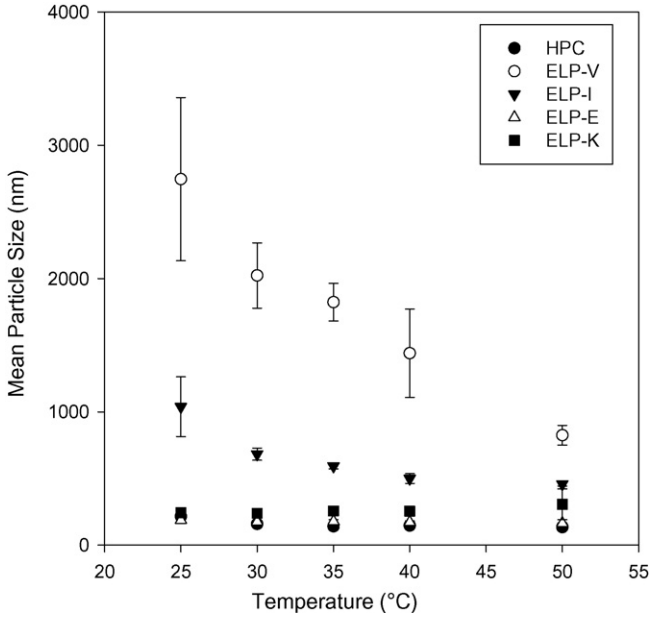


Fig. 2. Particle size reduction of naproxen in presence of ELP as a function of processing temperature. HPC was used as a control.

structures become more regular, and adopt spiral triple helix structures (Lee et al., 2001a; Urry, 1997). Below the critical temperature, they adapt random chain architecture. As a result, phase separation behavior similar to LCST behavior in petroleum-based polymers can be found. The major difference between the inverse transition temperature and LCST is that the chain architecture of ELP undergoes structural changes at the inverse transition temperature on top of phase separation behavior (Urry, 1997).

Fig. 2 shows that significant particle size reduction can be achieved in the ELP-E and ELP-K cases. The comminution processes using ELP-V and ELP-I could not produce significant particle size reduction at a low temperature, but a higher temperature helps the particle size reduction. The particle size of ELP-V case processed at 25 °C is above 2 μm, but the same system produces a particle size below 1 μm at 50 °C. The comminution processes using ELP-E and ELP-K successfully reduce the particle size of naproxen, and the temperature dependence of particle size is not significant.

In the cases of ELP-V, the decrease of particle size with an increase in temperature does not precisely reflect the existence of a critical temperature (ca. 37 °C). The decrease is relatively smooth and does not show any critical temperature point or transition in Fig. 2. All the four ELPs are known to have critical temperatures (Urry, 1997).

The particle sizes in Fig. 2 may reflect the surface adsorption ability of ELPs. Following the method previously reported (Lee, 2003), TGA measurements provided the amounts (g) of adsorbed polymers on the surface of 1 g drug particles, which were 0.96, 0.76, 1.17, 0.36, and 0.45 g/g at 25 °C for HPC, ELP-V, ELP-I, ELP-E, and ELP-K, respectively. Interestingly, the EPL-V and ELP-I cases had larger amounts of polymers on the surface of drugs, but their stabilization abilities were poor. Their adsorption amounts were similar to that of HPC, but the particle sizes of the systems were much larger than that of HPC case. ELP-E and ELP-K cases had smaller amounts of polymer chains on the surface of drugs, but they seem to be more effective stabilizers (possibly stronger adsorption and steric repulsion).

The particle sizes in Fig. 2 do not directly reflect the amounts of adsorbed polymers on the surface of drug particles. Therefore, it might be the case that we can have stronger adsorption with a full coverage and slower desorption kinetics if specific interactions between polymer chains and the functional groups on the surface of drugs exist (Ploehn and Russell, 1990). The carboxylic acid and amine functional groups of polymers can interact with the carboxylic acid, amide, hydroxyl, ether, and amine functional groups of drugs (Fig. 1).

The differences in the amounts of adsorbed polymers might be influenced by the difference in molecular weight, since ELP-E and ELP-K are 14 or 21 kg/mol lighter than ELP-V and ELP-I. As the molecular weight of polymer increases, stronger surface adsorption can be expected by an increased thermodynamic driving force, but the diffusion of polymer for the adsorption takes more time (Morrison and Ross, 2002; Ploehn and Russell, 1990).

It could be conjectured that the hydrophobic moiety of an amino acid copolymer provides a driving force for chain adsorption onto the hydrophobic surface of drugs. This is why the amphiphilic nature of polymeric stabilizers has been considered to be beneficial (Berglund et al., 2003a; Hanson et al., 2008; Ploehn and Russell, 1990). However, in this study, the existence of lysine or glutamic acid seems to provide a driving force for chain adsorption and steric stabilization. Indeed, among the ELP cases in Fig. 2, the most hydrophobic ELP-I could not produce the smallest particle size. ELP-V having no ionic functional groups could not produce drug nanoparticles at a temperature between 25 and 40 °C. An incorporation of a small amount of K or E into ELP-V improved the steric stabilization ability of ELP, resulting in a significant particle size reduction in the cases of ELP-E and ELP-K.

When only ELP-V and ELP-I are compared (two ELPs having no polar side functional groups), more hydrophobic ELP-I performed better. As temperature increases, the polymers undergo phase separation (Tt transition), resulting in an increase in their hydrophobicity. This increase in hydrophobicity induces more particle size reduction in nano-comminution (Fig. 2). Thus, the more hydrophobic, the better performance in nano-comminution was observed. However, ELP-I could not produce a size comparable to those of ELP-E and ELP-K. This point indicates that the existence of polar functional groups seems to be strongly helpful for the surface adsorption and the steric repulsion of polymer chains.

An interesting and important result was obtained when ELP-K was mixed with ELP-E by 1:1 molar ratio. It has been known that the mixture of ELP-K and ELP-E produced an ionic complex due to the structural complexation between the amine and carboxylic acid functional groups (Urry, 1997; Lee et al., 2001c). The complexation mixture had a critical temperature similar to ELP-V (ca. 37 °C). When the complex mixture was used in the nano-comminution of naproxen at room temperature (under the same total polymer concentration and the other experimental conditions), the volume-averaged particle size was increased to 0.52 (±0.18) μm. The structural complexation makes the two amino acid polymer chains move together, and the chain complex overall behave more hydrophobic than the each chain before complexation (ELP-E and ELP-K). However, the specific interactions between polymers and drug surfaces will be greatly prohibited. An increase in particle size due to the complexation leads us to speculate that the specific interactions are more important than hydrophobic interactions in nano-comminution. The complexation may also reduce the flexibility of polymer chains, which makes the discussion rather less conclusive.

It might be good to mention that the effect of temperature is not directly related with the hydrophobicity of polymers. An increase in temperature will increase the hydrophobicity of ELP, but the chain structure and flexibility of ELP changes too. The chain becomes more structured above the critical inverse temperature (Tt), which is not helpful for steric stabilization.

3.3. Microscopy investigation

Figs. 3 and 4 show the SEM micrographs of drug particles. The nifedipin case is given as a typical example, and the ibupro-

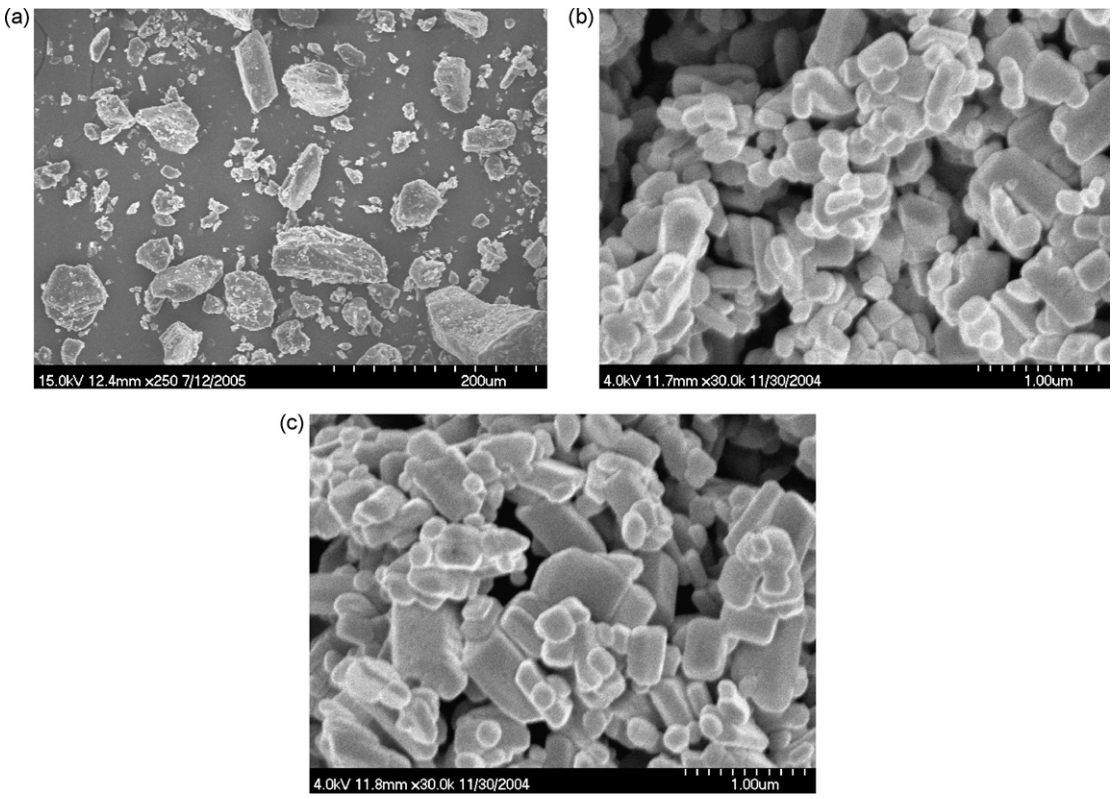


Fig. 3. SEM micrographs of nifedipin particles before and after nano-comminution. The results of DSC thermal analysis are in the parenthesis. (a) Nifedipin as received (T_m = 174.3 °C, ΔH = 98.3 ± 1.24 J/g); (b) nifedipin + K8L2 (T_m = 168.9 °C, ΔH = 58.7 ± 0.35 J/g); (c) nifedipin + K7F3 (T_m = 170.4 °C, ΔH = 105.0 ± 0.58 J/g).

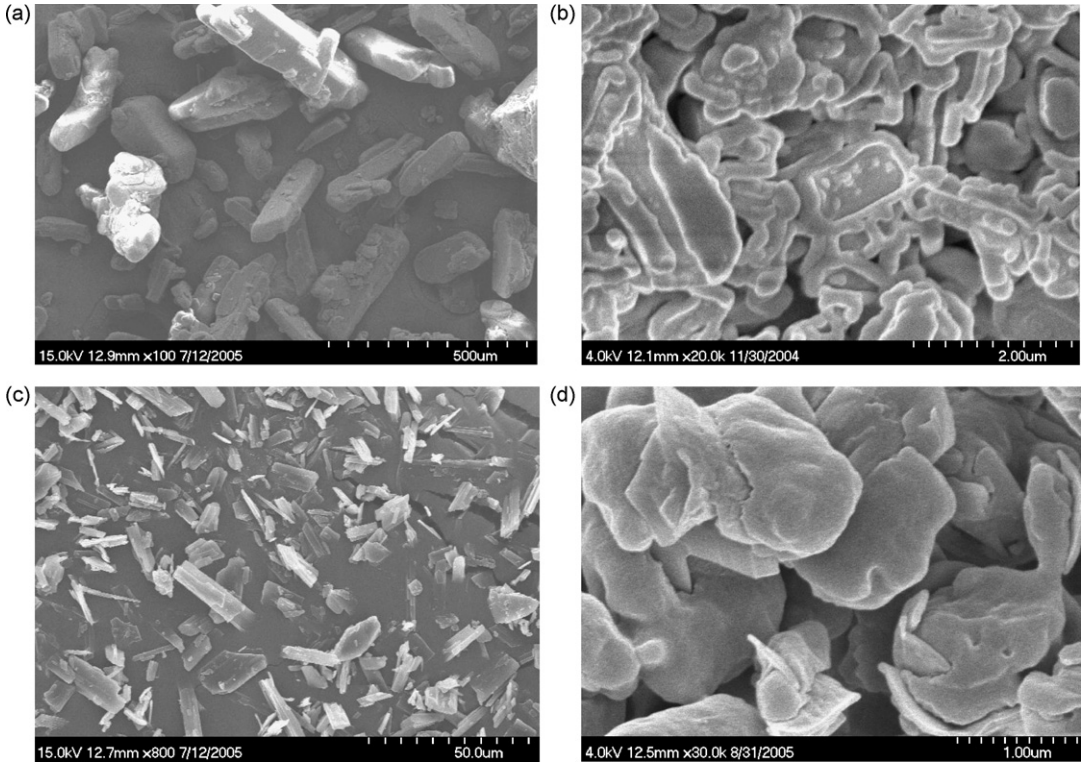


Fig. 4. SEM micrographs of ibuprofen and itraconazole particles before and after nano-comminution. The results of DSC thermal analysis are in the parenthesis. (a) Ibuprofen as received (T_m = 79.8 °C, ΔH = 175.0 ± 0.42 J/g); (b) ibuprofen + K7F3 (T_m = 78.5 °C, ΔH = 154.8 ± 0.81 J/g); (c) itraconazole as received (T_m = 169.0 °C, ΔH = 90.8 ± 0.45 J/g); (d) itraconazole + K7F3 (T_m = 163.0 °C, ΔH = 50.7 ± 0.22 J/g).

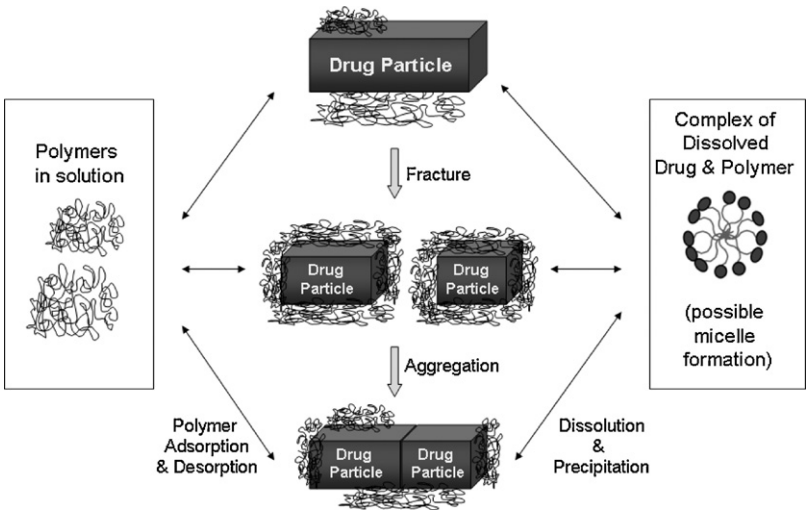


Fig. 5. Schematic diagram of particle fracture and assembly between drug particles and polymers during nano-comminution.

fen and itraconazole cases are the exceptions. The nifedipin particles as received show a common crystalline habit. After nano-comminution, nanoparticles of low aspect ratios were produced from significant attrition and fracture. Fig. 3b and c have particle sizes above and below 1 μm, respectively (Table 2). The DSC results show that Fig. 3b sample has smaller and more imperfect crystalline phases than Fig. 3c. However, the difference in particle size could not be easily discerned in the SEM micrographs. All the primary particle sizes seem to be below 1 μm, and the aggregation of primary particles appears to induce the increase in particle size.

The difference in particle size was relatively discernable only in the cases of ibuprofen. The melting temperature of ibuprofen was 79.8 °C. The nano-comminution was performed at room temperature, and the temperature increase during comminution was measured to be +4 °C. However, attrition and friction between media and drug particles in a viscose polymer solution could generate a significant local heating. Fig. 4b indeed shows ibuprofen particles partially molten during the comminution. Ibuprofen might re-crystallize during and after comminution, but its ΔH was significantly smaller than that of particles as received. The melting of drug particles might be the reason why successful particle size reduction could not be achieved in the cases of ibuprofen.

The particle size results of itraconazole in Table 2 are similar to those of ibuprofen, but SEM investigation shows that the reason for the insufficient particle size reduction might be different (Fig. 4c and d). The melting temperature of itraconazole was relatively high, and as a natural consequence, no significant melting of itraconazole crystals was expected. The DSC results indicated that significant damages in crystalline phases have occurred during comminution. Even the primary particles in Fig. 4d seem to be relatively large compared to the cases of nifedipin in Fig. 3c. Therefore, there might be a mechanism of crystallinity deterioration and related aggregation.

3.4. Amino acid copolymers for nano-comminution

Comminution cannot infinitely reduce the particle size of drugs, since it is related with the fracture characteristics of a material (Kendall, 1978; Lee, 2003). It is normally impossible to reach a size far below 100 nm. With the use of a proper polymeric stabilizer, a volume average size near or slightly above 100 nm can be achieved. In amino acid copolymer, various amino acids can be used to tailor the properties of polymers, while the flexibility of helical chain backbone can be kept largely constant. Therefore, the effects of polymer chemical properties can be investigated without considering the steric repulsion issues related with chain flexibility.

In this study, the cases of both the relatively hydrophilic and hydrophobic copolymers were successful. The smallest particle size results came from the systems of relatively hydrophobic polymers having a small amount of carboxyl or amine functional groups. An increase in the hydrophobicity of polymer by changing monomer composition or increasing processing temperature could not dramatically alter particle size. However, the addition of a small amount of carboxyl or amine functional groups into relatively hydrophobic ELPs generated dramatic effects in particle size reduction. All the drugs used in this experiment have polar functional groups that can interact with the polar functional groups of polymer chains (Fig. 1). Therefore, the specific interactions between polymers and drugs appear to be critical in designing a proper polymer for the nano-comminution of a drug.

Fig. 5 shows the possible mechanisms of nano-comminution. Solid drug particles can be fractured with aid of a polymeric stabilizer, but they can go back to bigger particles through aggregation and fusion (ibuprofen case). Dissolved drug molecules can have complex structures with polymers, although the population of this structure is limited by the poor water solubility of drug. Polymers could be in pseudo-equilibrium through reversible adsorption and desorption. The complexity of the mechanism is the reason why the selection of a stabilizer is not straightforward, compared to the common nano-emulsion cases.

4. Conclusions

Poorly water-soluble drugs were processed into nanoparticles in presence of amino acid copolymers. The stabilization ability of amino acid copolymers was greatly dependent on the type of drugs. Both hydrophilic polymers (polylysine and its copolymers) and relatively hydrophobic polymers (elastin-like polymers) could serve as the proper stabilizers for nano-comminution. The existence of polar functional groups in polymer chains (hydrophilic amino acids) was critical for successful nano-comminution. The specific interactions of polymer chains with drug crystal surfaces seemed to improve steric stabilization efficiency. The particle sizes of nanosuspensions processed with ELP-V and ELP-I had temperature dependence, but the other ELPs having a small amount of lysine and glutamic acid did not have significant temperature

dependence. These results show the possible use of amino acid copolymers for nano-comminution and their requirement to be successful stabilizers, which can provide a basis for the future preparation of intelligent drug delivery systems using protein or peptide stabilizers.

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