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# Relative physical stability of the solid forms of amiloride HCl

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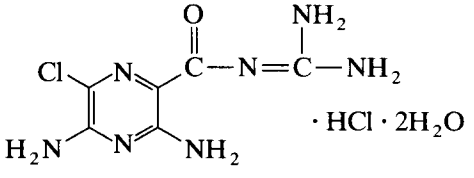
## Summary

Amiloride HCl is available in two polymorphic dihydrate forms, either of which can be dehydrated to an anhydrous crystalline form. The anhydrate rapidly rehydrates to polymorph A of the dihydrate upon exposure to ambient relative humidity. The polymorph A and polymorph B dihydrates have similar melting points, FTIR spectra, and solubilities. X-ray powder diffraction can differentiate the two polymorphs, and quantitative estimates for mixed phase samples can be obtained. This technique was used to show that the composition of USP grade material varies by both vendor and lot number. Polymorph A was found to be more physically stable than polymorph B by using X-ray powder diffraction to follow solid-state transformations upon milling or compressing both forms.

## Introduction

The moderate base amiloride ($pK_a$ 8.7) is an oral diuretic which acts by enhancing sodium ion excretion. It is now available from generic sources as the stable dihydrate of the monohydrochloride salt. Inhaled solutions of amiloride HCl are being investigated as a palliative therapy in cystic fibrosis (App et al., 1990). Cystic fibrosis is an inherited disorder in Caucasians characterized by an increased viscosity in the bronchial mucous, which makes breathing labored and promotes infection. The increase in airway water content after amiloride therapy (associated with excreted sodium ions) appears to enhance mucociliary clearance. Glaxo Inc. has acquired the marketing rights to inhaled sterile solutions of amiloride HCl from the University of North Carolina and is developing it as an orphan drug for cystic fibrosis.



amiloride HCl dihydrate

It has been reported in the literature (Mazzo, 1986; Brittain and Newman, 1990) that amiloride HCl dihydrate can be produced in at least two distinct polymorphic crystalline forms, termed

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polymorph A and polymorph B. A review of the general polymorph literature (Haleblian and McCrone, 1969, Byrn, 1982) shows that amiloride HCl polymorphs have two relatively uncommon properties:

(1) The two polymorphs are of the solvated form (dihydrate). Many drugs have multiple non-solvated polymorphs (Byrn, 1982; Borka and Haleblian, 1991) or are able to form many types of solvates (Chapman et al., 1968, Byrn, 1982; Ashizawa et al., 1989; Agafonov et al., 1991) but few examples of pharmaceutical solvates exhibiting polymorphism have been reported.

(2) X-ray powder diffraction is noted as the only common technique which is able to differentiate the two forms (Mazzo, 1986). The FTIR patterns, DSC/TGA scans, crystal habits, solubilities, and densities of the two dihydrate forms are not sufficiently different to identify the polymorphic form.

When formulating a solution dosage form, most of the physical property differences between polymorphs are irrelevant, the exceptions being equilibrium solubility and dissolution rate. Formulation at a concentration below the solubility of the most stable form at the appropriate storage temperature will prevent precipitation of a less soluble polymorph upon storage. Significant differences in the rates of dissolution of polymorphs have been seen (Tuladhar et al., 1983; Salole and Al-Sarray, 1985) which may impact the ease of manufacture of bulk solutions. Preliminary preformulation work on amiloride solutions for inhalation found that different vendors were providing the dihydrate of the hydrochloride salt as either pure polymorph A, pure polymorph B, or a mixture of the two forms (Jozwiakowski et al., 1991). The objectives of the present investigations were:

(1) To compare the physical properties of these polymorphs which might affect the solution dosage form (equilibrium solubility and dissolution rate).

(2) To compare suppliers of amiloride HCl USP XXII in terms of their ability to reproducibly supply a given polymorphic form.

(3) To characterize the relative physical stability of the dihydrate phase relative to the anhydrate of the HCl salt at different conditions of temperature and relative humidity.

(4) To determine whether the two polymorphs could be assigned relative physical stabilities by inducing transformations in the solid state under stressed conditions. The usual method of assigning metastability or stability of the forms by DSC melting behavior is not applicable to this system, since the polymorphs dehydrate to the same anhydrate below the melting point, thus showing the same melting point (Mazzo, 1986).

## Materials and Methods

*Sources of materials*

Amiloride HCl was used as received from five different vendors, with multiple lots purchased from two of those vendors. Single lots were received from Sigma (lot 78F-0024, Sigma Chemical Co., St. Louis, MO, U.S.A.), Merck (lot 001T051, Merck & Co., Inc., Rahway, NJ, U.S.A.), and Bioindustria (lot 149247, Bioindustria Farmaceutici S.p.A., Fresonara, Italy). The Sigma material was for laboratory use only, the Merck material met USP XXI specifications, and the Bioindustria material was tested to BP 1988 standards. Multiple lots meeting USP XXI specifications were received that were manufactured by Siegfried (Siegfried AG, Zofingen, Switzerland) and Sifavitor (Sifavitor S.p.A., Milan, Italy). The lot numbers used in this study can be found in Table 3. Sodium chloride used for the solubility studies was USP grade material from Mallinkrodt (lot 7532KBMJ, Mallinkrodt, Inc., Paris, KY, U.S.A.). Water used in solubility and dissolution experiments was purified distilled water from a Milli-Q water system (Millipore Corp., Bedford, MA, U.S.A.).

*Preparation of the anhydrous form*

Drying the dihydrate form at 150°C in a convection oven (Baxter Scientific Products, U.S.A.) produced anhydrous amiloride HCl in 30–45 min (as verified by TGA), which remained crystalline (as verified by X-ray powder diffraction). Drying

at 100°C did not remove all of the crystalline water after 4 h, and reduced the crystallinity of the sample (Brittain and Newman, 1990). The relative humidities at which each hydration state of amiloride HCl was physically stable and the hygroscopicities of each polymorphic form were studied by placing 0.5 g samples in desiccators containing saturated salt solutions and weighing them periodically for 3 weeks on a Sartorius Research balance. Reagent grade chemicals were used to produce the following chambers: low humidity (dry $CaSO_4$), 15% RH (LiCl), 33% RH ($MgCl_2$), 52% RH ($Na_2Cr_2O_7$), 66% RH ($NaNO_2$), 75% RH (NaCl), 84% RH (KBr), and 97% RH ($K_2SO_4$). Material from Bioindustria was used as the polymorph A drug and Siegfried lot 9007H902 was used as the source of polymorph B drug.

*Polymorph characterization procedures*

FTIR patterns were taken as dry powder samples on a $BaF_2$ window using a Perkin-Elmer 1720X FTIR equipped with a SpectraTech FTIR microscope. Scanning electron photomicrographs were taken on gold-coated specimens using a Zeiss DSM960 SEM and printed on Polaroid 52 film. Thermogravimetric analysis (TGA) was performed on 10 mg samples using a Perkin-Elmer TGA-7, with nitrogen purge, and a 10°C/min heating rate. Differential scanning calorimetry (DSC) was carried out in triplicate on 4–6 mg samples in sealed aluminum pans on a Perkin-Elmer DSC-7 system using a nitrogen purge and a heating rate of 1.5 or 15°C/min. Intrinsic dissolution rates were measured after compressing powder at 3000 lb/inch$^2$ for 30 s into a custom-designed Wood's die with an area of 1 cm$^2$. This was the minimum compression force which would hold the tablet together for the duration of the 15 min run at 50 rpm and produce linear data. The medium was 900 ml of 37°C distilled water, and the drug concentration was monitored at 282 nm using a flow-through dissolution system (Beckman DU-65). Equilibrium solubility was measured in 0.15% NaCl/water at 5, 15, 25, 35, and 45°C after equilibration for 3–4 days (determined experimentally to be sufficient). This medium was chosen because the common ion effect reduces the drug solubility in NaCl solutions, and pure polymorph A was in short supply. Samples were filtered (Gelman SUPOR-200 0.2 $\mu$m membrane filters) soon after removal from the bath and diluted for assay at 362 nm on a Cary 2351 spectrophotometer. A linear Beer's Law plot was obtained from 0 to 0.05 mg/ml of amiloride HCl. Qualitative X-ray powder diffraction patterns of slurries stored for 4 days at 5, 25, and 45°C showed no evidence of transformation of the excess solid to the other polymorphic form. These characterization studies used Bioindustria material for polymorph A and Siegfried lot 9007H902 for polymorph B.

*X-ray powder diffraction*

Samples were run on a Scintag XDS2000 diffractometer using Cu-K$\alpha$ radiation, 45 kV, 40 mA, and a germanium detector. Zero background quartz plates were coated with a thin layer of petroleum jelly and sprinkled with powder (lightly triturated in a mortar and pestle to uniform fine particles) in random orientations; these disks were then spun during data acquisition to further reduce preferred orientation effects. The samples were scanned in steps of 0.03° $2\theta$ from 2 to 50° $2\theta$ using slits of 2, 4, 1, and 0.3 mm. The calibration of the $d$-spacings was verified prior to each run using a $LaB_6$ standard powder. Instrument software was used for Cu-K$\alpha_2$ stripping, background subtraction, peak location, and graphics. Quantitative estimates of mixed phases were done from a standard curve prepared from triplicate measurements on known mixtures of the pure phases, and using the peaks at 8.9° $2\theta$ (polymorph A, Bioindustria) and 9.5° $2\theta$ (polymorph B, Siegfried lot 9007H902).

*Methods to induce solid-state transformations*

Samples of each dihydrate polymorph were subjected to ball-milling in a porcelain mill (U.S. Stoneware, Mahwah, NJ. U.S.A.) with 10 balls of 1/2 inch diameter for 60 min. Powders were also compressed at different pressures and dwell times on a laboratory press (Fred S. Carver, Inc., Menomonee Falls, WI, U.S.A.) and crushed to powder form in a mortar and pestle for X-ray powder diffraction. Polymorph B was micronized

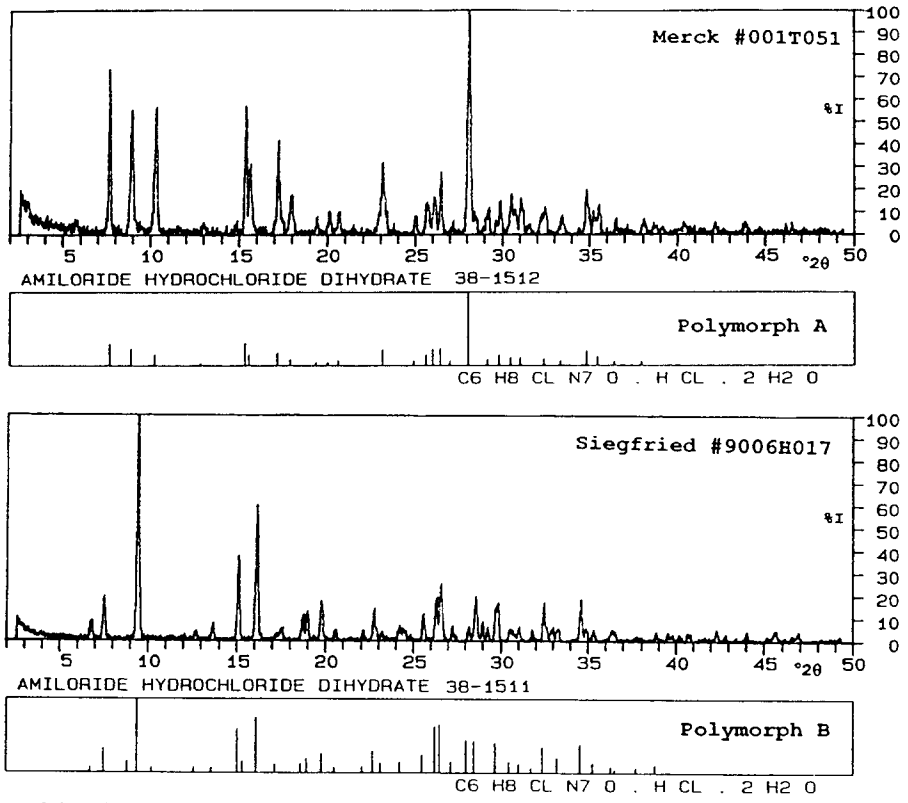


Fig. 1. Comparison of the JCPDS patterns for polymorph A and polymorph B of amiloride HCl dihydrate with a pure polymorph A lot (Merck) and a pure polymorph B lot (Siegfried).

TABLE 1

*X-ray powder diffraction data for the 10 most intense peaks in the patterns for the solid phases of amiloride HCl*

| Polymorph A [a] | | | Polymorph B [b] | | | Anhydrate [c] | | |
|---|---|---|---|---|---|---|---|---|
| $2\theta$ (°) | $d$ | $I/I_0$ | $2\theta$ (°) | $d$ | $I/I_0$ | $2\theta$ (°) | $d$ | $I/I_0$ |
| 7.72 | 11.45 | 35 | 7.55 | 11.71 | 19 | 10.54 | 8.38 | 79 |
| 8.96 | 9.86 | 36 | 9.46 | 9.35 | 100 | 14.50 | 6.10 | 11 |
| 10.31 | 8.58 | 29 | 15.14 | 5.85 | 36 | 15.33 | 5.78 | 28 |
| 15.42 | 5.74 | 28 | 16.17 | 5.48 | 56 | 15.55 | 5.69 | 42 |
| 15.66 | 5.65 | 16 | 19.79 | 4.48 | 16 | 21.13 | 4.20 | 32 |
| 17.27 | 5.13 | 19 | 26.33 | 3.38 | 18 | 24.06 | 3.70 | 29 |
| 23.18 | 3.83 | 18 | 26.59 | 3.35 | 24 | 25.35 | 3.51 | 25 |
| 26.49 | 3.36 | 24 | 28.60 | 3.12 | 18 | 25.91 | 3.44 | 20 |
| 28.13 | 3.17 | 100 | 29.84 | 2.99 | 16 | 27.30 | 3.26 | 100 |
| 34.91 | 2.57 | 10 | 32.50 | 2.75 | 16 | 31.41 | 2.85 | 29 |

[a] Merck lot 001T051 as received.

[b] Siegfried lot 9006H017 as received.

[c] Siegfried lot 9007H902 dried at 150°C.

in an air impact micronizer (Trost, Garlock Inc., Newton, PA, U.S.A.). The effect of temperature was gauged by two heating regimens, 100°C for 30 min (minimal loss of dihydrate water by TGA) and 150°C for 45 min (complete dehydration by TGA). These samples were cooled in a 75% RH chamber prior to X-ray powder diffraction.

## Results and Discussion

*Basic characterization of the dihydrate polymorphs*

X-ray powder diffraction patterns on the samples of amiloride HCl dihydrate from different suppliers showed that the Merck and Bioindustria samples matched the Joint Committee for Powder Diffraction Standards (JCPDS) file no. 38-1512, for polymorph A. Three lots from Siegfried matched JCPDS file no. 38-1511, for polymorph B of amiloride HCl dihydrate. Fig. 1 illustrates this match for one lot of each pure polymorphic dihydrate phase, and Table 1 gives the positions and relative intensities of these peaks. Comparative physical properties were measured using these pure phases, since all other lots tested were mixtures of these two phases.

The SEM photomicrographs in Fig. 2 are of representative particles from the pure polymorphs and show that there are no morphological differences between polymorph A and polymorph B. Each consists of rods averaging 5–10 $\mu$m in length by 1–2 $\mu$m in width which aggregate into the bundles which are responsible for the bulk properties of the solid. FTIR scans on individual crystals of each form were indistinguishable; both polymorphs had identical infrared spectra corresponding to a previously published spectrum (Mazzo, 1986). TGA on all lots showed the expected 12% weight loss associated with the loss of dihydrate amounts of water. DSC scans showed an endotherm at 117–118°C due to this water loss, a melting peak at approx. 295°C, and subsequent decomposition. Fig. 3 depicts both the TGA and DSC curves for polymorph B amiloride HCl dihydrate. The onset temperatures for the endothermic peaks from repetitive analysis of polymorph A dihydrate, polymorph B dihydrate, and the anhydrate are given in Table 2, for

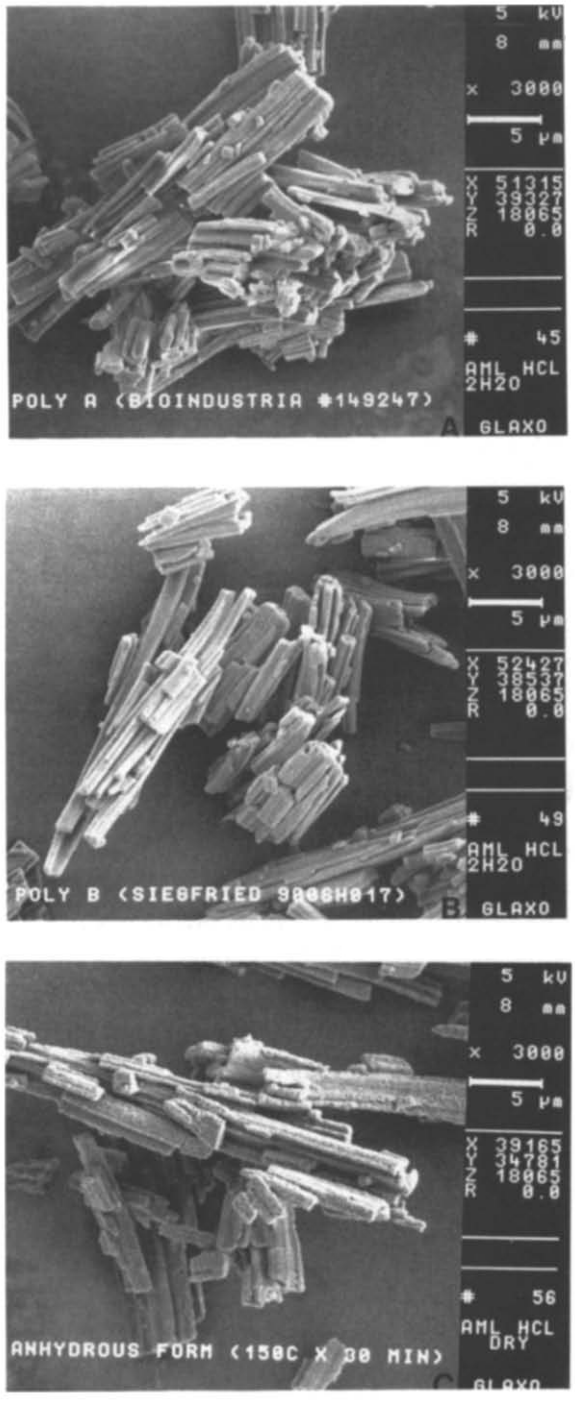


Fig. 2. Scanning electron micrographs of: (A) polymorph A amiloride HCl dihydrate from Bioindustria, (B) polymorph B amiloride HCl dihydrate from Siegfried, and (C) amiloride HCl dried to the anhydrous form by heating at 150°C for 30 min.

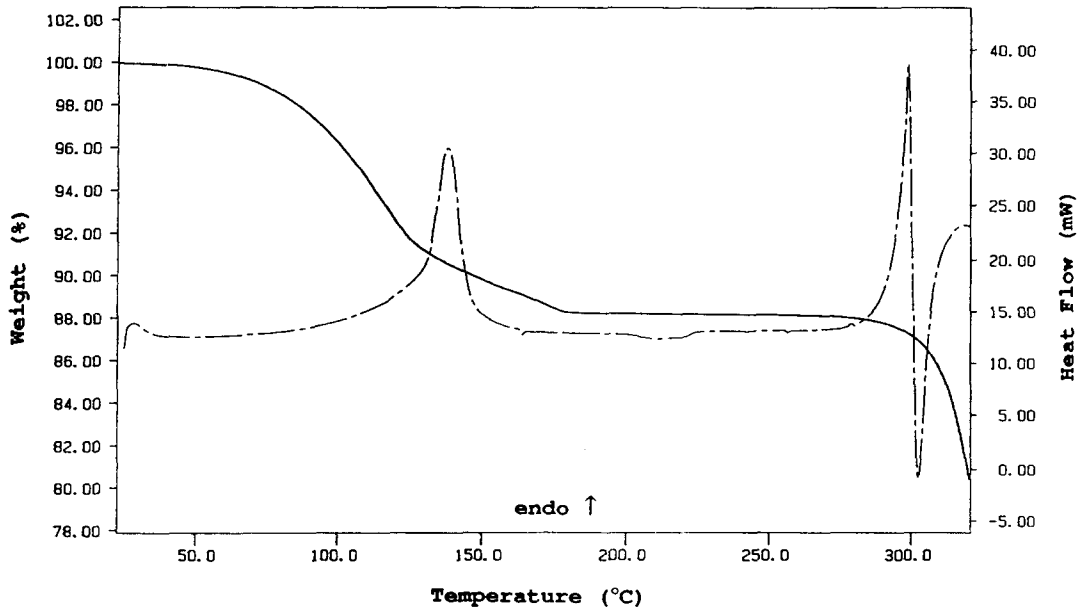


Fig. 3. DSC/TGA scans for amiloride HCl dihydrate polymorph B (Siegfried lot 9007H902). Scan rates: TGA, 10°C/min (———) (in open pans); DSC 15°C/min (— · —) (sealed pans).

two different scan rates. The heat of fusion was not reproducible due to the subsequent decomposition. There was no detectable difference in the dehydration or melting temperature between the two dihydrate polymorphs at either heating rate. That the melting points are identical is not surprising, since the polymorphs are of the hydrated crystal form, which dehydrates 150°C lower than the melting point. This does indicate that the two polymorphs may dehydrate to the same anhydrous form under these conditions. When the anhydrate is used as the starting material, a melting point 3–5°C higher is obtained. Since the sample pans are sealed, this may mean that the water lost from the dihydrate affects the material properties of the remaining anhydrate, lowering its melting point.

The equilibrium solubilities of the two dihydrate polymorphs in 0.15% NaCl were found to be the same (no significant differences at the 95% confidence level) from 5 to 45°C (Table 3). There was no change in the excess solid state during the experiment throughout that temperature range when starting with either dihydrate phase. A van 't Hoff treatment of the data using unitary quantities (mole fraction units) gave apparent enthalpies of solution of +9.32 (0.64) kcal/mol for polymorph A and +8.98 (0.32) kcal/mol for polymorph B. These are not significantly different at the 95% confidence level by

TABLE 2
*Onset temperatures for the endothermic events of amiloride HCl solids in DSC* [a]

| Event | Rate (°C/min) | Poly A ($2H_2O$) | Poly B ($2H_2O$) | Anhydrate |
|---|---|---|---|---|
| Solvate loss | 15 | 118.0 (1.0) | 117.0 (0.3) | – |
| Melting | 15 | 294.9 (0.6) | 294.9 (0.1) | 297.7 (1.2) |
| Melting | 1.5 | 278.9 (1.9) | 276.6 (0.4) | 282.9 (1.1) |

[a] $n = 3$, one standard deviation in parentheses.

TABLE 3

*Equilibrium solubilities of amiloride HCl dihydrate polymorphs in 0.15% NaCl (expressed as mg/ml of the HCl salt; one standard deviation for n = 5 in parentheses)*

| Temperature (°C) | Polymorph A | Polymorph B |
|---|---|---|
| 5 | 0.64 (0.03) | 0.55 (0.07) |
| 15 | 0.95 (0.06) | 0.95 (0.11) |
| 25 | 1.63 (0.06) | 1.58 (0.03) |
| 35 | 2.77 (0.16) | 2.43 (0.09) |
| 45 | 5.68 (0.42) | 4.40 (0.60) |

Student's *t*-test. Fig. 4 shows the data for the two polymorphs, and only the data at 35°C ($1/T = 0.03245$) does not show overlap of the error bar regions, due in part to the low standard deviation of that particular polymorph B point. It cannot be concluded from these data that the equilibrium solubilities of the two polymorphs are different. A combined line for the data ($r = -0.993$) yields an apparent enthalpy of solution of +9.15 kcal/mol.

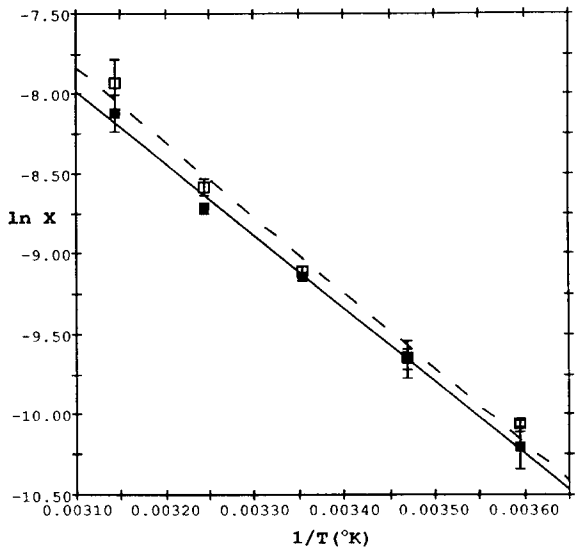


Fig. 4. Van 't Hoff plots for the equilibrium solubility of amiloride HCl dihydrate polymorphs in 0.15% aqueous NaCl. Solubilities of polymorph A (□ — — — □) ($r = -0.993$) and polymorph B (■ ——— ■) ($r = -0.998$) are expressed as mole fractions. Error bars indicate one standard deviation ($n = 5$).

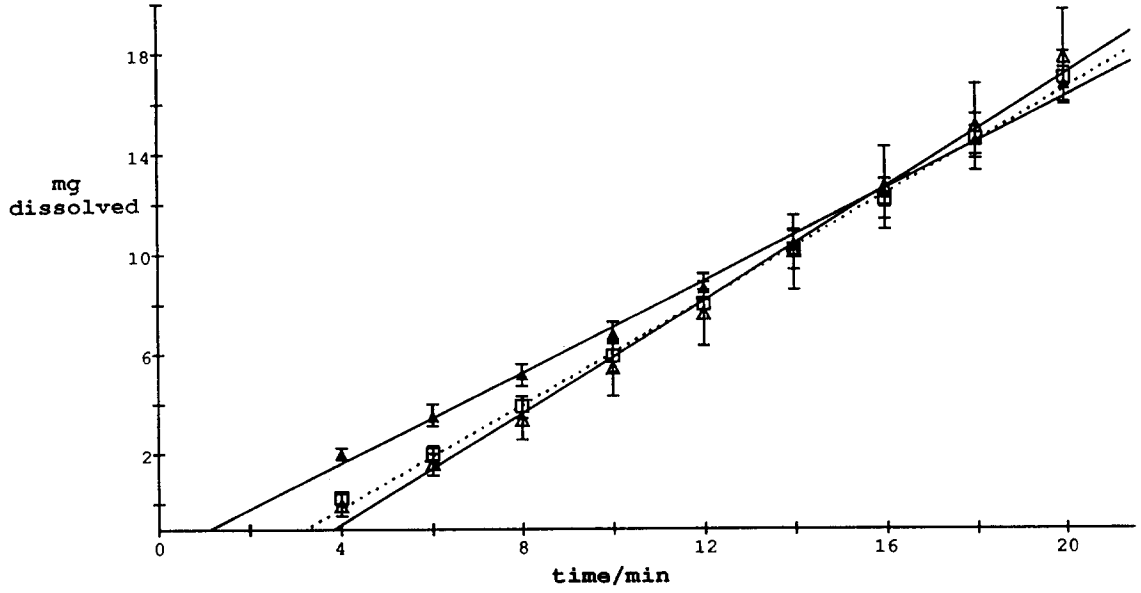


Fig. 5. Intrinsic dissolution plots for the solid-state forms of amiloride HCl in water at 37°C. Rates obtained from the slopes of these lines (in mg/min per $cm^2$): polymorph A dihydrate, 1.1 (△ ——— △); polymorph B dihydrate, 0.9 (▲ ——— ▲); anhydrate 1.1 (□ ... □). Error bars represent one standard deviation of 6 cells tested. The polymorph B disks contained some polymorph A due to conversion during compression (see text).

The intrinsic dissolution data is given in Fig. 5. Both polymorphic dihydrates and the anhydrous form give similar slopes ($1.0 \pm 0.1$ mg/min per $cm^2$) and the data remain linear for at least 20 min after contact with the dissolution medium. As described in the later section on solid state transformation, the polymorph B disks in this study were later found to have partially converted to polymorph A during compression. Nevertheless, if the intrinsic dissolution rates were very different, it would still be detected by this method (only the sensitivity would be greater). When this is taken in concert with the solubility data, the DSC data, and the FTIR patterns, it is apparent that only X-ray powder diffraction is a reliable method of distinguishing these forms.

*Hygroscopicity and the anhydrous form*

Dehydration of the dihydrate under the stated conditions resulted in a crystalline anhydrous phase, defined by the X-ray powder diffraction data given in Table 1. Fig. 2 shows that the anhydrate is composed of the same bundles of rods as the dihydrate phase, but the loss of the water molecules resulted in an increased surface roughness. This may indicate a fracturing of the crystals due to the rapid, high temperature removal of the water from the lattice structure. Fig. 6a shows the X-ray powder diffraction pattern of the anhydrous form obtained upon heating polymorph B (Siegfried lot 9007H902) at 150°C for 40 min. Both dihydrate polymorphs dehydrated to produce the same crystalline anhydrate, confirming the hypothesis from the DSC data. Regardless of the starting material polymorphic composition, subsequent storage of this anhydrate in a 75% RH chamber always caused it to rehydrate to the polymorph A form of the dihydrate. Fig. 6b shows the X-ray powder diffraction pattern of the anhydrate from polymorph B after rehydration for one week. Comparison to Fig. 1 reveals the match to the pattern for polymorph A of amiloride HCl dihydrate.

Fig. 7 plots the number of moles of water per

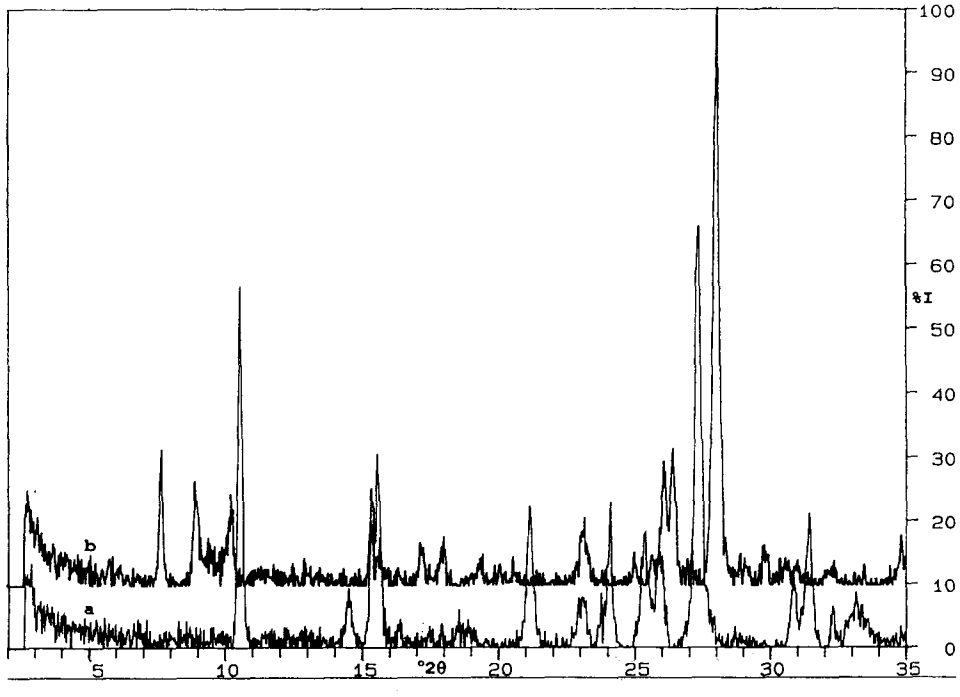


Fig. 6. X-ray powder diffraction patterns of: (a) crystalline anhydrous amiloride HCl made by drying polymorph B dihydrate, and (b) its pattern after rehydration for 1 week at 75% RH (matching polymorph A).

amiloride molecule after 21 days of storage over saturated salt solutions. The dihydrate was stable to weight gain or loss from 15 to 84% RH (both polymorphs behaved similarly, polymorph B only is shown for simplicity). In the dry $CaSO_4$ chamber, there was a gradual loss of water of crystallization, but no separate monohydrate phase could be identified by X-ray powder diffraction. At the highest relative humidity (97%), there was additional uptake of water beyond that required for a dihydrate. The anhydrate was physically stable to weight change only in the $CaSO_4$ chamber. It partially rehydrated at 15 and 33% RH (with less than monohydrate water equivalent) and rehydrated to the dihydrate (polymorph A by X-ray powder diffraction) from 52 to 84% RH. At 97% RH, it showed the same additional water uptake as did the sample starting from the dihydrate phase.

*Analysis of mixed phases by X-ray powder diffraction*

Fig. 8 illustrates the change in the 7 to 11° $2\theta$ portion of the X-ray powder diffraction pattern with composition for mixtures of polymorph A and polymorph B. The trio of peaks characteristic of polymorph A grows in relative intensity to the large peak at 9.5° $2\theta$ due to polymorph B as the mixture is enriched with polymorph A. Changes in the intensity ratios between a crystalline form and an internal standard (Imaizumi et al., 1980; Chrzanowski et al., 1984) have often been used to estimate the amount of a crystalline form in a mixture of solid phases. These estimates are more prone to error when less than 10% of one phase is present, due to problems in defining pure phases or intensity ratios approaching zero. When two crystalline forms are present, the log of their intensity ratio versus composition (actually a sig-

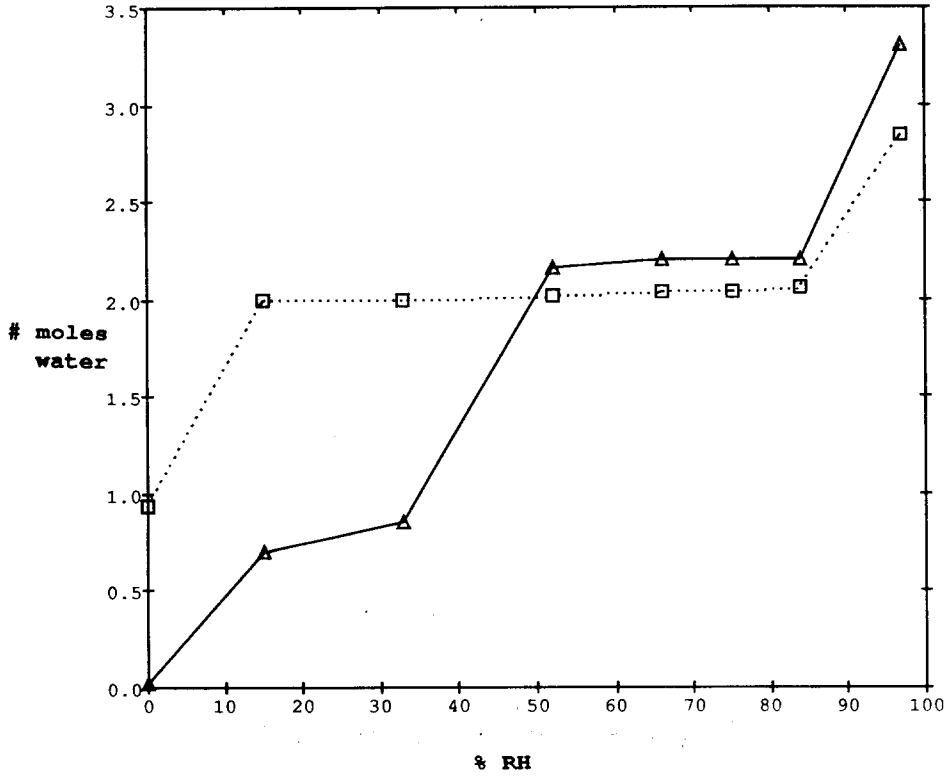


Fig. 7. Equilibrium water content of amiloride solids after 21 days equilibration over saturated salt solutions. Anhydrate (△ ——— △) stored after drying at 150°C for 45 min; dihydrate (□ ... □) was polymorph B as received. Lines are drawn point to point to aid in visualizing the trends only.

moidal function) can be estimated by a linear approximation in the region where appreciable amounts of both forms are present. This technique has been used successfully to estimate the relative percentage of each phase once the region of log-linearity is established (Black and Lovering, 1977; Kaneniwa and Otsuka, 1985; De Villiers et al., 1991).

Fig. 9 shows the log-linear relationship obtained when the intensity of the center peak of the polymorph A trio to the main polymorph B peak is plotted against the percent polymorph A in the mixture, using three replicates of the standard mixtures shown in Fig. 8. The equation defining the straight line from 10 to 90% polymorph A was used to estimate the percentage of each polymorphic dihydrate in amiloride HCl lots received from the five manufacturers (see Table 4). This region provided an acceptable correlation ($r = 0.998$), which can be used to yield an estimate of the relative amount of polymorph A in the mixture. Outside of this region, where the error in this estimate increases, the composition was only estimated to the nearest 5%. The composition of amiloride HCl purchased as USP grade material varies not only by vendor but also by specific lot number. A slight variation in the temperature, rate, or solvent mixture used during the recrystallization of this drug profoundly affects the reproducibility of the polymorphic form. This is not surprising given the similarity in properties observed for these two forms, nor is it of pharmacological significance. However, this does demonstrate that there are aspects of the recrys-

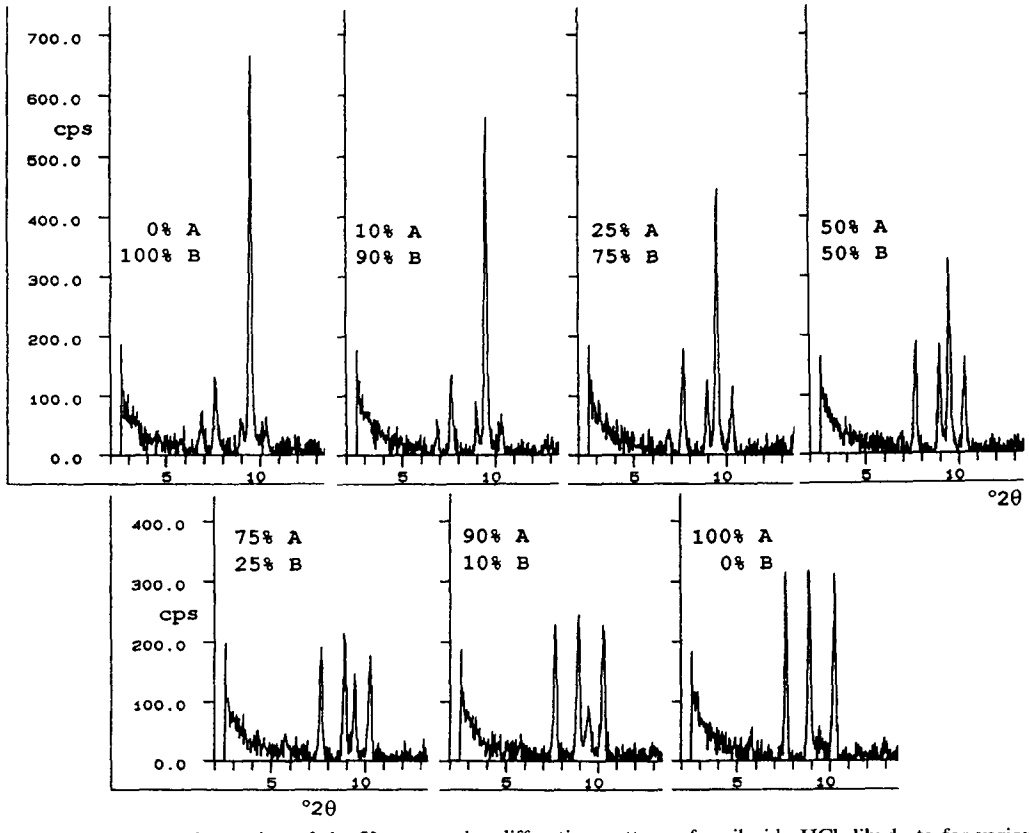


Fig. 8. Change in the low-angle portion of the X-ray powder diffraction pattern of amiloride HCl dihydrate for various physical mixtures of polymorph A and polymorph B.

tallization process that are not completely understood by all of the manufacturers of amiloride HCl USP.

*Solid-state transformations*

X-ray powder diffraction patterns were run on the drug substance after it was subjected to the stresses described previously, and the amount of each polymorph present was estimated using the log-linear relationship given by Fig. 9. The percent conversion by solid-state transformation is presented in Table 5. Polymorph A was unaffected by ball milling, compression, or heating. Polymorph B was partially converted to polymorph A by ball milling (60%), compression (6–69%), or drying to the anhydrate and rehydrating (83%), but was unaffected by air impact micronization. Grinding (Lee and Hersey, 1977, York, 1983; Kaneniwa and Otsuka, 1985) and compression (York, 1983; Chan and Doelker,

TABLE 4

*Relative amounts of each polymorph found by manufacturer and lot number using X-ray powder diffraction*

| Manufacturer | Lot No. | Polymorph mixture |
|---|---|---|
| Merck | 001T051 | 100% A |
| Bioindustria | 149247 | 100% A |
| Sigma | 78F-0024 | 31% A |
| Sifavitor | 214LA87 | 42% A |
| Sifavitor | 099LA89 | 50% A |
| Sifavitor | 064LA90 | < 10% A |
| Sifavitor | 089LA90 | < 5% A |
| Sifavitor | 100LA90 | 56% A |
| Siegfried | 8510H103 | 85% A |
| Siegfried | 9007H902 | 100% B |
| Siegfried | 9007H001 | 100% B |
| Siegfried | 9006H017 | 100% B |
| Siegfried | 9101H100 | < 5% A |
| Siegfried | 9102H102 | > 95% A |
| Siegfried | 9102H104 | 82% A |

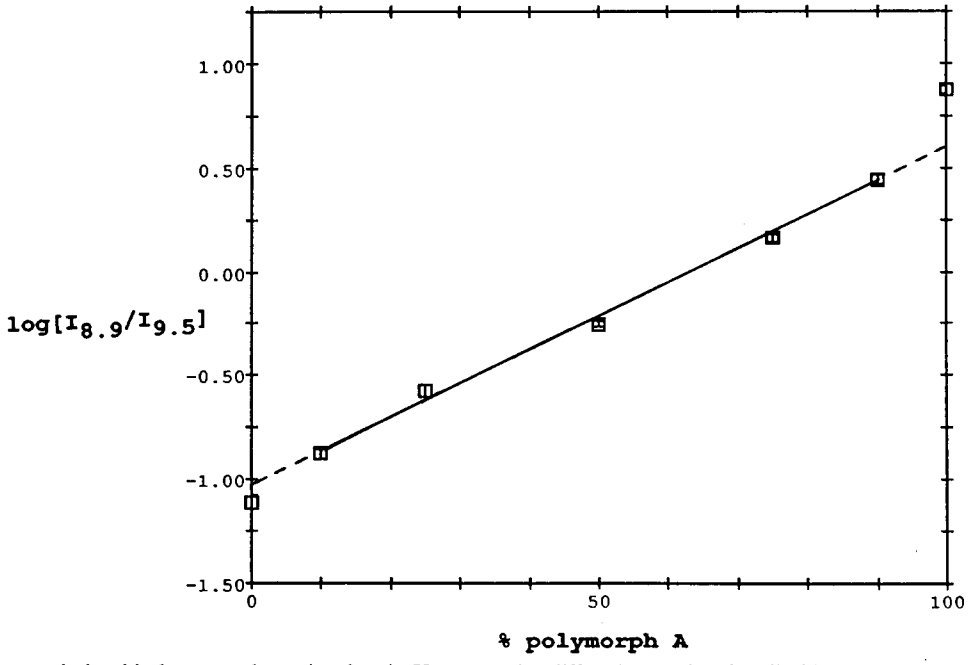


Fig. 9. Log-linear relationship between the ratio of main X-ray powder diffraction peaks of amiloride HCl dihydrate polymorphs and the percent polymorph A used to prepare a standard physical mixture. Error bars based on one standard deviation of three replicates; line valid for 10–90% polymorph A ($y = 0.016x - 1.019$) with $r = 0.998$.

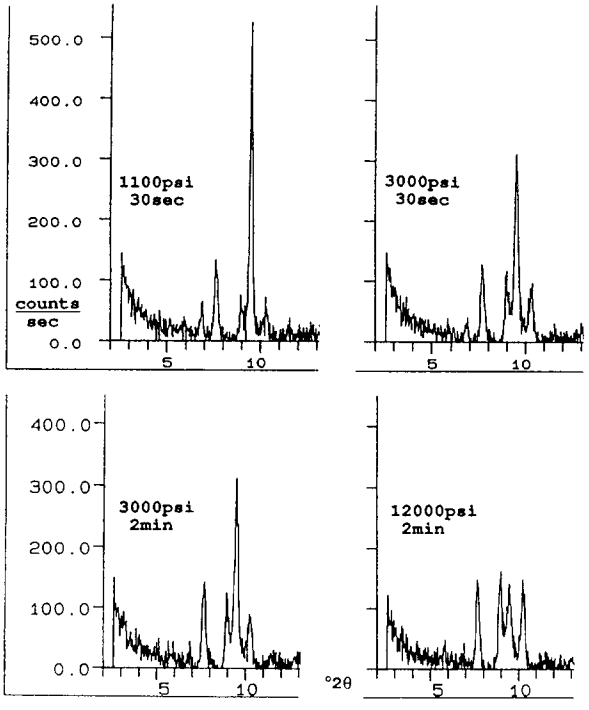


Fig. 10. Effect on compression force and dwell time on the X-ray powder diffraction pattern of polymorph B (Siegfried lot 9007H902).

1985; Lefebvre et al., 1986) have been shown to cause such transformations in other drug substances. Fig. 10 shows the effect of compression force and total time under pressure (dwell time) on the amount of polymorph B which is transformed to polymorph A. The force greatly affected the amount converted, but a dwell time increase from 30 s to 2 min did not cause more conversion at 3000 lb/inch$^2$. For intrinsic dissolution measurements, 3000 lb/inch$^2$ was found to be necessary for the tablet to hold together, so the data for polymorph B actually represents the dissolution of a tablet with 40% polymorph A by weight. This was also the reason why FTIR spectra were taken by the IR microscope, since KBr pellets or grinding in mineral oil might have masked any difference in FTIR spectra that may have been present.

Drying polymorph B at 150°C for 40 min and rehydrating under 75% RH caused 83% conversion to polymorph A. The same treatment did not change polymorph A, and polymorph B was physically stable to long term storage at 75% RH. Drying polymorph B at 100°C for 30 min, resulting in only a 1% decrease in weight, did not cause any conversion to polymorph A after rehydration at 75% RH. Therefore, it appears to be necessary to go through the anhydrous state when transforming polymorph B to polymorph A by heating. Although the physical properties of the two dihydrate forms are very similar, transformations under stressed conditions show that polymorph A is the thermodynamically stable form at room temperature.

TABLE 5

*Solid-state transformations induced in amiloride HCl dihydrate powders by various physical stresses*

| Initial polymorph | Stress treatment | % conversion |
|---|---|---|
| A | ball milling 60 min | 0 |
| B | ball milling 60 min | 60 |
| A | compression 12000 lb/inch$^2$/2 min | 0 |
| B | compression 12000 lb/inch$^2$/2 min | 69 |
| B | compression 3000 lb/inch$^2$/2 min | 35 |
| B | compression 3000 lb/inch$^2$/30 s | 41 |
| B | compression 1100 lb/inch$^2$/30 s | 6 |
| B | micronization (air impact) | 0 |
| A | 150°C/45 min, rehydrate 75% RH | 0 |
| B | 150°C/45 min, rehydrate 75% RH | 83 |
| B | 100°C/30 min, expose to 75% RH | 0 |

## Conclusions

Amiloride HCl is physically stable under normal storage conditions only in the dihydrate state, and this is the commercially available form. Either polymorphic dihydrate may be received when purchasing USP grade material, and this can vary by both choice of vendor and manufacturing date of the batch. The two polymorphs can be distinguished by X-ray powder diffraction, but are identical with respect to solubility, dissolution rate, FTIR spectra, melting points, and hygroscopicity. The percent of each polymorph in mixed systems can be estimated by X-ray powder diffraction, and this can be used to show that polymorph B transforms in the solid state to

polymorph A under conditions of stress (grinding, compression and heating to the anhydrate).

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