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Research Article

Amorphization Alone Does Not Account for the Enhancement of Solubility of Drug Co-ground with Silicate: The Case of Indomethacin

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Abstract. The solubility advantage of indomethacin amorphized by co-grinding with Neusilin US2 in various media was investigated. Physical mixtures of γ-indomethacin and Neusilin US2 (in the ratios 1:1, 1:4 and 1:5) were amorphized at room temperature employing 75% RH in a porcelain jar mill using zirconia balls. The crystallinity of the samples was determined using ATR-FTIR and PXRD. The solubility and dissolution profiles of co-ground powders and crystalline counterparts were evaluated in 0.1 N HCl, water and phosphate buffer (pH 6.8) in a USP type II dissolution apparatus at 250 rpm and 37 °C. Very high concentrations of dissolved indomethacin as compared to the solubility of γ-indomethacin (~500 times in water and ~ 3.7 times in phosphate buffer) were attained. However, the presence of other polymorphs detected by PXRD and a change in the pH of the medium made interpretation of the results difficult. In 0.1 N HCl the solubility (i.e., the peak in a concentration *versus* time plot) of the amorphized drug in a 1:5 ratio with Neusilin increased to 109 times the solubility of crystalline γ-indomethacin alone. An increase in amount of drug and Neusilin in the same ratio added to the dissolution medium also increased peak and plateau dissolution concentrations. The presence of silicic acid and ions (Mg^{2+} and Al^{3+}) in the dissolution media were found to cause the increase in the plateau concentration of indomethacin. Amorphization alone does not account for all of the dissolution enhancement; acidity, ions, and silicic acid are major contributors to dissolution enhancement.

KEY WORDS: amorphization; co-grinding; dissolution; indomethacin; silicate; solubility.

INTRODUCTION

Historically amorphous materials have been the domain of ceramists and material scientists. However recently, amorphous forms of drugs have received considerable attention from pharmaceutical scientists. Combinatorial chemistry and high throughput screening seem to have led to an increase in the number of poorly water-soluble drug candidates coming out from the discovery pipeline. This necessitates an improvement in the aqueous solubility (and bioavailability) of these poorly water-soluble compounds. The amorphous form of a compound is a more highly energetic state in comparison to its crystalline counterpart and therefore should provide an advantage in terms of solubility, dissolution and bioavailability (1,2). Hancock and Parks (3) reported at least two to four times increase in the experimental solubilities of amorphous solids in comparison to their crystalline counterparts. Kinoshita *et al.* (4), reported higher solubility (1.7 times that of its crystalline counterpart) and bioavailability (2.5 times that of its crystalline counterpart) of 3-*bis*(4-methoxyphenyl) methylene-2-indoline made amorphous by melt adsorption on amorphous calcium silicate. Watanabe *et al.* (5,6), found increased apparent equilibrium solubility of indomethacin (2.7 times) when co-ground with silica (as compared to its solubility in physical mixture). A ten-fold higher aqueous solubility and bioavailability of an amorphous solid dispersion of ritonavir with PEG have been reported (7).

Several methods have been employed to produce amorphous drugs. The most common method is melting the crystalline drug followed by its solidification. Solidification of the melt has been accomplished using different techniques such as rapid quenching over liquid nitrogen (8,9), slow cooling at room temperature (10) or cooling the melt at −5 °C/min (11). Other methods to produce amorphous solids include solvent deposition (12), solvent evaporation (9, 13), spray drying (14–16), freeze drying (9,13), and spray-freeze drying (17). Another method that has been successfully used to produce amorphous solids is co-grinding crystalline materials with excipients. Boldyrev *et al.* (18), used a planetary ball mill to prepare amorphous co-ground mixture of sulfathiazole with polyvinylpyrrolidone. Amorphization of indomethacin was achieved by milling it with silica or polyvinylpyrrolidone (6). Forni *et al.* (19), amorphized chloramphenicol stearate with microcrystalline cellulose using a grinder comprised of an agate mortar and pestle. Aspirin has been amorphized by physically mixing with controlled pore glass (CPG) or Aerosil (20). Amorphization of ketoprofen, indomethacin, naproxen and progesterone by

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co-grinding with Neusilin US2 have been reported by Gupta *et al.* (21). Konno *et al.* (22), reported spontaneous amorphization of aspirin and phenacetin when mixed with Neusilin and stored at room temperature for 7–14 days. The transformation of crystalline drugs to amorphous states upon storage as physical mixtures with colloidal silicon dioxide has also been well documented by Kim *et al.* (23).

Yang *et al.* (12), showed that dissolution of drugs co-ground with silica could either increase or decrease in comparison to the dissolution of neat crystalline drugs. In fact the dissolution was silicate and drug specific (12). Kinoshita *et al.* (4), reported an increase in dissolution and solubility of TAS-301 melt adsorbed on amorphous calcium silicate. An improvement in dissolution and/or solubility of drugs such as nitrendipine (24), tolbutamide (25), indomethacin (15), phenytoin (26), and meclozine hydrochloride (27) co-processed with silicates have been well documented. In a previous study, we reported physical and chemical stability of indomethacin amorphized by co-grinding with Neusilin US2 (28). The objective of the present study was to evaluate solubility and dissolution profile of indomethacin in its crystalline and co-ground amorphous state. We report the effect of ratio of indomethacin: Neusilin US2, percent crystallinity of the resulting co-ground powder, quantity of excess solid, and pH of the media on dissolution rate and "solubility" (i.e., peak and plateau concentrations) of the co-ground amorphous indomethacin.

MATERIALS AND METHODS

Materials

Indomethacin USP, 1-(*p*-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid, as the γ-polymorph, was purchased from Spectrum Chemicals (New Brunswick, NJ). Indomethacin exists predominantly in two polymorphic forms (α and γ) with γ polymorph (mp 162 °C) being the stable form. The pK_a of indomethacin is 4.5.(29). Neusilin US2, amorphous magnesium aluminosilicate, was obtained as a gift sample from Fuji Chemicals (Englewood, NJ). Aerosil-200 (colloidal silicon dioxide) was supplied by Degussa AG (Frankfurt, Germany). For preparation of buffers, certified ACS grade potassium phosphate (monobasic) and hydrochloric acid was purchased from Fisher Chemicals (Fair Lawn, NJ) and J. T. Baker (Philipsburg, NJ), respectively. A Barnstead/Thermolyne NANOpure deionization system (Dubuque, Iowa) was used as a source of ultrapure water for preparation of the dissolution media. Magnesium chloride (certified ACS grade) and aluminum reference solution (1 mg of Al/ml) were purchased from Fisher Chemicals (Fair Lawn, NJ). Silicic acid (MP Biomedicals Inc., Solon, OH) was used as received.

General Description of the Co-grinding Process

A rolling jar mill (Model no. 202421, Paul O. Abbe Inc., Little Falls, NJ) consisting of a cylindrical porcelain jar (outer diameter=5.25 in.; internal volume=1,000 ml) and zirconia balls (outer diameter=0.25 in.) was used to affect conversion of the physical mixtures of indomethacin and Neusilin US2. The cylindrical jar was filled with zirconia balls up to 600 ml of its total internal volume which gives a ball charge of ~ 40% v/v. The gasket between the lid and the jar ensured a well-sealed system during co-grinding. The speed of rotation of the cylindrical jar was 85 rpm. In each experiment 48 g of powder (indomethacin-Neusilin US2) was added to the jar. No increase in the temperature was noted within the sensitivity of ±1 °C during the grinding process.

Indomethacin, Neusilin US2, the porcelain jar and zirconia balls were equilibrated at room temperature at 75% RH in a glove box for 36 h prior to co-grinding. The humidity inside the glove box was maintained using a saturated NaCl solution. A hygrometer (Model LAM 880D, Mannix, Lynbrook, NY) was used to monitor the humidity inside the glove box.

Preparation of Co-ground Amorphous Indomethacin with Neusilin US2

Powder mixtures of indomethacin to Neusilin US2 (n=1) in ratios of 1:1, 1:4 and 1:5 by weight were co-ground at 75% RH for 8, 8 and 5 days, respectively, to complete amorphization (28). The respective percent crystalline fraction remaining in the powders quantified using ATR-FTIR as described elsewhere (28) was 1, 1 and 0%, which is essentially amorphous within the limit of detection (2%) for this method. Powder X-ray diffraction (PXRD) studies as previously described (28) and the absence of birefringence under cross-polarized light further confirmed the amorphous state of the co-ground powders. Samples of co-ground indomethacin with Neusilin US2 (in the ratio 1:4 at 75% RH) corresponding to 26% crystallinity (determined by previously reported ATR-FTIR method (28)) were obtained by co-grinding for 3 days. No significant chemical degradation was confirmed by an assay value of 97.4% using a stability-indicating HPLC assay on a sample of indomethacin co-ground with Neusilin US2 in the ratio 1:5 for 12 days.

Solubility and Dissolution Rate Studies

Solubility and dissolution profiles were evaluated using powders in a USP type II (paddle) dissolution apparatus (Vanderkamp 600, Vankel Industries, Chatham, NJ). Preliminary dissolution studies using crystalline indomethacin or co-ground amorphous indomethacin in the various media using the usual rotation speed of 100 rpm and paddle position resulted in cone formation at the bottom of the dissolution vessel. The powder was more evenly distributed in the dissolution medium by adjusting the paddle height to be 0.7 in. from the bottom of the vessel and increasing the paddle rotation speed to 250 rpm. Either crystalline indomethacin or co-ground amorphous indomethacin was introduced into the dissolution medium at 37°C. Preliminary experiments were conducted in water, phosphate buffer as well as in 0.1 N HCl using 900 ml of the dissolution medium. However, the solubility of co-ground amorphous indomethacin in water and phosphate buffer was very high and the amount of co-ground powder needed to conduct dissolution experiments (in triplicate) was insufficient from one batch of co-ground powder (maximum yield from one batch was 46 g). Therefore, to perform dissolution with the same batch of co-ground material, the dissolution media used were: (1) 300 ml of water, (2) 900 ml

of 0.1 N HCl and (3) 150 ml of phosphate buffer (pH 6.8). Aliquots (2 ml for water, 5 ml for 0.1 N HCl and 1 ml for phosphate buffer) were withdrawn through a 0.22 μm acetate filter and analyzed by measuring absorbance at 318 nm. The volume withdrawn was not replaced and the concentration of drug in the aliquot was accounted for in calculating the amount dissolved at and prior to the peak in concentration *versus* time. The crystallinity in the co-ground powders after dissolution and solubility studies was investigated by PXRD. The suspensions were filtered through 0.22 μm acetate filters employing vacuum and the recovered solids (partially wet) were pressed lightly to form discs for the measurements. Except where otherwise specified in the results, the recovered solids were found to be amorphous.

A sample of 500 mg of Neusilin US2 as received and one ground for 5 days at 75% RH were dispersed separately in 900 ml of 0.1 N HCl as described above. The pH at the end of the study was monitored and compared to the initial pH. Aliquots were withdrawn and filtered through 0.22 μm filter. The concentration of magnesium was determined by analyzing aliquots at 285.2 nm using atomic absorption spectrophotometry (Spectra AA-200, Varian Inc., Victoria, Australia) with an aluminum/calcium/magnesium hollow cathode lamp and an air–acetylene flame. The presence of dissolved silicic acid was detected using silicomolybdate method as described by Iler (30).

RESULTS AND DISCUSSION

Previous groups have reported an increase in drug solubility when drug is co-ground with silicates (5,31). This increase has been attributed to the drug being in the amorphous state. The following results show that there are other contributing factors.

Effect of Dissolution Medium

The solubility of crystalline γ-indomethacin in water was determined to be 2 μg/ml (Fig. 1). The concentration of indomethacin dissolved from excess drug (co-ground with Neusilin US2 in the ratio 1:5) in water peaks within 5 min and declines to a plateau. The peak and plateau concentrations of amorphous indomethacin co-ground in the ratio 1:5 were 998 and 575 μg/ml, respectively (Fig. 1). Similarly, the peak and plateau concentrations of amorphous indomethacin co-ground in the ratio 1:1 were 818 and 735 μg/ml, respectively. The pH of the medium, determined at the final time point (Fig. 1), differed. The pH was 4.5 for γ-indomethacin and 6.7 and 8.1 for indomethacin co-ground with Neusilin US2 in the ratio 1:1 and 1:5, respectively. Neusilin US2 is reported to behave as a buffer with the pH of its 4% (w/v) suspension being 7.4 (21). Therefore an enhancement in the peak concentration and solubility of the co-ground indomethacin could have been due to the buffering effect of Neusilin US2. As a control, dissolution of crystalline γ-indomethacin was studied in the presence of Neusilin US2 in the ratio 1:1 and 1:5 (referred as 1:1 PM and 1:5 PM, respectively, in the Fig. 1). Indeed the presence of Neusilin US2 leads to an increase in the concentration of the dissolved indomethacin but does not result in the same peak seen for co-ground indomethacin:Neusilin US2 (1:5). Further PXRD studies on the solids recovered at the end of the dissolution detected the presence of α and γ polymorphs of indomethacin. The variation in pH and the detection of different polymorphs made further interpretation of the results difficult. Previous studies reporting an increase in the solubility of poorly water soluble drugs by processing with silicates (4,5,31,32) have concluded that the increase is due to amorphization. However, pH variations and polymorphic transformations have not been explicitly accounted for in these studies. In the light of above findings the results of these studies should be interpreted with care. Furthermore, the effect of surface area is a complicating factor. However, particle size or surface area measurements would not be as useful in these composite (i.e. drug and silicate) powders as they would be for neat powders, since the surface may be either drug or silicate.

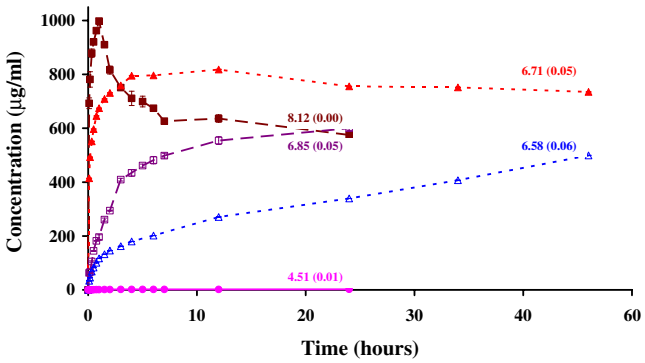

Fig. 1. Dissolution profiles of indomethacin (300 mg) in water: Crystalline γ-indomethacin, indomethacin co-ground with Neusilin US2 (1:1), 1:1-PM, indomethacin co-ground with Neusilin US2 (1:5), 1:5-PM

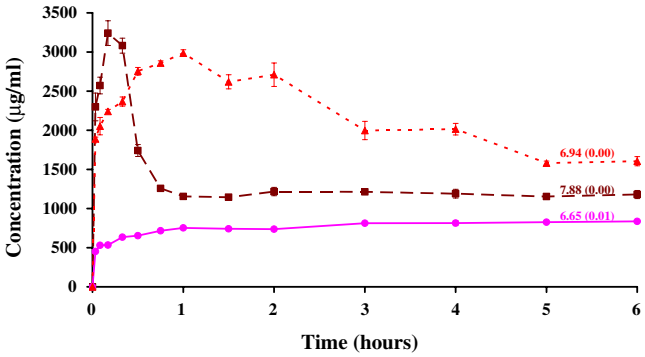

Fig. 2. Dissolution profiles of indomethacin (500 mg) in pH 6.8 phosphate buffer: Crystalline γ-indomethacin, indomethacin co-ground with Neusilin US2 (1:1), indomethacin co-ground with Neusilin US2 (1:5)

Dissolution profiles in phosphate buffer at pH 6.8 (Fig. 2) lowered the variation in the pH of the medium and resulted in lower solubility enhancement as compared to that seen in water. The solubility of crystalline γ-indomethacin in phosphate buffer (pH 6.8) was 835 μg/ml. The peak and plateau concentrations of indomethacin co-ground with Neusilin US2 in the ratio 1:1 were 2,985 and 1,580 μg/ml, respectively. For 1:5 ratio, corresponding peak and plateau concentrations were 3,081 and 1,180 μg/ml, respectively. Similar to the findings in water as a dissolution media, the presence of other polymorphs of indomethacin complicated

the interpretation of the results. In addition, a change in pH of phosphate buffer pH 6.8 (for 1:5 co-ground powder) was also observed. Therefore dissolution of indomethacin co-ground with Neusilin US2 was further evaluated in 0.1 N HCl as described below.

Crystalline γ-indomethacin is white in color while amorphous indomethacin is yellow. During dissolution studies (in 0.1 N HCl), up to 5 min the color of the dissolution media was yellow. However, at about 5 min a color change from yellow to an opaque whitish-yellow corresponding to a decrease in the concentration of indomethacin (as determined by UV) was observed. Assuming other polymorphs are also white, this visual observation gave direct evidence of crystallization of indomethacin in the dissolution medium. The solids recovered from the dissolution medium at 60 min were whitish-yellow in color indicating the presence of some amorphous indomethacin. In addition to this visual evidence, detection of γ-indomethacin by PXRD of the solids recovered at the end of the 60 min dissolution study confirmed the absence of any polymorphic transformation. In addition, no change in the pH of the dissolution medium was observed at the end of the study. These data show the importance of monitoring pH (of the dissolution media) for solubility estimation of ionizable drugs co-ground with Neusilin US2.

Effect of Quantity of Excess Powder

The dissolution profiles of crystalline γ-indomethacin and various amounts of indomethacin amorphized by co-grinding with Neusilin US2 in the ratio 1:1 for 8 days in 0.1 N HCl are compared in Fig. 3. The co-ground amorphous indomethacin shows higher maximum transient concentration (MTC) and maximum sustained concentration (similar to Watanabe's apparent equilibrium solubility (5) and hereafter referred to as MSC) in comparison to crystalline indomethacin which showed no peak and a lower MSC or true solubility, in this case. The table in Fig. 3 quantifies the effect of excess amounts of indomethacin (co-ground amorphous) used in the dissolution study on peak (MTC) and plateau (MSC). The peak and plateau ratios were calculated by dividing MTC and MSC, respectively, by the solubility of γ-indomethacin. The larger the amounts of amorphous indomethacin in the dissolution vessel resulted in higher peak and

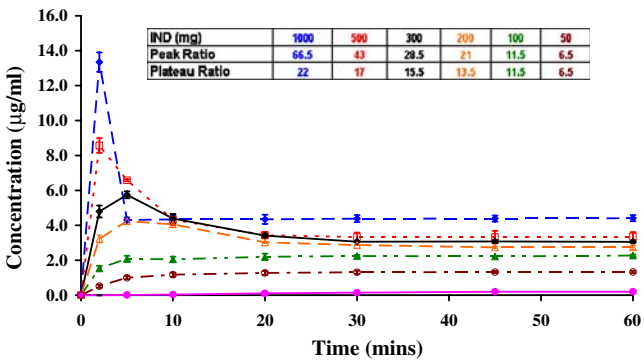

Fig. 3. Dissolution profiles of indomethacin co-ground with Neusilin US2 (1:1) in 0.1 N HCl: 50, 100, 200, 300, 500, 1,000 mg, crystalline γ-indomethacin (100 mg)

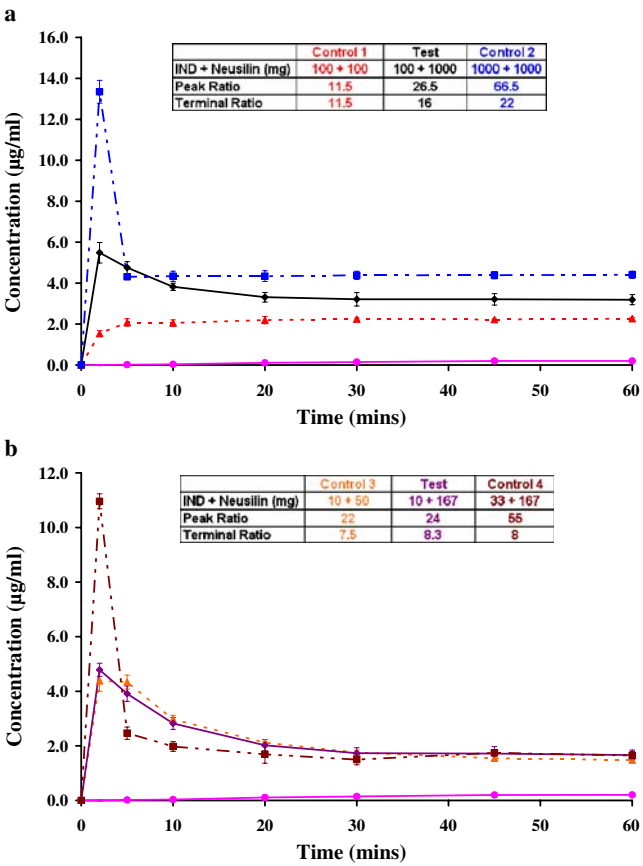

Fig. 4. Dissolution profile of indomethacin co-ground with Neusilin US2 in 0.1 N HCl—a 1:1, b 1:5: Control 1, Control 2, Test 1, Control 3, Control 4, Test 2, crystalline γ-indomethacin (100 mg)

plateau concentrations. We also note that the higher the peak, the quicker is the decline in concentration of indomethacin to a plateau. The decline in the concentration is due to crystallization of indomethacin. Others have reported on the effect of amount of excess solid on solubility (31,33,34). One such study was specific for salt forms (mono and dihydrochloride) of an experimental basic drug (E2050) (33). An increase in the amounts of excess of dihydrochloride salt lowered its solubility due to precipitation of the lower solubility monohydrochloride salt (in pH regions where solubility is controlled by monohydrochloride salt). Kawakami *et al.* (34), reported the impact of the amount of excess solid on apparent solubility of neat crystalline free acids and free bases. With an increase in the amount of excess indomethacin, the apparent solubility decreased at pH 5 and 6 and yet increased at pH 6.5 and 7. Using co-ground mixtures rather than neat amorphous indomethacin we report an increase in the MTC and MSC of co-ground amorphous indomethacin (in 0.1 N HCl where drug is unionized) with an increase in the amount of drug added to the dissolution medium. Another study reports an increase in the solubility of drugs (mixed with glass beads or sodium chloride) with an increase in the amount of drug (31). However, contradictory to the other two reports (33,34), the solubility for the corresponding crystalline drugs was reported to be independent of the amount of excess (31).

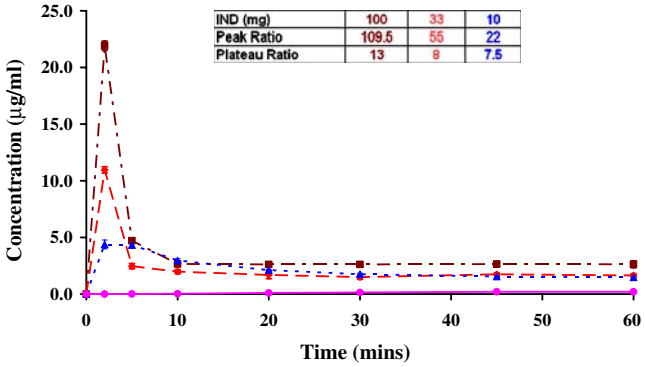

Fig. 5. Dissolution profiles of indomethacin co-ground with Neusilin US2 (1:5) in 0.1 N HCl: 10, 33, 100 mg, crystalline (100 mg)

As the amount of co-ground amorphous indomethacin in dissolution studies was increased, there was a proportional increase in the amount of Neusilin US2. In order to study the effect of Neusilin US2, dissolution of co-ground amorphous indomethacin (in the ratio 1:1) was evaluated in the presence of an additional amount of Neusilin US2 (Fig. 4a). The powder weights of two controls (indomethacin co-ground with Neusilin US2 in the ratio 1:1) used in the study were 200 mg and 2,000 mg, which were equivalent to 100 and 1,000 mg of indomethacin (referred to as control 1 and control 2, respectively). The test sample (referred to as test 1) consisted of 200 mg of co-ground amorphous indomethacin powder (equivalent to 100 mg of indomethacin) to which 900 mg of additional Neusilin US2 was added giving this powder the same amount of indomethacin as control 1 and the same amount of Neusilin US2 as control 2. The addition of Neusilin US2 to the co-ground sample led to a peak and plateau intermediate between the two controls (Fig. 4a).

Maximum transient concentrations for 1:5 co-ground indomethacin samples were higher than those attained for 1:1 co-ground samples. Similarly, MSCs or plateaus for amounts equivalent to 10, 33 and 100 mg of co-ground amorphous indomethacin were also higher than those for 1:1 co-ground samples at 1.5, 1.6 and 2.6 µg/ml, respectively. Again, there is an increase in MTC and MSC with increase in excess co-ground mixture (Fig. 5). Again, at this ratio the effect of excess Neusilin US2 on the dissolution and solubility of indomethacin (co-ground in the ratio 1:5) was evaluated using two controls (control 3 and control 4) and a test sample (test 2). The controls 3 and 4 were comprised of 60 (equivalent to 10 mg of indomethacin) and 200 mg (equivalent to 33 mg of indomethacin) of co-ground amorphous powder (indomethacin with Neusilin US2 in the ratio 1:5), respectively. The test 2 consisted of 60 mg of co-ground amorphous indomethacin powder (equivalent to 10 mg of indomethacin) to which 117 mg of additional Neusilin US2 was added giving this powder the same amount of indomethacin as control 3 and the same amount of Neusilin US2 as control 4. No improvement in peak concentration for test *versus* control 3 was achieved (Fig. 4b). The MSC of test was higher than that of control 3 ($p<0.05$). It should be noted that the difference in MSCs of test and control 4 is not statistically significant ($p>0.05$). The results suggest that additional Neusilin US2 added to the co-ground amorphous indomethacin further increased its MSC.

Effect of Ratio of Indomethacin: Neusilin US2 and Degree of Crystallinity

Despite the similarity of co-ground amorphous samples (co-ground in the ratios 1:1, 1:4 and 1:5) with respect to their percent crystallinity, PXRD and FTIR scans, their dissolution profiles are different from each other (Fig. 6). There is an increase in the peak concentration with an increase in the amount of Neusilin US2 (or decrease in the ratio of indomethacin to Neusilin US2). A small increase in the MSC of indomethacin co-ground with Neusilin US2 in the ratio 1:4 over that co-ground in the ratio 1:1 was observed (Fig. 6). However, difference in MSCs (of samples co-ground in the ratios 1:4 and 1:5) is not statistically significant ($p>0.05$). Higher peak concentrations with increased amounts of Neusilin suggest that the bioavailability of co-ground amorphous drugs could also be dependent upon drug to silicate ratio (particularly for BCS class II compounds).

The effect of the degree of crystallinity of co-ground indomethacin on dissolution and solubility was evaluated. 500 mg of co-ground indomethacin powder in the ratio 1:4 (equivalent to 100 mg of indomethacin) corresponding to 0 and 26% crystallinity was used for dissolution studies. The dissolution profiles are shown in Fig. 7. The peak concentration (MTC) for the sample corresponding to 0% crystallinity (15.4 µg/ml) is higher than that for the sample corresponding to 26% crystallinity (4.5 µg/ml). Similarly, the MSCs for samples corresponding to 0 and 26% crystallinity are 2.7 and 2.2 µg/ml, respectively. The results (table inserted in Fig. 7) suggest higher solubility and dissolution advantage with decrease in the degree of crystallinity of drug in the co-ground powder.

Underlying Factors Affecting Peak (MTC) and Plateau (MSC) Concentration

The plateau concentration of indomethacin co-ground with Neusilin US2 (an amount equivalent to 100 mg of indomethacin) in the ratio 1:5 at 60 min of dissolution study was 13 times higher than the solubility of γ-indomethacin (Fig. 5). The dissolution study of co-ground amorphous indomethacin (in the ratio 1:5) was continued for 2 days with no change in the plateau concentration. Furthermore, seeding

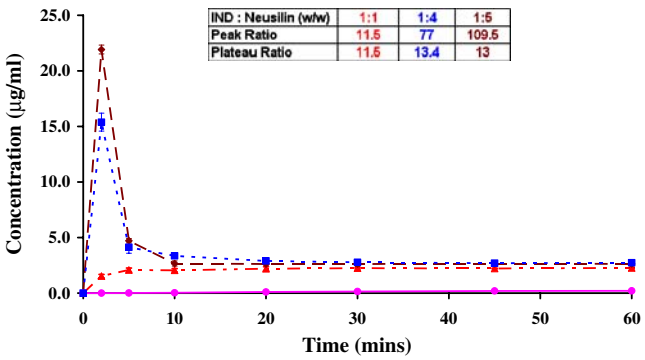

Fig. 6. Effect of ratio of indomethacin to Neusilin US2 on dissolution of co-ground amorphous indomethacin (100 mg) in 0.1 N HCl: 1:1, 1:4, 1:5, crystalline γ-indomethacin

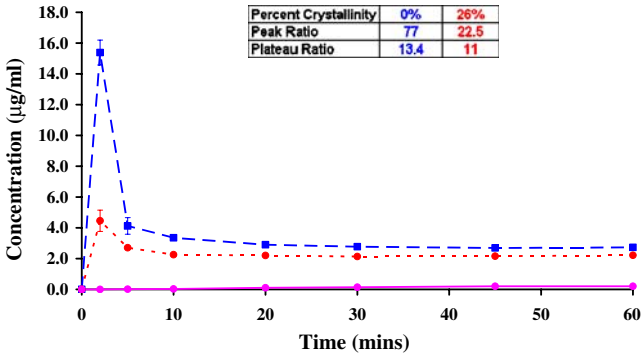

Fig. 7. Effect of percent crystallinity on dissolution of indomethacin co-ground with Neusilin US2 (1:4) at 75% RH in 0.1 N HCl: 0%, 26%, crystalline γ-indomethacin

of the dissolution medium with a suspension of γ-indomethacin did not result in a change in concentration when monitored for 4 h after seeding. In a separate study, the dissolution medium at the end of 60 min study was filtered through 0.22 µm acetate filter and stored at room temperature for 2 days. No drop in the concentration at the end of 2 days (in comparison to the concentration at 60 min) showed the stability of dissolved indomethacin in the apparently supersaturated state. In addition at the end of a 60 min dissolution study a sample was subjected to a temperature cycling of 37±20 °C for three cycles. No change in concentration was observed. Stability of apparently supersaturated solutions of methylprednisolone for 120 days (35) and griseofulvin for 50 days (36) at room temperatures and of indomethacin for 2 days at 37 °C (5) have also been reported in the literature.

The reason for the stability of apparently supersaturated solutions of co-ground amorphous indomethacin and dependency of the MSCs on the amount of co-ground amorphous indomethacin added to dissolution media (Figs. 3 and 5) was further investigated. Neusilin US2 (500 mg) was added to 900 ml of 0.1 N HCl at 37°C and stirred at 250 rpm. At 60 min, 100 mg of crystalline γ-indomethacin was added to the dissolution media. A higher rate and greater extent of dissolution of crystalline indomethacin in the presence of Neusilin US2 as compared to that of crystalline indomethacin in the absence of Neusilin US2 was observed (Fig. 8). While the dissolution rate was slower than that of co-ground indomethacin with absence of a transient peak, both samples attained the same plateau concentrations (Fig. 8). This suggests that the presence of Neusilin US2 in the medium somehow influences the MSC of indomethacin. Dissolution of unprocessed Neusilin US2 (n=3) in 900 ml of 0.1 N HCl at 37 °C was allowed to proceed for 60 min. The dissolution medium was centrifuged for 4 h at relative centrifugal force of 3,700×g and filtered through a 0.22 µm. Then dissolution of crystalline γ-indomethacin (100 mg) was evaluated in this centrifuged and filtered dissolution medium. The dissolution profile (Fig. 8) was similar to that of previously determined unfiltered crystalline γ-indomethacin in the presence of Neusilin US2 indicating that an increased solubility of indomethacin could be due to a component in Neusilin US2 that was released into the dissolution medium.

To determine whether ions dissolved from the surface of Neusilin US2, dissolution of controls (ground or unground Neusilin US2) and indomethacin co-ground with Neusilin US2 in the ratio 1:5 was evaluated at 37 °C. A sample equivalent to 500 mg of Neusilin US2 was added to 900 ml of 0.1 N HCl for 60 min. The concentration of dissolved magnesium ions was determined by AA spectrophotometry. The results (Table I) showed no difference in the dissolved concentrations of magnesium from the co-ground indomethacin sample over the controls. This dissolved concentration of magnesium ions (1.22 µM) is equivalent to 2.4 mmol of Mg^{2+} per gram of Neusilin US2. Thus out of a total of 3.6 mmol of Mg^{2+} reportedly present per gram of Neusilin US2, 67% dissolves in 0.1 N HCl. In a separate experiment, crystalline γ-indomethacin (100 mg) was added to the dissolution medium containing 1.22 µM magnesium (added as magnesium chloride). The plateau concentration of dissolved indomethacin (0.6 µg/ml) in presence of 1.22 µM of Mg^{2+} was triple that of neat crystalline γ-indomethacin (0.2 µg/ml; Fig. 8). However, that concentration was still less than one fourth of the MSC for indomethacin (2.6 µg/ml) amorphized by co-grinding with Neusilin US2 in the ratio 1:5 (Fig. 8). In the presence of 122 µM of Mg^{2+}, the concentration of dissolved indomethacin was proportionately higher (2.4 µg/ml). A proportionate increase in concentration of dissolved Mg^{2+} was confirmed by dissolving a sample of Neusilin US2 equivalent to 200 and 1,000 mg, respectively, in 900 ml of 0.1 N HCl and determining amounts of Mg^{2+} present in solution (Table I). With an increase in amount of co-ground mixture added to the dissolution medium, a corresponding increase in amount of Mg^{2+} going into solution (dissolution medium) could partially explain an increase in plateau concentrations of dissolved indomethacin.

Neusilin US2 is chemically magnesium aluminometasilicate. Therefore the possibility of Al^{3+} dissolving and increasing the concentration of dissolved indomethacin was also studied. The amount of Al^{3+} (from 500 mg of Neusilin US2 dissolved in 900 ml of 0.1 N HCl for 60 min) was below the limit of detection. In the presence of very high concentrations 110 µM of Al^{3+} in 0.1 N HCl (Fig. 8), dissolution of 100 mg of γ-indomethacin increased (2.8 µg/ml).

The concentration of Mg^{2+} and Al^{3+} present in solution only partially accounted for an increase in the dissolved

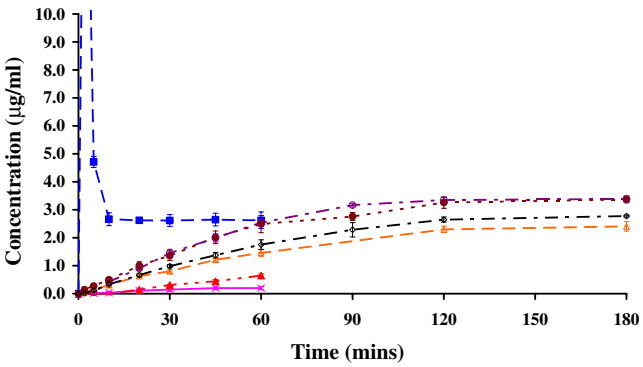

Fig. 8. Dissolution profiles of indomethacin in 0.1 N HCl: crystalline, co-ground amorphous (1:5), crystalline + Neusilin (500 mg), crystalline + filtered-centrifuged Neusilin US2 (500 mg), crystalline + Mg^{2+} (1.22 µM), crystalline + Mg^{2+} (122 µM), crystalline + Al^{3+} (110 µM)

Table I. Determination of the Concentration of Mg^{2+} Dissolved in 0.1 N HCl (900 ml) at the End of 60 min Dissolution Study by Atomic Absorption (AA) Spectrophotometry

Sample	Amount of Neusilin (mg)	Grinding Time at Room Temperature and 75% RH	Concentration of Mg^{2+} by AA Spectrophotometry (μm)[a]
Neusilin US2	500	As is (unground)	1.22 (0.01)
Neusilin US2	500	5 days	1.22 (0.01)
Indomethacin: Neusilin US2 (1:5)	500	5 days	1.22 (0.2)
Neusilin US2	200	As is (unground)	0.48 (0.01)
Neusilin US2	1000	As is (unground)	2.22 (0.04)

[a] Standard deviation of three readings is in parentheses

concentrations of indomethacin. Therefore, we further evaluated dissolution of crystalline γ-indomethacin in the presence of colloidal silicon dioxide (Aerosil-200), a silicate that does not contain Mg^{2+} or Al^{3+}. Aerosil-200 (500 mg) was added to 900 ml of 0.1 N HCl at 37 °C using 250 rpm. At 60 min, 100 mg of crystalline γ-indomethacin was added to the dissolution vessel. The dissolution profile (Fig. 9) showed an increase in the dissolved concentration of indomethacin (3.2 μg/ml) over that for crystalline γ-indomethacin in the absence of Aerosil-200 (0.2 μg/ml). Similar to the experiments conducted previously for Neusilin US2, we evaluated dissolution of crystalline γ-indomethacin (100 mg) in the centrifuged and filtered Aerosil-200 dissolution medium (900 ml of 0.1 N HCl). There was no difference in the MSC of indomethacin in filtered-centrifuged Aerosil-200 (3.1 μg/ml) and unfiltered-non-centrifuged Aerosil-200 dissolution medium (3.2 μg/ml; Fig. 9). The results suggested that a component from Aerosil-200 was released into the dissolution vessel and interacted with indomethacin. The presence of silicic acid in the medium was detected by a colorimetric method (30).

The solubility of 100 mg of crystalline γ-indomethacin in the presence of 500 mg of silicic acid was 14.7 times greater than in the absence of silicic acid (Fig. 9). Thus the presence of silicic acid in the medium also contributed to an increase in the solubility of indomethacin. Raman spectroscopy was used to investigate the interaction between indomethacin and silicic acid in solution. However, the dissolved concentration

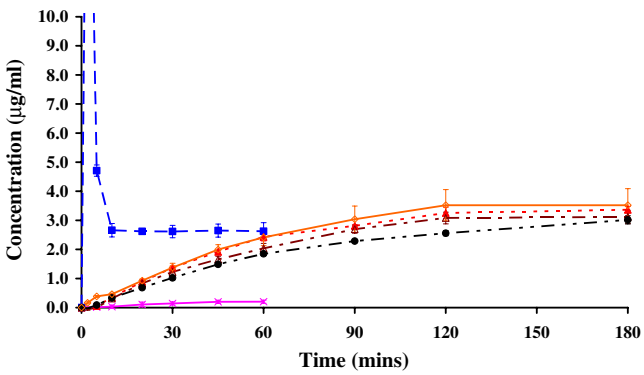

Fig. 9. Dissolution profiles of indomethacin in 0.1 N HCl: crystalline, co-ground amorphous (1:5), crystalline + Aerosil-200 (500 mg), crystalline + filtered-centrifuged Aerosil-200 (500 mg), crystalline + silicic acid (500 mg), crystalline + silicic acid (500 mg) + Mg^{2+} (1.22 μM)

was below the limit of detection. Thus the nature of the interaction between indomethacin and silicic acid remains unknown. In a separate experiment, silicic acid (500 mg) and Mg^{2+} (1.22 μM) were added to 900 ml of 0.1 N HCl dissolution medium at 37 °C. At 60 min, 100 mg of crystalline γ-indomethacin was added to the dissolution media. Further enhancement in the solubility (3.5 μg/ml) as compared to that observed in the presence of silicic acid alone (3.0 μg/ml) was observed (Fig. 9). It should be noted that the solubility of indomethacin in Neusilin US2 was 3.3 μg/ml (Fig. 8). Thus it was concluded that the presence of silicic acid and ions (Mg^{2+} and Al^{3+}) contributed to enhance the solubility of crystalline γ-indomethacin. The individual contribution of these components followed the order silicic acid>Mg^{2+}>Al^{3+}. Our findings explain an increase in plateau concentrations with increasing amount of indomethacin (co-ground with Neusilin US2 in the ratio 1:1) added to the dissolution media due to the presence of Neusilin. However, results reported in Fig. 6 at first seem inconsistent with the above findings as MSCs of indomethacin co-ground with Neusilin US2 in the ratios 1:4 and 1:5 are similar. In percentage terms, the proportion of Neusilin US2 (80 and 80.3%) is similar and could explain their experimentally observed similar plateau concentrations.

Others have observed an increase in apparent solubility of griseofulvin when co-ground with glass beads (31) and an increase in what the authors' refer to as "apparent equilibrium solubility" of indomethacin when co-ground with Aerosil (5). In both studies water was used as a dissolution medium. We separately evaluated dissolution of 500 mg of Neusilin US2 and Aerosil-200 in 900 ml of water at 37 °C. At 60 min, the presence of silicic acid (for Neusilin US2 and Aerosol-200) was confirmed by a colorimetric method (30). In addition, the presence of dissolved magnesium ions (for Neusilin only) was confirmed by AA spectrophotometry. The presence of silicic acid and dissolved ions coupled with ionization of drug in water (dissolution medium) offer an alternate explanation to the enhancement of solubility of griseofulvin found by Mosharraf *et al.* (31) and enhancement of solubility of indomethacin found by Watanabe *et al.* (5).

CONCLUSIONS

An increase in solubility of drugs amorphized by co-processing with silicates has been attributed to the presence of drug in amorphous state (4,5,15,26,31,32). Amorphization alone does not account for all of the dissolution enhancement

of co-ground amorphous indomethacin. Factors such as pH of the dissolution medium, release of ions and silicic acid from Neusilin are major contributors to dissolution enhancement. Silicates cannot be considered as largely inert excipients.

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