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The use of near-infrared spectroscopy to monitor the mobility of water within the sarafloxacin crystal lattice

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Abstract

Near-infrared spectroscopy (NIRS) is proposed as a technique to study the mobility of water within the sarafloxacin crystal lattice. An investigation of two samples of sarafloxacin revealed that NIRS can distinguish between acceptable and unacceptable batches for formulation purposes. X-ray powder diffraction (XRPD), mid-infrared (mid-IR) spectroscopy, differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) could not detect any differences between the two samples. NIRS detected differences in the location or orientation of the water molecules within the crystal lattices. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Sarafloxacin; Near-infrared spectroscopy; Water mobility

1. Introduction

Escherichia coli is a natural component of the gut microflora of poultry such as chickens and turkeys. A number of serotypes of *E. coli* are pathogenic and are the most common cause of respiratory infection and bacterial disease in these animals.

The fluoroquinolones including sarafloxacin are synthetic broad-spectrum antibiotics which show activity against both gram-positive and gram-negative organisms. Sarafloxacin is currently marketed for the treatment of *E. coli* infections in chickens and turkeys [1] by water medication, i.e. added to water, but not dosed directly to the animals. The antimicrobial spectrum and mode of action for fluoroquinolones has been reviewed in detail [2–5]. Sarafloxacin is administered for veterinary use as an aqueous formulation involving an aqueous granulation process.

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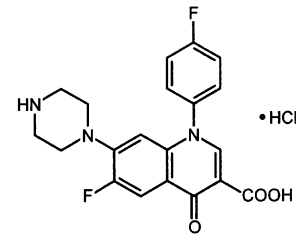
We have found that several of the fluoroquinolones exhibit hygroscopocity similar to sodium cromyln [6] wherein the water contained interstitially in the solid equilibrates with atmospheric humidity.

Sarafloxacin differs somewhat from other fluoroquinolones studied in that it forms multiple



Sarafloxacin

$C_{20}H_{17}F_2N_3O_3 \cdot HCl$

M.W. 421.83

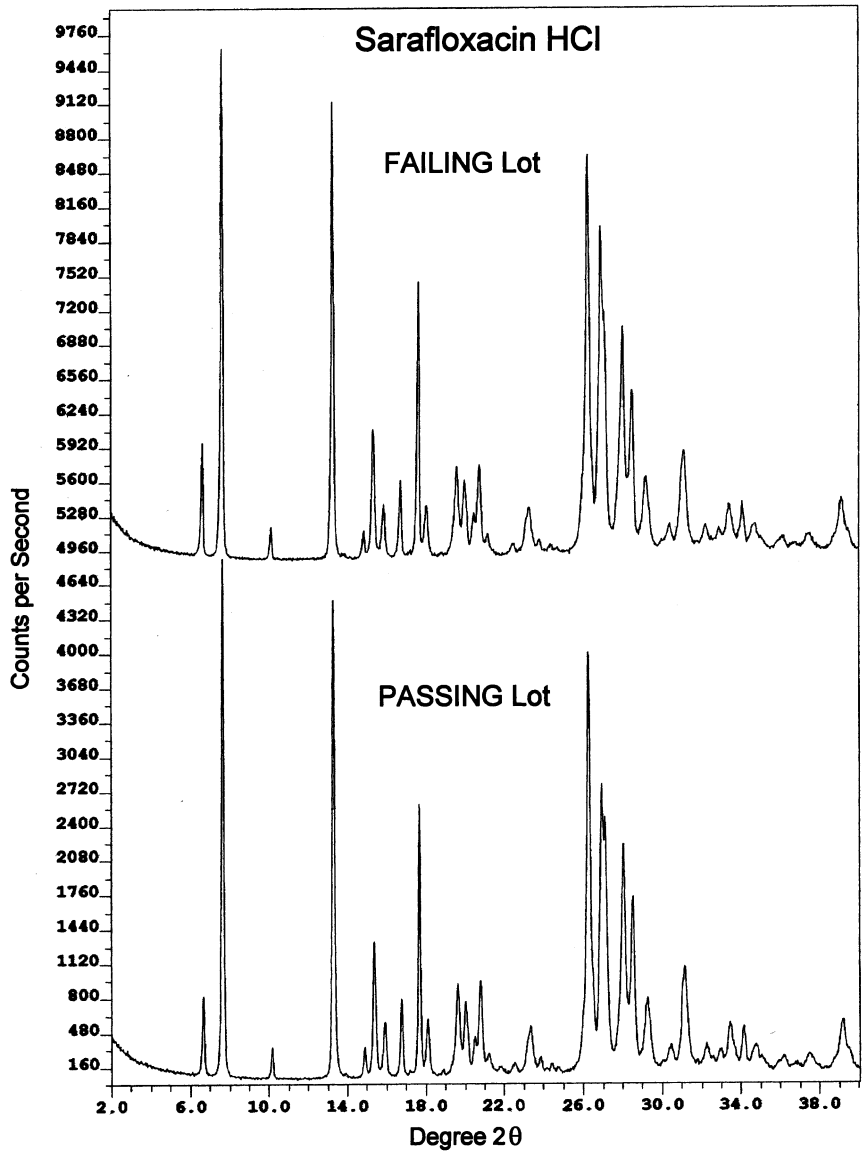


Fig. 1. XRPD diffraction patterns of sarafloxacin lots with passing and failing granulation properties.

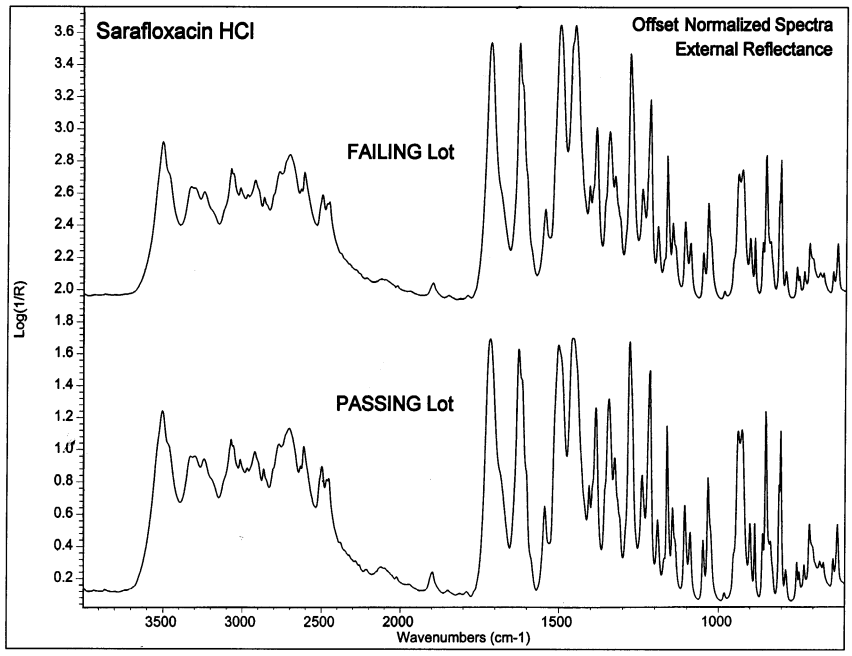


Fig. 2. Mid-IR spectra of sarafloxacin passing and failing lots.

hydrates. Byrn stated that in some cases water molecules may assume positions in the crystal lattice in a regular pattern but with relatively weak interactions and with more of a space filing role. In such situations upon heating or exposure to the atmosphere, water molecules have been observed to move out of the solid [7]. This paper reports near-infrared spectroscopic studies which demonstrate the mobility of water within the crystal as it is absorbed and the subsequent effect on granulation processability.

2. Experimental

Near infrared (NIR) spectra were generated using a Nicolet model 750 spectrometer Magna-IR™ spectrometer with a CaF_2 beamsplitter, and with a Nicolet SabIR near-infrared (NIR) diffuse reflectance fiber optic probe accessory equipped with a PbS detector. All NIR spectra were acquired at a resolution of 8 cm^{-1} with either 16 or 64 scans. Bulk material was added into either a clear glass one dram vial or an HPLC autosampler vial, and then placed on the probe tip. The NIR spectrum of the bulk material was an average spectrum of four spectra, each at 16 scans, acquired after manually mixing the sample for about 10–15 s followed by manual tapping to remove air gaps.

Mid-infrared (mid-IR) spectra were obtained using a Nicolet model 750 spectrometer Magna-IR™ spectrometer with a KBr beamsplitter, and with a Spectra-Tech InspectIR video and microanalysis accessory equipped with a liquid nitrogen cooled MCT/A detector. All mid-IR spectra were acquired at a resolution of 4 cm^{-1} with 16 scans in the external reflection mode. Bulk material was placed on an aluminum coated slide, and then manually pressed on the slide with a metal micro spatula. Samples were exposed to ambient atmosphere at all times.

X-ray powder diffraction (XRPD) patterns were obtained using a Scintag model X1 diffraction unit with a copper target (1.54060 Angstroms wavelength radiation: 45 kV and 40 ma), a scan rate of 1° per min continuous; and a scan range of 2–40° 2-theta at room temperature using a Peltier cooled detector tuned for cooper radiation. The X-ray tube used a 2.0 and 1.0° divergence slit. All XRPD samples were ground to a fine powder in a mortar and pestle prior to analysis.

Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) were performed using a TA Instruments Model 3100 Thermal Analyst with model 2910 DSC module and model 2950 Hi Resolution TGA module, respectively. All DSC samples were prepared in uncrimped aluminum pans. Typical DSC sample

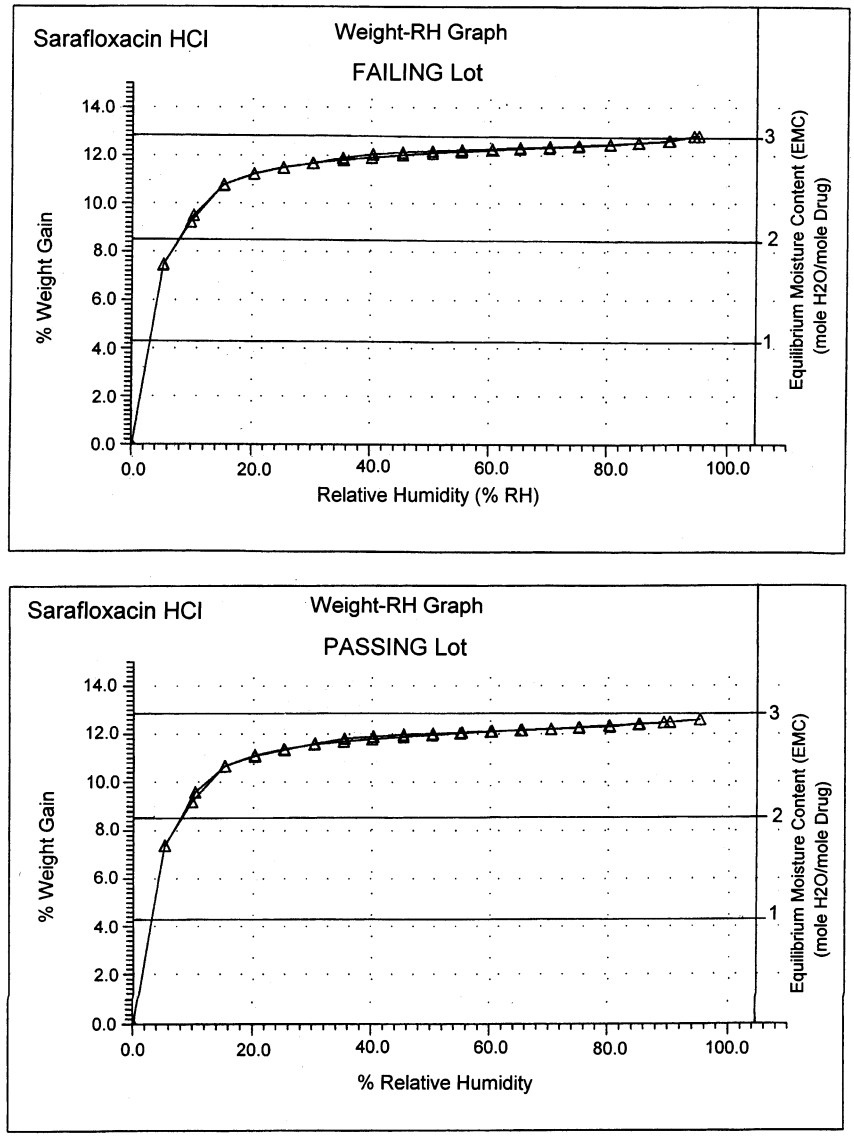


Fig. 3. DMSG sorption/desorption isotherms of sarafloxacin passing and failing lots.

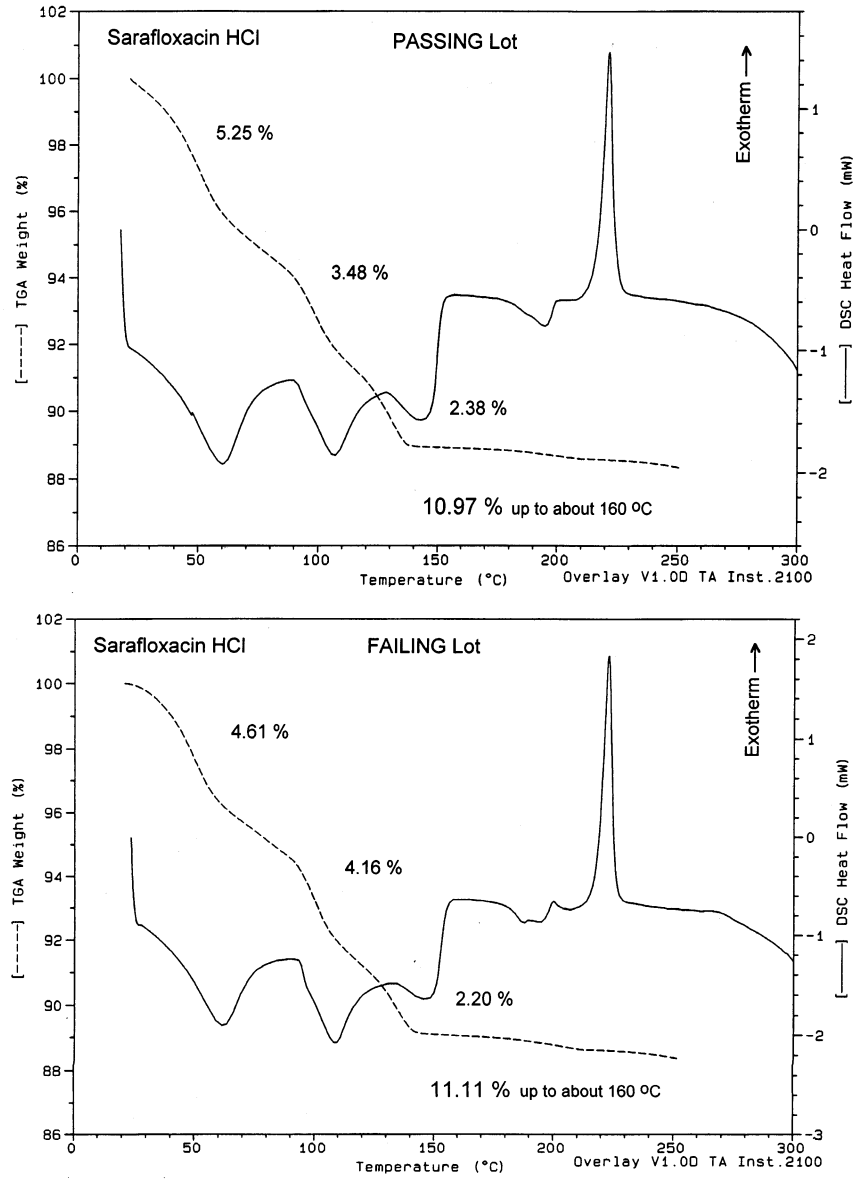


Fig. 4. Overlay of DSC and TGA thermograms of sarafloxacin passing and failing lots.

weights were about 2–5 mg, and typical TGA sample weights were about 15–20 mg. Both the DSC and TGA were performed at 5° per min, under a nitrogen purge with a flow rate of about 40 ml per min.

Dynamic moisture sorption gravimetry (DMSG) was performed on VTI Corp. Model MB300G sorption microbalance using vacuum to control the relative humidity (RH). The automated system controlled the RH and temperature to which the sample was exposed, while continually recording sample weight changes. Sorption and desorption isotherms were performed at 25 ± 0.1°C with 5 ± 1% RH step intervals from 0–95% RH. Samples of about 25–30 mg were dried with a vacuum for up to 180 min (approximate RH =

0–1% RH) before each experiment. The weight loss observed during the drying period was used to estimate how 'tightly' each sample held on to the water. After the drying period, sorption isotherms started at 5% RH. A weight equilibrium criteria of less than 5 μg weight change over three 5 min periods was used to move to the next RH step. When the equilibrium conditions were achieved for the 95% RH step, the desorption isotherm started.

Optical video microscopy was performed using a Nikon Microphot-FXA polarizing light microscope in a differential interference contrast set-up mode at a magnification of 800×. All samples were immersed in mineral oil.

Hot stage microscopy was performed with a Mettler model FP2 with a FP1 controller hot stage and a Nikon Microphot-FXA polarizing light microscope at 100×. Samples were immersed in silicon oil and heated at a rate of 2° per min.

Karl Fischer titrimetry was performed with a Metrohm model 701 KF Titrino titrimeter and Metrohm model 703 Ti stand using Hydranal-Composite 5 as Karl Fischer reagent, and analyzed in methanol. Typical sample weight was about 400 mg.

Samples of bulk sarafloxacin were supplied by Abbott Laboratories (passing lot) and CHEMO (failing lot). Both lots had greater than 98% purity, as determined by high performance liquid chromatography (HPLC).

Sarafloxacin drug product was manufactured using a wet granulation process in a low intensity masser. The wet mass was dried and sized into a granular form. The active ingredient, sarafloxacin hydrochloride, along with the other powder ingredients, such as edetate sodium dihydrate, sodium carbonate monohydrate, and povidone (binder) were mixed and then granulated using granulating fluid (water and dye). Typically, granules start to form rather quickly as the granulating fluid is slowly dispersed throughout the powder mass.

Two lots of sarafloxacin were evaluated to investigate the use of an alternate supplier for sarafloxacin HCl. These two lots of sarafloxacin were evaluated in a product development pilot plant facility using a sample of current material as a control (passing lot). The failing lot of

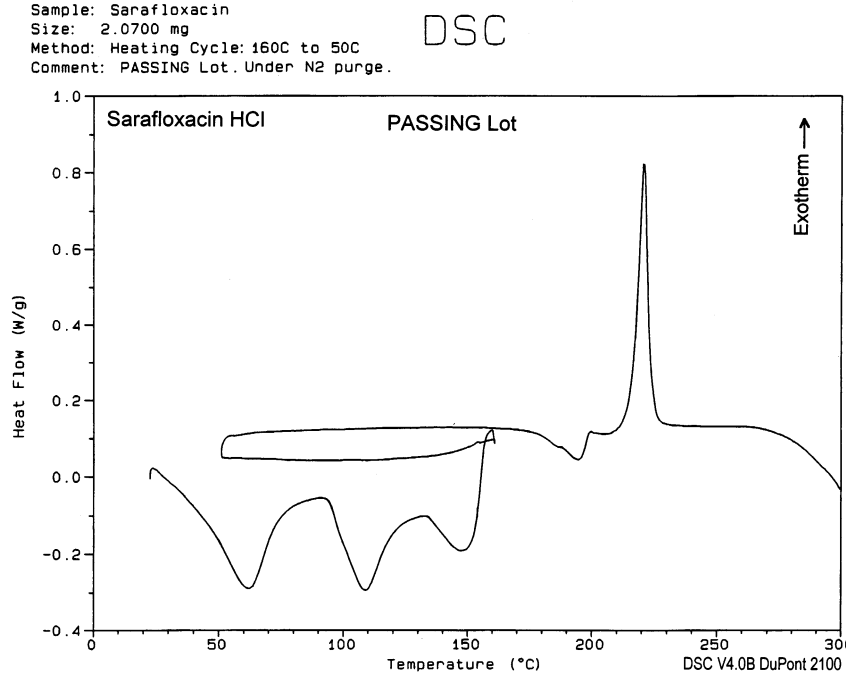


Fig. 5. DSC thermogram of heating and cooling cycle of sarafloxacin passing lot.

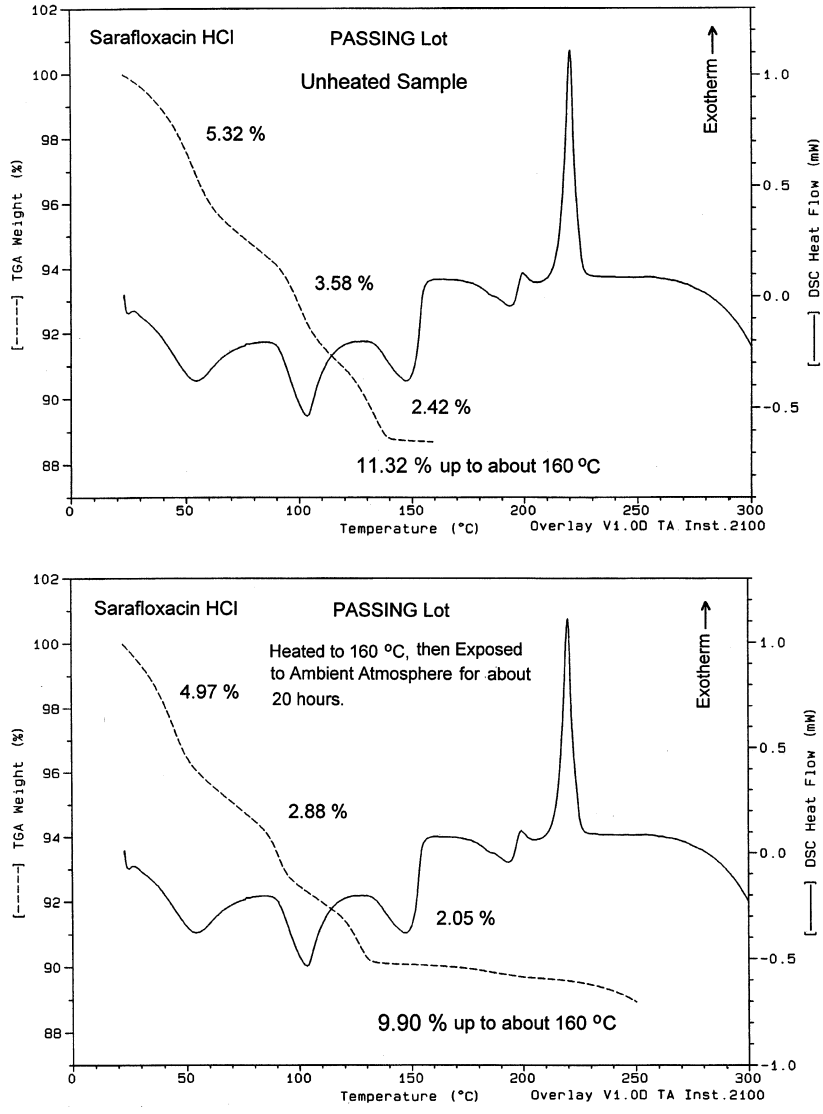


Fig. 6. Overlay of DSC and TGA thermograms of sarafloxacin passing lot heated to 160°C and then exposed to ambient atmosphere for 20 h.

sarafloxacin performed significantly different than the passing lot. During the granulation of the formulation containing the failing lot, it was noticed that during the gradual addition of the water and dye mixture, there was no evidence of granule formation. The powder mass appeared unaffected by the addition of the granulation solution, basically remaining a dry powder. When approximately 90% of the solution had been added, the powder mass suddenly became very pasty in appearance. Within a couple of seconds, the pasty mass became a hard mass which stopped and seized the masser. Examination of the hardened mass revealed a mass of solidified powder held in a matrix formed by a stringy, elastic material thought to be povidone. It appeared as though the powder mass was able to absorb the granulating solution within the structure of the powder

and then, when an equilibrium state was exceeded, the water was released to bind with the povidone and form a stringy lattice type structure.

3. Results and discussion

Two lots of sarafloxacin (passing and failing lots) were analyzed by HPLC and were greater

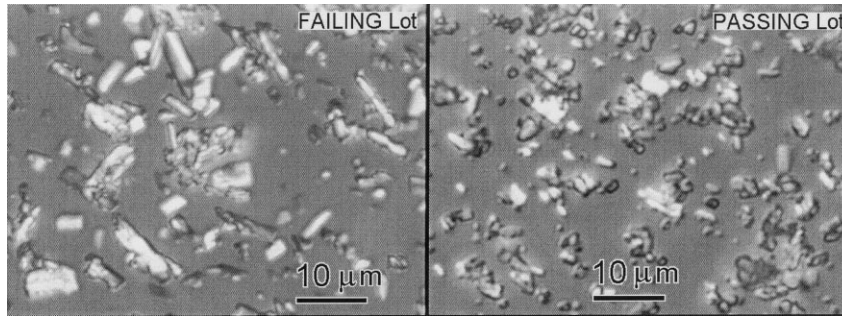


Fig. 7. Comparison of the video micrographs of sarafloxacin passing and failing lots at a magnification of 800 × .

Table 1
Comparison of the particle shape and size of sarafloxacin passing and failing lots

Lot	Particle shape characterization	Particle size characterization (μm)	Typical particle size (μm)
Failing	Irregularly shaped particles	<2–16	8
Passing	Irregularly shaped particles	<2–8	4

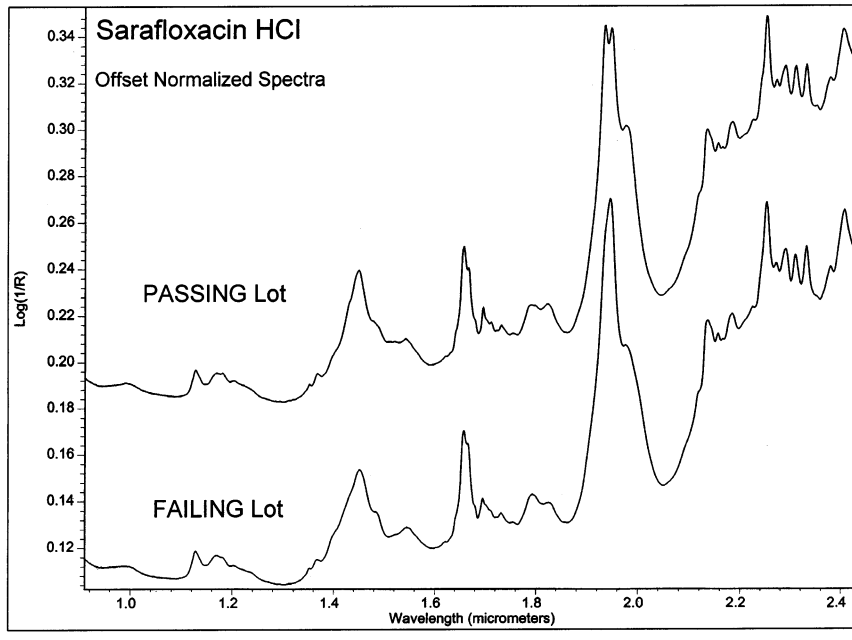


Fig. 8. NIR spectra of sarafloxacin passing and failing lots.

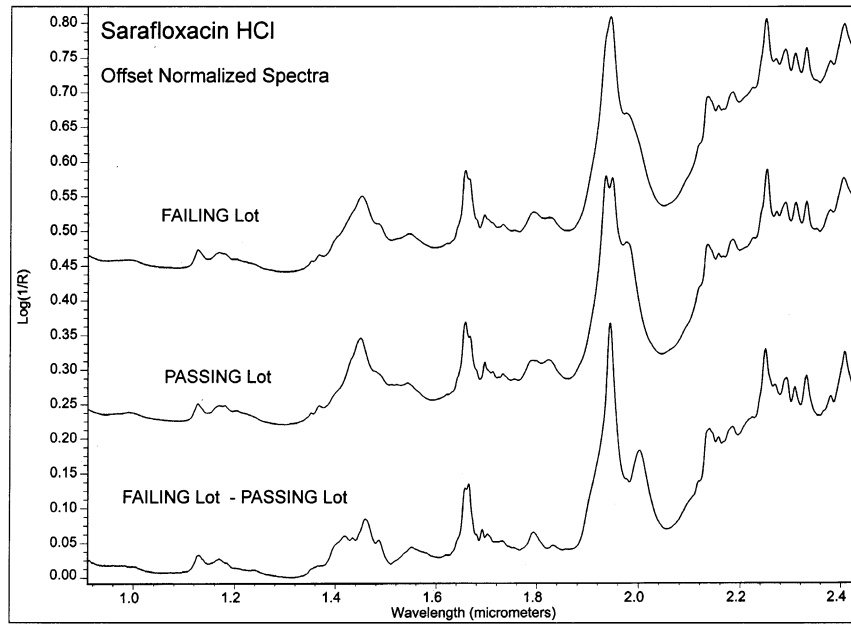


Fig. 9. NIR spectra of sarafloxacin failing and passing lots, and the subtraction spectrum of failing minus passing lots.

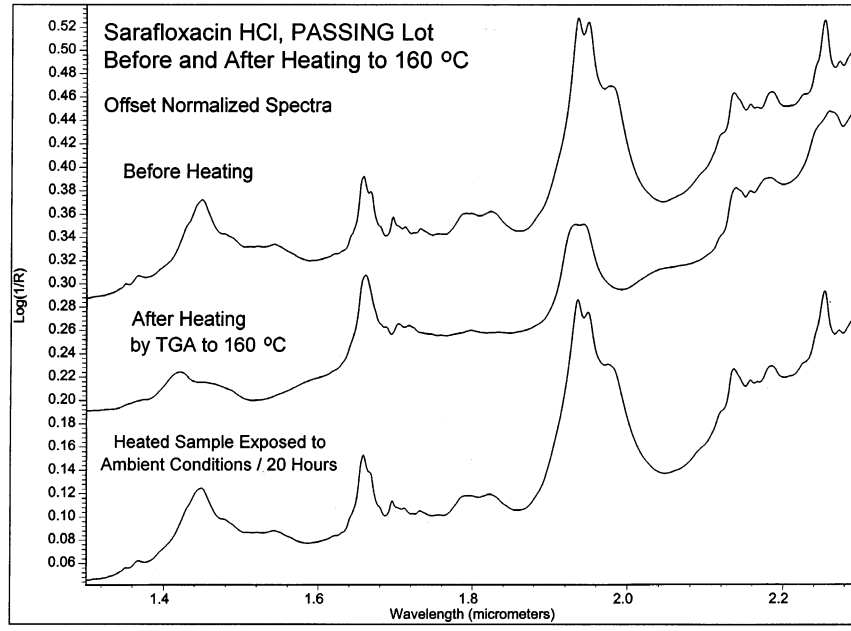


Fig. 10. Spectra of sarafloxacin passing lot before and after heating to 160°C, and exposed to ambient atmosphere for 20 h.

than 98% pure. However, when these lots were used to formulate the veterinary formulation, the two lots granulated differently. The failing lot could not be formulated well on a laboratory scale due to massing between each addition of the granulating liquid. This process would not have transferred very well to a commercial scale. The XRPD patterns of sarafloxacin indicate that the crystal forms of the two lots are identical in Fig. 1.

The mid-IR spectra of the failing and passing lots were also identical and are shown in Fig. 2.

Water content was about 11.39% and about 11.48% (w/w) by Karl Fischer titrimetry, for the passing and failing lots, respectively. This was in good agreement with a trihydrate stochiometry

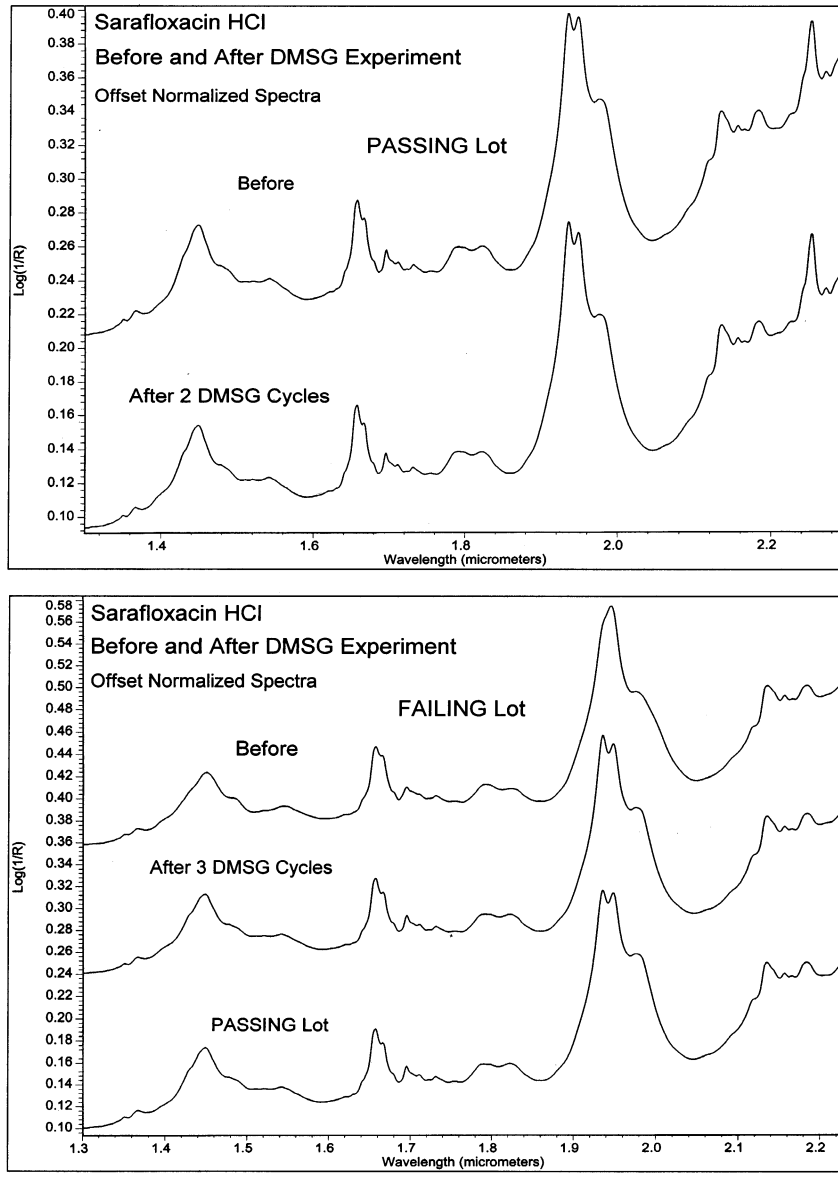


Fig. 11. Comparison of the NIR spectra of sarafloxacin passing and failing lots before and after DMSG experiments.

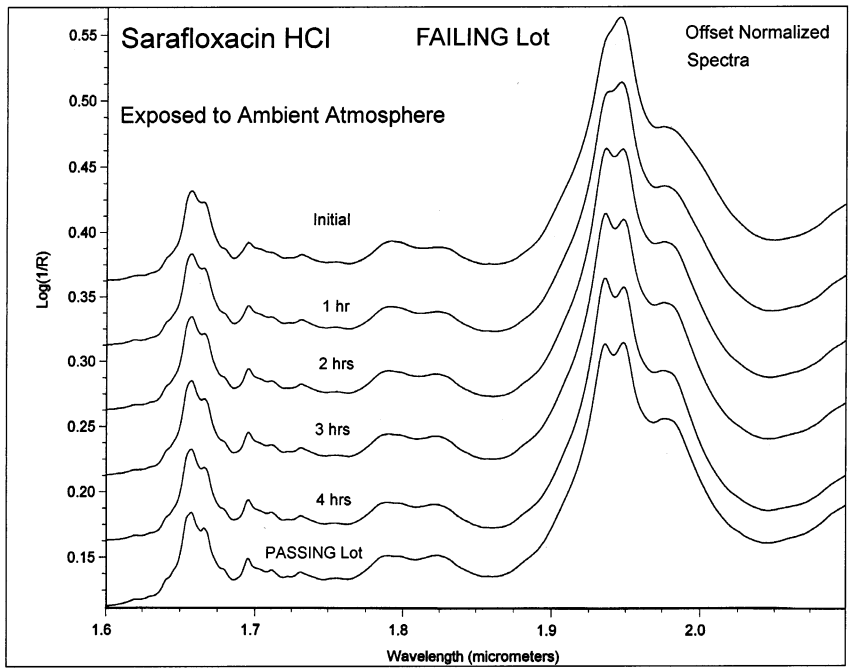


Fig. 12. Comparison of NIR spectra of sarafloxacin failing lot exposed to ambient atmosphere.

(11.36% (w/w) theoretical weight loss). As shown in Fig. 3, the crystal form was confirmed to be a trihydrate by DMSG, since the equilibrium moisture content (EMC) was equivalent to about 3 moles of water per mole of drug, or about 12.8%. In addition, the sorption and desorption DMSG isotherms were identical for both lots, indicating there were no differences in how the two lots pick up water. Also, there was no hysteresis between the sorption and desorption isotherms, indicating that the sorption and desorption processes were identical.

Fig. 4 represents the DSC and TGA thermograms for the two lots. The DSC thermograms indicate three endothermic stages of water loss up to about 155°C corresponding to about an 11.0% weight loss by TGA. A heating and cooling cycling DSC experiment was performed by heating the passing lot to about 160°C followed by cooling to about 50°C, and then re-heating to about 300°C, this revealed that while the three endothermic events were non-reversible, the other thermal events after about 160°C, remained unchanged as displayed in Fig. 5. This indicates that the three endothermic events are due to de-hydration and not to a loss of HCl. To further prove that the first three DSC endotherms were due to de-hydration, a sample of the passing lot was heated to 160°C by TGA, with a corresponding 11.32% weight loss, or 3 moles of water, as shown in Fig. 6. The heated sample was then exposed to ambient atmosphere for about 20 h. The DSC and TGA thermograms of the exposed or re-hydrated sample, were identical to the unheated sample; confirming that the first three DSC endotherms could only be due to loss of water. In addition, hot stage microscopy of a sample immersed in silicon oil, heated at a rate of 2° per min, showed water droplet formation, confirming this stepwise water loss. The stepwise loss, as determined by TGA for the passing lot, is equivalent to $1.437[WD1][WD1] \pm 0.015$ ($N = 5$) mole of water for the first weight loss, $0.870[WD2][WD2] \pm 0.033$ ($N = 5$) mole of water for the second weight loss and $0.630[WD3] \pm 0.006$ ($N = 5$) mole of water for the third weight loss.

The particle size and size distribution for the two lots were qualitatively identical, as shown in Fig. 7 and Table 1.

NIR spectra of the two samples, as displayed in Fig. 8, were comparable, however, there were observable differences in the water hydroxy combination region at about 1.95 μm, and to a lesser extent in the alkyl overtone region at about 1.65 μm, as well as the hydroxy first overtone region at about 1.45 μm. Subtraction of the failing lot minus the passing lot revealed significant differences only in the water combination band region, as shown in Fig. 9. Thus, the only significant differences by NIR spectroscopy between the two lots was how water interacts with sarafloxacin in the lattice. Also, there were no broad bands observed in the subtraction spectrum, indicating that there was little or no difference in the amount of amorphous material between the lots. These NIR data combined with the fact that the XRPD pat-

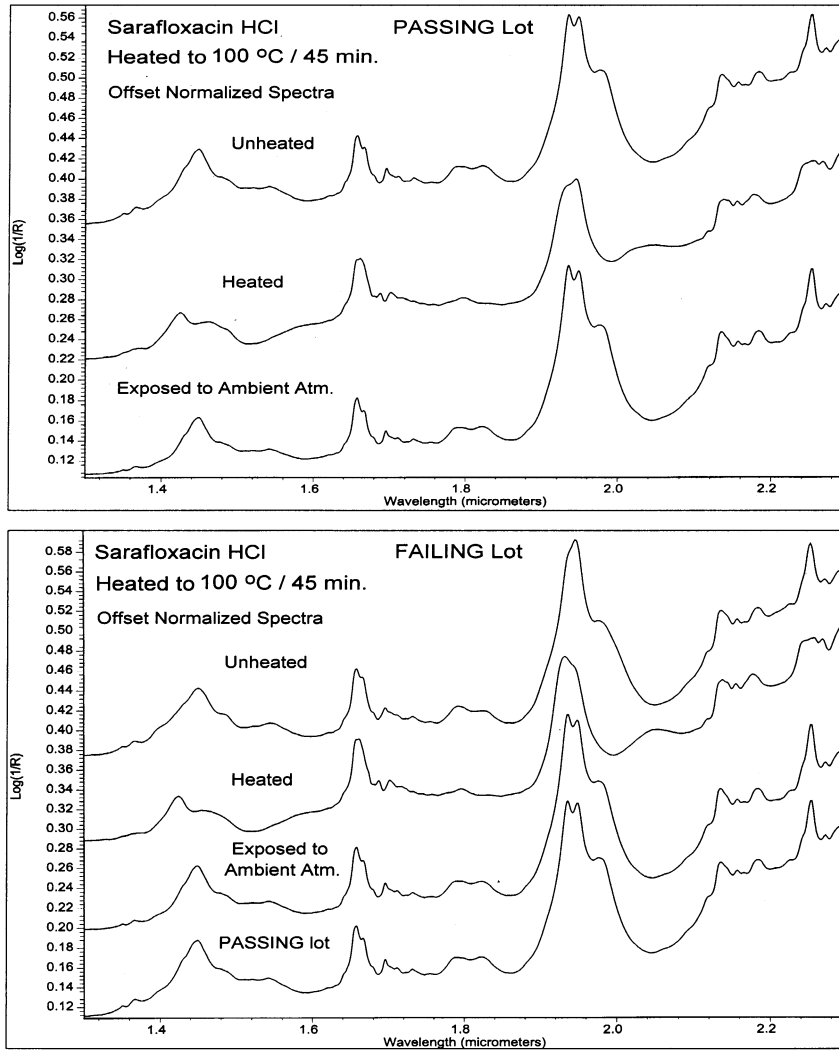


Fig. 13. Comparison of the NIR spectra of the passing and failing lots of sarafloxacin unheated, heated samples to 100°C for about 45 min, and exposed samples to ambient atmosphere.

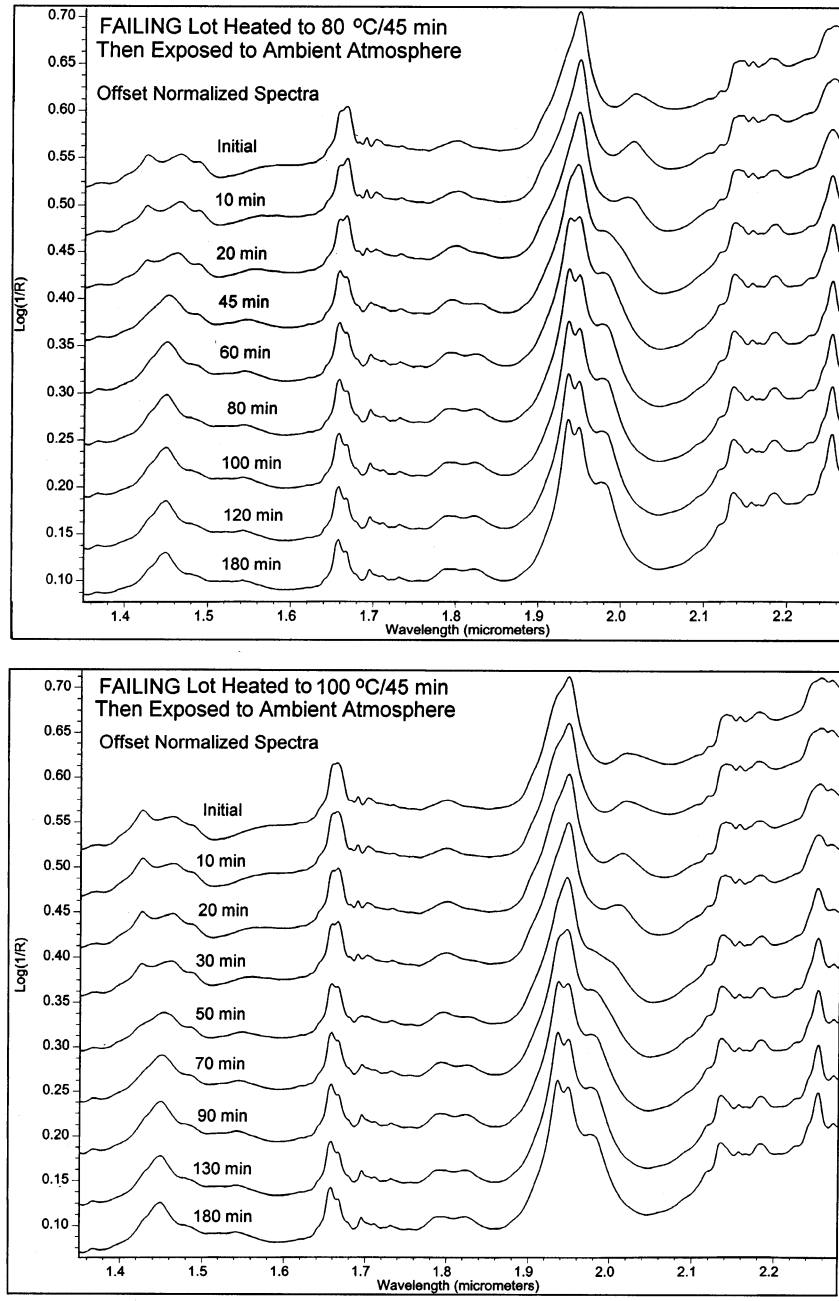


Fig. 14. Comparison of the NIR spectra of sarafloxacin failing lot heated to 80 and 100°C for about 45 min and then exposed to ambient atmosphere.

terns were identical, indicate that these isomorphic crystal lattices are the same crystal form, but have differences in the chemical environments around the water active sites.

NIR analysis of the samples in Fig. 6 revealed that the NIR spectra of sarafloxacin before heating to 160°C (initial sample), and the same sample re-hydrated were identical; whereas, the NIR

spectrum of the heated sample displayed a loss of water as shown in Fig. 10. Thus, heating the sample to about 160°C, or until complete loss of the three moles of water, followed by exposure of the heated sample to ambient atmosphere for about 20 h, indicate that the re-hydrated sample

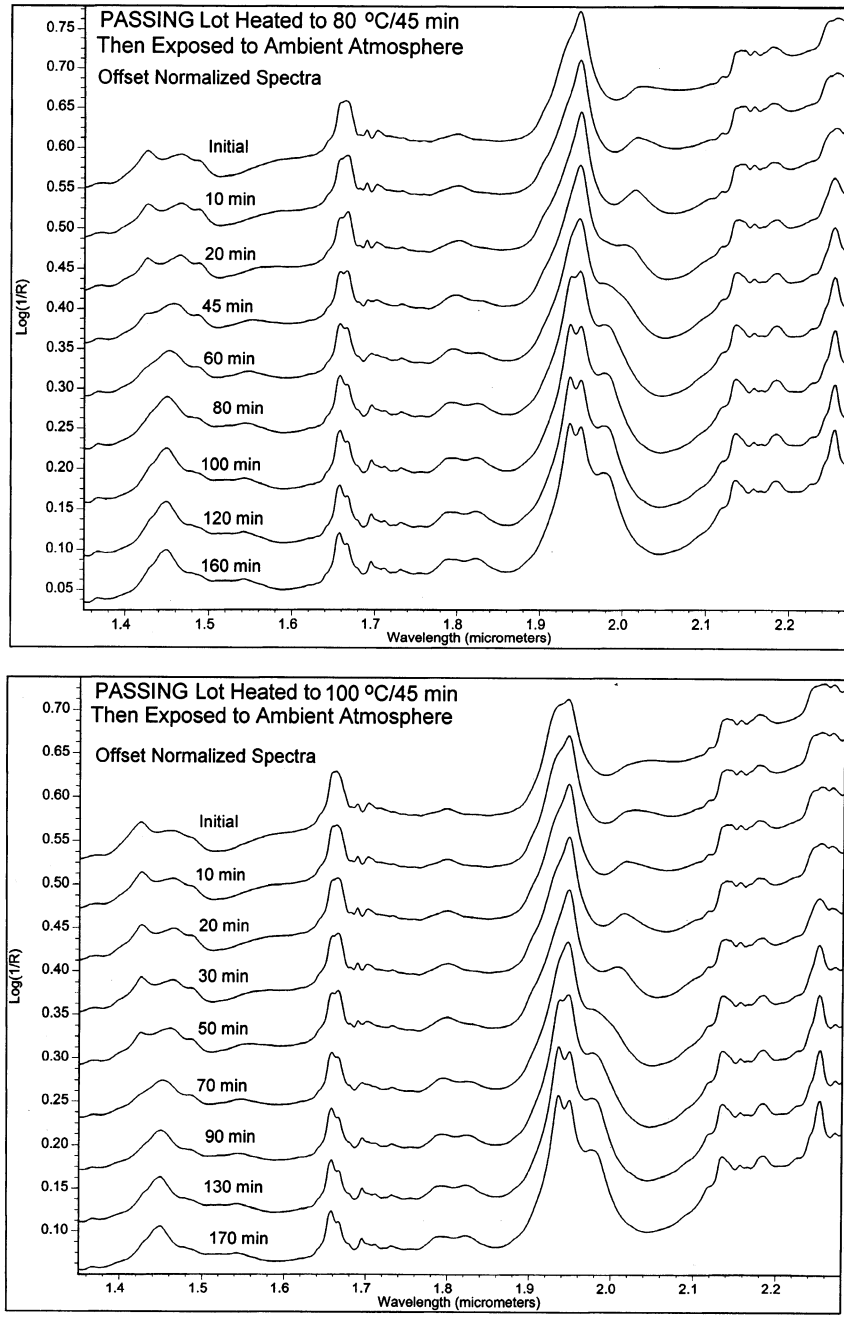


Fig. 15. Comparison of the NIR spectra of sarafloxacin passing lot heated to 80 and 100°C for about 45 min and then exposed to ambient atmosphere.

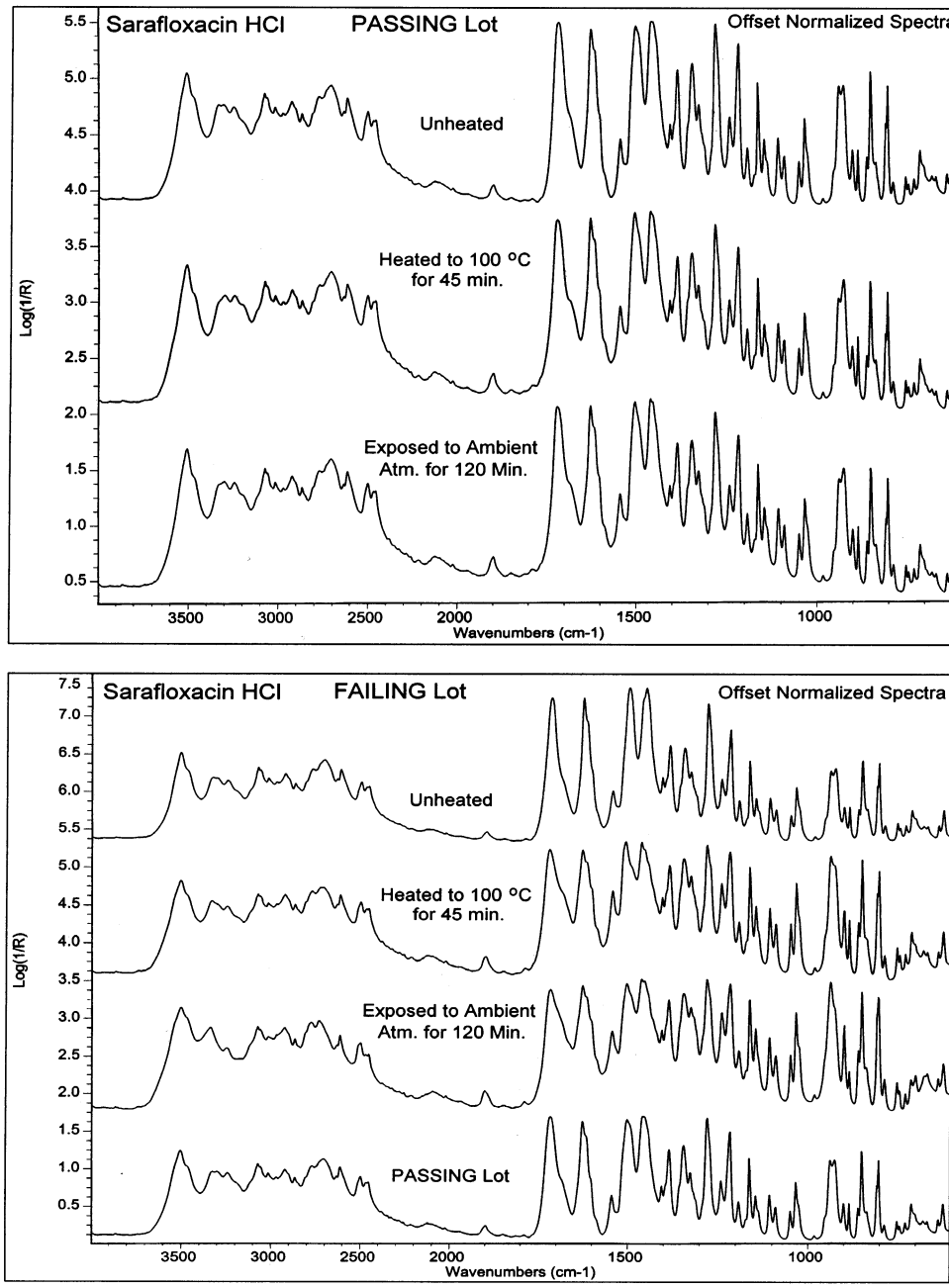


Fig. 16. Mid-IR spectra of sarafloxacin failing lot, heated sample to 100°C for about 45 min, and exposed sample to ambient atmosphere.

reabsorbed the water lost during the initial heating to return to the crystal form of the heated sample.

Similar NIR analysis of the passing and failing lots, before and after the DMSG experiments, revealed that the NIR spectra were identical to

the passing lot, indicating no change in the crystal form after the DMSG experiment, as shown Fig. 11. Also, the NIR spectrum of the failing lot after the DMSG experiment was identical to the NIR spectrum of the passing lot.

Curiously, the sites of occupancy by the water molecules in the crystal lattice are dependent on the water activity or relative humidity (RH) in the atmosphere. It appears that the degree or position of occupancy is not continuous with increasing RH. As can be observed from the DMSG isotherms in Fig. 3, there is a transition point at about 5% RH, where the hydrated state is preferred over the anhydrous state. The presence or absence of water in the crystal lattice can effect the physical properties and processing ability of the material in such pharmaceutical operations as milling and granulation. It was first suspected that the water content of the two lots was responsible for the differences in wetting or massing during granulation.

Although the total amount of water in the two lots was essentially the same by TGA and Karl Fischer titrimetry, differences in the NIR water hydroxy combination band region indicated that the location or orientation of the water molecules within the lattices was different. Interestingly, exposure of the failing lot to ambient atmosphere

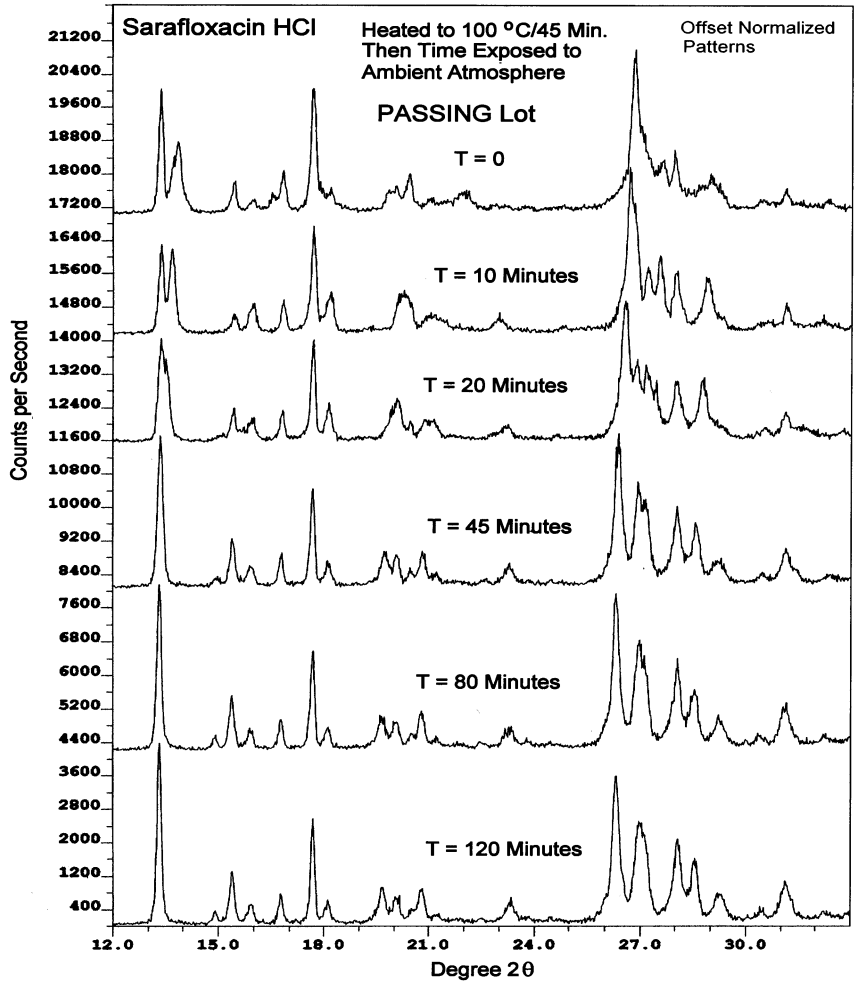


Fig. 17. XRPD patterns of sarafloxacin passing lot heated to 100°C for about 45 min and then exposed to ambient atmosphere.

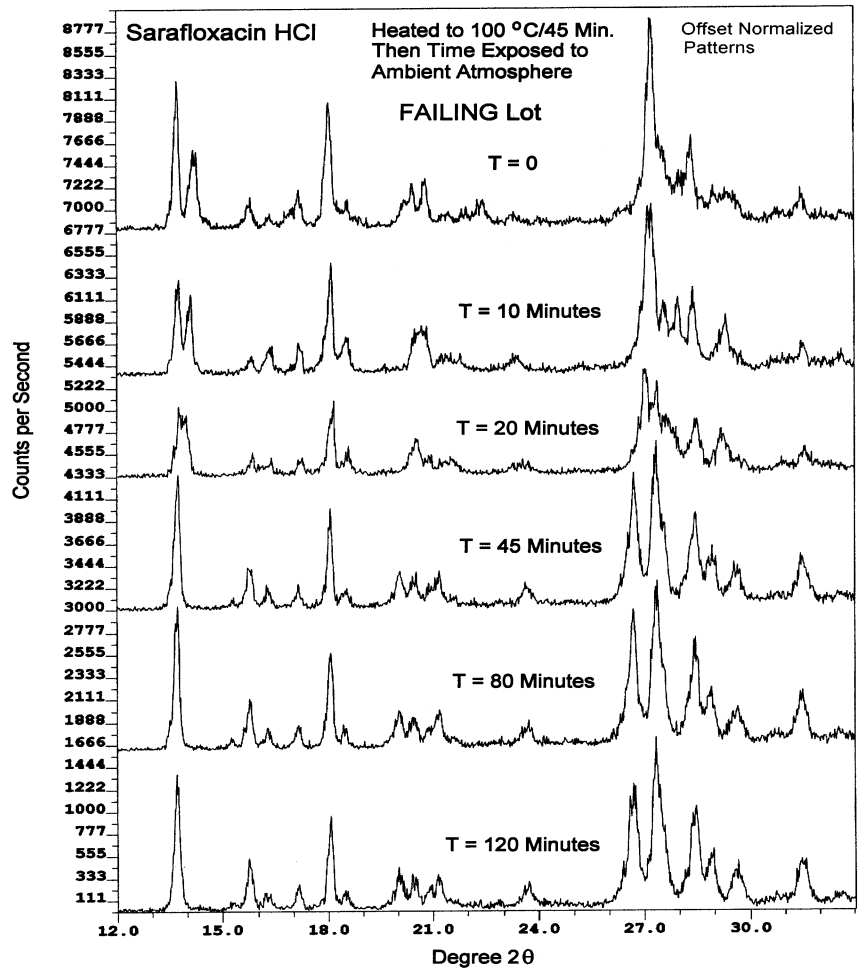


Fig. 18. XRPD patterns of sarafloxacin failing lot heated to 100°C for about 45 min and then exposed to ambient atmosphere.

for approximately 3 h, changed the NIR spectrum to that observed for the passing lot (Fig. 12).

To determine if these orientations reflect intermediate stages of water absorption by the sarafloxacin crystal lattice, the failing and the passing lots were dried, by TGA at 80 and 100°C isothermally for about 45 min and then exposed to ambient atmosphere. The TGA weight loss for the failing lot was 8.17 and 8.82%, for the 80 and 100°C heated samples, respectively. The two temperatures were chosen to minimize thermal degradation of the samples. The heated samples reabsorbed approximately 95% of the starting or initial level of water, as determined by TGA, after 10 min of exposure to ambient atmosphere. However, the NIR spectra obtained of the heated samples, were different from both that of the initial failing and the passing lots, as shown in Fig. 13. The NIR spectra of the heated failing lot continuously changed for a period of about 100 min, even though little or no additional water was absorbed (Fig. 14). The changes in the NIR spectra progress through the spectrum observed for the initial failing lot, however the NIR spectrum matched that of the initial, or unheated passing lot (Figs. 13 and 14). The passing lot was also heated under identical conditions and was found to have similar drying and re-absorption properties as the failing lot (Figs. 13 and 15).

However, the mid-IR spectra of the heated samples, at 100°C for 45 min, were identical to both the initial or unheated samples, and the exposed or re-hydrated samples, both for the failing and passing lots, as demonstrated in Fig. 16. Hence, mid-IR spectroscopy was found not to be useful in this study in examining how the water molecules interact with the crystal lattice.

To determine the solid-state phase of heated sarafloxacin, after the isothermal heating at 100°C, XRPD patterns were acquired, in 10–30 min intervals, similar to the time intervals in the NIR experiment described in Figs. 14 and 15. Figs. 17 and 18 show the overlay of XRPD patterns for the passing lot heated to 100°C for 45 min, and then exposed to ambient atmosphere for up to about 120 min, and the initial or unheated sample in Fig. 19. As can be observed in the XRPD patterns from the $T=0$ to the $T=45$ min time points, there is a change in the distance between the diffraction lines at about 13° 2 theta indicating a change in the d-spacing and thus a change in the dimension of the unit cell. The XRPD patterns stop changing after 45 min exposure to ambient atmosphere. This implies that the unit cell dimension changes up to about the 45 min time point. However, the NIR spectra for the exposed sample after 45 min exposure continue to change until about 120 min. Therefore, it is apparent, after taking into account the XRPD and

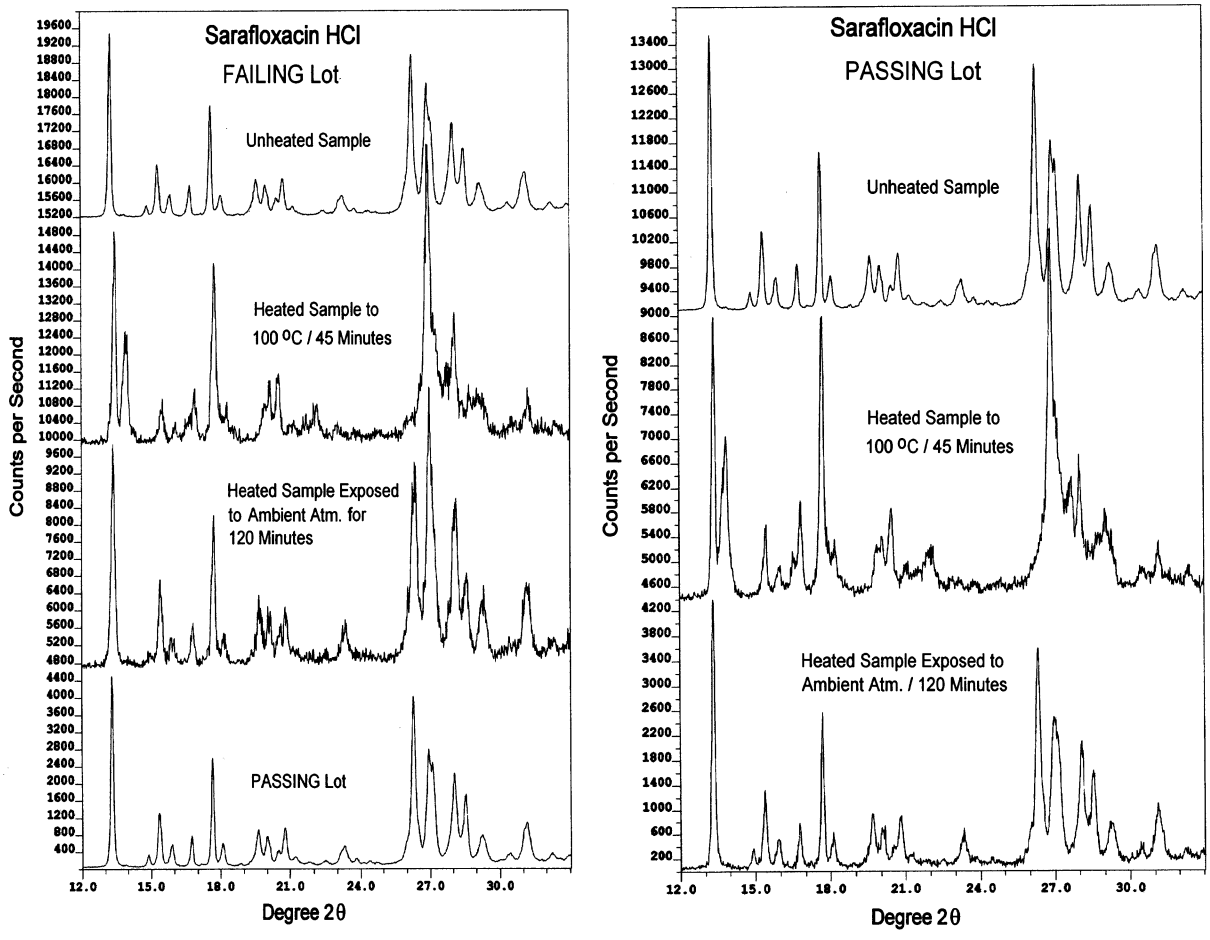


Fig. 19. Comparison of the XRPD Patterns of sarafloxacin passing and failing lots of the unheated samples, heated samples to 100°C for about 45 min and exposed samples to ambient atmosphere.

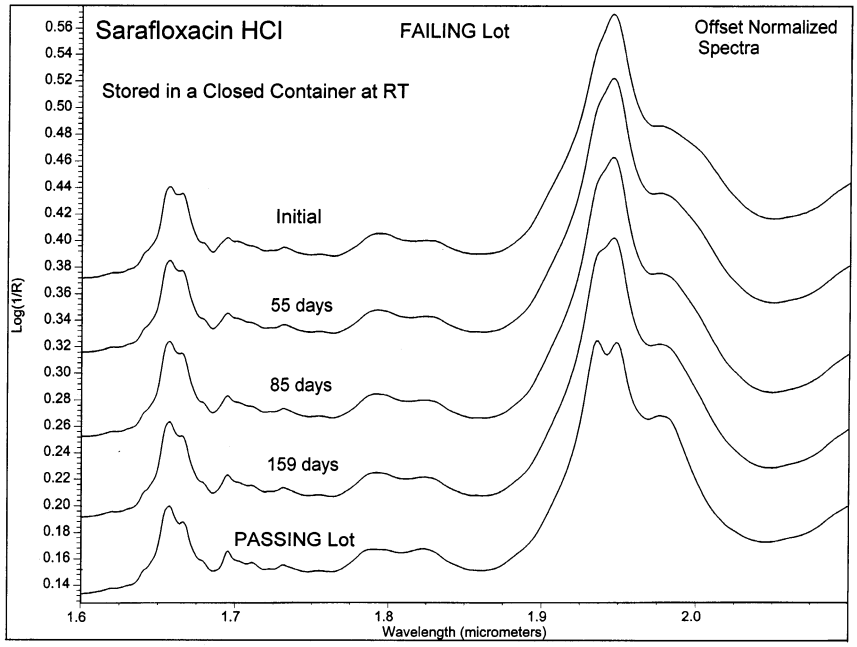


Fig. 20. Comparison of the NIR spectra of sarafloxacin failing lot stored in a closed container at room temperature and the passing lot.

NIR data, that the water chemical environment in the crystal lattice changes without a change in the unit cell dimension.

Similar XRPD analysis of failing lot, heated to 100°C and then exposed to ambient atmosphere, revealed similar XRPD patterns to the passing lot after similar exposure times (Fig. 18). In addition, the XRPD patterns were identical for the passing lot re-hydrated sample (120 min exposure) and the unheated sample, indicating no change in crystal form, as shown in Fig. 19. However, the XRPD pattern of the heated sample has a different d-spacing, indicating a different unit cell dimension. Though the XRPD patterns of the heated samples of both lots indicated different unit cell dimensions from the initial samples, there was no evidence of formation of an amorphous material.

When the exposure to ambient atmosphere was interrupted, by storage in a closed container, before the final water static state was achieved, the NIR spectrum stopped changing, indicating that continuous exposure to ambient relative humidity is critical for complete conversion. Fig. 20 displays the NIR spectra of the failing lot store in a closed container for up to 159 days.

As described in Zografi and Kontny [8], two forms of 'bound' water can exist within a crystal lattice, one involving hydration directly with the molecule via hydrogen bonding, and the other a more non-specific intermediate type of bonding. In this study, it is apparent that atmospheric moisture plays a major role in facilitating a redistribution of water molecules between these types of binding in the sarafloxacin crystal lattice.

4. Conclusions

Two lots of sarafloxacin with identical XRPD patterns, mid-IR spectra, DSC and TGA thermograms and particle size had different properties during granulation. Both of these isostructural samples also had identical water sorption profiles by DMSG, and showed approximately trihydrate stoichiometry from about 30–95% RH. They also had identical water content by Karl Fischer titimetry with about 11.4% (w/w) water, or a tri-

hydrate stoichiometry. However, by NIR spectroscopy, these isomorphic structures had observable differences in the water hydroxy regions with less pronounced differences in the alkyl region. It is obvious that these isomorphic crystal lattices are the same form but with small differences in the chemical environments around the water active sites. This lack of complete conversion of the failing lot is related to the difficulty in processing, in this case, wetting of the drug substance, by restricting the sites available for solvent interaction.

Even though the water content of the two samples was identical, exposure of the failing lot to ambient humidity for about 3 h converted its NIR spectrum to that of the passing lot. Interestingly, this conversion was not observed when the failing lot was exposed to low relative humidity, as in a closed container, even after 159 days. It is apparent that orientation, as well as the occupancy of the water molecules in the lattice is dependent on water activity.

The NIR spectrum of the failing lot can be reproduced by heating either the passing or the failing lot to 80 or 100°C for about 45 min followed by exposure to ambient humidity. The isothermally heated samples reabsorb more than 95% of the moisture lost during heating after only about 10 min exposure, yet the NIR spectra continuously change for up to about 100 min. At this point, the NIR spectra of both heated lots were identical to that of the passing lot. Therefore, the failing lot could have resulted from an insufficient period of exposure to ambient humidity upon storage. However, the failing lot could be converted to a material that is identical to the passing lot (using NIR spectroscopy); by simply exposing it to ambient atmosphere.

These studies demonstrate for the first time, the use of NIR spectroscopy to detect and monitor the mobility of water within the crystal lattice of crystalline sarafloxacin.

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