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Raman microspectroscopic mapping or thermal system used to investigate milling-induced solid-state conversion of famotidine polymorphs

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Confocal Raman microspectroscopy was used to nondestructively determine the polymorphic conversion of famotidine in the course of the milling process. A mapping system was applied to assess the blending uniformity of each polymorphic component in the milled mixture. Raman microspectroscopy combined with a thermal analyzer was also used to investigate the synergistic co-effects of milling and heating on the polymorphic conversion of famotidine polymorphs. Famotidine has two polymorphs, forms A and B, the raw material of famotidine used was proved to be of form B. The Raman peak intensity ratio of the 2920 cm^{-1} band for form A and 2897 cm^{-1} band for form B was used to act as an indicator to evaluate the polymorphic conversion of famotidine form B to form A after different milling courses. The results indicate that the peak intensity at 2897 cm^{-1} gradually decreased with the milling time, whereas the peak intensity at 2920 cm^{-1} slowly enlarged, suggesting the polymorphic conversion of famotidine from form B to form A. The longer milling process might strongly induce and promote this polymorphic conversion of famotidine. Both polymorphic forms of famotidine were found to be well uniformly distributed within the milled samples due to their smaller varieties by using the Raman microscopic mapping system. The temperature effect could synergistically accelerate the polymorphic conversion of famotidine from form B to form A in the milled sample. The thermal-dependent critical temperature for sharply enhancing the content of famotidine form A in each milled sample was also identified. Copyright © 2007 John Wiley & Sons, Ltd.

KEYWORDS: famotidine; polymorphic transformation; milling time; confocal Raman spectroscopy; mapping; thermal analyzer

INTRODUCTION

A lot of pharmaceuticals have been reported to possess more than one polymorphic form in the solid state.[1,2] Different pharmaceutical solid polymorphs can significantly influence the physico-chemical properties, formulation development and manufacturing production of drugs, leading to the changes in dissolution, stability and bioavailability of drug substance.[3,4] Selecting a stable polymorph of drug can ensure the stability and efficacy of a final pharmaceutical product. The most stable polymorph is preferred to include into a marketed formulation to prevent the polymorphic transformation during delivery or storage. The polymorphic transformation of several drugs has been found to cause the problem of bioavailability,[3,5,6] thus stringent regulatory requirement of FDA application has been imposed to identify and specify the polymorphs for particular drug materials as part of the quality assurance process. Generally, it is necessary to ensure and keep the stable polymorph in the final drug products to go to market.

Famotidine is a representative third-generation of a histamine H_2-receptor antagonist, which is commonly used to treat the stomach and duodenal ulcers.[7] Famotidine has two polymorphs (forms A and B) that differed by the arrangement of intra/intermolecular hydrogen bonds.[8–11] FDA has convinced that famotidine with poor solubility was one of the generic drug products having different polymorphic forms.[2,3] Although the characteristics and crystal structure of these two famotidine polymorphs have been investigated,[8–10] the effect of pharmaceutical manufacturing process on the polymorphic transformation of famotidine was scanty studied.[12] The milling process as a particle size reduction technique is well known to be one

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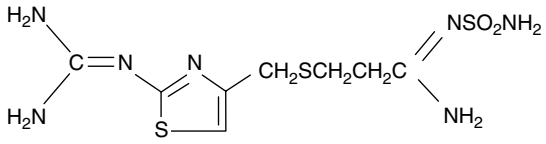
of the possible manufacturing procedures in pharmaceutical industry.[13] The energetic input of solid-state milling has been reported not only to alter the particle size and surface area of the drug but also to induce a variety of solid-state conversion of drug, including crystalline to amorphous, amorphous to crystalline and polymorphic transformation, resulting in the modification of physical and chemical properties of drugs[14,15] and the influence on bioavailability of a drug through the rate of dissolution.[16]



Although the grinding effect on the polymorphic transformation of famotidine has been reported in our previous study by using Fourier transform infrared (FT-IR) microspectroscopy,[12] the compression effect (using KBr pellets) may possibly further cause polymorphic conversion of drugs during sample preparation.[17,18] Thus the FT-IR technique seems not an optimal method to examine the polymorphic transformation of drug, particularly for the pressure-sensitive drug compounds.[19,20] Recently, Raman spectroscopy has been widely recommended to nondestructively investigate the changes in drug polymorphism at different conditions.[21,22] In addition, the combination of Raman spectroscopy with optical microscopy can further extend the capability of Raman technique to study the minute material in the heterogeneous systems. Thus Raman microspectroscopy is a highly suitable method for the examination of drug polymorphs because it is lack of sample preparation to reduce or eliminate the risk of polymorphic modification during sample preparation, as compared with FT-IR analytical technique.[23,24] Moreover, the easy and fast detection of Raman microspectroscopy enable rapid screening of correct polymorph in drug development or in quality control of drug products.[25,26] However, the single spectrum from one sampling point of Raman spectroscopic determination cannot essentially reflect the spatial distribution of the polymorphic components in the mixture. Thus in this study, confocal Raman microspectroscopy was used instead of FT-IR microspectroscopy to explore the milling effect on the polymorphic conversion of famotidine. A mapping technique was applied to assess the blending uniformity of each spatially resolved polymorphic component in the milled mixtures. Moreover, the milling process-induced solid-state polymorphic conversion of famotidine during milling was also investigated by using confocal Raman microspectroscopy combined with thermal analyzer.

EXPERIMENTAL

Materials

Famotidine was of pharmaceutical grade and purchased from China Chem. Synthesis Ind. Co., Ltd (Shu-Lin, Taipei, Taiwan, ROC). The organic solvents were of analytical reagent grade (Nacalai Tesque, Kyoto, Japan).

Preparation of two polymorphs of famotidine

According to the reports of Hassan *et al*.,[9] and Lin *et al*.,[12] forms A and B of famotidine were prepared by recrystallization from acetonitrile and methanol, respectively. The raw material of famotidine used in this study was proved to be a metastable form B.[12]

Preparation of various milled samples

A certain amount of famotidine form B was respectively milled for different times (ranging from 5–30 min) in an oscillatory ball mill (Mixer Mill MM301, Retsch GmbH & Co., Germany) at 15 Hz. A 0.2 g powder sample was placed in a 25 ml volume stainless steel milling jar containing two 12 mm diameter stainless steel balls.

Confocal Raman microspectroscopic mapping study

Each famotidine sample with or without milling was flatten on the glass slide and then determined by a confocal micro-Raman spectrophotometer (Ventuno, Jasco Co., Tokyo, Japan) equipped with a 30 mW green (532 nm) solid-state laser as an excitation source.[27] An appropriate sample area was selected, and the Raman spectra were collected successively from the actual analysis area by a mapping process. An automated X–Y stage for mapping was employed in order to obtain full Raman spectra from the sample. The spectra manager software for instrument control and data handling was used. The Raman peak intensity ratio of 2920 cm^{-1} for form A and 2897 cm^{-1} for form B was applied to act as an indicator to evaluate the polymorphic conversion of famotidine polymorphs after different milling courses.

Confocal Raman microspectroscopic study combined with thermal analyzer

Each famotidine form B, 10 min-, 20 min- or 30 min-milled sample was directly placed onto a temperature controlled microscope cell (THMS 600, Linkam Scientific Instruments Ltd, Surrey, UK), respectively. This hot–cold cell was then set in a confocal micro-Raman spectrometer. The laser beam was directly focused on each sample by an Olympus long-working-length objective. The cell temperature was controlled by a temperature controller (TMS 94, Linkam Scientific Instruments Ltd, Surrey, UK). The heating rate of the controller assembly was maintained at 10 °C/min under ambient conditions. At each prescribed temperature, the sample was isothermally maintained for 5 min before recording its Raman spectra.

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Statistical analysis of the distribution variability of milled samples

The blend uniformity of the milled samples was estimated from the mapping spectra by evaluating the consistency of the Raman peak intensity ratio of 2920/2897 cm^{-1}. Spectral manager software for windows (Jasco Co., Japan) was used. The mean and standard deviation (SD) of the above Raman peak intensity ratio for each sample were calculated.

RESULTS AND DISCUSSION

Identification of famotidine forms B and A in the milled sample

The Raman spectra of both intact polymorphic forms A and B of famotidine are shown in Fig. 1. It is evident that the characteristic Raman bands for famotidine form B were indicated at 3406 and 3239 (NH stretching), 3105 (CH stretching of heterocyclic ring), 2938 and 2911 (CH asymmetric stretching) and 2897 (CH symmetric stretching) cm^{-1}, while the Raman spectra for famotidine form A were located at 3455 and 3247 (NH stretching), 3100 (CH stretching of heterocyclic ring), 2934 and 2920 (CH asymmetric stretching) cm^{-1}, respectively.[28] It clearly reveals that famotidine form B had several unique bands at 3406, 3377, 2978, 2911, 2897 cm^{-1} and exhibited markedly different from that of famotidine form A at 3455, 3432, 3324, 2997, 2966 and 2920 cm^{-1}. Since the unique Raman bands at 3455 and 2920 cm^{-1} for form A as well as 3406 and 2897 cm^{-1} for form B did not interfere with each other, and both peak intensities at 2920 and 2897 cm^{-1} were also more sharper than others, thus we selected both unique peaks as an indicator. This suggests that it is possibly to distinguish the Raman spectral difference from the milled mixture by determining the peak intensity ratio of the Raman bands of polymorphic form A (2920 cm^{-1}) and form B (2897 cm^{-1}) of famotidine.

Since the raw material of famotidine used in this study was proved to be a form B, the Raman spectral change of this form B was investigated when it was milled with time. Obviously, several unique Raman bands of form A at 3455, 3422, 2997 and 2920 cm^{-1} were clearly observed in the 20 min- and 30 min-milled samples. In particular, the unique indicator at 2920 cm^{-1} assigned to form A was enhanced its peak intensity with milling time whereas the peak intensity at 2897 cm^{-1} due to form B's indicator was dropped off. This strongly evidences that the milling process might induce the polymorphic conversion of famotidine from form B to form A.

Milling-dependent polymorphic transformation of famotidine determined by Raman microspectroscopic mapping system

In order to investigate the blend homogeneity of both polymorphic forms of famotidine in the milled sample, the confocal Raman microscopic mapping system was applied. Figure 2 shows one representative Raman spectrum of famotidine form B and 30 min-milled sample. Its Raman spectral maps after applying the spectral mapping system are also displayed. Obviously, the three-dimensional Raman spectral plots of both samples exhibited an even contour map. This clearly implies that the blend homogeneity of the milled sample was kept to a uniform manner even if the milling process was performed for 30 min. As an indicator, the variety of Raman peak intensity ratio of 2920 cm^{-1} (form A)/2897 cm^{-1} (form B) was calculated. Figure 3 illustrates the relationship between the Raman peak intensity ratio of 2920/2897 cm^{-1} and milling time. It is evident that the famotidine form B, 10 min- and 20 min-milled samples almost displayed a constant Raman peak intensity ratio after randomly determination of 49 sampling spots, suggesting that both polymorphic famotidine forms were well uniformly distributed within the milled samples. However, the less blend homogeneity was also obtained for the 30 min-milled sample by their longer milling time. The more the milling time performed, the higher the Raman peak intensity ratio obtained. This reveals that the content of famotidine form A was increased with the milling time, indicating the successive promotion of polymorphic conversion of famotidine from form B to form A.

The effect of milling time on the changes of Raman spectra for all the milled samples is displayed in Fig. 4(A). Here, each representative Raman spectrum was the averaged spectrum ($n = 49$) from Fig. 3 by using spectral manager software for windows. The mean values and SD of Raman peak intensity

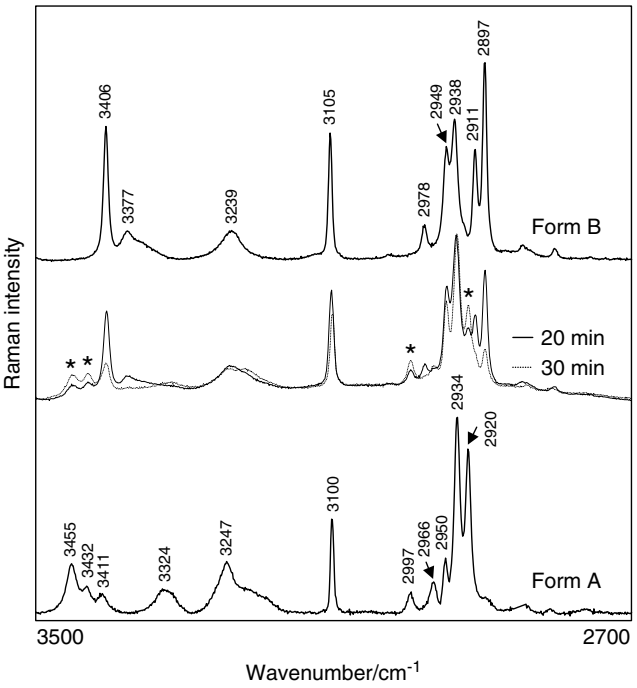

Figure 1. Raman spectra of polymorphic forms A and B of famotidine, as well as the milled samples of intact famotidine form B after milling for 20 or 30 min.

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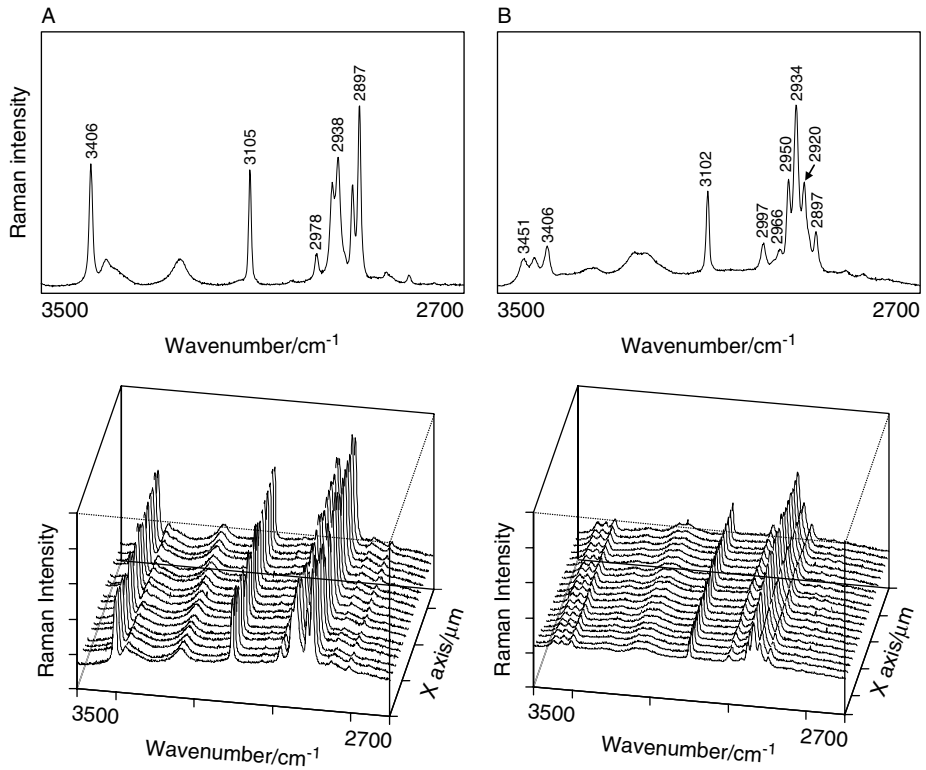

Figure 2. The representative Raman spectrum of famotidine form B (A) and 30 min-milled sample (B) and its three-dimensional Raman spectral maps. Key: *: indicates the formation of famotidine form A.

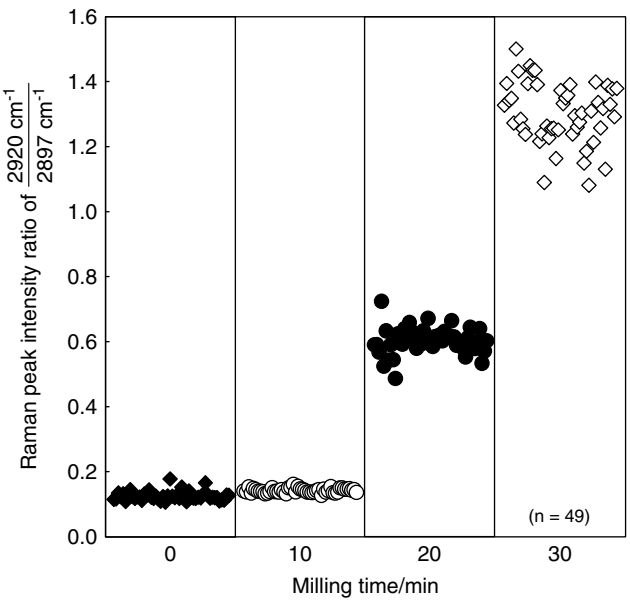

Figure 3. The relationship between the Raman peak intensity ratio of 2920/2897 cm^{-1} and milling time. Key: Total sampling points: 49.

ratio of 2920/2897 cm^{-1} with milling time are also shown in Fig. 4(B). Obviously, a unique Raman band at 2897 cm^{-1} for form B gradually reduced its peak intensity with the increase milling time, whereas the peak intensity of Raman band at 2920 cm^{-1} for form A slowly enhanced. Moreover, the value of the Raman peak intensity ratio of 2920/2897 cm^{-1} was only slightly increased before 15 min-milling time. B eyond a 15 min-milling course, however, the value of this Raman peak intensity ratio was abruptly increased. This indicates that the longer milling process might strongly induce and promote the polymorphic conversion of famotidine from form B to form A. However, the present result was somewhat different from our previous study.[12] In this study, the polymorphic change of the milling-dependent Raman peak intensity ratio was an upward curve, but the grinding-related IR peak intensity ratio in the previous study was a linear plot. The marked difference between both results might be due to that the peak at 2920 cm^{-1} in the Raman spectrum was not completely resolved its neighboring peaks and led to less detectable with small form A concentrations. Moreover, the ball milling used in the present study differed from the hand milling in the previous study might be also responsible for this reason.

Thermal effect accelerated the polymorphic transformation of famotidine from form B to form A in the milled mixture

The polymorphic transformation of many drug compounds have been reported to be easily caused by several factors such

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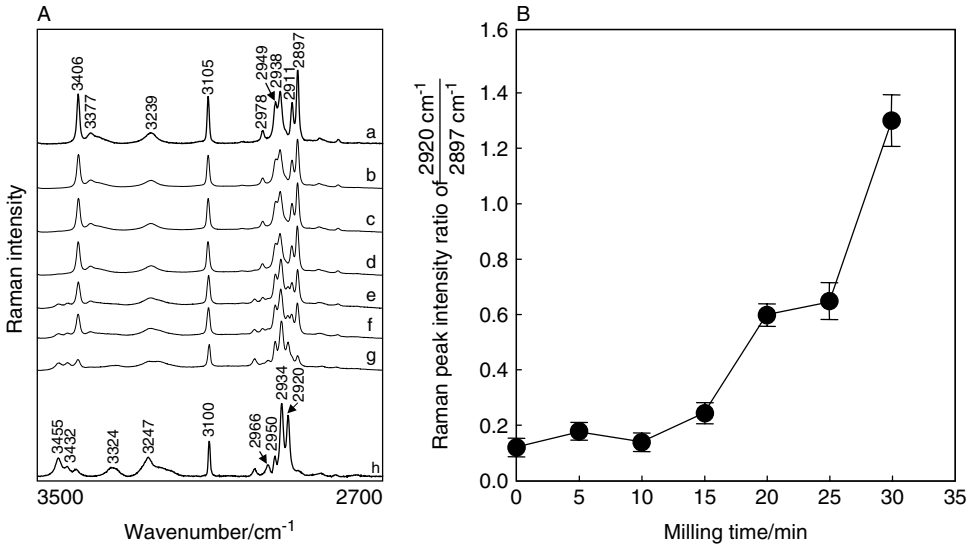

Figure 4. (A)Milling time-dependent changes in the averaged Raman spectra and (B) the averaged Raman peak intensity ratio of 2920/2897 cm^{-1} for all famotidine milled samples. Key: intact famotidine form B before milling (a), famotidine form B after milling for 5 min (b), 10 min (c), 15 min (d), 20 min (e), 25 min (f) and 30 min (g), intact famotidine form A (h).

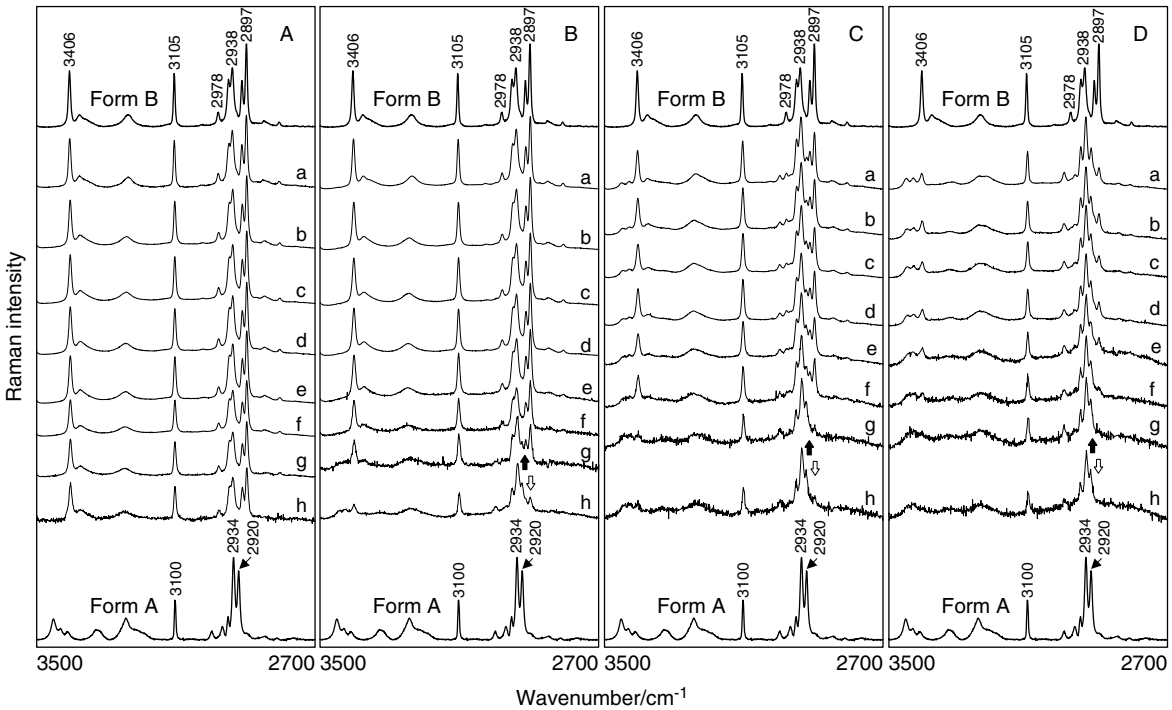

Figure 5. Temperature-dependent Raman spectral changes of intact famotidine form B and different milled samples. Key: Intact famotidine form B before milling (A), famotidine form B after milling for 10 min (B), 20 min (C) and 30 min (D). Heating temperatures: a, 30 °C; b, 90 °C; c, 100 °C; d, 110 °C; e, 120 °C; f, 130 °C; g, 140 °C; h, 150 °C.

as pressure, heat, solvent, excipient added and manufacturing methods.[3,4,25,29–31] The fact that two factors co-affecting the polymorphic conversion of drug are always occurred in the practical manufacturing operation. For example, the milled sample may be dried to remove the residual water. Here, the confocal Raman microspectroscopy with thermal analyzer was combined to simultaneously investigate the thermally milling-induced polymorphic conversion of famotidine.

Figure 5 shows the temperature-dependent Raman spectral changes of the famotidine form B, 10 , 20 and 30 min-milled samples. It is clearly evident that the Raman spectra of

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famotidine form B without milling did not alter the spectrum even heating to 150 °C (Fig. 5(A)). There was no evidence for the thermal-induced polymorphic conversion of famotidine form B even heating to higher temperature, strongly suggesting that the famotidine form B was independent of thermal effect. On the other hand, a thermal-dependent Raman spectral change of the Raman spectra for all the milled samples was clearly observed (Fig. 5(B–D)). All the milled samples exhibited a polymorphic conversion behavior of famotidine from form B to form A. By increasing the temperature of each milled sample, the Raman peak intensity at 2920 cm^{-1} of each milled sample was more enhanced accompanied with the reduction of the Raman peak intensity at 2897 cm^{-1}. This indicates that the thermal effect could more accelerate the original polymorphic conversion of famotidine from form B to form A.

The synergistic co-effects of milling and heating on the polymorphic conversion of famotidine from form B to form A are displayed in Fig. 6. Obviously, the Raman peak intensity ratio of the 2920/2897 cm^{-1} band almost maintained a constant level for the famotidine form B even heating to higher temperature, indicating there was no polymorphic conversion. However, the above Raman peak intensity ratios for the milled samples exhibited a different behavior. The Raman peak intensity ratio of 2920/2897 cm^{-1} slightly increased its value with the temperature for both 10 min- and 20 min-milled samples, but the ratio values sharply enhanced beyond 130 °C for 10 min-milled sample or 110 °C for 20 min-milled sample, respectively. The temperature at 110 or 130 °C was the critical point to accelerate the polymorphic conversion of famotidine from form B to form A. Although the critical temperature at 110 °C was also found for the 30 min-milled sample, its ratio values in the temperature range of 30–110 °C were more increasingly ascended with the temperature, as compared with that of the constant level for 10 min- or 20 min-milled sample in the same temperature range. This strongly demonstrates that the thermal effect could synergistically accelerate the polymorphic conversion of famotidine from form B to form A in the milled sample. The accelerated thermal-related polymorphic conversion of famotidine after milling was similar to our previous study.[12] However, a turning temperature point from form B to form A accelerated by thermal effect in the present study was lower than that of the phase transition temperature found in the previous study. This might be due to that the ball milling was more energized than the hand milling.

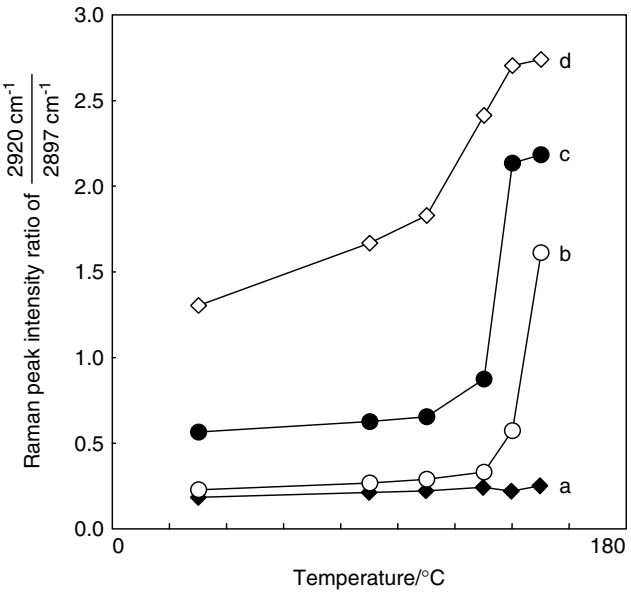

Figure 6. The relationship between the Raman peak intensity ratio of 2920/2897 cm^{-1} and heating temperature. Key: Intact famotidine form B before milling (a), famotidine form B after milling for 10 min (b), 20 min (c) and 30 min (d).

CONCLUSIONS

Polymorphic conversion of famotidine from form B to form A in the milling process was easily evidenced by nondestructive confocal Raman microspectroscopy. The longer milling process might strongly induce and promote this polymorphic conversion of famotidine. Both polymorphic forms of famotidine were good uniformly distributed within the milled samples by determination with Raman microspectroscopic mapping system. The synergistic co-effects of milling and heating on the polymorphic conversion of famotidine from form B to form A were also found by using Raman microspectroscopy combined with a thermal system. The thermal-dependent critical temperature for sharply enhancing the content of famotidine form A in the milled sample was also determined as follows: 130 °C for 10 min-, 110 °C for 20 min- and 30 min-milled samples, respectively.

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