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Increasing the Dissolution Rate of a Low-Solubility Drug Through a Crystalline-Amorphous Transition: A Case Study with Indomethicin

Xiaohong Pan
School of Pharmacy, University of Maryland, Baltimore, USA

Thomas Julian
School of Pharmacy, University of Maryland, Baltimore, and Genta, Berkeley Heights, NJ, USA

Larry Augsburger
School of Pharmacy, University of Maryland, Baltimore, USA

The purpose of this research is to further the understanding of the crystalline to amorphous phase transition (amorphization) that occurs when some crystalline drugs are dry blended with porous adsorbents. Indomethacin (IMC) and three grades of silica gel (SGs) were used in the study. Amorphization of crystalline IMC occurs rapidly during dry mixing with SG and was independent of mixing intensity and time. Extent of amorphization increases with lower ratios of IMC:SG and with decreased IMC and SG particle size. Blocking H-bonding silanol groups on SG by chemical modification reduced the extent of amorphization. IMC-SG mixtures showed improved dissolution rates over crystalline IMC, the improvement being directly related to the extent of amorphization. To preserve the improved dissolution rate, mixtures should be protected from moisture and heat. This approach holds promise as a mean of improving the dissolution rate of sparingly soluble drugs such as IMC.

Keywords amorphization; quantitative analysis; phase transition; differential scanning calorimetry; dissolution; X-ray powder diffractometry; indomethacin; silica gel; amorphous; crystalline; hydrogen bonding

INTRODUCTION

For poorly water-soluble drugs, improving dissolution is key to achieving the desired bioavailability. Most active pharmaceutical ingredients in products are in their stable form (crystalline form) for physical and chemical stability considerations. In more recent years, the use of high energy state materials, namely the amorphous materials, to improve the dissolution and hence the bioavailability of poorly water soluble drugs has been garnering increased interest (Hancock, 2002). However, maintaining the physical and chemical stability of amorphous active drugs during processing and storage is a substantial challenge.

Amorphous drug substance could be obtained by a number of different techniques (Hancock & Zografi, 1997). But, since the 1980s, there have been numerous publications on the formation of amorphous forms through the interaction of crystalline drugs with porous adsorbents (e.g., magnesium aluminum silicate, porous glass beads, calcium silicate, etc.) during dry blending and storage. For example, crystalline drugs were found partially or completely converted to the amorphous state after being dry blended with certain porous adsorbents (Hanawa, Ikoma, & Sugihara, 1997; Konno, Kinuno, & Kataoka, 1986; Nakai, Yamamoto, & Terada, 1984). Crystalline benzoic acid mixed with nonporous glass powder did not lose crystallinity during mixing or storage. It is believed that the porous nature and the pore size of the adsorbents played an important role in the crystalline to amorphous phase transition (amorphization) in the mixtures (Nakai, Yamamoto, & Izumikawa, 1989). Decreased pressure and increased temperature within the mixing chamber, as well as increased rotating speed and mixing time, favored the amorphization of flufenamic acid in mixtures with magnesium aluminum silicate (Konno, 1990; Konno & Kinuno, 1989). These observations led to the suggestion that amorphization took place via the gaseous phase (Konno, 1990). Hydrogen bonding between drugs and porous adsorbents has been postulated to be responsible for the loss of crystallinity of the drugs in mixtures (Konno, 1990; Konno & Kinuno, 1989). Mixtures of maltol and porous calcium silicate have exhibited improved dissolution profiles compared with the crystalline drug alone (Hanawa et al., 1996). The improved dissolution was believed to be the result of the amorphization

Address correspondence to Xiaohong Pan, School of Pharmacy, University of Maryland, 20 N. Pine Street, Baltimore, MD 21201, USA. E-mail: laugsbur@rx.umaryland.edu

of crystalline drugs in mixtures since the amorphous drugs usually exhibit higher apparent solubilities than their crystalline counterparts. The reported increase in dissolution rate resulting from the amorphization of crystalline drugs in dry blended drug-adsorbent mixtures may provide a new approach to improving the dissolution and hence the bioavailability of poorly water-soluble drugs.

To determine whether the amorphization of crystalline drugs in dry blended drug-adsorbent mixtures could be exploited to improve the dissolution of poorly water-soluble drugs, a further understanding of the mechanism that causes amorphization of crystalline drugs in crystalline-adsorbent mixtures is necessary. In previous papers, although hydrogen bonding between drug and adsorbent is believed to be the cause of amorphization of crystalline drugs in dry blended drug-adsorbent mixtures, no hydrogen bonding evidence was provided. Furthermore, the stability of amorphous materials formed in dry blended crystalline-adsorbent mixtures during storage has not been investigated. Previous papers only reported amorphization of crystalline drugs as "partial" or "complete" in dry blended mixtures, and none of them developed a quantitative method to determine the extent of amorphization or how much crystalline drug converted to its amorphous form in the mixtures.

Thus, the objectives of present study were to investigate the factors (i.e., mixing condition, drug-to-adsorbent ratio, particle size, moisture content, etc.) affecting the extent of amorphization of crystalline drugs in dry blended mixtures of crystalline drugs and porous adsorbents; to probe the possible mechanism of such amorphization; to evaluate the potential improvement in the dissolution of a model drug, and to evaluate the physical and chemical stability of the amorphous drugs formed in drug-adsorbent mixtures upon storage. In this work, crystalline indomethacin (IMC), a Biopharmaceutics Classification System (BCS) class II drug that has hydrogen binding groups (Figure 1) was selected as the model drug. Three different pore size grades of silica gels (SGs), which are porous and amorphous materials, were selected as the adsorbents. Amorphization of indomethacin in IMC-SG mixtures was evaluated by quantitative differential scanning calorimetry (DSC), as previously described (Pan, Julian & Augsburger, 2006).

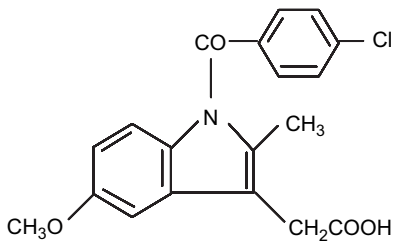


FIGURE 1. Structure of IMC.

EXPERIMENTAL

Materials

Three different grades of SG (60–200 μm) with pore sizes of 40, 60, and 100 Å were purchased from Sigma-Aldrich (St. Louis, Missouri). Dichlorodimethylsilane (Dow Corning®, Midland, MI) was obtained from Sigma-Aldrich (St. Louis, Missouri). Poloxamer (LUTROL® F68 NF Prill surfactant) was obtained from BASF (Florham Park, New Jersey). Acid-washed glass beads (60–200 μm) and dimethyldichlorosilane (DMCS) were purchased from Sigma-Aldrich (St. Louis, Missouri).

Crystalline and Amorphous IMC

IMC has two known major polymorphs: γ-form and α-form. The γ-form is the stable form and shows a melting endotherm at approximately 159°C to 162°C (O'Brien, McCauley & Cohen, 1984). The α-form is a metastable form and shows a melting endotherm at approximately 151°C to 154°C (O'Brien et al., 1984).

Crystalline (γ-form) indomethacin was obtained from Sigma and was used without further treatment. It has a melting point of 162°C and a heat of fusion of 106 J/g, similar to the reported values (O'Brien et al., 1984). The α-form IMC was prepared by dissolving 10 g of γ-form IMC in 10 ml of ethanol at 80°C, then 20 ml of room temperature distilled water was added. The precipitate was α-form IMC, which was collected by filtration and vacuum drying as reported by Kaneniwa, Otsuka, and Hayashi (1985). The prepared α-form IMC has a melting temperature of 152°C and a heat of fusion of 97 J/g, which is similar to the reported values (O'Brien et al., 1984).

Amorphous IMC was prepared by melting 2 to 5 g of crystalline IMC (γ-form) at 162°C to 165°C in a 2-inch aluminum foil pan using a heating plate and quench cooling the melt in liquid nitrogen. The glassy amorphous IMC was ground with a mortar and pestle. Powders with particle size < 44 μm were collected by sieving through a #325 mesh screen. Polar light microscopy and X-ray powder diffractometry confirmed that the prepared samples were amorphous. The prepared amorphous IMC has a glass transition temperature of 41°C as measured by DSC, which is similar to the reported values (O'Brien et al., 1984). Figure 2 shows the XRPD profiles of SG, γ-form, α-form, and amorphous IMC.

X-Ray Powder Diffraction (XRPD)

A Rigaku Geigerflex X-ray diffractometer (model 4037V1, Tokyo, Japan) with Ni-filtered Cu-Kα radiation was used under the following experiment conditions: voltage, 40 kV; current, 40 mA; divergence slit, 1/2 degrees; scatter slit, 1/2 degrees; receiving slit, 0.3 mm; receiving slit, monochrometer, 0.6 mm; scanning speed, 3.0 degrees/minute; scanning range, 3 to 50 degrees (2θ). A powder bed of 1 mm thickness was prepared using 70 to 90 mg of samples on the glass holder.

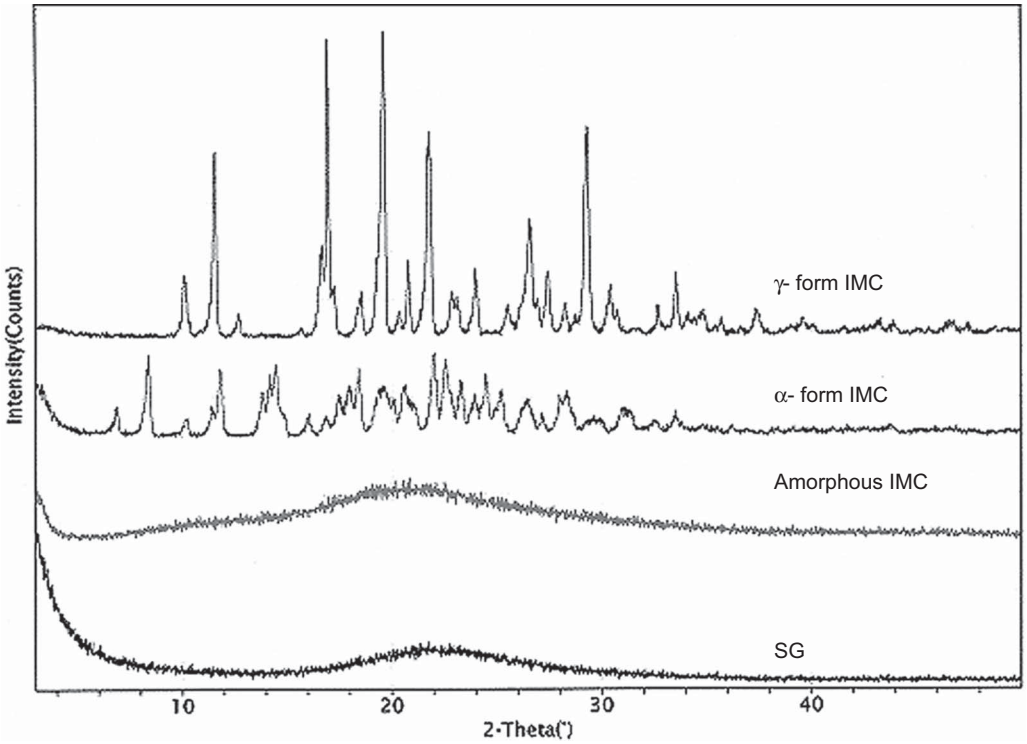


FIGURE 2. XRPD patterns of pure γ-form crystalline IMC, α-from crystalline IMC, amorphous IMC and SG (60 Å),

Differential Scanning Calorimetry (DSC)

Samples of 4 to 7 mg were carefully weighed in the aluminum pans, and the pans were sealed with a pinhole on the top. The amount of crystalline drug in the mixture was determined by using a Perkin Elmer DSC-7 (model 7719, Boston, Massachusetts) apparatus at a scan rate of 5°C/min from 25°C to 170°C under a flow of dry N_2 gas (50 ml/minute). A quantitative DSC method was developed (Pan et al., 2006). The crystallinity of IMC, which was presented as the percentage of crystalline IMC remaining in the mixtures, was estimated using following equation:

$$\text{Crystallinity of IMC} = [(A/Wt)/S] \times 100\%$$

$$\text{Extent of Amorphization of IMC} = 100 - \text{Crystallinity of IMC}$$

A = area under the melting endotherm; Wt = amount of IMC in the mixture as measured by HPLC (the crystallinity of IMC in the sample before mixing was assumed to be 100%); S = slope of DSC calibration curve (also the heat of fusion).

High Performance Liquid Chromatography (HPLC), Thermal Gravimetric Analysis (TGA), and Microscopic Studies

An HPLC system (Agilent, HP 1100, Palo Alto, CA) was used with a Phenomenex C_{18} column (Luna, 5μm analytical column, 150 × 3.0 mm). Absorbance was measured by a UV detector at 254 nm. The mobile phase was 60:40 of 0.01 M phosphate buffer (pH 6.8) and acetontrile. Buffer was prepared according to the USP suggested phosphate buffer preparation method, using potassium phosphate (monobasic) and adjusting pH by NaOH. The flow rate was 1 ml/minute. Sample injection volume was 20 μl. The total amount of IMC (both crystalline and amorphous forms) in the samples was measured using HPLC analysis. Samples were prepared by adding 3 to 5 mg of mixtures to 10 ml of 0.01 M phosphate buffer (pH 6.5), sonicating for 5 minutes, and then filtering through 0.45 μm filters (Acrodisc, CR PTFE). Dilution with buffer might be required. The analytical concentration of IMC samples ranged from 0.01 to 0.15 mg/ml. This analysis was performed to eliminate the errors associated with mixing and sampling. All reported values are the means of three independent measurements.

The amount of moisture in the mixtures was determined by a thermal gravimetric analyzer (TGA-7, Perkin Elmer, Boston, Massachusetts). Weight loss was monitored in the temperature range from 22°C to 150°C at a scan rate of 5°C/minute. All reported values are the means of three independent measurements.

Mixtures were examined by an optical microscope (Leitz Laborlux, Wetzlar, Germany) with and without crossed-polar filters. Samples were prepared by immersing in mineral oil. The magnification level was 10x and light source was at the bottom.

Effect of Mixing Time and Intensity

Mixtures containing 30% IMC and 70% SG (60 Å) were prepared in amber glass vials using a Turbula™ T2C shaker-mixer

at room temperature. The mixing times studied included 5, 15, 30, and 60 minutes. Mixing intensity was controlled by changing the motor rpm on the shaker-mixer. The mixing intensities studied included 30, 62, and 90 rpm. Crystalline IMC (γ-form) alone was mixed in an amber glass vial at 90 rpm for 60 minutes as control. The amount of crystalline IMC remaining in the samples was determined by DSC. Data are reported as the means of three determinations.

Effect of Reduced Pressure

Mixtures were stored for up to a week in a vacuum oven (Napco, model 5831, Precision Scientific, Chicago, Illinois) under reduced pressure (50–100 mm Hg) at both controlled room temperature and 42°C. Samples were taken at different time points. The amount of crystalline IMC remaining in the samples was determined by DSC. Data are reported as the means of three determinations.

Effect of IMC:SG Ratios

Mixtures containing different known amounts of crystalline IMC (γ-form) (5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% IMC) and SG (40, 60, and 100 Å) were mixed at 62 rpm for 15 minutes using a T2C Turbula™ shaker-mixer. The amount of crystalline IMC remaining in the samples after the mixing was determined by DSC. Data are reported as the means of three determinations.

Effect of Particle Sizes of IMC and SG

Crystalline IMC (γ-form) and SGs (40, 60, and 100 Å) were fractionated into different particle size groups by sieving (U.S.A. Standard Testing Sieves, Mesh sizes are #80 and #140 for SG, #100 and #325 for IMC). Specific surface area of silica gel was measured with a surface analyzer (Gemini 2360, Micromeritics Instrument Corporation, Norcross, Georgia) using the BET method. Samples (2–3 g) were degassed for at least 24 hours before the measurement. H_2 and N_2 were used as the gases. Tests were conducted in triplicate. Data are reported as the means of three determinations. Table 1 lists the surface area of different size groups of SG and IMC.

TABLE 1
Specific Surface Areas of SGs and IMC. Values Represent the Mean and Standard Deviation of Three Measurements

	Specific Surface Area (m^2/g)		
Material	As Received	< 106 μm	> 180 μm
SG (40 Å)	565.7 ± 1.2	566.5 ± 1.6	556.5 ± 1.4
SG (60 Å)	462.2 ± 1.6	464.6 ± 2.1	458.5 ± 0.9
SG (100 Å)	329.9 ± 1.8	329.5 ± 0.8	330.9 ± 0.6
	As Received	< 44 μm	> 149 μm
IMC	0.308 ± 0.010	0.430 ± 0.055	0.171 ± 0.039

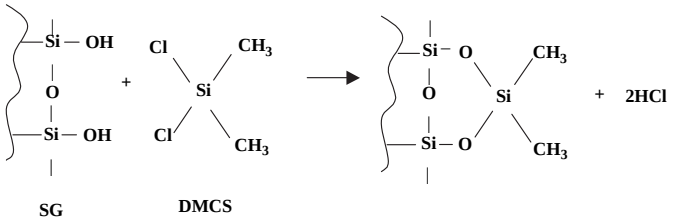
Mixtures containing 30% IMC and 70% SG from different size groups were prepared by dry blending at 62 rpm for 15 minutes using a T2C Turbula™ shaker-mixer. The amount of crystalline IMC remaining in the mixtures was determined by DSC. Data are reported as the means of three determinations.

Effect of Moisture Content in SG

SGs of different pore sizes (40, 60, and 100 Å) were pretreated before mixing with crystalline IMC (γ-form). The treatments included: (1) drying the SGs in an oven (Isotemp oven, model 750G) at 110°C overnight; (2) storing the SGs in a desiccator at 50% relative humidity (RH) for 7 days (RH maintained with a saturated sodium dichromate dihydrate solution); (3) storing the SGs in a desiccator at 100% RH for 7 days (RH maintained at 100% with distilled water). The treated SGs were then dry-blended with crystalline IMC at a ratio of 30:70 (IMC:SG) in amber glass vials at 62 rpm for 15 minutes using a T2C Turbula™ shaker-mixer. Mixtures with untreated SG (used as received) and crystalline IMC were also prepared in the same manner. The amount of moisture in the mixtures was determined by a thermal gravimetric analyzer (TGA-7, Perkin Elmer). The amount of crystalline IMC remaining in the mixtures was determined by DSC. Data are reported as the means of three determinations.

Effect of Silanol Groups of SGs

The goal of this study was to probe the hypothesis that amorphization of crystalline IMC in IMC-SG mixtures was related to the hydrogen bonding between IMC and silanol groups of SGs. To investigate the existence of hydrogen bonding between IMC and SG, free silanol groups on the surface of SG were chemically modified to prevent forming hydrogen bonds with functional groups on IMC. Dimethyldichlorosilane (DMCS), which reacts with silanol groups, was used to treat SG (Gilpin & Burke, 1973; Hair & Hertl, 1969). After silanization, SG was further treated with 99.8% ethanol to remove unreacted Cl groups of DMCS. The reaction, if carried out to completion, would eliminate any hydrogen bonding potential of SG. One of the possible reactions between DMCS and SG is:



The treated SG was then dried in the desiccator under the vacuum. Mixtures containing 30% IMC and 70% SG (either treated or untreated) were prepared using the Turbula™ T2C shaker-mixer. The mixtures were dry-blended for 15 minutes at 62 rpm. The amount of crystalline IMC remaining in the

mixtures was determined by DSC. Data are reported as the mean of three determinations.

Dissolution Study

Dissolution tests were conducted using a DISTEK dissolution system (model 2100A, North Brunswick, New Jersey) with USP paddle method. The dissolution medium (900 ml) was 0.01 M phosphate buffer solution (pH 6.5) with 0.01% poloxamer (LUTROL® F68 NF Prill Surfactant). The poloxamer was used to improve the wetting of the sample powder. Buffer was prepared according to the USP suggested phosphate buffer preparation method, using potassium phosphate (monobasic) and adjusting pH by NaOH. Dissolution studies were carried out at 37°C ± 0.5°C with paddle speed of 100 ± 1 rpm. A sample corresponding to 50 mg of IMC was added to the dissolution medium. Aliquots (10 ml) of the dissolution media were withdrawn and filtered through a 0.45 μm (Acrodisc, CR PTFE) membrane filter at appropriate time intervals (5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes). The amount of IMC dissolved was determined by UV analysis (Agilent HP 8453 spectrometer, Palo Alto, CA) at a wavelength of 318 nm.

Dissolution analysis was carried out on mixtures containing 10% and 70% IMC. The samples were prepared using the Turbula™ T2C shaker-mixer at 62 rpm for 15 minutes. Dissolution profiles of the mixtures were compared with that of the pure crystalline IMC (γ-form) and amorphous IMC.

Stability Study

Fifteen grams (15 g) of mixtures containing 30% IMC and 70% SG were prepared by dry blending at 62 rpm for 15 minutes with a T2C Turbula™ shaker-mixer at room temperature. Amorphous IMC, pure crystalline IMC (γ-form), and SG samples were used as controls. Pure γ-form IMC was obtained from supplier. Amorphous IMC was prepared as described previously. Storage conditions included controlled room temperature (22°C) under desiccation (anhydrous calcium sulfate was used as the desiccant), 25°C / 60% RH and 40°C / 75% RH. Samples were stored in 120-ml HDPE bottles without caps in the stability chambers (25°C / 60% RH and 40°C / 75% RH). Samples stored at room temperature under desiccation were in capped 20-ml amber glass vials. Samples were tested at selected time points of initial, 1, 2, and 3 months.

RESULTS AND DISCUSSION

Effect of Mixing Time and Intensity

Figure 3 shows the XRPD patterns of γ-form crystalline IMC (A) and a mixture of 30% IMC and 70% SG dry blended at 90 rpm

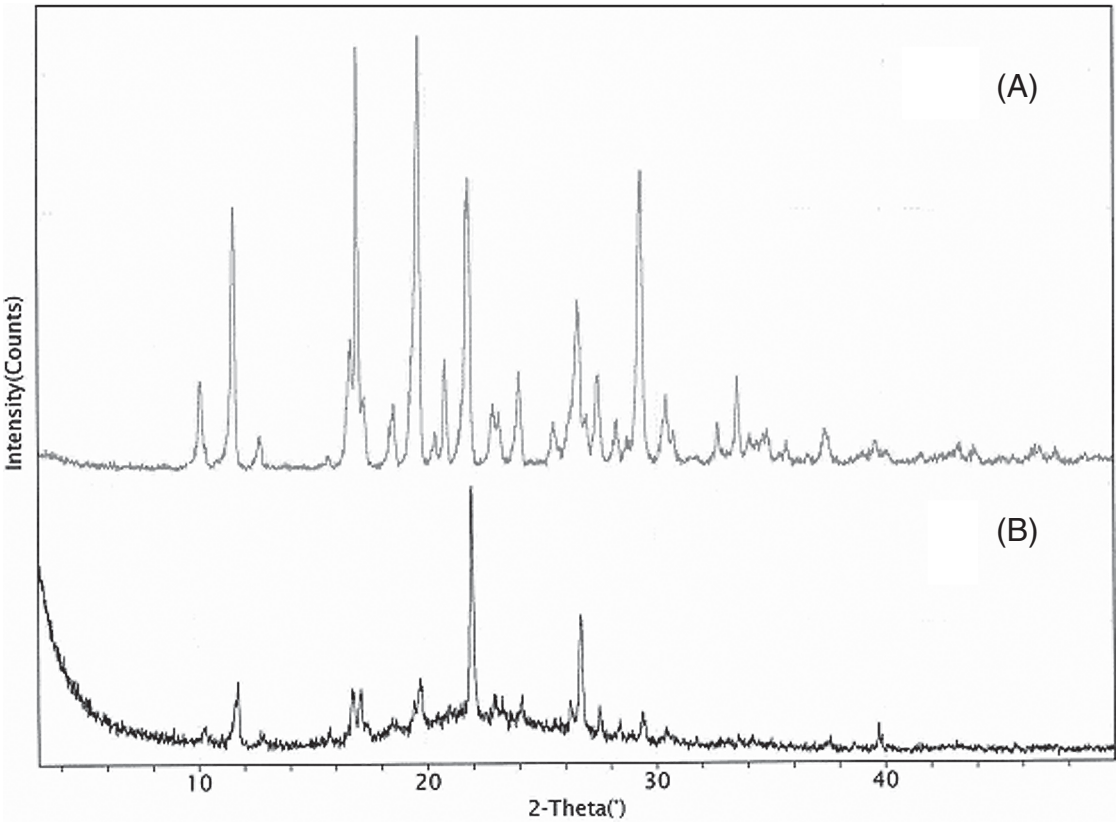


FIGURE 3. XRPD patterns of pure γ-form crystalline IMC (A) and a mixture of 30% IMC and 70% SG (B) mixed at 90 rpm for 1 hour.

for 60 minutes (B). Only the γ-form of IMC was present in the mixture as determined by XRPD since all diffraction peaks of the mixture match those of pure γ-form crystalline IMC. This has also been confirmed by DSC analysis because only one melting peak at 162°C corresponding to the melting point of γ-form crystalline IMC was observed for the mixture. It can be seen from this study that polymorphic conversion of IMC did not occur in IMC-SG mixtures. Mixing crystalline IMC (γ-form) by itself at 90 rpm for 60 minutes did not cause any amorphization of crystalline IMC.

Figure 4 shows the effect of mixing time (5, 15, 30, 60 minutes) and intensity (30, 62, 90 rpm) on the extent of amorphization of crystalline IMC in mixtures containing 30% IMC and 70% SG (60 Å). The amorphization of crystalline IMC in IMC-SG mixtures occurred within the first 5 minutes of mixing. The extent of the amorphization was found to be independent of mixing time and mixing intensity in this study. Only about 40% of the initial 30% crystalline IMC remained as the crystalline form in the mixtures after being dry-blended with SG. This observation is contradictory to the findings of other reports that, with increased mixing time and intensity, increased extent of amorphization of crystalline drugs was found (Konno & Kinuno, 1989; Konno, 1990).

Our findings could be explained by the low Tg (≈ 42°C) of IMC. It is suggested that the surfaces of the crystalline drug particles become activated and disordered during the dry-blending process (Mosharraf & Nystrom, 1999). This facilitates the amorphization of crystalline drug in the mixtures. However, dry blending also generates heat on the surface of the crystalline particles due to particle–particle interactions. If the temperature in the outer peripheral layer is increased to above Tg during dry blending, the disordered regions of crystalline particles will recrystallize (Mosharraf & Nystrom, 1999). In IMC-SG mixtures, both amorphization and recrystallization processes might exist and they are in balance during dry blending at different mixing times and intensities. This explains why the extent of amorphization of crystalline IMC in IMC-SG mixtures is independent of mixing time and intensities in our study.

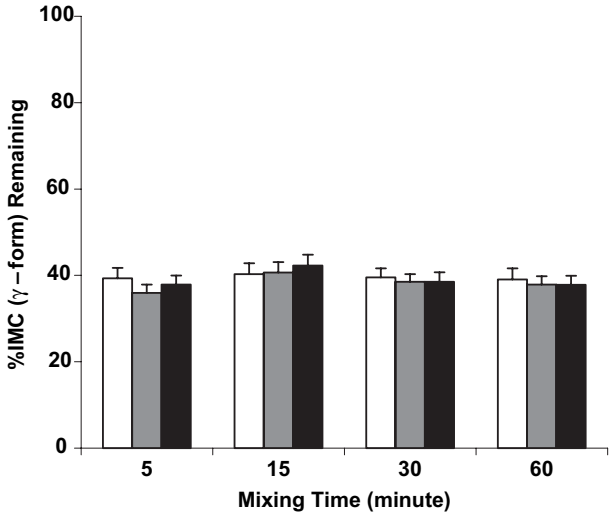


FIGURE 4. Effect of mixing time and intensity on the extent of amorphization of IMC (γ-form) in mixtures containing 30% IMC and 70% SG (60 Å). Mixing intensity: □: 30 rpm; ■: 62 rpm; ■: 90 rpm.

Effect of Reduced Pressure

There are reports that decreased pressure and increased temperature within the mixing chamber favored amorphization of drugs in their mixtures with porous adsorbents. It is suggested that the loss of crystallinity of drugs takes place via the gaseous phase and is induced by the vapor pressure of the drugs. Drug with a higher vapor pressure showed a faster amorphization rate in the mixtures (Konno, 1990; Konno & Kinuno, 1989; Konno et al., 1986).

In this study, IMC and SG mixtures stored in a vacuum oven at room temperature and 42°C under vacuum for a week showed no further change in IMC crystallinity. The crystalline IMC remaining in the mixtures during storage was γ-form IMC as determined by both DSC and XRPD. No effect of reduced pressure on the amorphization of IMC in the mixtures in our study was believed to be due to the extremely low vapor pressure of IMC (9.89×10^{-11} mm Hg, [SRC PhysProp Database, http://esc.syrres.com/interkow/webprop.exe?CAS=53-86-1]). It is suggested that the amorphization of IMC in IMC-SG mixtures might occur via routes other than going through the gaseous phase. The particle–particle interactions between IMC and SG during dry blending increase the disorder of the crystal lattice on the surface of the drug particles. It is believed that drug molecules in the disordered crystal lattice were removed from the drug surface by forming hydrogen bonds with silanol groups on the surface of the SG during the blending process, which resulted in the loss of crystallinity of IMC in the mixtures.

Effect of IMC:SG Ratio

Figure 5 shows the effect of IMC:SG ratio on the extent of amorphization of IMC in the mixtures. Mixing ratio has a significant effect on the amorphization of crystalline IMC in the mixtures. The extent of amorphization of crystalline IMC decreased with an increased ratio of IMC:SG (Figure 5 A). This is due to the fact that as the ratio of IMC:SG increases, there is relatively less SG to convert the crystalline IMC in the mixtures. This was true for all three grades of SGs (40, 60, and 100 Å). Mixtures containing medium pore size SG (60 Å) converted more crystalline IMC to its amorphous form than the other two grades of SGs at similar mixing ratios. Maximum amorphization occurred at the mixing ratio of 5:95 (IMC:SG) for all three grades of SGs. The maximum amorphization of crystalline IMC in the mixtures was about 70% for mixtures containing SGs with small and medium pore sizes (40 Å and 60 Å) and 55% for mixtures containing SG with a large pore size (100 Å). Complete amorphization of IMC was not

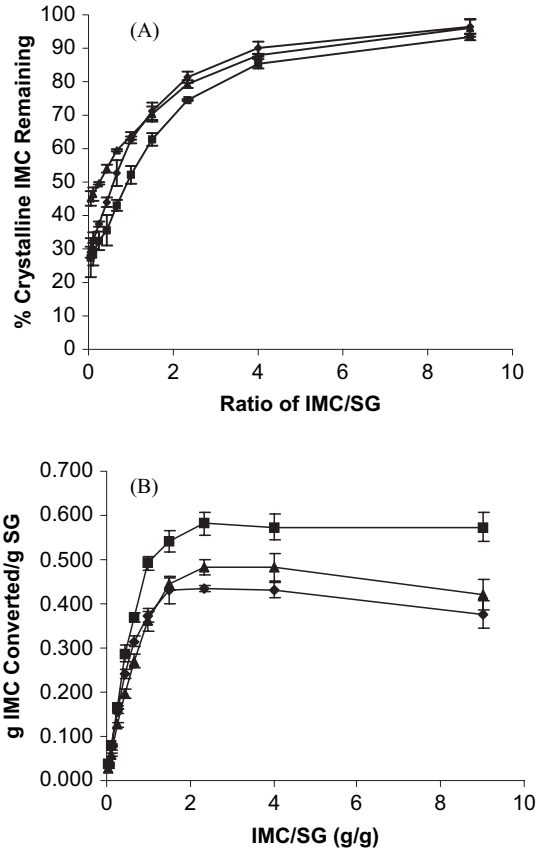


FIGURE 5. Effect of IMC:SG ratios on the amorphization of IMC (γ-form) in the mixtures. IMC/SG mixtures contained 30% IMC and 70% SG and were mixed at 62 rpm for 15 minutes. Mixing ratios (IMC:SG) include: 5:95, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10. ◆: SG (40 Å); ■: SG (60 Å); ▲: SG (100 Å). (A): Percent crystalline IMC remaining in the mixtures; (B): Amorphization efficacy and capacity of SG.

observed in the present study even at mixing ratios as low as IMC:SG = 5:95. This is probably due to the recrystallization of IMC during dry blending.

The effect of IMC:SG ratio on the amorphization efficiency and capacity is shown in Figure 5b. The amorphization efficiency refers to the amount of crystalline IMC that has been converted by each gram of SG. The amorphization capacity refers to the maximum amount of crystalline IMC that has been converted by each gram of SG. For all three grades of SGs, as the IMC:SG ratio increased, the amorphization efficiency also increased until the amorphization capacity was reached. The amorphization capacity was achieved at the mixing ratio of IMC:SG = 70:30 (or 2.3 g/g). The amorphization capacity was about 0.60 g of IMC per g of SG (60 Å); 0.50 g of IMC per g of SG (40 Å); and 0.45 g of IMC per g SG (100 Å).

SG with medium pore size (60 Å) and medium specific surface area showed a significantly greater ($P < .05$) amorphization efficiency at different mixing ratios and a larger amorphization capacity than the other two grades of SGs for mixtures containing more than 40% IMC. Comparing the specific surface area of the mixtures (IMC:SG = 30:70) before and after dry blending, the specific surface area after blending was reduced by 4%, 20%, and 3% in mixtures containing small, medium, and large pore size SG, respectively. It is postulated that IMC molecules moved from the surface of SG into the pores by diffusion. From computational chemistry, the molecular diameter of IMC was calculated to be about 14 Å. Although SG with smaller pores has a larger specific surface area, some of the surface inside the pores may not be accessible to IMC molecules if the pores are too small; pore openings are too narrow or pore passages are torturous. Whether the pores are available or not for hydrogen bonding with IMC in the mixtures is likely dependent upon pore size and pore morphology of SG.

Effect of Particle Sizes of IMC and SG

Figure 6 shows the effect of particle sizes of IMC and SG on the extent of amorphization of IMC in the mixtures. Table 1 shows the specific surface areas of SGs and IMC. Mixtures containing smaller particles of both IMC and SG (IMC < 44 μm, SG < 106 μm) exhibited the largest extent of amorphization (about 80%). Mixtures containing larger particles of both IMC and SG (IMC > 149 μm, SG > 180 μm) were found to have the least extent of amorphization (about 35%). It is suggested that as particle size reduced, surface area increased, which led to increased particle–particle interactions and an increased extent of amorphization. The particle size of IMC exhibited a greater effect on the extent of amorphization than the particle size of SG. This is due to the fact that reducing the particle size of IMC has significantly increased the specific surface area of

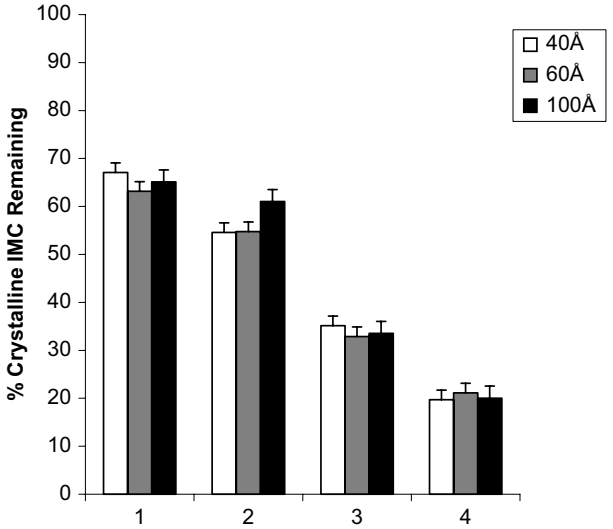


FIGURE 6. Effect of particle size of IMC and SG on the amorphization of IMC in the mixtures. Samples containing 30% IMC and 70% SG were prepared by mixing at 62 rpm for 15 minutes. Data are the means of three measurements. Mixture compositions: 1: IMC >149 μm, SG >180 μm; 2: IMC > 149 μm, SG < 106 μm; 3: IMC < 44 μm, SG > 180 μm; 4: IMC < 44 μm, SG < 106 μm.

IMC. However, within the same grade of SG, the reduction of the particle size of SG had little or no effect on the specific surface area of SG. This might be due to fact that most of the specific surface area is contained within the pore structure. Therefore, the size of the pores has a greater effect on the specific surface area of SG than the particle size.

Effect of SG Moisture Content

Porous SG is very hygroscopic and could take up a fairly large amount of moisture depending on environmental conditions. The adsorbed/absorbed moisture could also hydrogen-bond with silanol groups of SG and subsequently interfere with the amorphization of crystalline materials in the mixtures. In the present study, the effect of moisture content of porous adsorbents on the loss of crystallinity of drugs in drug-adsorbent mixtures has been investigated.

Different grades of SG were pretreated by storing them in desiccators with different controlled relative humidities before blending with crystalline IMC. Table 2 shows the effect of the amount of moisture in SG on the loss of crystallinity of IMC in the mixtures. At the same storage condition, the amount of water adsorbed/absorbed by SG increased as the surface area of SG increased. When the moisture level in the mixture was below 7% (w/w), the amount of crystalline IMC remaining in the mixtures increased as the amount of moisture in the mixture increased. However, when the moisture level in the mixture was greater than 7% (w/w), the amount of crystalline IMC remaining in the mixtures decreased as the amount of moisture in the mixture increased. This was true for all three grades of SG (40, 60, and 100 Å).

It is suggested that when the moisture level in the SG is low (< 7% w/w), water might compete with IMC for hydrogen binding sites (silanol groups) on SG and result in a decreased extent of amorphization. However, as the moisture content in the mixture increased to more than 7% (w/w), some of the IMC molecules on SG may have diffused through absorbed water into pores that previously were not accessible for hydrogen bonding and resulting in a decrease in the amount of crystalline IMC in the mixtures.

Effect of Silanol Groups of SG

Table 3 shows the results of amorphization of crystalline IMC in mixtures containing 30% IMC and 70% SG with and without DMCS treatment. In mixtures containing SG without DMCS treatment, more than 50% of the crystalline IMC lost its crystallinity, while in mixtures containing SG with chemically modified silanol sites, less than 10% of the crystalline IMC lost its crystallinity. This probably was due to the incomplete blockage of silanol groups on SG by DMCS. This observation suggests that the silanol groups of SG are involved in the amorphization of IMC in the mixtures, probably by forming hydrogen bonds with the carboxyl group of IMC. The results of our study thus strongly supports that hydrogen bonding between IMC and SG is responsible for the amorphization of crystalline IMC in the IMC-SG mixtures.

Dissolution Study

Figure 7 displays the dissolution profiles of two mixtures of IMC and SG (40Å) (10% IMC/90% SG and 70% IMC/30% SG), as well as those of amorphous and crystalline IMC. All dissolution samples contain 50 mg of IMC. From Figure 7, the time required for dissolving 80% (t_{80}) of pure crystalline IMC was about 75 minutes, and it was 28 minutes for pure amorphous IMC. The t_{80} was 44 minutes for the 10% IMC / 90% SG mixture and 62 minutes for the 70% IMC / 30% SG mixture. IMC dissolved significantly ($P < .05$) faster from mixtures than from pure crystalline IMC, and this is due to the amorphization of crystalline IMC in the mixtures during blending.

Figure 8 shows the relationship between the amount of amorphous IMC in the samples and t_{80} of dissolution. It was found that the t_{80} decreased linearly as the amount of amorphous IMC in the mixture increased. The increase in dissolution

TABLE 2
Effect of Moisture Content in the SG on the Amorphization of Crystalline IMC in IMC-SG Mixtures

SG (40 Å)		SG (60 Å)		SG (100 Å)	
Moisture in SG (% w/w)	Crystallinity (%)	Moisture in SG (% w/w)	Crystallinity (%)	Moisture in SG (% w/w)	Crystallinity (%)
1.56[a]	36.21	0.93[a]	37.13	1.20[a]	44.27
3.99[b]	39.74	2.12[b]	49.49	4.32[b]	49.25
13.91[c]	25.79	8.12[c]	41.66	6.53[c]	54.54
36.36[d]	17.25	35.95[d]	20.60	27.54[d]	21.51

[a]SG was stored at 110°C overnight.
[b]SG as received.
[c]SG was stored at RT in desiccator with 50% RH for a week.
[d]SG was stored at RT in desiccator with 100% RH for a week.

TABLE 3
Amorphization of Crystalline IMC in Mixtures Containing 30% IMC and 70% SG with and without DMCS Treatment

SG Pore Size (Å)	% Crystalline IMC Remaining	
	SG Treated	SG Untreated
60	92.8 ± 1.3	38.0 ± 2.4
100	91.3 ± 2.1	48.8 ± 2.3

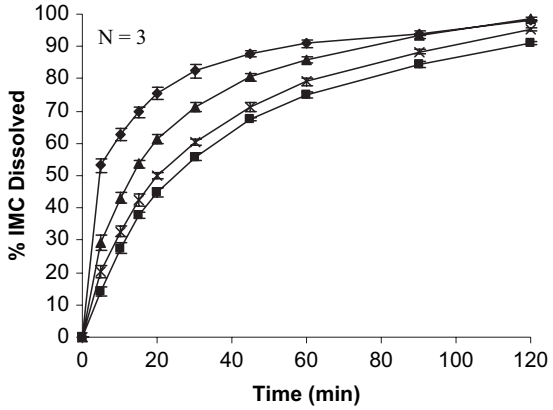


FIGURE 7. Dissolution profiles of IMC from mixtures of IMC and SG (40 Å). Dissolution medium: 0.01 M phosphate buffer with 0.01% poloxamer, pH 6.5, 37 ± 0.5 °C. Paddle method: 100 ± 1 rpm. Data are the mean of three measurements. ♦: amorphous IMC; ■: crystalline IMC (γ-form); Δ: mixture (IMC:SG = 1:9); ×: mixture (IMC:SG = 7:3).

rate resulting from the amorphization of IMC in IMC-SC mixtures could lead to an improved bioavailability compared with the use of pure crystalline IMC. An increased maximum plasma level and area under the concentration-time curve (AUC) following administration of amorphous form of IMC to rabbits compared with those following the administration of crystalline IMC was reported (Fukuoka, Makita, & Yamamura, 1987).

Stability Study

Table 4 summarizes the stability results.

1. Crystallinity

The amount of crystalline IMC remaining in the mixtures was unchanged when stored at controlled room temperature under desiccation and 25°C / 60% RH for up to 3 months. Amorphous IMC formed in IMC-SG mixtures did not recrystallize during storage. This might be due to the inhibition of crystallization of amorphous IMC by hydrogen bonding between IMC and SG. However, for samples stored at 40°C / 75% RH, the amount of crystalline IMC decreased slightly over time for mixtures containing small- and medium-pore-size SG (40 and

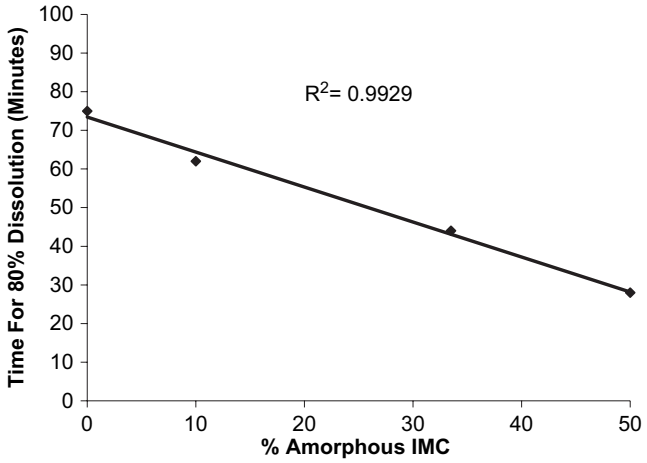


FIGURE 8. Relationship between the times required for 80% dissolution of the IMC and the percentage of amorphous IMC in the samples. All samples contained 50 mg of IMC.

60 Å). However, mixtures containing large-pore-size SG (100 Å) were unchanged. It was also found that mixtures containing small- and medium-pore-size SG absorbed significantly more moisture than mixtures containing large-pore-size SG during storage at 40°C / 75% RH. It is suggested that as the moisture content in the mixtures increased during storage, IMC molecules could have diffused into the pores of SG that were not previously accessible and formed hydrogen bonds there. This resulted in a further decrease of crystallinity of IMC in the mixtures during storage. Only the γ-form of IMC was found in the mixture during the 3 months of storage, and this was confirmed by both DSC and XRPD studies. Pure crystalline IMC (γ-form) did not change its crystallinity regardless of the storage conditions during the stability study. Amorphous IMC crystallized into a mixture of α-form and γ-form IMC during storage.

2. Degradation

No degradation was found in IMC-SG mixtures stored at controlled room temperature under desiccation for up to 3 months. When mixtures were stored at 25°C / 60% RH, there was less than 1% degradation in IMC-SG mixtures at the end of 3-month storage. When mixtures were stored at 40°C / 75% RH, there was up to 2.5% degradation in the mixtures during storage. No degradation was found in pure crystalline IMC samples when stored at controlled room temperature under desiccation or at 25°C / 60% RH for up to 3 months. Up to 0.3% of degradation was found in pure crystalline IMC stored at 40°C / 75% RH at the end of 3 months of storage. The degradations of amorphous IMC were about 0.5%, 3.2%, and 7.4% at the end of 3 months of storage at controlled room temperature under desiccation, 25°C / 60% RH, and 40°C / 75% RH, respectively. From the above observations, crystalline IMC is much more stable than its amorphous form. It is believed that

TABLE 4
Summary of Stability Study

	IMC-SG (40△)	IMC-SG (60△)	IMC-SG (100△)	Crystalline IMC	Amorphous IMC
Controlled RT Under Desiccation					
Crystallinity	No change	No change	No change	No change	α and γ forms
Degradation	No	No	No	No	0.5%
Dissolution	No change	No change	No change	No change	No change
Color	No change	No change	No change	No change	No change
25°C / 60% RH					
Crystallinity	No change	No change	No change	No change	α and γ forms
Degradation	0.6%	No	0.2%	No	3.2%
Dissolution	Deteriorated	Deteriorated	Deteriorated	No change	Deteriorated
Color	Light brown	Light brown	Light brown	No change	Brown
40°C / 75% RH					
Crystallinity	Decreased	Decreased	No change	No change	α and γ forms
Degradation	2.5%	1%	1.5%	0.3%	7.4%
Dissolution	Deteriorated	Deteriorated	Deteriorated	No change	Deteriorated
Color	Dark brown	Dark brown	Dark brown	No change	Dark brown

the degradation in the mixtures was mainly due to the degradation of amorphous IMC formed in the mixtures. However, comparing the stability of IMC-SG mixtures with amorphous IMC samples, it seems that IMC-SG mixtures are more stable than the amorphous IMC samples at all storage conditions in the study. It is suspected that hydrogen-bonding between IMC and SG might have helped to stabilize the amorphous IMC in the mixture and reduced the degradation.

3. Dissolution

Dissolution profiles of mixtures stored at controlled room temperature under desiccation did not change during storage. Dissolution profiles of mixtures stored at 25°C / 60% RH (Figure 9a) and 40°C / 75% RH (Figure 9b) were deteriorated during storage. Microscopic examination indicated that a small amount of agglomerates was formed in the mixtures stored at 25°C / 60% RH, while extensive agglomeration was found in the mixtures stored at 40°C / 75% RH. The agglomeration in the mixtures might be responsible for the deteriorated dissolution profiles of the mixtures in the stability study. Dissolution of pure crystalline IMC did not change in the study regardless of the storage conditions. Dissolution of amorphous IMC stored at controlled room temperature under desiccation did not change, but dissolution of amorphous IMC stored at 25°C / 60% RH and 40°C / 75% RH was deteriorated and extensive agglomeration was found in amorphous samples.

4. Color

The appearance of IMC-SG mixtures stored at controlled room temperature under desiccation did not change in the study. The appearance of IMC-SG mixtures stored at 25°C / 60% RH and 40°C / 75% RH changed from white to light brown and dark brown in color at the end of 3 months of storage, respectively. Pure crystalline IMC and pure SG did not change color during storage regardless of storage conditions. Amorphous IMC did not change color when stored at controlled room temperature under desiccation, but changed to brown (25°C / 60% RH) and intense dark brown colors (40°C / 75% RH) at the end of the 3-month study.

Pictures of IMC-SG mixtures taken by microscope with crossed-polar filters showed that SG particles, which were amorphous and colorless at initial time point, remained amorphous but turned to light (25°C / 60% RH) and dark brown colors (40°C / 75% RH) at the end of 3 months of storage. Since SG and crystalline IMC did not change color, while amorphous IMC did change color during storage, it is suggested that this color change of SG particles in the mixtures is due to the amorphous IMC molecules on the surface of SG particles. This observation indirectly indicates that amorphization of IMC occurred on the surface of SG.

CONCLUSIONS

This study has proved that dry blending crystalline IMC and porous SG led to amorphization of crystalline IMC in the mixtures, probably by forming hydrogen bonds between IMC and silanol groups on SG. Mixing time and intensity did not affect the extent of amorphization of crystalline IMC in IMC-SG mixtures in this study. Factors such as the ratio of IMC to SG, moisture content in the SG, and particle sizes of both IMC and SG affected the extent of amorphization of crystalline IMC in the mixtures. Such amorphization phenomenon was shown to improve the

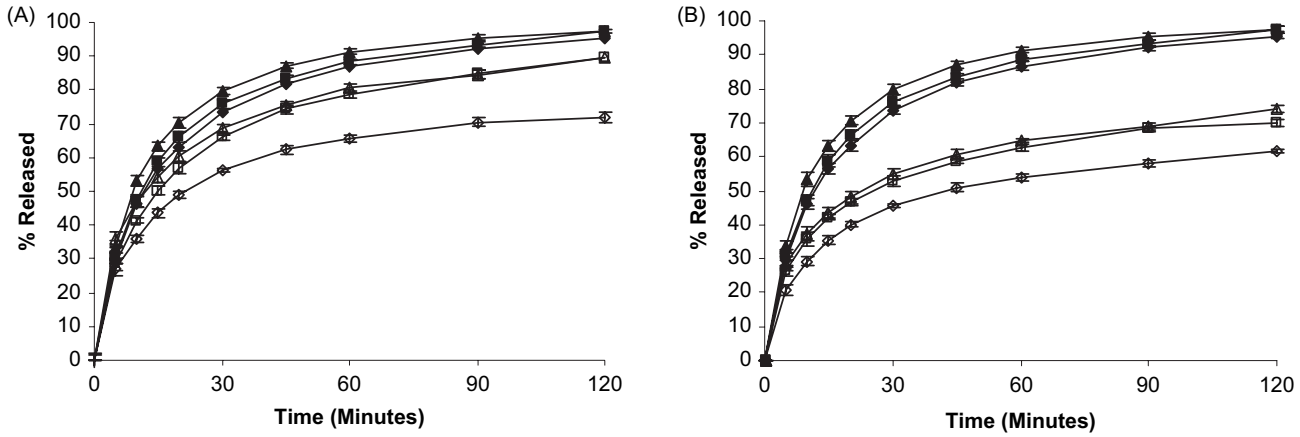


FIGURE 9. Dissolution profile of mixtures containing 30% IMC and 70% SG stored for 3 months at (A) 25°C / 60% RH and (B) 40°C / 75% RH. N = 3. Initial: ♦: 40 Å ■: 60 Å, Δ: 100Å; 3 months: ◇: 40 Å, □: 60 Å, Δ: 100 Å.

dissolution rate of IMC in IMC-SG mixtures compared with the pure IMC crystalline form. The increased dissolution rate observed for the IMC-SG mixtures was found to be a function of the amount of amorphous IMC in the mixtures. Storage conditions significantly affect the stability of the mixtures as well as their dissolution profiles. In order to preserve the improved dissolution rate and maintain the stability of the mixtures, mixtures should be protected from heat, humidity, and light.

This work has demonstrated the potential of using SG as a means to induce crystalline to amorphous phase transition of sparingly soluble drugs such as IMC to improve in vitro dissolution. It may provide an alternative approach to enhance the bioavailability of some poorly soluble drugs that exhibit dissolution-rate limited absorption. Since this study was only carried out on the IMC-SG binary system, further work is needed to determine the mechanisms for the occurrence of this phenomenon and any possible effect that formulation and manufacture of the final dosage form may have on improved dissolution and stability.

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