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Effect of Amorphous Content on Dissolution Characteristics of Rifampicin

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Rifampicin, one of the main first line anti-TB drugs, shows variable bioavailability in different marketed preparations and reasons cited include physiological, degradation, manufacturing/ processing, solid state, and bioavailability assessment procedure. Although the amorphous form of a drug is expected to exhibit higher solubility, the amorphous rifampicin has been reported to have a solubility disadvantage as compared to crystalline form II, which is used in marketed preparations. Amorphous form was generated and characterized by solid-state characterization techniques. Physical powder mixtures of form II with varying amounts of amorphous form were prepared, which were then subjected to solid-state characterization techniques and further evaluated for their dissolution behavior. Differential scanning calorimetry (DSC) scans show that area enclosed by integral of melting endotherm can be used for quantification of crystalline component, which can then be used to estimate amorphous content. No definite trend was evident in powder dissolution of mixtures that could implicate solubility difference of amorphous form. Intrinsic dissolution rate (IDR) results indicate that amorphous content has no effect on dissolution profiles of crystalline rifampicin.

Keywords rifampicin; amorphous; solid-state characterization; intrinsic dissolution rate

INTRODUCTION

Solubility and permeability define the extent and rate of entry of the drug (and hence bioavailability) from a peroral dosage form into the body. Quality control apart, rifampicin shows variable bioavailability between different manufacturers (Agrawal & Panchagnula, 2004; Pahkla et al., 1999) Furthermore, rifampicin has been shown to exist in various crystalline forms (polymorphs—forms I and II, various solvates, and mono-/di-/pentahydrates) (Henwood, Liebenberg, Tiedt, Lotter, & de Villiers, 2001; Pelizza, Nebuloni, Ferrari, & Gallo, 1977). Form II is the one most predominantly found in marketed generic rifampicin. Thus, purity of a drug can be a major concern in a pharmaceutical setup (Bauer, de Leede, & Van Der Waart, 1998). Amorphous form of rifampicin is reported to have a significantly lower dissolution rate (Henwood, de Villiers, Liebenberg, & Lotter, 2000). Another report indicates that polymorphism has no effect on intrinsic dissolution rate (IDR) of rifampicin, and the key is the influence of particle size on dissolution rate, which contributes to variable bioavailability of rifampicin (Agrawal, Ashokraj, Bharatam, Pillai, & Panchagnula, 2004). During the formulation of rifampicin into dosage forms, it is subjected to pharmaceutical processes and conditions that can increase its amorphous content. Rifampicin on the basis of its solubility and permeability is classified as borderline (Agrawal et al., 2004) class II (Lindenberg, Kopp, & Dressman, 2004) of the Biopharmaceutic Classification System (BCS), and this solubility difference might have significant implication from a clinical and regulatory perspective. Till now, there is no reported study on dissolution behavior of rifampicin containing varying proportions of amorphous form. The powder dissolution for hydrophobic drugs such as rifampicin is a problematic task because the powder is poorly wetted by the aqueous dissolution medium and the powder either settles at the bottom forming lumps or floats at the surface. Based on the above discussion, the presented study was designed to check the effect of amorphous content playing a role in the dissolution characteristics of rifampicin in alone or fixed dose combinations.

MATERIALS AND METHODS

Materials

Standard forms I and II of rifampicin were obtained from Lupin (Mumbai India). Commercial samples of rifampicin for preparation of amorphous form were obtained from Macleods (Mumbai India). Other chemicals and solvents were of analytical or laboratory grade.

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Generation and Characterization of Amorphous Form

Preparation

For the preparation of amorphous form, a 1.1% wt/vol solution of rifampicin in tertiary butanol was frozen in a chiller and subjected to vacuum in a freeze drier for 18–20 h. An attempt was also made to prepare the amorphous form by quench-cooling method; however, because of decomposition of rifampicin, it was not continued further.

Characterization of the Amorphous Form

The entire freeze-dried product from different batches was pooled and passed through sieve 60 (mesh size 250 μm) to aid handling and subsequently stored in light-resistant containers kept in a desiccator. Its solid-state characterization was done as per a set protocol developed in our laboratory (Agrawal et al., 2004; Gandhi et al., 2002, Panchagnula, Prakash, Pillai, Agrawal, & Ashokraj, 2004).

Fourier Transformed Infrared Spectroscopy. The samples were analyzed [FTIR spectrophotometer; Perkin Elmer (Norwalk, CT, USA) using Spectrum software version 3.02] in KBr pellets in the region of 4,000–600 cm^{-1}. Each spectrum, corrected for background noise was derived from an average of at least four scans and Fourier transformed.

Differential Scanning Calorimetry. Thermograms of the powder samples were recorded on a differential scanning calorimetry (DSC) (Mettler Toledo 821^e, Schwerzenbach, Switzerland) using Mettler Star system. The temperature axis and cell constant of DSC were calibrated with indium. Temperature range used was 25–300°C at an incremental rate of 10°/min, with nitrogen purging at a rate of 80 mL/min (Panchagnula et al., 2004).

Thermogravimetric Analysis. Powder samples kept in alumina crucibles were subjected to thermogravimetric analysis (TGA) (Mettler Toledo TGA/SDTA 851^e, Switzerland) over a temperature range of 25–300°C at a rate of 10°C/min under nitrogen purge of 20 mL/min (Gandhi et al., 2002).

Powder X-Ray Diffraction. The samples, passed through sieve 60, were analyzed by an X-ray powder diffractometer (Philips PW 1710, Eindhoven, The Netherlands) using CuKα radiation (tube operated at 35 kV, 20 mA). Data were collected over a range of 5–50° 2 θ in continuous scan mode at step size of 0.02° utilizing 1 s for each step.

Preparation and Characterization of Powder Mixtures

As form II is the one predominantly used commercially, the study was carried out for powder mixtures of amorphous form with polymorph type II only. The maximum extent to which the amorphous form can be expected to be present in the crystalline form, as an impurity, was set at 50%. Amorphous powder was physically mixed with the standard crystalline form II in proportions of 10, 20, 30, 40, and 50%. Weighed amounts of the two forms were mixed in geometric proportion with the help of a stainless steel spatula. Resulting blends were then passed through sieve 60 and again mixed gently with a spatula. The blends were stored in light-resistant containers and kept in a desiccator until used. The powder blends were subjected to Fourier transformed infrared spectroscopy (FTIR) and DSC to study the effect of amorphous impurity in crystalline samples.

Dissolution Studies

Powder Dissolution

Powder dissolution was carried out in an USP apparatus II (paddle) supplied by Electrolab (Mumbai India). The powder drug has wetting problems and sticks to the walls of the dissolution vessel. Five grams of glass beads of diameter 1–1.5 mm were used to disperse the powder per dissolution vessel. Dissolutions were carried out for 45–60 min, depending on the dissolution medium, in 900 mL of dissolution media at 50 rpm and at a temperature maintained at 37 ± 0.5°C.

IDR

Rotating disc assembly at 100 rpm using 900 mL dissolution medium was used. The IDR was found out by compressing the powder into a pellet of diameter 8 mm (area approx. 0.5 cm^2) at a pressure of 500 pounds per square inch (psi), maintained for 60 s (Agrawal et al., 2004). The amount of drug going into the solution from the 100-mg pellet was recorded online (colorimetrically at 475 nm) over a period of 50 min.

RESULTS AND DISCUSSION

Generation and Characterization of Amorphous Form

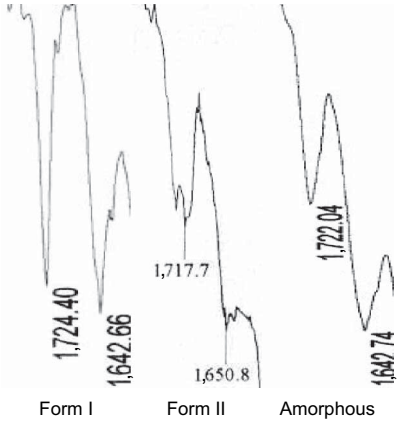
Generation of Amorphous Form

Amorphous form was generated by the modification of a freeze-drying process developed earlier in our laboratory (Agrawal et al., 2004). Water was excluded from the above method without affecting the nature of the product. As the solvent requirement in this method was high, the amount of drug dissolved was increased.

Characterization of the Amorphous Form

The following characterization studies were carried out on the freeze-dried product.

FTIR. Figure 1 shows the comparative spectra of forms I, II, and amorphous form. Dissimilarities in the FTIR spectra for forms I and II have been based on the differences in the intramolecular hydrogen bonding (Henwood et al., 2001; Pelizza et al., 1977). The area of interest is mainly in the region of 1,700–1,750 cm^{-1}. In form II, the C-21 hydroxyl is hydrogen-bonded to C-23 hydroxyl, which itself is hydrogen-bonded to the C-25 acetyl (Figure 1, lower panel). In form I, the C-23 hydroxyl is not hydrogen-bonded to the C-25 acetyl group, which is evident by the free acetyl group frequency at 1,724 cm^{-1}. The amorphous form also shows a single peak indicating that the acetyl group is not involved in hydrogen bonding. In form I, a single peak at 1,721–1,725 cm^{-1} was observed,



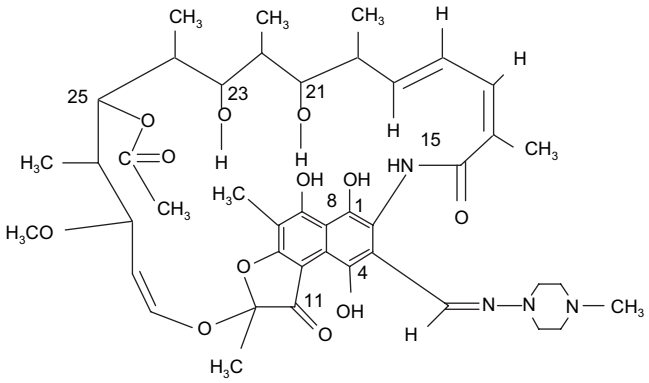


FIGURE 1. Fourier transformed infrared spectrometry (FTIR) spectra of forms I, II, and amorphous forms of rifampicin in the region 1,800–1,600 cm^{-1}(upper panel). Amorphous rifampicin resembles the form I in having a sharp peak at approximately 1,725 cm^{-1} because of free C-25 acetyl group, whereas the form II shows two peaks in the region. Form II differs in having a hydrogen-bonded acetyl group showing stretching frequency at approximately 1,715 cm^{-1} and the second peak at approximately 1,740 $cm^{-1}$is because of non-hydrogen-bonded furanone carbonyl (lower panel).

whereas two close peaks were seen in the similar region for form II because of hydrogen-bonded C-25 acetyl (1,715 cm^{-1}) and the free furanone carbonyl (around 1,740 cm^{-1}). In form I and amorphous rifampicin, the furanone carbonyl is hydrogen-bonded to the C-4 hydroxyl group. Amorphous form also showed a single peak around 1,720 cm^{-1}, with a very small shoulder on the lower frequency side. Thus, the spectrum of the amorphous form is similar to that of form I. No noticeable differences were found in the other regions of the spectra. This provides an important insight into the role of acetyl group into the packing arrangement of unit cells of rifampicin, one particular conformation favoring packing in such a way that it provides the most stable polymorph (only two polymorphs are known for rifampicin, form I being more stable) and the other preventing any regularity in the structure at all, thus giving rise to the amorphous form (Pelizza et al., 1977).

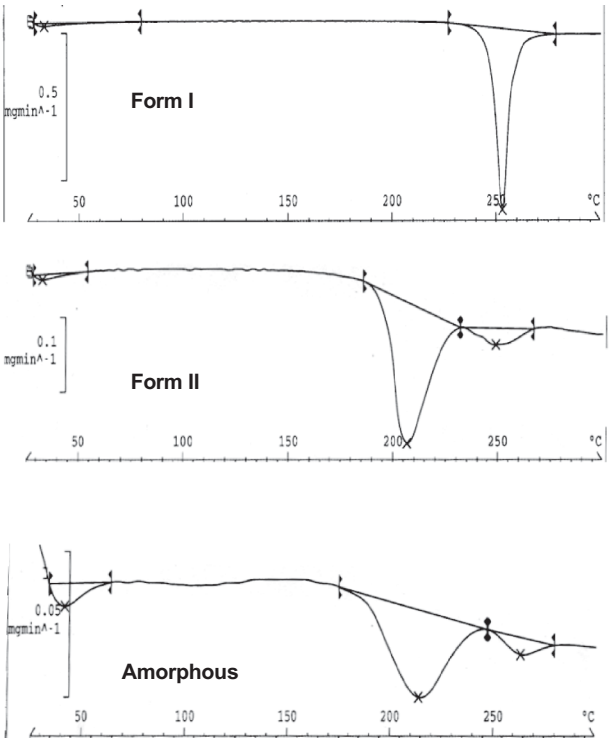


FIGURE 2. Thermogravimetric analysis (TGA) curves for forms I, II, and amorphous rifampicin. Form I shows a single weight loss at 250°C, whereas the amorphous form resembles the form II with respect to weight loss peaks position.

TGA. The comparative TGA curves are shown in Figure 2. It can be seen from the figure that the form I of rifampicin shows a single and rather sharp weight loss phase with a peak at around 250°C and has been reported to decompose on melting. The weight loss above the melting temperature (melting point of form I is reported to be around 230–240°C) supports the said fact (Pelizza et al., 1977). In comparison, form II of rifampicin shows two weight loss phases, the first starts at around 193°C and shows a peak at 204°C, whereas the second peaks at around 246°C. This observation is consistent with an earlier report that form II, with a melting point at 193°C, recrystallizes following melting to form I that then decomposes at around 240°C (Pelizza et al., 1977). Form II, upon melting, not only starts recrystallizing to form I but also starts decomposing, evident from the first weight loss phase. Then recrystallized form I shows the second weight loss phase following its melting. Overall, the consistency of weight loss shows that not all the molten form II degrades or recrystallizes. A fraction keeps on decomposing. It might also be possible that the continued weight loss is because of further decomposition of the degradation product. A precise estimation of the progress of events is difficult because of the simultaneous occurrence of different processes. TGA curve of amorphous form shows resemblance to that of form II although the first weight loss phase starts at a somewhat lesser temperature, at around 170°C. The following behavior is similar to that as described

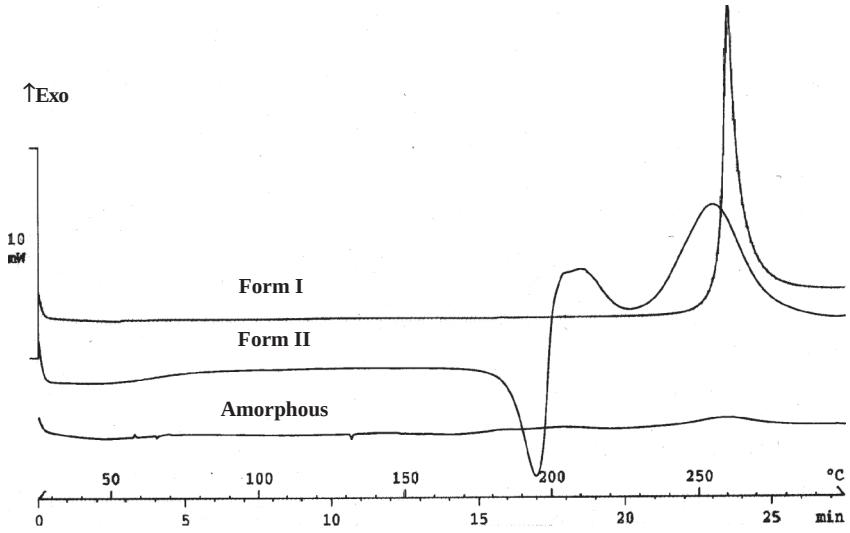


FIGURE 3. Differential scanning calorimetry (DSC) thermograms for forms I, II, and amorphous rifampicin. A single exotherm is shown by form I, whereas the form II shows a melting endotherm and a recrystallization exotherm followed by another exotherm because of decomposition of the recrystallization product. Amorphous thermogram resembles that of form II but has a much lesser intensity so that in comparative figure it appears as a flattened curve.

for form II. Hence, it suggests that even amorphous form after melting undergoes the same sequence of events of decomposition, a simultaneous recrystallization followed by the melting and decomposition of the recrystallized form. The amorphous form resembles form I in FTIR studies and form II in TGA behavior. Thus, both these techniques used together can differentiate amorphous form from either of the crystalline forms.

DSC. DSC thermograms of the polymorphic (forms I and II) and amorphous rifampicin are shown in Figure 3. Form I shows a single exotherm with a peak at 261°C. No melting endotherm is observed, and correlation with TGA weight loss at this temperature range suggests decomposition of the polymorph. On the contrary, form II shows an endotherm starting at 185°C with a peak at 194°C suggesting melting, which is followed by a recrystallization exotherm to some other polymorph, possibly form I. This is overlapped by a decomposition exotherm followed by yet another exotherm starting at 235°C, which might be due to simultaneous melting and decomposition of the recrystallized product. The initial decomposition phase starts with the melting of form II, evident by the TGA weight loss and continues till 222°C, eclipsing the melting endotherm and the following exotherm at least partially.

The enthalpy changes in amorphous form are comparatively weak, but a faint melting endotherm is evident starting around 150°C, which is eclipsed like in form II by a decomposition phase, and is followed by yet another decomposition phase starting at 239°C. These results are consistent with the TGA results discussed in the previous section.

Powder X-Ray Diffraction. Conclusive evidence of generation of amorphous form was provided by powder X-ray diffraction (pXRD). A comparison of spectra of forms I, II, and amorphous rifampicin is shown in Figure 4. While the crystalline forms show characteristic peaks (at 13.65 and 14.35° 2 θ for form I and at 9.93 and 11.10° 2 θ for form II), the amorphous form shows a halo, with no well-defined peaks and a distinctly low intensity as compared with the peaks of the former.

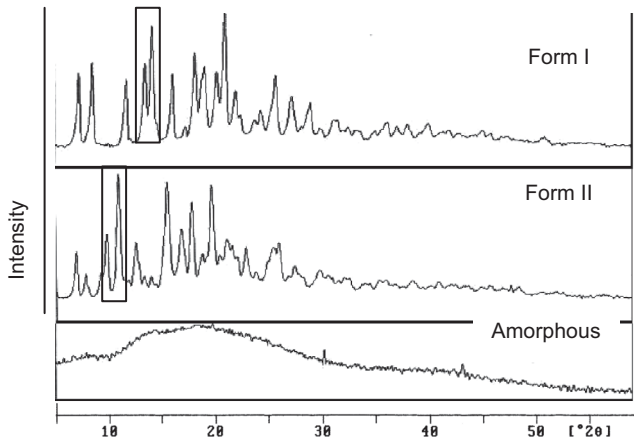


FIGURE 4. Powder X-ray diffraction (pXRD) patterns of forms I, II, and amorphous rifampicin. Although sharp peaks are seen for the crystalline forms, the amorphous form shows a halo, with no peaks. The area shown in boxes highlights the characteristic peaks for forms I and II.

Results of the solid-state characterization studies are summarized in Table 1.

Solid-State Characterization Studies on Powder Mixtures

FTIR

FTIR spectra for the different powder mixtures are shown in Figure 5. A high amorphous content sample shows appearance

TABLE 1
Summary of Solid-State Characterization of Forms I, II, and Amorphous Rifampicin

Sample	DSC[a]			TGA[b]	FTIR (Values in cm^{-1})	XRD
	Endotherm	Exotherm 1	Exotherm 2			
Form I	–	–	257–265 (154.66)	Single-weightloss phase at 244–256 (15.4%)	Single peak at 1,724	Sharp form I peaks
Form II	185–198 (–36.11)	198–222 (51.04)	234–273 (98.89)	Two-weight loss phases at 194–223 (6.22%) and 236–262 (0.75%)	Double peak at 1,735 and 1,718	Sharp form II peaks
Amorphous	186–193 (–0.66)	193–216 (9.92)	238–277 (64.12)	Two-weight loss phases at 186–234 (6.44%) and 244–276 (0.69%)	Single peak at 1,722	Halo

XRD, X-ray diffraction.

The amorphous form resembles form II in thermogravimetric analysis (TGA) behavior and form I in Fourier transformed infrared spectrometry (FTIR) spectrum. Thus, a combination of the two techniques can be used to differentiate the amorphous form either of the two crystalline forms. In differential scanning calorimetry (DSC), the form I does not show a melting endotherm like form II and amorphous. Form I shows a single exotherm compared to the two shown by form II and amorphous. The crystalline forms show sharp characteristic peaks, whereas the amorphous form shows a diffuse pattern.

[a]Values signify the range of temperature in degrees Celsius over which the transition was observed. The values in the parentheses denote the change in enthalpy in J/g.

[b]Values signify the range of temperature in degrees Celsius over which the weight loss phase lasted. The values in the parentheses denote the percentage change in weight with respect to the initial sample weight taken.

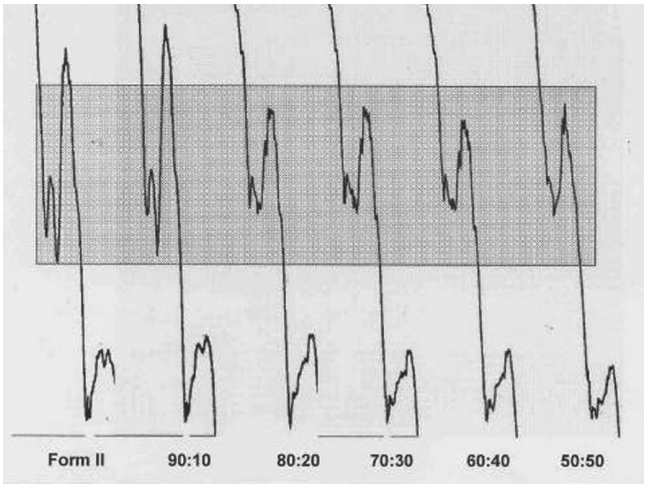


FIGURE 5. Fourier transformed infrared spectrometry (FTIR) spectra of mixtures of form II with the amorphous form in various proportions in the region 1,800–1,600 cm^{-1}. The C-25 acetyl group stretching vibration is highlighted to show the gradual shift from hydrogen-bonded state in form II to non-hydrogen-bonded state of amorphous form. The ratio of form II to amorphous (wt/wt) is shown at the bottom of each spectrum.

of a single peak, though rather broad and stunted. Although the FTIR study could not conclusively ascertain the presence of amorphous regions in the sample, the mixtures show a definitive shift of acetyl group behavior from the hydrogen-bonded (high crystalline content) to the non-hydrogen-bonded (higher amorphous content) state.

DSC

The DSC thermograms of the powder mixtures are shown in Figure 6A. The integral of the area enclosed by the melting endotherm of form II shows a very good correlation (Figure 6B) with the amorphous content and can be used for estimation of amorphous content as an impurity in polymorph II of rifampicin. However, the method might not be suitable for estimating small amounts of amorphous form in the crystalline bulk.

Dissolution Studies

Dissolution Using Paddle Method (USP Apparatus II)

Rifampicin is a hydrophobic drug showing problems in wetting, which leads to formation of lumps in the dissolution media. The powdered drug either settled at the bottom or floated on the surface of the dissolution medium or was stuck to the walls of the dissolution vessel. The results did not

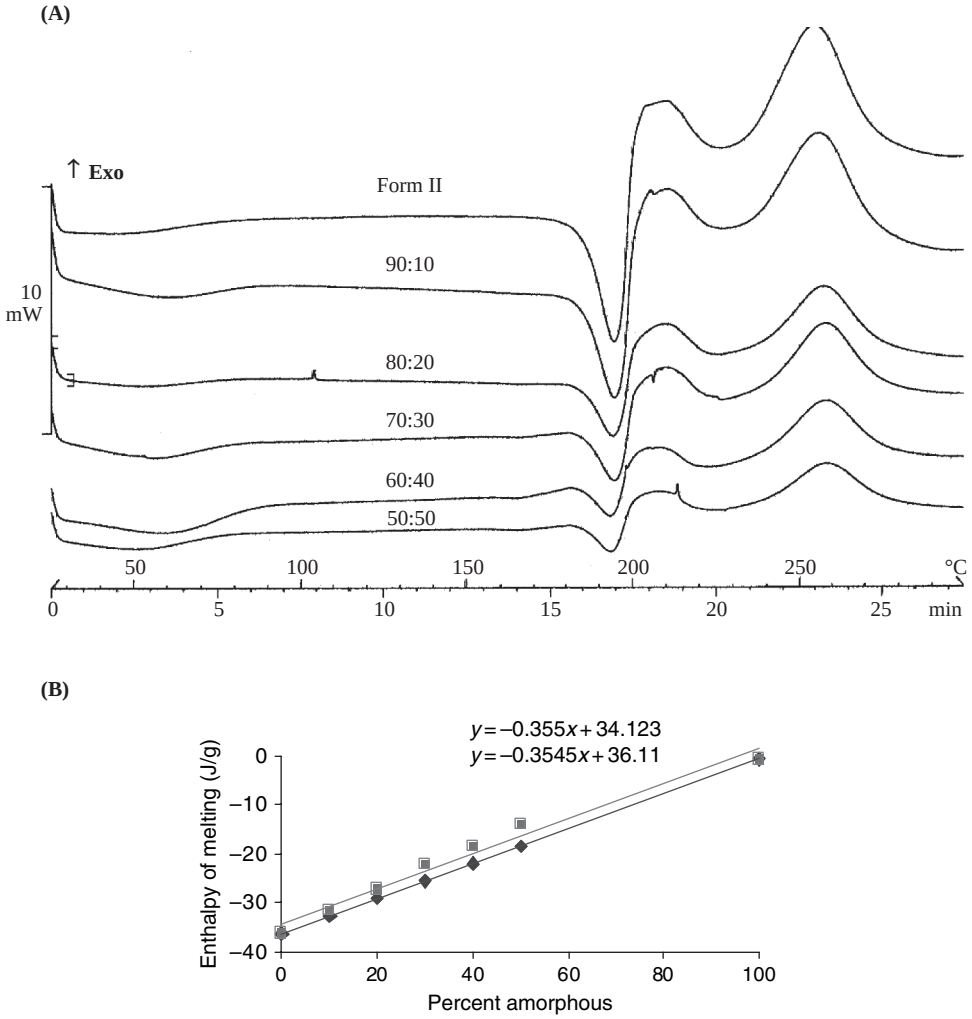


FIGURE 6. (A) Differential scanning calorimetry (DSC) thermograms for powder mixtures. The area under the melting endotherm decreases as the amorphous content in the mixtures with form II increases. The figures represent the wt/wt ratio of form II and amorphous. (B) Theoretical (shown in blue) and actual (shown in red) plots of enthalpy of melting of form II versus amorphous content in powder mixtures. Theoretical curve was generated based on normalized enthalpies of pure crystalline and amorphous forms.

improve even on dispersion with glass beads, signifying the severity of the problem of wetting. Moreover, it was observed that the powder aggregates form a water-impervious layer on the surface, which altogether prevents entry of dissolution medium across this layer. Even after being submerged for more than an hour in the dissolution medium, dry powder was recovered from the lumps formed at the bottom of the dissolution vessels.

Rifampicin degrades in both acidic as well as alkaline solutions (Gallo & Radaelli, 1976). Amount of rifampicin dissolved over 60 min in phosphate buffer (pH 6.8) and 45 min in 0.01 N HCl is shown in Figure 7. In phosphate buffer, less than 50% of the drug was dissolved at the end of study time of 60 min. No particular trend was evident from the dissolution, with some mixtures showing dissolution lesser than form II and others between form II and amorphous. Although some difference was seen, it was so small that it cannot prove a solubility advantage of amorphous form over form II. These small differences could have arisen because of the freeze-dried nature of the former as the particle size and particle size distribution play an important role in powder dissolution and freeze-dried forms show a faster dissolution. In 0.01 N HCl, form II and pure amorphous rifampicin showed similar dissolution. The dissolution of mixtures differed only during the initial stages, with the total amounts in solution approximating each other after 30 min (Figure 7). Thus, the powder mixtures failed to show any significant trend in dissolution at both these physiologically relevant pH media. No direct correlation was observed between the amorphous content and the dissolution profiles, which is attributed to variability of the wetting of powder.

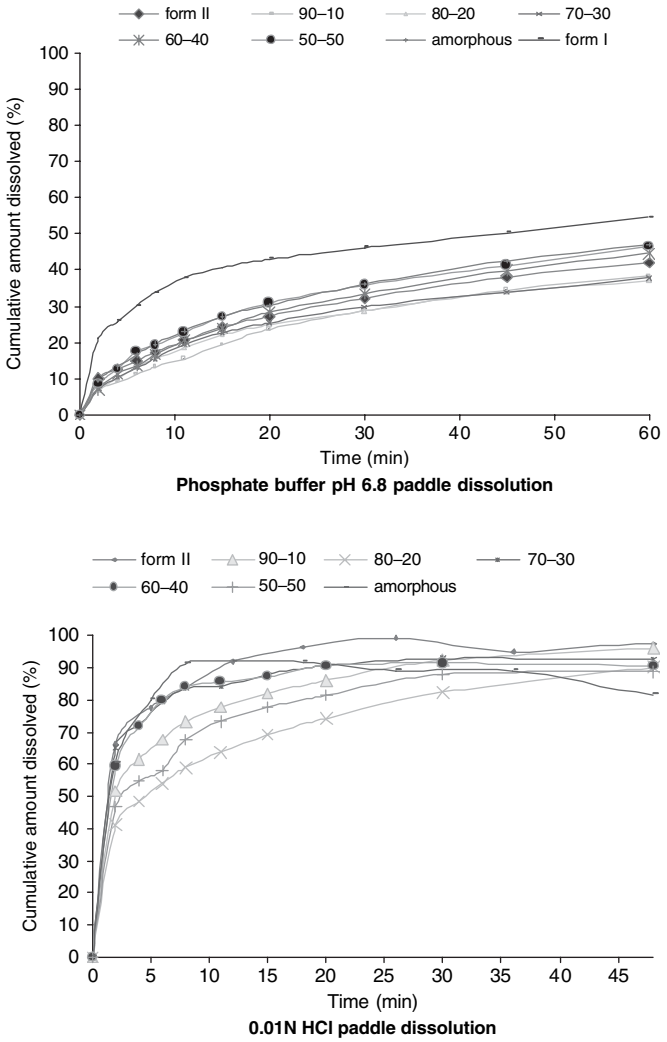


FIGURE 7. Paddle dissolution results of powder mixtures of varying proportions of form II and amorphous form of rifampicin in phosphate buffer (pH 6.8) and 0.01 N HCl.

While dispersing the powders with glass beads in the dissolution vessels before exposure to dissolution medium, it was observed that because of high surface charge of amorphous form, it completely coated the glass beads, whereas the crystalline form covered the beads only partially with the larger particle fractions staying away.

Another factor that might be playing a role is the difference in the density of pure crystalline and the freeze-dried amorphous form. The amount of glass beads required could have been different depending on the density of the blend. Although the amorphous form had a higher bulk volume (bulk densities of freeze-dried amorphous forms I and II were 0.12 and 0.70 g/mL, respectively), it had a higher sticking affinity toward the glass beads, and hence a constant amount (wt/wt) of glass beads (5 g for 150 mg of drug) was used. There is a possibility that the mixtures could have been separated, to different degrees, in attempts to disperse them using glass beads, because of the amorphous form preferentially sticking and coating the beads. These opposing factors, which could not be controlled, balanced each other out for pure crystalline and amorphous forms to give equivalent dissolution profiles but developed some disparity in the mixtures resulting in variable dissolution.

The results of this study also point toward the difficulties associated with this method for studying powder dissolution. This methodology can be applied for such a study only after all the factors influencing the dissolution of powders in a poorly wetting medium are completely understood and controlled. In light of the above discussion, the methods currently being used to study powder dissolution cannot be used for poorly wettable drugs. Therefore, there is a need for development of a methodology that can be applied for studying the dissolution behavior of such poorly wettable drugs or polymorphic forms of drugs falling in class II of BCS as solubility-borderline cases. The conclusion drawn is that the amorphous form does not affect the dissolution of form II, and wetting of the powder sample needs to be ensured for dissolution studies on powders.

IDR Studies

Keeping the surface area constant by compressing the drug sample into pellet form and exposing only this surface area to dissolution medium by means of IDR assembly and by maintaining the sink conditions, intrinsic dissolution properties can be found out, ruling out the influence of particle size. Dissolution can serve as a surrogate marker for bioavailability of rifampicin from alone or fixed dose combination formulations (Agrawal & Panchagnula, 2004). Phosphate buffer (pH 6.8) and 0.01 N HCl are the two media recommended for this purpose and were used to study the dissolution behavior of form II and amorphous rifampicin. The results from this study are summarized in Figure 8.

An interesting finding during these studies was that as the amorphous form was compressed under a pressure of 500 psi, it darkened to a reddish-black color. The FTIR spectrum of the blackened amorphous rifampicin showed no significant difference from the uncompressed sample, implying that the compound might not have decomposed, which has been further confirmed by DSC and high-performance liquid chromatography (HPLC). This blackening of the pellet was also seen for the mixtures containing amorphous form. However, this observation did not affect the dissolution profile in these two media.

To conclude the results of the IDR study, it can be said that in both 0.01 N HCl and phosphate buffer (pH 6.8), there is no significant effect of amorphous content on the dissolution characteristics of form II. These results clearly indicate that amorphous content has no influence on dissolution behavior and is in complete contradiction with the previously reported results (Henwood et al., 2000), where the difference is probably because of wetting problem.

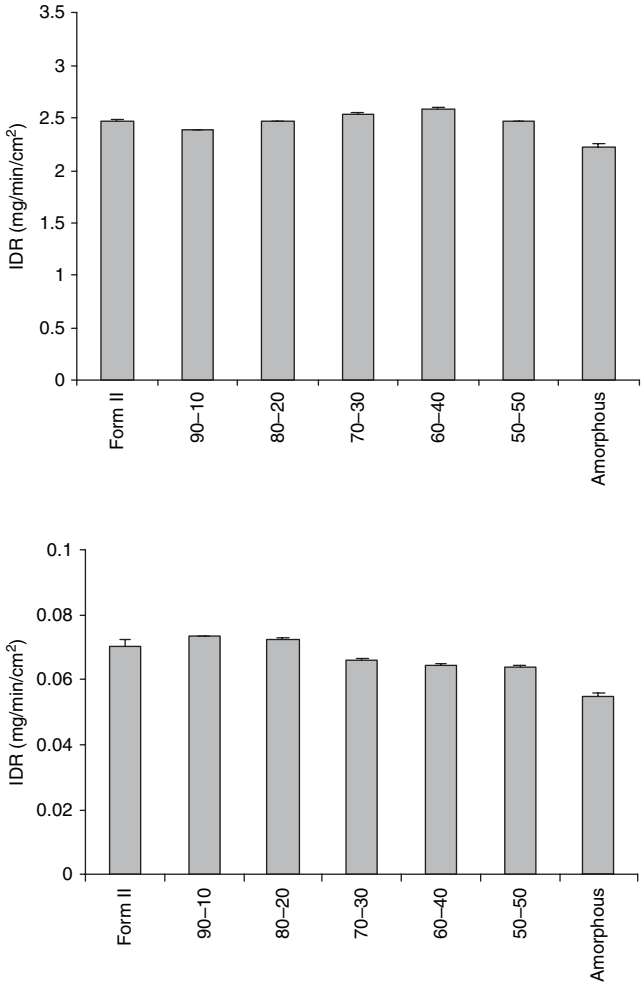


FIGURE 8. Intrinsic dissolution rates (IDRs) of powder mixtures of form II and amorphous rifampicin in 0.01 N HCl (upper panel) and phosphate buffer, pH 6.8 (lower panel).

CONCLUSIONS

No suggestive pattern was evident in the dissolution of powder mixtures of rifampicin containing varying proportions of amorphous form in phosphate buffer pH 6.8 and 0.01 N HCl. Furthermore, the study highlights the problems associated with carrying out dissolution of powders, especially of hydrophobic and poorly wettable drugs in the paddle apparatus. Neither the FTIR nor the TGA alone could, but together, both these techniques complemented each other to differentiate amorphous from either of the two crystalline forms of rifampicin. The DSC thermograms showed a good correlation between the enthalpy of melting and the proportion of form II in powder mixtures that can be used for quantification of the amorphous form when present as an impurity in large proportions in crystalline bulk. The results of the study highlight the fact that amorphous content is inconsequential in the dissolution of form II of rifampicin and therefore the possibility of it playing a role in the variable bioavailability of the drug from different marketed preparations can be safely ruled out.

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