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Prediction of apparent equilibrium solubility of indomethacin compounded with silica by ^{13}C solid state NMR

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Abstract

The apparent equilibrium solubility (AES) of indomethacin increased by co-grinding with silica. Change in the long- and short range disorder of indomethacin by co-grinding was examined by X-ray powder diffraction and ^{13}C solid state NMR, respectively, to elucidate the increased AES. Since the increase in AES was particularly marked after complete disappearance of X-ray diffraction peaks, we attributed the enhanced AES primarily to the short range disorder on the molecular basis. This was confirmed by a high correlation between the standardized full width at half maximum (SFWHM) of the specific peaks observed by ^{13}C solid state NMR and log (AES). The correlation enables the prediction of AES as well.

Keywords: Apparent equilibrium solubility; Indomethacin; Short range order; Co-grinding; Solid state NMR

1. Introduction

A drug compounded with a carrier solid is called as a solid dispersion system. The system is widely applied to enhance the apparent solubility and bioavailability of sparingly soluble drugs (Shakhtshneider et al., 1996; Okonogi et al., 1997; Van den Mooter et al., 1998). By compounding, drugs turn quite often into amorphous and hence less stable (Sugimoto et al., 1998). These structural changes are closely related to the crystalline imperfection detectable by X-ray powder diffractometry (XRPD). However, when the crystallite size decreases less than 10^{-7} m, or when lattices are severely disordered, the XPRD profile becomes too diffuse to evaluate precision crystallographical properties (Markovich et al., 1997).

Elamin et al. (1994) reported that an increase in the free energy and a decrease in the enthalpy of

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solution of a ground hydrophobic drug increase the apparent solubility due to disordering of the solid structure. The term 'apparent solubility' is defined as the metastable or dynamic solubility of the materials (Mosharraf et al., 1999). It has been reported that the presence of an outer particle layer disordered by mechanical stress increases the apparent equilibrium solubility (AES; Elamin et al., 1994). The term 'AES' is defined here as the concentration of the solution in apparent equilibrium with the solid, whose structure is not in its most stable state. This value is, in practice, important to describe the state of drugs after administration, although it is thermodynamically indefinable.

As we previously reported (Watanabe et al., 2001) incomplete co-grinding of the indomethacin (IM)–SiO_2 mixture made the amorphous state considerably unstable since the remaining crystallite served as seeds for recrystallization. The remaining crystallites are also likely to affect the dissolution properties, such as the apparent solubility. Crystallization of the amorphous drugs will occur during storage, resulting in a decrease in the apparent solubility, and hence the bioavailability. The amorphous state stability of ursodeoxycholic acid, when prepared by melt-quenching, is reported to be higher than that prepared by grinding, because molecules are arranged much more randomly in the quenched sample (Yonemochi et al., 1997, 1999). In order to describe the disordered arrangement of molecules of the drug in a solid, solid state nuclear magnetic resonance (NMR) spectroscopy is regarded as a versatile tool (Middleton et al., 1997). Solid state cross-polarization magic-angle-spinning (CP/MAS) NMR for ^{13}C is used not only to evaluate drug polymorphs (Stephenson et al., 1997; Kimura et al., 1999; Matsunaga et al., 1999), but also to assess amorphous materials (Gustafsson et al., 1998), since the disorder on the molecular basis gives rise to broader resonance than in the crystallized form (Gao, 1998; Grossmann et al., 2000; Medek and Frydman, 2000).

The purpose of this study is to elaborate the relationship between the IM profiles in the ^{13}C CP/MAS NMR spectra and AES of amorphous IM compounded with silica, and to examine whether and to what extent the relationship serves to predict AES from structural analysis without actually measure the concentration after prolonged dissolution.

2. Materials and methods

2.1. Materials

A commercial reagent (Sigma Chemical Co., USA) was used as the source of indomethacin (IM, γ-indomethacin: 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid). As a carrier for co-grinding, fumed SiO_2 (Aerosil 200, specific surface area: ca. 200 $m^2\ g^{-1}$; Nippon Aerosil Co., Japan) were used. Both reagents were used in our previous study (Watanabe et al., 2001, 2002).

2.2. Preparation

IM and SiO_2 were mixed in a mortar in a weight ratio of 1:1 to obtain a physical mixture. Amorphous IM was prepared by grinding or melt-quenching with SiO_2. The mixture was co-ground by a laboratory size vibration mill (Hi-speed Vibrating Sample Mill Model TI-100, CMT MFG. Co., Japan). The same physical mixture was heated up to 165 °C, which is slightly above the melting point of γ-IM (162 °C) and left standing for 5 min. The heated mixture was placed into a cool trap (Neo Cool Trap, Yamato Co., Japan) at −101 °C. Detailed grinding and melt-quenching conditions for the mixture are given elsewhere (Watanabe et al., 2001). Specific surface areas of the mixtures were determined by a BET method with N_2 gas adsorption (Quantasorb, Quantachrome Co., USA).

2.3. Characterization

2.3.1. X-ray powder diffraction (XRPD)

X-ray diffraction patterns for all the samples were measured by a Geiger Flex Rint-2200 (Rigaku Co., Japan) diffractometer with Cu Kα radiation at 40 kV/40 mA. The samples were step-scanned at 0.02° interval from 5.00 to 40.00° (2θ) at the rate of 4.00° min^{-1}.

2.3.2. Solid state nuclear-magnetic-resonance

CP/MAS NMR spectra for ^{13}C were obtained using a 300 MHz spectrometer (CMX-300, Chemagnetics Inc., USA). The following conditions were adopted: frequency of 75.6 MHz, 90° proton pulse 5 μs, contact time 1.5 ms, pulse delay 5 s, spinning rate 3500 ± 10 Hz, spectral width 25 kHz, acquisition time 40 ms and fixed sample weight for each measurement. The chemical shifts were calibrated indirectly to the higher field adamantane peak (29.5 ppm relative to tetramethylsilane, TMS).

2.3.3. Apparent equilibrium solubility

The AES of IM was determined in water at 37.0 ± 0.5 °C. A mixture containing 10 mg of IM was added to 100 ml of water in a beaker. The suspension was vigorously agitated with a stirrer. After 24 h, aliquots were then filtered through a 0.45-μm membrane filter. The drug concentration ($n = 6$) was analyzed with an UV spectrometer (UV-3100, Shimadzu Co., Japan) to determine the AES.

3. Results and discussion

3.1. Changes in the long and short range order of indomethacin by co-grinding

The changes in relative crystallinity (RC) with grinding time determined by a modified Herman's method using XRPD (Usui et al., 1998) are shown in Fig. 1. In the following discussion, the RC estimated by XRPD is denoted as RC. The RC became 0 after co-grinding for 30 min and so the state was assigned as non-crystalline or X-ray amorphous. Non-crystalline states contain different kinds of disordered states, for which XRPD becomes inapplicable. Solid state NMR enables the examination of short range order for the assessment of amorphous materials (Gustafsson et al., 1998). We therefore employed ^{13}C CP/MAS NMR and analyzed the relationship between the spectra and the dissolution characteristics of IM.

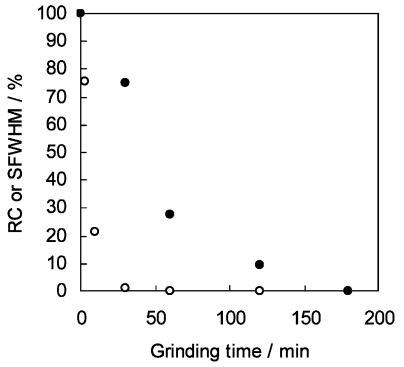

Fig. 1. Changes in the RC (○) and the SFWHM (●) estimated by XRPD and solid state NMR, respectively.

The changes in the ^{13}C CP/MAS NMR spectra due to co-grinding are shown in Fig. 2, in addition to the data obtained in our previous report (Watanabe et al., 2001). We observed sharp peaks in the ^{13}C CP/MAS NMR spectra of the sample co-ground for 30 min as well as in that of the physical mixture. We therefore concluded that the mixture ground for 30 min loses the long range order but maintains a regular molecular arrangement, giving sharp peaks in the NMR spectrum. NMR peak broadening occurred due to an extended short range disorder resulting from a

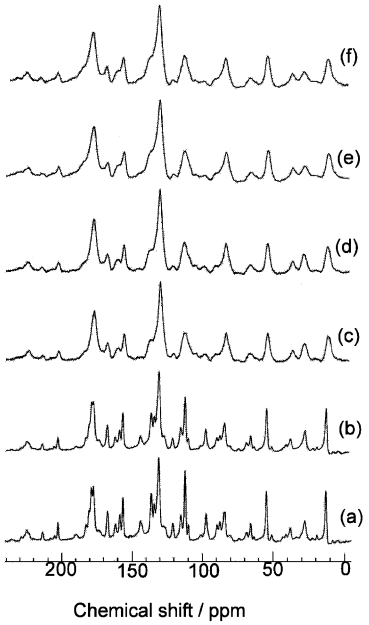

Fig. 2. ^{13}C-CP/MAS-NMR spectra of indomethacin–SiO_2 mixtures; (a) physical mixture, (b) ground for 30 min, (c) 60 min, (d) 120 min, (e) 180 min and (f) melt-quenched mixture.

broader distribution of the molecular orientation, i.e. disordering of IM molecules (Markovich et al., 1997). Vanderhart et al. (1981) reported that a variation in the conformation, bond distortion or nearest-neighbor distance gives rise to a chemical shift. Due to the mechanical stress during grinding, the atomic arrangement in a solid becomes slightly altered. In consequence, peak broadening in the NMR spectra was observed. Therefore, peak broadening of the solid state NMR spectrum may serve as a quantitative measure of any changes in the molecular arrangement of the solid, after prolonged grinding, in the present study.

3.2. Determination of changes in the short range ordering

Spinning side bands (SSBs) marked by asterisks in Fig. 3 are observed in the solid state CP/MAS NMR spectrum. Peak overlap makes quantitative analysis ambiguous. Since the presence of SSB affects the peak broadening, the total suppression of side bands (TOSS) method (Dixon, 1982) was employed. The CP/MAS and the CP/MAS/TOSS spectra in Fig. 3 are interpreted on the basis of

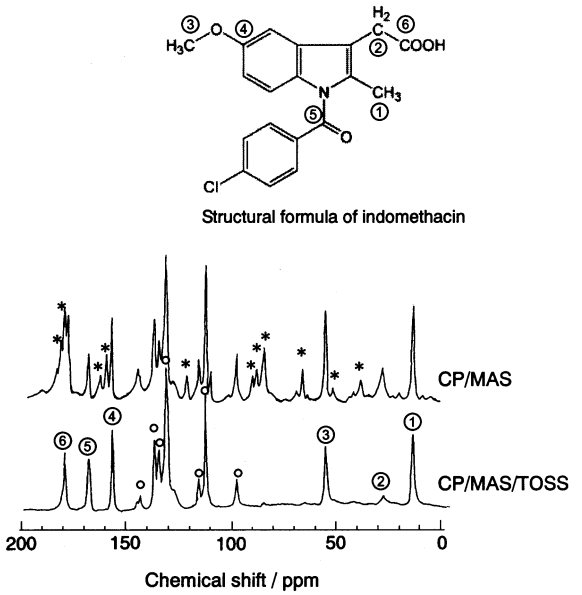

Fig. 3. ^{13}C-CP/MAS and CP/MAS/TOSS NMR spectra comparison of sieved indomethacin; open circle and asterisk denote an aromatic carbon and an SSB, respectively.

liquid phase ^{13}C-NMR (Lin, 1992; Rusu et al., 1998). As shown in Figs. 2 and 3, the peaks (4) and (6) overlapped with SSBs due to peak broadening by prolonged co-grinding, and the intensity of peak (2) was weak. Moreover, peaks (4), (5), and (6), which were assigned as those of methoxy, carbonyl and carboxyl groups in the IM molecules, respectively, were confirmed to have changed as a result of a mechanically induced interaction between these functional groups and SiO_2 (Watanabe et al., 2001, 2002). Therefore, based on the broadening of peak (1) or (3), it is appropriate to evaluate the disorder of IM.

Partial least-squares (PLS) analysis (Gustafsson et al., 1998), and a calculation based on the scan number, the area and the signal height in ^{13}C-NMR (Hu and Schmidt-Rohr, 2000) were used to estimate the change in atomic arrangement, or short range disorder. We considered that the standardized full width at half maximum (SFWHM) of peak (1) or (3) was a useful parameter for estimating the disorder of a drug in a multi-component, e.g. a solid dispersion. Since the intensity of peak (3) was slightly stronger than that of peak (1) in the NMR spectrum of the mixture ground for 180 min, we chose peak (3) for this purpose. The changes in the SFWHM of peak (3) with grinding time in the NMR spectra are also shown in Fig. 1. The calculation of SFWHM is based on Eq. (1):

$$\mathrm{SFWHM}_X\ (\%) = \left(\frac{\mathrm{FWHM}_{180} - \mathrm{FWHM}_X}{\mathrm{FWHM}_{180} - \mathrm{FWHM}_0}\right)100 \qquad (1)$$

where FWHM is the full width at half maximum of peak (3) in the NMR spectra, and the subscript number is the grinding time in minutes. The experimental settings, which may affect the ^{13}C line broadening in organic solid (Vanderhart et al., 1981), were kept constant for all the measurements. The reproducibility of SFWHM was $\pm 1\%$. Simultaneous decreases in the RC and the SFWHM suggest a disruption of the ordered structure of IM as a result of applying mechanical stress. It is important to note that the RC decreased rapidly due to grinding but that only the SFWHM decreased gradually and in two

discontinuous steps. This indicated that the long range order was lost by applying mechanical stress, while the short range order was maintained, even after X-ray amorphizaton. As we reported previously (Watanabe et al., 2001), the ground IM–SiO_2 mixture showed exothermic peak in the DSC profile, which was ascribed to the crystallization. Since the area of crystallization peak was increased linearly with decreasing RC estimated by XRPD, we considered that DSC was not an appropriate tool to examine the short range disordering by mechanical stress.

Note that the grinding time to cancel RC was similar to the time until the point of inflection was reached in the changes of SFWHM shown in Fig. 1. This indicated that the mechanical energy applied at the early stage of grinding was used mainly for disrupting the long range order of IM, leading to the disappearance of the XRPD peaks for crystalline IM. Almost all the rest of the mechanical energy was consumed for disrupting the short range order. Similar examples were given in the case of mechanical activation (Schrader et al., 1966; Heinicke and Beyer, 1981) or mechanical alloying (Amils et al., 1999). On the other hand, the RC and SFWHM of the melt-quenched mixture were similar to those of the mixture ground for 180 min. This indicates that a similar loss of the short and long range order occurs by melt-quenching as well.

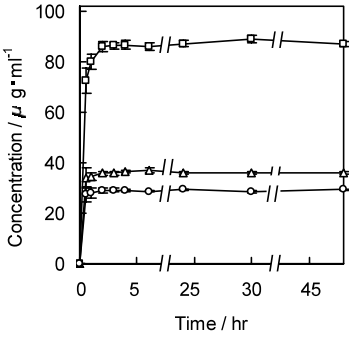

Fig. 4. Concentration–time profiles of indomethacin in indomethacin–SiO_2 mixture (bars = S.D.). Symbols: ○, physical mixture; △, mixture ground for 30 min; □, mixture ground for 180 min.

3.3. Changes in the AES by co-grinding

As shown in Fig. 4, 2 or 3 h after dissolution, the drug concentration attained eventually the constant value at least up to 48 h. This may be regarded as the apparent solubility of the drug in equilibrium. It is clear, however, that the solid IM is not in the most stable state, so that the value is safely regarded as AES. Elamin et al. (1994) reported that the decrease in the metastable solubility of griseofulvin was slow, and rate-limiting step was not controlled by solute molecular, but was a solid state transition on the surface of particles. In the present study, no changes in the apparent solubility of IM for 48 h seemed to be explained by the same token. From the time span mentioned above and the reproducibility, we defined in this paper the value of the concentration 24 h after dissolution as AES. As shown in Fig. 5, the AES of crystalline IM in the physical mixture of IM and SiO_2 was about 30 μg ml^{-1} at 37 °C. The reproducibility of AES was ±2%. Grinding the mixture increased the AES of IM particularly after the inflection point of SFWHM, as shown in Fig. 1. In addition to the lattice disorder, AES may also be increased as a result of particle size reduction and generation of reactive crystal faces by grinding (Suryanarayanan and Mitchell, 1985). As shown in Fig. 6, however, the specific surface area of the mixture was decreased and reached a plateau as a consequence of cohesion of SiO_2 which has a high specific surface area. This indicates that the size reduction of IM particles

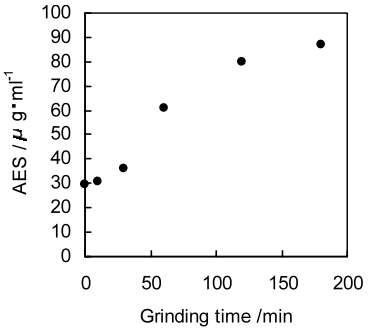

Fig. 5. Changes in the AES of indomethacin due to co-grinding.

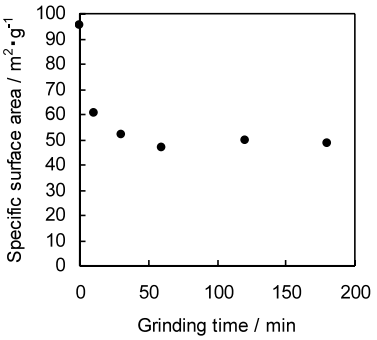

Fig. 6. Changes in the specific surface area due to co-grinding.

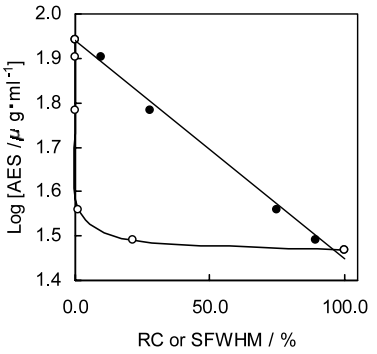

Fig. 7. Relationship between the RC estimated by XRPD or the SFWHM estimated by NMR and the logarithm of AES. Symbols: ○, RC of ground indomethacin (IM)–SiO_2 mixture; ●, SFHWM of ground IM–SiO_2 mixture.

which might increase the surface area, is not play a significant role in increasing the AES.

The relationship between the logarithm of AES and the RC or the SFWHM is shown in Fig. 7. The AES increased after the RC became 0. By grinding the mixture, on the other hand, the logarithm of AES increased linearly with decrease in the SFWHM, with a correlation coefficient of 0.995. The persistent increase in the AES by grinding even after X-ray amorphization must, therefore, be attributed to the short range disordering. We therefore suggest an appropriate method to predict the AES based on the SFWHM of solid state NMR peaks. The predicted AES of the melt-quenched mixture was 87.2 μg ml^{-1} according to the relationship shown in Fig. 7, which was close to the actually observed value of 89.3 ± 1.8 μg ml^{-1} (the mean ± S.D., $n = 6$). Thus, the AES of IM in the solid dispersion could be predicted by solid state NMR even if the calibration curve (logarithm of AES vs. SFWHM) was drawn based on the results obtained from another preparation method.

4. Conclusion

The short range order of solid IM on the molecular basis was evaluated from the peak broadening (SFWHM) of the solid state NMR. The linear relationship between SFWHM and the logarithm of AES established in this study serves as a tool to predict AES of the drugs with different preparation method without actually measuring the concentration after prolonged dissolution.

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