Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 305 (2005) 37–51
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Inhibition of a solid phase reaction among excipients that accelerates drug release from a solid dispersion with aging

Masayasu Mizuno [a], Yutaka Hirakura [a,*], Ikuro Yamane [b], Hideo Miyanishi [a], Shoji Yokota [a], Munetaka Hattori [b], Atsushi Kajiyama [a]

[a] *Pharmaceutical Research and Development Laboratories, Astellas Pharma Inc., 180 Ozumi, Yaizu, Shizuoka 425-0072, Japan*
[b] *Pharmaceutical Technology Laboratories, Astellas Pharma Inc., 180 Ozumi, Yaizu, Shizuoka 425-0072, Japan*

Received 17 June 2005; received in revised form 5 August 2005; accepted 20 August 2005
Available online 13 October 2005

Abstract

Hydrophobic drug substances can be formulated as a solid dispersion or solution using macromolecular matrices with high glass transition temperatures to attain satisfactory dissolution. However, very few marketed products have previously relied on solid dispersion technology due to physical and chemical instability problems, and processing difficulties. In the present study, a modified release product of a therapeutic drug for hypertension, Barnidipine hydrochloride, was developed. The drug product consisted of solid dispersion based on a matrix of carboxymethylethylcellulose (CMEC), which was produced using the spray-coating method. An enteric coat layer was sprayed on the surface of the solid dispersion to control drug release. Interestingly, the release rate accelerated as the drug product aged, while there were no indications of deceleration of the release rate which was due to crystallization of the drug substance. To prevent changes in the dissolution kinetics during storage periods, a variety of processing conditions were tried. It was found that not only use of non-aqueous solvents but also a reduction in coating temperatures consistently resulted in stable solid dispersions. The molecular bases of dissolution of the drug substance from those matrices were investigated. The molecular weight of CMEC was found to be a dominant factor that determined dissolution kinetics, which followed zero-order release, suggesting an involvement of an osmotic pumping mechanism. While dissolution was faster using a higher molecular weight CMEC, the molecular weight of CMEC in the drug product slowly increased with aging (solid phase reaction) depending on the processing conditions, causing the time-induced elevation of dissolution. While no crystalline components were found in the solid dispersion, the amorphous structure maintained a degree of non-equilibrium by nature. Plasticization by water in the coating solution relaxed the amorphous system and facilitated phase separation of the drug substance and CMEC upon production. The solid phase reaction advanced differentially in the solid dispersion depending on the degree of phase separation set initially. The use of non-aqueous solvents and/or a decrease in the coating temperatures inhibited the occurrence of phase separation upon production, thereby preventing the formation of CMEC-rich phases where the solid phase reaction occurred during storage.

Keywords: Solid dispersion; Phase separation; Dissolution; Solid phase reaction; CMEC; Aging

* Corresponding author. Tel.: +81 54 627 7294; fax: +81 54 629 6455.
E-mail address: yutaka.hirakura@jp.astellas.com (Y. Hirakura).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.08.021

1. Introduction

The number of poorly water-soluble compounds has rapidly increased after high throughput screening and combinatorial chemistry were introduced into the development processes for pharmaceuticals (Leuner and Dressman, 2000). To ensure oral bioavailability, solubility behavior as well as permeability in the gastrointestinal tract is of critical importance. Accordingly, the need for a solid dispersion system as an alternative in formulation selection has increased (Serajuddin, 1999). Macromolecules used to create a solid dispersion are divided into several groups including polyethyleneglycols (PEG) (Guyot et al., 1995), polyvinylpyrolydones (PVP) (Tantishaiyakul et al., 1996), and cellulose derivatives, such as hydroxypropylmethylcellulose (HPMC) (Okimoto et al., 1997; Kohri et al., 1999). Since most drugs that are administered orally are absorbed through the intestinal wall instead of the stomach, carboxymethylethylcellulose (CMEC) (Kai et al., 1996), and polymethacrylates (Shukla, 1994) are sometimes selected as a matrix to absorb water and disintegrates only in the intestine where the pH of the solution is neutral. Water-insoluble ethylcellulose has been used to form a rate-controlling membrane on the outermost layer of a drug product (Porter, 1989).

While drug substances are dispersed in the macromolecular matrix in solid dispersion, fine particles and amorphous forms of drug substances are chemically unstable and liable to generate degradation products during storage. It has also often been observed that solid dispersions are subject to structural change with aging due to their physical instability (Six et al., 2004). The physical instability reflects the fact that solid dispersions remain in a non-equilibrium state. Crystallization of drug substance has even been observed at below glass transition temperatures (Liu et al., 2002). Solid dispersions should therefore be processed with special care. For instance, if a tablet made of a solid dispersion is entirely amorphous and glassy, use of aqueous solvents and/or exposure to high temperatures upon film coating can plasticize the solid structure and allow phase separation and/or recrystallization to take place in the tablet. It is therefore of great importance to extensively examine the robustness of processing conditions and to conduct physical chemical characterizations of the solid dispersions formed by possible production schemes.

In the present study, processing conditions and long-term increases in the dissolution rate of a modified release product of a therapeutic drug for hypertension, Barnidipine hydrochloride, were investigated. The drug substance is only sparingly soluble in water and needs to be formulated as a solid dispersion to enhance its dissolution in the intestinal fluid. Solid dispersion was produced by the spray-coating (evaporation) method using carboxymethylethylcellulose as a macromolecular matrix and Tween 80 as a solubilizer. Since modified release was required in terms of the profile of the drug concentration in blood, a rate-controlling thinner layer was spray-coated on the surface of the solid dispersion using ethylcellulose (EC) and CMEC. Drug release from the drug product became significantly faster but not slower after long periods of storage. It is interesting that drug release was accelerated instead of decelerated with time, the latter of which is usually observed with drug release from a solid dispersion due to the crystallization of a drug substance. An objective of this study is to obtain the processing conditions under which stable products which show no increase in the dissolution rate with aging are consistently produced. The other objective is to elucidate the release mechanism and the nature of long-term increases in the dissolution rate. For these purposes, molecular weight measurement, compatibility testing, differential scanning calorimetry, scanning electron microscopy, swelling measurement, and moisture content determination were used.

2. Materials and methods

2.1. Reagents

Carboxymethylethylcellulose of different kinematic viscosities (mm^2/s) which comply with Japanese Pharmaceutical Excipients (JPE) was purchased from Sanyo Kasei Co., Ltd. (Aichi, Japan). Ethylcellulose was supplied by Dow Chemical (Midland, MI, USA). Nonpareil was obtained from Freund Corporation (Tokyo, Japan). Bardinipine hydrochloride was purchased from Sankyo Chemical Co., Ltd. (Tokyo, Japan). Polysorbate 80 (Tween 80) was supplied by Kao Corporation (Tokyo, Japan). Methanol and

dichloromethane for production were purchased from Nippon Kasei Chemical Co., Ltd. (Tokyo, Japan) and Asahi Glass Co., Ltd. (Tokyo, Japan), respectively. All the other inorganic and organic reagents were supplied by Kanto Chemical Co., Ltd. (Tokyo, Japan).

2.2. Production of the drug product

Barnidipine hydrochloride, CMEC, and Tween 80 (10:20:2.3, v/v) were dissolved in dichloromethane and methanol (4:1, v/v). The solution was spray-coated on nonpareils, purified sucrose spheres about 710–850 μm in diameter, in a fluid bed granulator (FLO-200, Freund Corporation) for 5 h (first coating process). After the coating process, granules were dried at 40 °C. Then, CMEC and EC (1:4, v/v) were dissolved in pure methanol or a mixture of methanol, dichloromethane, and water (5:4:1). The solution was spray-coated on the first coat granules (product temperature, 40 °C) in the same fluid granulator for 3 h (second coating process). After the coating process, granules were dried at 40 °C. They were then blended with talc in a double corn blender (YKS071003, Ishida-tec Co., Ltd. Shizuoka, Japan). Granules produced using the mixture of solvents and pure methanol for the second coating process were named "standard-coated" and "methanol-coated" granules, respectively. The product temperature during the first coating process was 40 °C in both cases. The other granules with a product temperature of 35 °C or less during the first coating process were named "low temperature-processed" granules regardless of the second coating solvents. The low temperature-processed granules were also dried at 40 °C after the first coating process. Diameters of the granules selected for use ranged between 500 and 1180 μm. Granules were placed in gelatin capsules, packed in a double aluminum blister sheet, and stored at 25 °C 60%RH unless otherwise stated.

2.3. Dissolution testing

Dissolution testing was performed with the European Pharmacopoeia (EP) paddle method with a sinker using commercial dissolution apparatus equipped with six dissolution beakers (VK7000 dissolution tester, Vankel, CA, USA). Experiments were carried out at 37 °C in 500 ml with constant stirring at 100 rpm. Granule contents of a capsule were put into a dissolution beaker containing 500 ml of dissolution medium 1 (DM1, 34.2 mM NaCl, adjusted to pH 1.2 using hydrochloric acid). After 2 h, DM1 was replaced with dissolution medium 2 (DM2, 100 mM sodium phosphate containing 0.3% Tween 80 adjusted at pH 7.5 using hydrochloric acid). Part of the dissolution medium was circulated every 15 or 30 min with a plunger pump to flow-through cells (quartz, 1 cm) where absorbance was measured at 360 nm using an ultra-violet spectrophotometer (Lambda 20, Perkin-Elmer, Inc., Boston, USA) to quantify the percentage of the drug substance dissolved from the granules. Results were evaluated according to USP ⟨724⟩ through to level two.

2.4. Gel permeation chromatography (GPC)

Mobile phase (5 ml) for chromatographic analyses was added to a centrifugal tube containing 130 mg of fresh or aged granules or samples from compatibility testing. The centrifugal tube was then shaken for 20 min using a commercial shaker (SA300, Yamato Scientific Co., Ltd., Tokyo, Japan). After the supernatant was decanted, the sediment was re-dispersed in 5 ml of the same solvent and shaken for 20 min using the shaker. Supernatant was decanted to mix with previously decanted supernatant. This solution was subjected to centrifugation (2500 rpm, 10 min) and the supernatant was filtered through a 0.45 μm membrane filter (Millex-LH, 0.45 μm, Bedford, USA) to form a sample solution.

The sample solution was injected into a gel permeation column, and CMEC in the solution were detected by monitoring the refractive index (RI) change. Since CMEC was extracted from methanol, other macromolecular components such as ethylcellulose were likely to be included in samples, but the chromatograms represented the distribution of molecular weights of CMEC because the macromolecule predominated in quantity. The chromatograms were normalized in the vertical direction so that the peak areas became constant, independent of the trials. The peak areas derived from two different samples were compared and the shaded area (a.u.) between the peaks shown in Fig. 1 was defined as the magnitude of "molecular weight change". The chromatographic conditions were as follows: HPLC, LC-10AD, Shimadzu, Kyoto; Column, TSK-GEL α3000 (7.8 mm i.d.

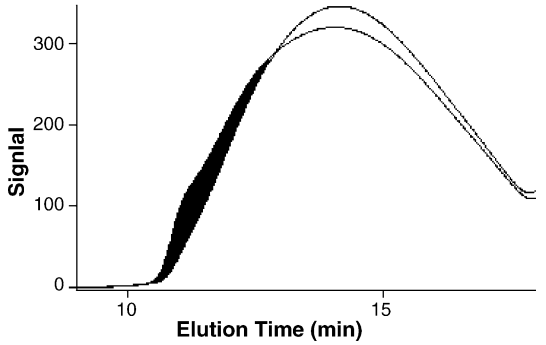


Fig. 1. Schematic view of how molecular weight increase was detected using gel permeation chromatography (GPC). Signal is the magnitude of refractive index (RI) change (arbitrary unit) caused by injection of a sample solution. Peaks were normalized in the direction of signal magnitude to have the same area. Elution time of a peak reflects molecular weights of CMEC in a sample solution. Two peaks were compared to each other, and the shaded area (a.u.) was defined as the magnitude of "molecular weight change".

30 cm, maximal exclusion molecular weight, 90,000 (PEG/PEO in water)), Tosoh, Tokyo; mobile phase, 10 mM LiBr in methanol; injection volume, 200 μl; flow rate, 0.5 ml/min; column temperature, 40 °C.

2.5. Compatibility testing

Samples were prepared as follows. Twenty milligrams of CMEC, 10 mg of Barnidipine hydrochloride (or Barnidipine free form), and/or 2.3 mg of Tween 80 (the first coat composition) were dissolved in 5 ml of methanol contained in a centrifugal tube. After the solvent was evaporated at 40 °C using a rotary evaporator and a vacuum pump for 30 min, the centrifugal tube was immediately used for gel permeation chromatography (initial) or sealed in an aluminum pouch and stored at 50 °C for 2 weeks (aged). Ten milliliters of methanol was added to the tube to dissolve the contents. After the solution was filtered through a 0.45 μm membrane filter, filtrate was subjected to gel permeation chromatography. Conditions of chromatographic analyses were the same as those for GPC of granules.

2.6. Swelling experiments

Swelling of granules was examined according to dissolution testing. Four grams of granules were weighed into a 15 ml plastic volumetric test tube (Falcon tube, Becton Dickinson, Franklin Lakes, NJ, USA). Nine milliliters of DM1 solution (pH 1.2) was added to the test tube, and immediately following, the volume of the granules in the solution was visually measured. After the test tube was gently shaken for 2 h using a commercial shaker (SA300, Yamato Scientific Co., Ltd.), increases in the volume of the granules were visually measured. Four milliliters of the supernatant was then replaced with the same volume of Japanese Pharmacopoeia disintegration test solution 2 (JP2 solution, pH 6.8, equivalent to DM2) containing or not containing 0.3% Tween 80 and the test tube was manually shaken to mix the contents. This procedure was repeated three times. One hour after the test tube was gently shaken using a commercial shaker, increases in the volume of granules were visually measured. Three milliliters of the supernatant was then replaced with the same volume of JP2 solution. One hour after the test tube was gently shaken using a commercial shaker, increases in the volume of the granules were again measured.

2.7. Preparation of the first coat casting films

Casting films of the first coat composition were prepared for differential scanning calorimetry (DSC) and scanning electron microscopy (SEM) experiments. Four grams of CMEC, 2 g of Barnidipine hydrochloride, and 0.47 g of Tween 80 were dissolved in 40 ml of a mixture of dichloromethane and methanol (4/1, v/v). Ten milliliters of the solution was cast on a laboratory dish made of Teflon, which was then left for about 1 h in an incubator (DP-23, Yamato Scientific Co., Ltd.) adjusted at 40 °C, and vacuum-dried for about 1 h to remove the solvent at the same temperature.

2.8. Differential scanning calorimetry

Thermal response from the granules and the first coat casting films was examined by differential scanning calorimetry (DSC6200, Seiko Instruments, Chiba, Japan). The sample (typically 8 mg) was placed in an aluminum pan, hermetically sealed, and placed in a furnace with a constant flow of nitrogen gas (flow rate, 60 ml/min). An empty hermetically sealed aluminum pan was used as a reference. The sample was heated and/or cooled at a rate of 10 °C/min. Liquid nitrogen was used to cool the samples.

2.9. Scanning electron microscopy

2.9.1. The first coat casting film

A casting film of the first coat composition was exposed to saturated vapor in a sealed vessel containing 4% osmium tetroxide aqueous solution for 24 h (or 1% solution for 72 h). Barnidipine hydrochloride in the casting film was selectively stained by osmium tetroxide, which preferentially binds to carbon–carbon double bonds. The stained film was retrieved and dried for 12 h under ambient conditions. The dried film was then lightly attached to a sample platform using double sided adhesive tape. Platinum was then sputtered on the dried film for 1 min (JFC-1600, automatic fine coater, JEOL, Tokyo, Japan) and images were observed (JSM-5510, scanning electron microscopy, 15 kV, WD 10 mm, JEOL) of the back scattering electrons (MP-64070, Si P-N type back scattering electron detector, JEOL), which mainly originated from the heavy metal, osmium, concentrated on the drug substance.

2.9.2. The granules

Intact granules and those treated with JP2 solution were used to observe their surface configurations. To treat granules with the solution, 50 mg of the granules were put into a glass centrifugal tube. After 50 ml of JP2 solution was added, the tube was shaken for 30 min using a commercial shaker (SA300, Yamato Scientific Co., Ltd.). Supernatant was decanted and the granules were re-dispersed in 50 ml of JP2 solution. The tube was again shaken for 30 min and the supernatant decanted. After they were filtered using a membrane filter, granules were washed with purified water and fully dried in dry air overnight. Intact and treated granules were then lightly attached to a sample platform using double sided adhesive tape. Platinum was sputtered on the dried granules for 1 min (JFC-1600, automatic fine coater, JEOL) and secondary electrons were detected to display images of the surface topography (JSM-6700F, field emission scanning electron microscopy, 3.0 kV, WD 3.0 mm, JEOL).

2.10. Moisture content determination

Moisture contents in the granules (about 260 mg) were measured using the Karl Fisher method (coulometric titration method, CA-100, Mitsubishi Chemical Corporation, Tokyo, Japan) with a water vaporizer (VA-100, Mitsubishi Chemical Corporation).

3. Results and discussion

3.1. The modified release drug product

Structure of the drug product is illustrated in Fig. 2A. Drug product is composed of three-layered granules filled in a gelatin capsule packed in a double aluminum blister sheet. A sucrose pill about 1 mm in diameter called nonpareil sits in the granule core, on which a solid dispersion is spray-coated consisting of carboxymethylethylcellulose, Tween 80, and the drug substance, Barnidipine hydrochloride (first coat layer, about 30% drug loading). Another solid dispersion consisting of ethylcellulose and CMEC is spray-coated on the first coat layer, forming a thinner layer that controls dissolution of the drug substance (second coat layer). Although it is sparingly soluble in acid solutions, CMEC is fairly soluble in neutral solutions due to the ionization of carboxyl groups. Since EC repels

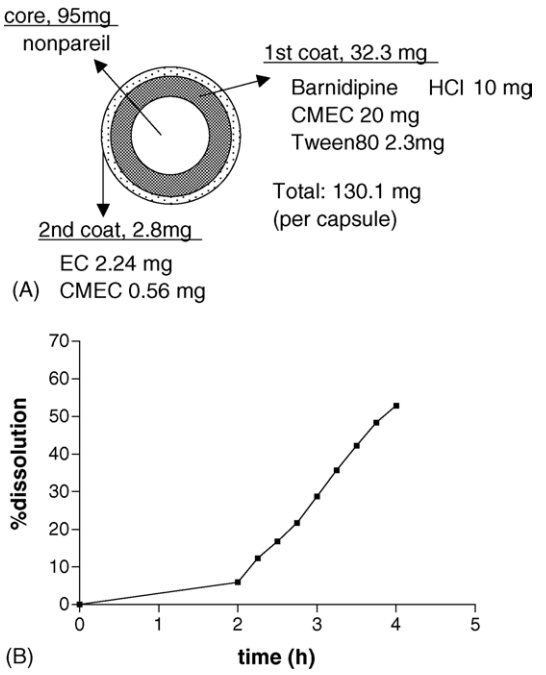


Fig. 2. (A) Illustration of a granule of the drug product. (B) A dissolution profile from granule contents of a capsule is shown.

water molecules and is hardly soluble at any pH, penetration of water into the granule can be controlled. Because this drug product was intended to dissolve in the small intestine, dissolution testing needs to be designed so as to simulate the gastrointestinal environment. For this reason, the granules were initially immersed in an acidic DM1 solution (pH 1.2) for 2 h, and subsequently in a neutral DM2 solution (pH 7.5). The upper and lower limits at 2 h in the acid solution were set for 10 and 0%, respectively. The lower limit was set for 46% at 2 h in the neutral solution (dissolution at 4 h). The upper limit at 4 h (dissolution at 4 h) also needed to be set for an appropriate level (66%) for the drug product to function as a modified release pharmaceutical. A dissolution profile of fresh standard-coated granules from a capsule is demonstrated in Fig. 2B.

3.2. Release mechanism

In an attempt to gain insight into the drug release mechanism of the granules, we first looked for parameters controlling drug release using fresh standard-coated granules. Positive correlation was found between viscosity of CMEC used to form the first coat layer and dissolution at 4 h from fresh granules. As shown in Fig. 3, using more viscous CMEC in the first coat layer resulted in higher dissolution ($R = 0.73$) whereas viscosity of CMEC in the second coat layer had nothing to do with dissolution (not shown). While correlation is evident between viscosity and dissolution, how the two parameters are linked to each other remains uncertain from a mechanistic point of view. Since CMEC can be a hydrophilic macromolecule, we hypothesized that swelling, which appears to be effected by viscosity, determines dissolution and examined swelling behavior of fresh granules produced using different lots of CMEC. While the granules were expanded to 1.2–1.3 times as much as the initial volumes in the acid solution in the first 2 h, swelling enhanced and diverged to 1.3–1.7 times as much as the initial volumes in the following neutral solution. Equilibrium was reached much faster than 1 h in the neutral solution. No difference was observed in the degree of volume expansion whether Tween 80 was included in the neutral solution or not. Fig. 4 demonstrates the dissolution at 4 h (A) and viscosity of CMEC (B) plotted as a function of the degree of volume expansion of granules in the neutral solution. Obviously, volume

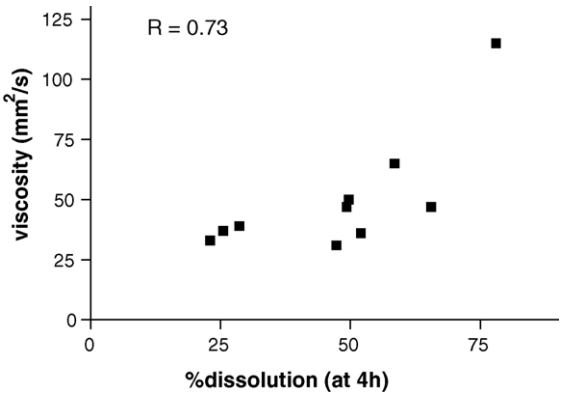


Fig. 3. Viscosity (mm^2/s) of CMEC plotted as a function of dissolution at 4 h (%). Correlation coefficient ($R = 0.73$) was calculated using all data points.

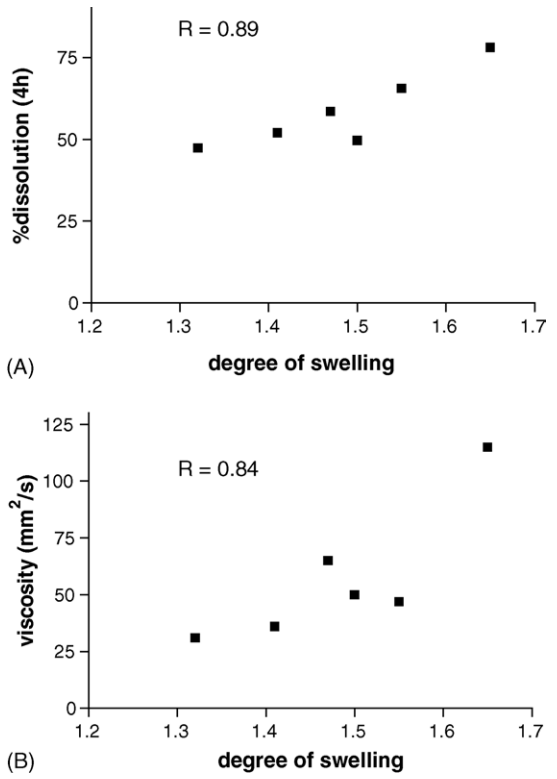


Fig. 4. Dissolution at 4 h (%) and viscosity of CMEC (mm^2/s) are shown as a function of the degree of swelling of granules in JP2 solution. (A) Correlation with dissolution at 4 h ($R = 0.89$). (B) Correlation with viscosity of CMEC ($R = 0.84$).

expansion showed good correlation with both of the two parameters. These results suggest that viscosity of CMEC determines the ability of swelling, and hence the rate of dissolution.

In order to examine how swelling leads to dissolution, we took scanning electron micrographs before and after dissolution testing in JP2 solution. Fig. 5A demonstrates a granule surface previously immersed in JP2 solution. The pores observed should be the spots where CMEC of the second coat layer was originally located because the macromolecule is fairly soluble in the neutral solution. The remaining porous matrix composed of EC would be elastic enough to sustain the tensile stress induced by volume expansion arising inside because integrity of the granule was preserved after the immersion. In striking contrast, however, a granule surface shows much smoother texture before immersion in JP2 solution (Fig. 5B), again suggesting that the pores are the remains of CMEC dissolved from the second coat layer. Reasonably, the drug substance passes through those pores upon dissolution.

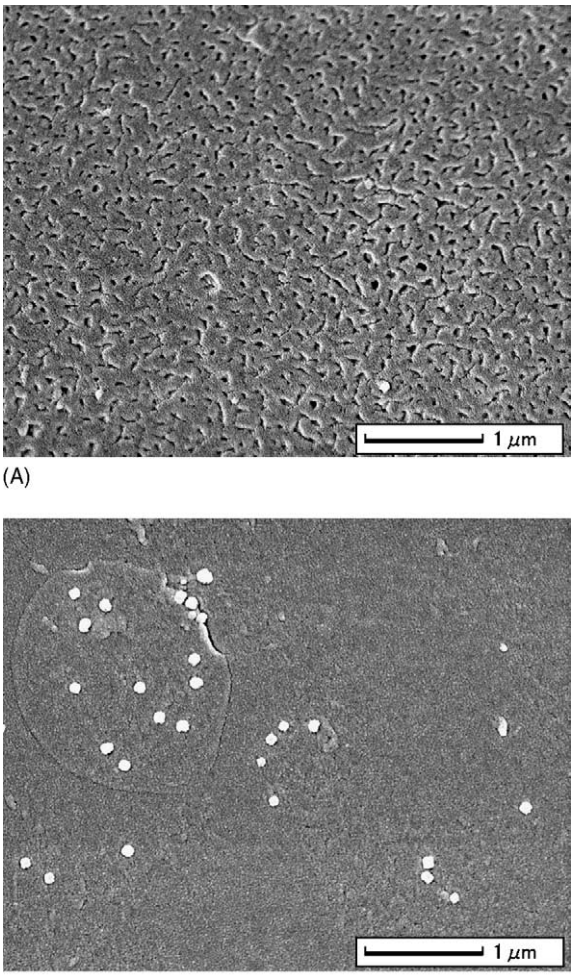


Fig. 5. SEM images obtained with granules before and after dissolution testing (×25,000). (A) Surface topography of a granule that was previously immersed in JP2 solution. Note that there are a lot of gaps and holes on the surface subject to dissolution. (B) Surface topography of an intact granule. A very smooth texture can be observed except for scattered white dots. It is unclear what the white dots are.

Osmotic pumping as well as a simple diffusion mechanism was probably involved in the dissolution process of the drug substance. This is supported by a finding that dissolution followed zero-order kinetics after granules were transferred to a neutral solution (Fig. 2B) where swelling occurred rapidly. A high osmotic pressure is expected for the inner first coat layer in a neutral solution due to the ionic dissociation of CMEC. Dissolution initiated by elementary osmotic pumping was earlier described by Theeuwes (1975), believed to be one of the major release mechanisms (Zentner et al., 1985), and is characterized by zero-order release kinetics. Previously, Hjartstam and Hjertberg (1998) reported that dissolution of metoprolol from a pellet coated with a rate-controlling film consisting of EC and HPMC (24% or less) was governed by the osmotic pumping mechanism. Since the second coat layer composition is equivalent to the rate-controlling EC/HPMC (24% or less) membrane (Hjartstam and Hjertberg, 1998) in a neutral solution, such as DM2, a simple diffusion mechanism alone would not account for the dissolution rate. Although permeability of the second coat layer to water or the drug substance has not been determined, it is certain that the dissolution rate is controlled by the water permeability of the second coat layer. Naturally, permeability of the second coat layer depends heavily on the swelling of granules because a stretched EC-rich matrix would include wider pores through it (Fig. 6). The second coat layer thus controls the penetration of water into granules, and hence the efflux of the drug substance, depending on the swelling behavior of the first coat layer (Fig. 4). The more swollen the granule is, the wider the pores will be, and the wider the pores are, the greater the permeability of water and/or the drug substance will be.

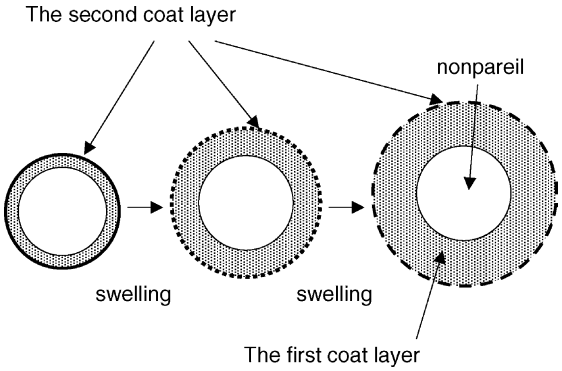


Fig. 6. Schematic view of swelling of the first coat layer is shown. When a granule is immersed in water, swelling occurs in the first coat layer depending on the viscosity of CMEC. Volume expansion stretches the second coat layer to create pathways (pores) permeable to water and the drug substance. Diameters of pores become larger as swelling advances, promoting penetration of water and dissolution of drug substance from granules.

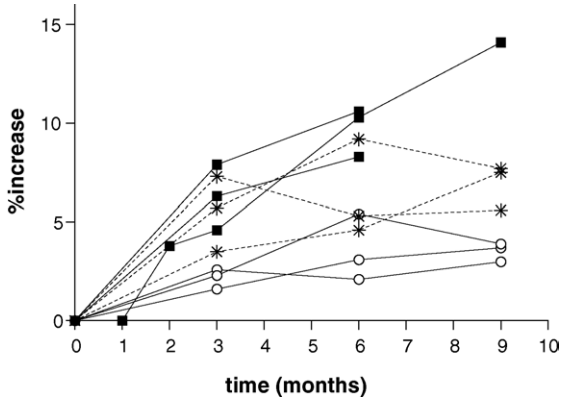


Fig. 7. Increases in dissolution at 4 h as a function of aging time (at 25 °C 60%RH). Percentage increase (vertical axis) was calculated according to the following formula: Percentage increase = dissolution percentage at 4 h at X months − dissolution percentage at 4 h at initial time. Symbols represent coating conditions (black square, standard-coated granules; star, methanol-coated granules; white circle, low temperature-processed granules (pure methanol for the second coating)). Results from three lots are shown for each coating condition.

3.3. Development of a processing condition to produce stable granules

Dissolution at 4 h was found to increase with aging of granules and was highly sensitive to the conditions of spray-coating in the production processes. Although it has to remain constant throughout storage periods, dissolution at 4 h gradually increased over time and sometimes reached an upper limit with standard-coated granules (Fig. 7). While dissolution at 4 h from fresh granules is easily controlled by trial and error such as changing the thickness of the second coat layer upon production, dissolution from aged granules is not easy to examine and control because it is time-consuming and unpredictable by nature. After repeated examinations, we succeeded in producing granules whose dissolution at 4 h remained below the upper limit over a year not only by using pure methanol (methanol-coated, star) instead of a mixture of solvents (standard-coated, black square) for the second coating but also by lowering product temperature in the first coating process (low temperature-processed, open square). Evidently, while the rate of increase in dissolution at 4 h depended on conditions of either the first or the second spray-coating, dissolution at 4 h almost leveled off at well below the upper limit with methanol-coated granules stored at 25 °C 60%RH. This is particularly true for low temperature-processed granules, with which the percentage increase of dissolution at 4 h became constant at a significantly lower level.

3.4. Mechanism of elevation of dissolution at 4 h by aging

We showed that viscosity of CMEC in the first coat layer correlates well with dissolution at 4 h of fresh standard-coated granules (Fig. 3). This correlation leads to the possibility that if viscosity of CMEC increases in granules with aging, dissolution at 4 h will increase as well. Therefore, molecular weights of CMEC in standard-coated, methanol-coated, and low temperature-processed granules of different ages were investigated using the gel permeation technique to obtain information on the viscosity of CMEC in fresh and aged granules in comparison to dissolution at 4 h of those granules. Gel permeation chromatography provides information on molecular weights of CMEC at first, and is instrumental in estimating a viscosity increase of the macromolecule because a positive correlation, if not proportionality, occurs between molecular weight and viscosity. Granules used for measurements were directly packed in an aluminum pouch for aging to resist accelerated and stressed storage conditions. Fig. 8A demonstrates leftward signal shifts observed in

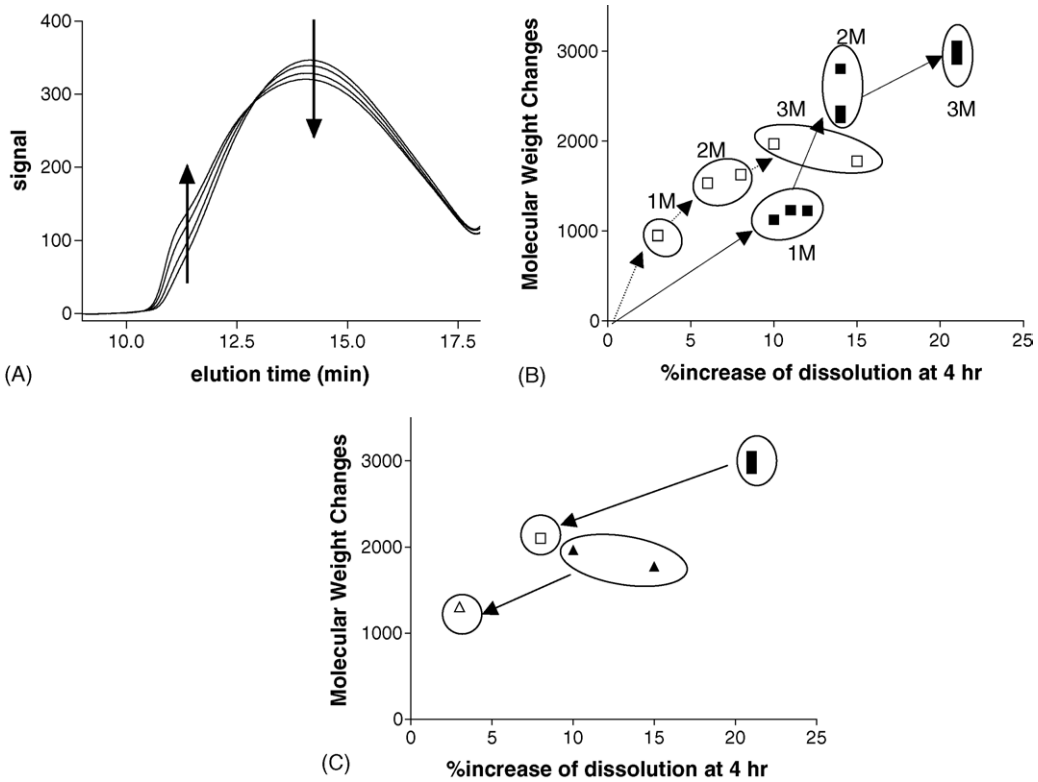


Fig. 8. Molecular weight increases of CMEC in granules over time are shown. (A) Leftward shifts of CMEC signals observed in GPC are shown. (B) Methanol- (open square) and standard-coated (closed square) granules. (C) Methanol- (triangle) and standard-coated (square) granules whose first coat layers were produced at a lower temperature (open symbols) as compared to standard temperature-processed granules (black symbols). (B and C) The horizontal axis denotes the percentage increase of dissolution at 4 h as defined in Fig. 7. The vertical axis denotes molecular weight increase as defined by "molecular weight change" shown in Fig. 1.

GPC using different ages of standard-coated granules (0, 2, 4, 6 weeks at 50 °C), indicating that molecular weights of CMEC actually increase in the granules over time. The increases are substantial, corresponding to viscosity increases in the order of 10^1 (mm^2/s). Fig. 8B indicates that the molecular weights of CMEC in granules stored at 40 °C increased over longer periods (1–3 months), showing a good correlation with the increases in dissolution at 4 h. Strikingly, the molecular weight increase as well as the percentage increase of dissolution at 4 h was lower with granules produced using pure methanol (methanol-coated, open square) than a mixture of solvents (standard-coated, black square), indicating that molecular weight increase can provide an excellent marker of the time-induced elevation of dissolution at 4 h observed with granules produced using different methods (Fig. 7). Importantly, those differences observed in molecular weight and dissolution between standard- and methanol-coated granules became more evident as the granules grew older, confirming the significance of the differences and the relevancy of the analytical method. Fig. 8C demonstrates the effects of product temperature set for the first coating process. As the adoption of a lower temperature (low temperature-processed) for the first coating process decelerated the rate of increase in dissolution at 4 h (Fig. 7), the increase over time (3 months at 40 °C) of the molecular weight of CMEC diminished accordingly (open square and triangle). These results suggest that the increase in dissolution with aging originated from a gradual increase in molecular weight (viscosity) of CMEC in the first coat layer of granules. Evidently, aging results in a molecular weight increase of CMEC in the first coat layer, which enables an increase of

the degree of swelling of granules, thus leading to enhanced dissolution at 4 h.

3.5. Variations in dispersion of the first coat layer components

While aging was found to cause increases of the molecular weights of CMEC in granules, an important question in terms of formulation development was why the molecular weights of CMEC increased differentially depending on the processing conditions (Fig. 8). In other words, the question was why methanol-coated and low temperature-processed granules were more stable than standard-coated granules. Because those granules have the same chemical composition, we first examined a possible difference in moisture content in granules by using the Karl–Fischer method. Table 1 demonstrates the moisture contents in standard-coated, methanol-coated, and low temperature-processed granules. No difference in moisture content was observed between standard- and methanol-coated granules, and the moisture contents in the granules remained constant over a year. Apparently, low temperature-processed granules contained a little less amount of moisture than the other ones but the difference may not be significant. The amounts of residual organic solvents including methanol and dichloromethane were also extremely low (not more than 0.01% or not detected) irrespective of the processing conditions (not shown). Since no significant differences were found in moisture content or residual organic solvent content, the granules stored in capsules have the same chemical composition including water molecules.

Table 1
Moisture contents in granules during storage at room temperature[a]

	1 month[b]	6 months[b]	12 months[b]
Std	–	0.69 ± 0.00	0.67 ± 0.03
Met	–	0.64 ± 0.04	0.63 ± 0.06
Low	0.58 ± 0.03	–	–

[a] Data are the weight percentages of moisture in the whole granules. Granules were stored at 25 °C 60%RH. Since moisture contents in granules equilibrate in several hours, these data represent stable moisture contents.

[b] Measurements were performed with standard-coated (Std) and methanol-coated (Met) granules at 6 and 12 months after production while measurements were conducted with methanol-coated low temperature-processed granules (Low) at 1 month after production.

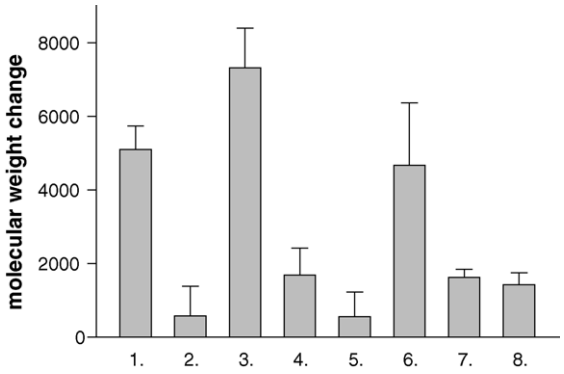


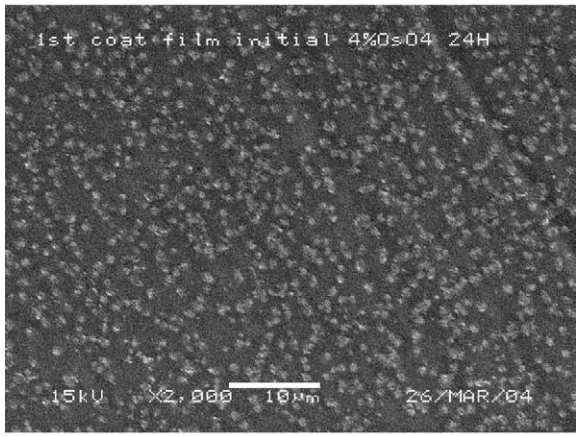
Fig. 9. Molecular weight increases of CMEC in granules and model films of the first coat layer and/or other compositions. Increases in molecular weight are shown with numbered columns and error bars: (1) CMEC:Barnidipine hydrochloride:Tween 80 = 20:10:2.3; (2) CMEC:Barnidipine free form:Tween 80 = 20:10:2.3; (3) CMEC:Tween 80 = 20:2.3; (4) CMEC:Barnidipine hydrochloride = 20:10; (5) CMEC:Barnidipine free form = 20:10; (6) CMEC only; (7) standard-coated granules; (8) methanol-coated granules. The vertical axis denotes molecular weight increase as defined by "molecular weight change" shown in Fig. 1.

Next, we designed compatibility testing to probe into factors controlling the rate of molecular weight increase of CMEC in the first coat layer of granules. Dry films that have the first coat and its relevant compositions were prepared by evaporating methanol at 40 °C using a rotary evaporator. Those films and granules (in an aluminum pouch) were then stored at 50 °C for 2 weeks to accelerate aging, and subjected to gel permeation chromatography to demonstrate increases in molecular weights of CMEC. Fig. 9 summarizes the results. CMEC by itself increased its molecular weight (column 6). Comparisons between columns 1 and 3 and between columns 4 and 6 indicate that the presence of Barnidipine hydrochloride inhibited molecular weight increase. Comparisons between columns 1 and 2 and between columns 4 and 5 showed that Barnidipine free form (instead of Barnidipine hydrochloride) further suppressed the molecular weight increase. A comparison between columns 1–3 and 4–6 shows that Tween 80 promoted molecular weight increase irrespective of composition. An emphasis should be placed on the finding that the molecular weight increases in granules (columns 7 and 8) are much smaller than that of a film of the same composition (column 1) whereas the difference between methanol- and standard-coated granules is only slight (columns 7 and

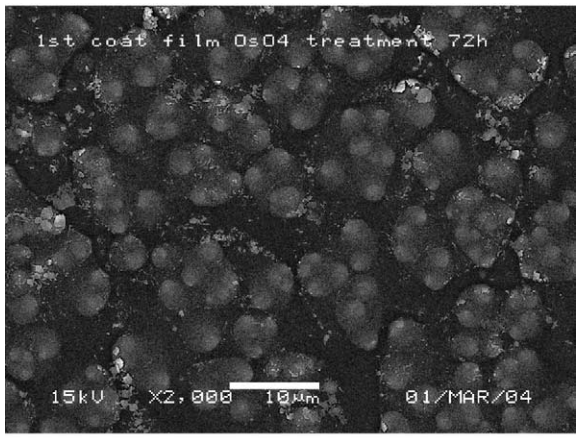
8). The significance of the difference between the granules became evident after granules aged (Fig. 8B, 2 and 3 months data). Since powder X-ray diffraction experiments using granules showed no indication of crystalline Barnidipine hydrochloride (not shown), a major fraction of the drug substance was amorphous in the first coat layer. CMEC is amorphous by nature. These results indicate that amorphous drug substance wedges in between CMEC molecules in the matrix, sterically interfering with chemical reactions among the macromolecules. The chemical composition being the same (Table 1), differences in molecular weight increase observed between granules produced using different production methods (Fig. 8B and C) are likely attributed to coarseness or fineness of the amorphous solid dispersion. Coarse dispersion would evade the steric hindrance by the drug substance and ensure a larger contact area among CMEC molecules, accelerating the rate of molecular weight increase of the macromolecule. In other words, it is possible that the rate of molecular weight increase is closely linked to distribution and/or disposition (phase structure) of the components in the first coat layer.

3.6. Visualization of phase separation in the first coat layer

In order to visualize phase variations suggested above, we examined scanning electron micrographs of first coat casting films. Since granules are covered with the second coat layer, visualization of the first coat layer is more easily attained using a first coat layer casting film. Specifically, the distribution and disposition of Barnidipine hydrochloride in the film was visualized by selectively staining the drug substance using osmium tetroxide and by observing the back scattering electrons which largely originated from the heavy metal. Staining was conducted with a casting film placed in an aqueous atmosphere (100%RH) in a sealed vessel containing a 1–4% osmium tetroxide aqueous solution for 24–72 h at room temperature. Staining advanced with time depending on the concentrations of osmium tetroxide. Fig. 10A shows an SEM image of a film stained for 24 h with a 4% osmium solution. A negative control experiment using a Barnidipine-free casting film demonstrated that the white parts represent the drug substance (not shown). Clearly, Barnidipine molecules gathered to create small domains and CMEC



(A)



(B)

Fig. 10. SEM images obtained with first coat casting films (×2000). White bars at the bottom of images indicate 10 μm. The white areas are the drug substance. (A) Casting films were stained for 24 h using a 4% osmium tetroxide solution. (B) Casting films were stained for 72 h using a 1% osmium tetroxide solution.

was isolated from the drug substance. In marked contrast, Fig. 10B shows another SEM image of a film stained for 72 h, where a 1% osmium solution was used instead to avoid the excessive staining of the film. The comparison with Fig. 10A indicates that the latter film contains much larger domains, suggesting that the drug substance tends to become separated from the CMEC matrix depending on the staining period. An X-ray experiment indicated that those samples contained no crystalline components (not shown), suggesting that the drug substance remained amorphous in the films. Six et al. (2004) formed solid disper-

sion of itraconazole-Eudragit E300 and observed that phase separation started from 13% drug loading, resulting in glassy clusters of pure drug. What occurs in a solid dispersion depends both on the composition of the solid system and the miscibility among the components due to the non-equilibrium nature of solid dispersion. In our system, relaxation under a very humid condition resulted in phase separation accompanying long-distance translational movements of molecules (from fine to coarse dispersion in the direction of Fig. 10A to B).

3.7. Glass transition of the first coat layer

In order to examine thermal response of the first coat layer, we used differential scanning calorimetry (Fig. 11). Chips of first coat casting films were used to eliminate possible interfering signals from components other than the first coat layer. Moisture content in the aluminum sample pan remained constant throughout the experiment because it was hermetically sealed. Fig. 11A shows repetition of downward and upward drifts of heat capacity responding to rising and falling temperatures, representing a glass transition. The DSC trace obtained with granules under the same experimental protocol was also characterized by the glass transition. No indication of a thermal response different from that observed with the first coat film was detected in the trace up to 120 °C. It has thus been postulated that DSC traces obtained with the film chips reflect the thermal response from the first coat layer of granules.

Fig. 11B demonstrates two consecutive experimental runs using a hermetically sealed aluminum pan containing chips of a first coat casting film. The aluminum pan was heated to 110 °C at a rate of 10 °C/min in the first run (dotted). After it was cooled to below 0 °C in the furnace, the sample was again heated to 110 °C at the same rate of 10 °C/min (solid). Note that, in the first run, an endothermic deflection was observed immediately after the glass transition temperature (T_g). This thermal response typically represents enthalpic recovery, being supported by the observation that the downward deflection disappeared in the second run. It should be noted that the T_g fell significantly in the second run. This marked contrast suggests that since long-range translational movement of molecules can occur above the T_g, the phase structure of the first coat film

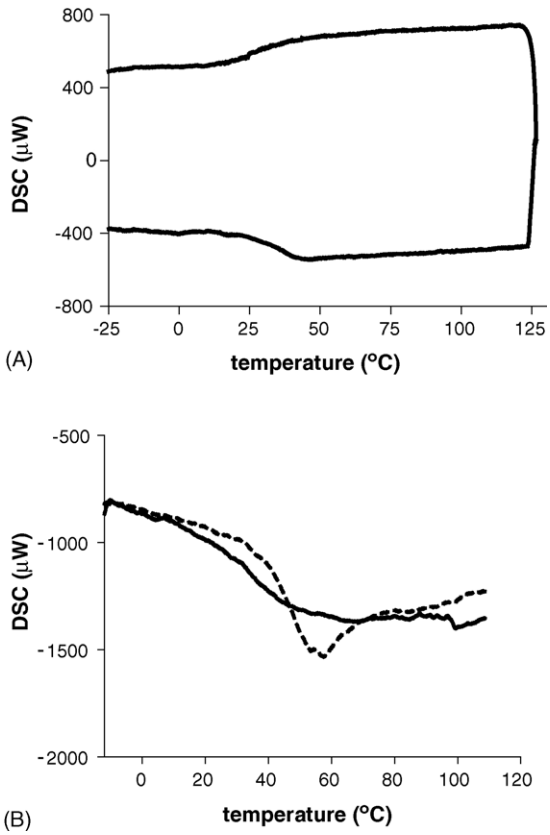


Fig. 11. Thermal analyses were performed using differential scanning calorimetry (DSC). (A) After equilibration at −30 °C, it was heated to 120 °C at a rate of 10 °C/min. Sample was immediately cooled to −30 °C at the same rate. (B) After equilibration at −30 °C, casting film was heated to 110 °C at a rate of 10 °C/min (dotted line). After cooling to −30 °C, sample was again heated to 110 °C at the same rate (solid line).

was relaxed at temperatures higher than the T_g observed during the first run, suggesting phase separation of the components (Fig. 10). This result indicates that the T_g represents phase structure of the first coat layer because the chemical composition of the film in the hermetically sealed aluminum pan remained unchanged throughout the experiment.

Since moisture contents in granules filled in capsules were uniformly about 0.7% (Table 1), we intended to determine the T_g of films and granules containing 0.7% moisture. The T_g were estimated to be 48.6, 45.5, and 24.4 °C for methanol-coated granules, standard-coated granules, and films, respectively. These results suggest that there is a small difference

in phase structure between standard- and methanol-coated granules whereas there is a great difference between films and granules, resulting in the differences observed in molecular weight increase among them (Figs. 8B and 9(columns 1, 7, and 8)). Reasonably, phase structure which shows a lower T_g (film for instance) is a coarse (separated) dispersion, which enables a faster increase in molecular weight because of its larger contact area among CMEC molecules. Coarseness of the phase structure of the film is also supported by the fact that upon film formation, there is enough time for the components to translate to the disposition which is thermodynamically favorable (phase separation) due to slow evaporation of the solvent while the first coat layer in the granule is vitrified before an advantageous disposition is reached due to fast evaporation of the solvent upon spray-coating. Another important point to note here is that the first coat layer of both methanol- and standard-coated granules stays in a glassy state during storage because their T_g are well above the room temperature. Since long-range translational movement does not occur in a glassy macromolecular matrix, phase separation does not advance as the T_g does not change with aging and, accordingly, those granules keep their own phase structure unchanged during storage in capsules. The molecular weights of CMEC in those granules thus increase differentially (Fig. 8) depending on the degree of phase separation set initially, thereby causing the dependence of the rate of increase in dissolution at 4 h on the production methods (Fig. 7).

3.8. Polymerization occurring below the T_g

Evidence supports the notion that molecular weight increase occurs with granules stored at room temperature. This suggests that polymerization proceeds among CMEC molecules in granules in a glassy state. This is consistent with the compatibility testing showing that CMEC by itself increased its molecular weight in a glassy state (Fig. 9, column 6) because the T_g of the macromolecule was much higher than the storage temperature (50 °C). Local molecular motions but not long-distance translational movements can occur at well below the T_g as enthalpic relaxation and crystalization were observed at the low temperatures (Andronis and Zografi, 1998; Rosilio et al., 1998; Yoshioka et al., 1994). It should also be noted that intramolecular cyclization (dehydration reaction between a carboxyl- and an amino-group) occurred with Quinapril stored at well below the T_g depending on the mobility of the molecule (Guo et al., 2000). It is therefore highly probable that those substituents such as carboxyl- and hydroxyl-groups in CMEC react with each other in a glassy state to form an ester bond to increase the molecular weight of the macromolecule. This is supported by the finding that molecular weight increase was reduced by using Barnidipine free form (Fig. 9). This implies the involvement of an acid catalyst in the esterifiying reaction. The facilitation of molecular weight increase in the presence of Tween 80 (Fig. 9) might suggest that the surfactant is included in the polymerization reaction due to its hydroxyl groups.

3.9. The origin of phase variations

Because phase structure remains unchanged during storage at room temperature, phase variations must occur in the production processes. Unlike the production of methanol-coated granules, water was used for the second coating process of standard-coated granules. It is therefore highly reasonable that water plasticized the non-equilibrium first coat layer of standard-coated granules upon second coating. Plasticization of the first coat layer above the T_g can result in phase separation as suggested by SEM (Fig. 10) and DSC (Fig. 11). It is true that the spray-coating was conducted in a drying environment but the second coating solvent was directly sprayed on the first coat layer and the solvent heavily contacted the first coat layer, particularly at an initial stage of second coating. Previously, Pourkavoos and Peck (1993) demonstrated that penetration of water into a tablet core in a film coating process modulates the solid structure and physical properties. The moisture contents in the granules immediately after the second coating process were found to be less than 0.85% (not shown). Since the T_g of the granule is 40 °C at 0.85%, the first coat layer is plasticized at the product temperature (40 °C) if the moisture content increases above 0.85% according to the Gordon–Taylor equation (Gordon and Taylor, 1952). Apparently, that observation might indicate that the granules stayed in a glassy state throughout the second coating process. However, this does not necessarily mean that plasticization or phase separation does not occur during the second coating process. Attention should be paid to the fact

that data available reflect the leveled moisture content throughout the process whereas a transient event of plasticization could promote phase separation upon second coating. It is even more reasonable to presume that changing the product temperatures of the first coating process creates phase variations in the first coat layer. It should be emphasized that there is no difference between standard temperature-processed (40 °C) and low temperature-processed granules except the first coating temperatures. Although clear explanations from a mechanistic point of view cannot be provided for the emergence of the phase differences between the granules, it is certain that temperature plays a great role in the phase formation because the first coat layer undergoes a liquid state upon first coating. In spite of the overwhelming importance of the controllability of phase structure, there is very little literature describing the relationship between processing conditions and the critical parameters concerning physical properties, probably reflecting the fact that it is usually difficult to monitor those parameters relevant to product quality. It is understandable that a great emphasis has been placed on process analytical technology (PAT), which would contribute to the detection of critical parameters such as water content in the production processes.

4. Conclusion

In the present study, we demonstrated that (1) stable granules are consistently obtained by use of pure methanol instead of a mixture of solvents for second coating and/or lowering product temperature for first coating; (2) dissolution at 4 h depends on swelling of granules which is effected by viscosity of CMEC used for first coating; (3) the age-induced elevation of dissolution at 4 h is ascribed to the increase of molecular weight of CMEC over time; (4) the dependence on production methods of the rate of increase in dissolution at 4 h is due to the phase differences set in the first coat layer of granules. It should be emphasized that those phase differences are marginal but still significant because they manifest themselves after long-term storage. It is this small difference that is critical in terms of quality control in pharmaceutical industry. The physical instability of the first coat layer rendered the rate of molecular weight increase highly susceptible to processing conditions. It should be noted that, in non-equilibrium systems, such as spray-coated layers, diverse amorphous solid dispersions with a variety of physical properties and compositions occur according to processing conditions. Solvents, such as water used for spray-coating processes, can greatly affect the system. Robustness of the processing conditions should carefully be ensured in terms of glass transition of the amorphous system. While pharmaceutical excipients with high T_g are often used for formulation development, formulation scientists should be aware of a possibility that chemical reactions can occur among them. Polymerization did in fact manifest itself with CMEC over months or a year under ambient conditions. While the macromolecule was highly advantageous in producing modified release products due to their carboxyl groups being sensitive to environmental pH, it was found liable to chemical reactions, causing instability during long-term storage. Since excipients are not necessarily an inert chemical species, their long-term chemical stability should be fully taken into account upon formulation selection.

Acknowledgements

We are grateful to Dr. Satoshi Kitamura, Dr. Takao Mizumoto, and Mr. Mitsuhiro Nakamura for their critically reading the manuscript.

References

Andronis, V., Zografi, G., 1998. The molecular mobility of supercooled amorphous indomethacin as a function of temperature and relative humidity. Pharm. Res. 15, 835–842.

Gordon, M., Taylor, J.S., 1952. Ideal copolymers and the second-order transitions of synthetic rubbers. I. Non-crystalline copolymers. J. Appl. Chem. 2, 493–500.

Guo, Y., Byrn, S.R., Zografi, G., 2000. Physical characteristics and chemical degradation of amorphous quinapril hydrochloride. J. Pharm. Sci. 89, 128–143.

Guyot, M., Fawaz, F., Bildet, J., Bonini, F., Lagueny, A.M., 1995. Physicochemical characterization and dissolution of norfloxacin/cyclodextrin compounds and PEG solid dispersions. Int. J. Pharm. 123, 53–63.

Hjartstam, J., Hjertberg, T., 1998. Swelling of pellets coated with a composite film containing ethyl cellulose and hydroxypropyl methylcellulose. Int. J. Pharm. 161, 23–28.

Kai, T., Akiyama, Y., Nomura, S., Sato, M., 1996. Oral absorption improvement of poorly soluble drug using solid dispersion technique. Chem. Pharm. Bull. 44, 568–571.

Kohri, N., Yamayoshi, Y., Xin, H., Iseki, K., Sato, N., Todo, S., Miyazaki, K., 1999. Improving the oral bioavailability of albendazole in rabbits by the solid dispersion technique. J. Pharm. Pharmacol. 51, 159–164.

Leuner, C., Dressman, J., 2000. Improving drug solubility for oral delivery using solid dispersions. Eur. J. Pharm. Biopharm. 50, 47–60.

Liu, J., Rigsbee, D.R., Stotz, C., Pikal, M.J., 2002. Dynamics of pharmaceutical amorphous solids: the study of enthalpy relaxation by isothermal microcalorimetry. J. Pharm. Sci. 91, 1853–1862.

Okimoto, K., Miyake, M., Ibuki, R., Yasumura, M., Ohnishi, N., Nakai, T., 1997. Dissolution mechanism and rate of solid dispersion particles of nilvadipine with hydroxypropylmethylcellulose. Int. J. Pharm. 159, 85–93.

Porter, S.C., 1989. Controlled-release film coatings based on ethylcellulose. Drug Dev. Ind. Pharm. 15, 1495–1521.

Pourkavoos, N., Peck, G.E., 1993. Evaluation of moisture sorption by tablet cores containing superdisintegrants during the aqueous film coating process. Pharm. Res. 10, 1212–1218.

Rosilio, V., Deyme, M., Benoit, J.P., Madelmont, G., 1998. Physical aging of progesterone-loaded poly(D,L-lactide-co-glycolide) microspheres. Pharm. Res. 15, 794–798.

Serajuddin, A.T., 1999. Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs. J. Pharm. Sci. 88, 1058–1066.

Shukla, A.J., 1994. Polymethacrylates. In: Wade, A., Weller, P.J. (Eds.), Handbook of Pharmaceutical Excipients. American Pharmaceutical Association/The Pharmaceutical Press, Washington, DC/London, pp. 362–366.

Six, K., Verreck, G., Peeters, J., Brewster, M., Van den Mooter, G., 2004. Increased physical stability and improved dissolution properties of itraconazole, a class II drug, by solid dispersions that combine fast- and slow-dissolving polymers. J. Pharm. Sci. 93, 124–131.

Tantishaiyakul, V., Kaewnopparat, N., Ingkatawornwong, S., 1996. Properties of solid dispersions of piroxicam in polyvinylpyrrolidone K-30. Int. J. Pharm. 143, 59–66.

Theeuwes, F., 1975. Elementary osmotic pump. J. Pharm. Sci. 64, 1987–1991.

Yoshioka, M., Hancock, B.C., Zografi, G., 1994. Crystallization of indomethacin from the amorphous state below and above its glass transition temperature. J. Pharm. Sci. 83, 1700–1705.

Zentner, G.M., Rork, G.S., Himmelstein, K.J., 1985. The controlled porosity osmotic pump. J. Pharm. Sci. 1, 269–282.