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Research Paper

General Trends in the Desolvation Behavior of Calcium Salts

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Purpose. This paper is concerned with the solid-state characterization of dehydrated calcium salts as well as the effect of dehydration on the physical properties of these salts.
Methods. The salts were analyzed by X-ray powder diffraction (XRPD), single crystal X-ray and polarized light microscopy (PLM).
Results. Our research was able to identify three general behaviors of the desolvation of calcium salts of carboxylic acids. Upon desolvation, ethanolate and pentahydrate calcium indomethacin (CaIndo and CaInd2, respectively) stayed crystalline, fenoprofen calcium (CaFEN), calcium ketoprofen (CaKTN), calcium salicylate (CaSAL), calcium mefenamate (CaMEF) and calcium tolfenamate (CaTOLF) became mesophases, while calcium diflunisal became partially crystalline. On the other hand, the solubility studies of CaFEN, CaKTN and CaSAL showed that all dehydrated calcium salts had higher solubility than their crystalline counterparts and amorphous CaKTN had higher solubility than mesomorphous CaKTN.
Conclusions. Several factors influence the desolvation behavior of calcium salts. We believe the flexibility of the benzene rings in CaKTN, CaFEN, CaMEF and CaTOLF was important for these products to become mesomorphous when they loose their crystalline water; meanwhile, CaDIF where the two benzene rings are coplanar remained crystalline when heated. Additionally, the existence of water channels and the hydrogen bonding networks in the crystals is hypothesized to play an important role in the desolvation behavior of these materials.

KEY WORDS: calcium salts; dehydration; liquid crystal; mesomorphous.

INTRODUCTION

Calcium salts are important pharmaceutical materials. In our laboratory we have begun a study of the structure and chemistry of calcium salts of organic drug molecules. Our strategy is to develop an understanding of these materials as a step in developing a fundamental understanding of a wide range of pharmaceutical materials.

A search of the 13th Edition of the Merck Index (Electronic Edition) reveals that there are 46 calcium salts listed of which about 29 are salts of carboxylic acids. One of these, fenoprofen calcium, has been previously studied and is believed to form liquid crystals upon dehydration. Additionally, a recent paper from our laboratory characterizes the mesophases (liquid crystals) produced by dehydrating calcium benzoate trihydrate (1). In the present paper we have looked at simple Ca salts of compounds in the Merck index and others that were not included in the Merck index.

According to the United States Pharmacopeia (USP), solids are either crystalline, non-crystalline (amorphous) or a mixture of the two (2). However, the USP also stated that "It is also possible for order to exist in only one or two dimensions, resulting in mesomorphic phases (liquid crystals)" (2). In recent years, a wide range of investigators realized that there were pharmaceutical solids with order between that of an amorphous material and that of a crystalline material. Moreover, additional insight into the structure of crystalline and amorphous compounds has been provided by Desiraju (3) who showed that in fact solids exist as a continuum between the amorphous and crystalline states.

Liquid crystals and mesophases occupy an intermediate point in this continuum. There are two major types of liquid crystals reported in the pharmaceutical literature, lyotropic, the most common, formed upon dilution of the crystals with a solvent, usually water, and thermotropic formed upon heating and desolvating the original crystals. It has been estimated that 5% off all organic compounds exhibit thermotropic mesomorphism (4).

Examples of pharmaceutical liquid crystals include salvarsan (nematic mesophase) (4), disodium chromoglycate or cromolyn sodium (thermotropic hexagonal and nematic mesophases) (4,5), L-660, 711, a Leukotriene D4 receptor antagonist (lyotropic) (6), nafoxidine HCl (hexagonal, cubic, lamellar mesophase), flufenamic acid (lamellar mesophase). Several non-steroidal anti-inflammatory drugs (NSAIDs) including ketoprofen and diclofenac form lyotropic lamellar mesophases in binary systems with water at a certain temperature range (4), fenoprofen calcium (thermotropic reversed hexagonal) (7) and cyclosporine (thermotropic) (8).

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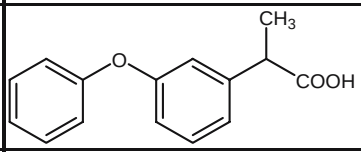
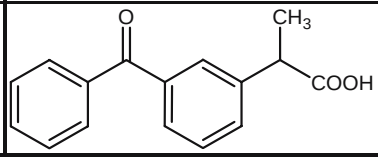
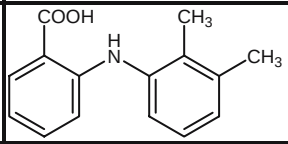
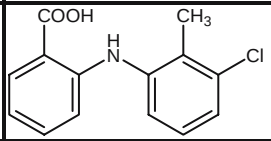
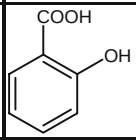
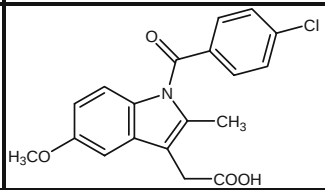
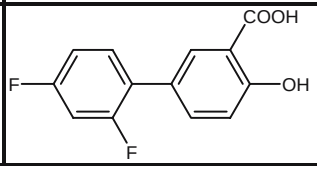
Table I. Elemental Analysis of Ca Salts Investigated

Name	C calculated/actual	H calculated/actual	MW	Percent purity based on Carbon
CaKTN-crystals	65.98/65.88	5.19/5.22	582.58	99.84
CaKTN-anhydrous	70.32/69.71	4.80/4.75	546.54	99.13
CaFEN-anhydrous	68.95/68.68	5.01/5.22	522.60	99.61
CaFEN-crystals	As is (Sigma)	As is (Sigma)		
CaSAL-crystalls (Wako)	48.0/47.57	4.03/3.99	350.34	99.10
CaTOLF-anhydrous	59.90/59.14	4.23/3.81	561.46	98.73
CaMEF-anhydrous	69.21/68.88	5.42/5.39	520.64	99.52
CaDIF-anhydrous	58.00/56.71	2.62/2.71	538.47	97.78
CaInd-pentahydrate	54.10/54.28	4.78/4.46	843.72	100.3

As exposure of pharmaceuticals to heat and energy cannot be avoided in many operations in pharmaceutical manufacturing and handling, it is very important to identify and investigate pharmaceutical materials that undergo a transformation to thermotropic mesophases and explore their physical and chemical properties.

Many papers in the pharmaceutical literature discussed different methods to characterize liquid crystals and these methods include XRPD, SAXS, (8,9) MDSC, PLM, tetrahertz pulsed spectroscopy, (10) and FTIR (11). However, very few articles discussed the physical and chemical properties of liquid crystalline drugs (12,13). Patterson *et al.*

Table II. The Calcium Salts of these Acids are Investigated in this Paper

Salt's Name	Acid's Structure
Fenoprofen Calcium	
Calcium Ketoprofen	
Calcium Mefenamate	
Calcium Tolfenamate	
Calcium Salicylate	
Calcium Indomethacin	
Calcium Diflunisal	

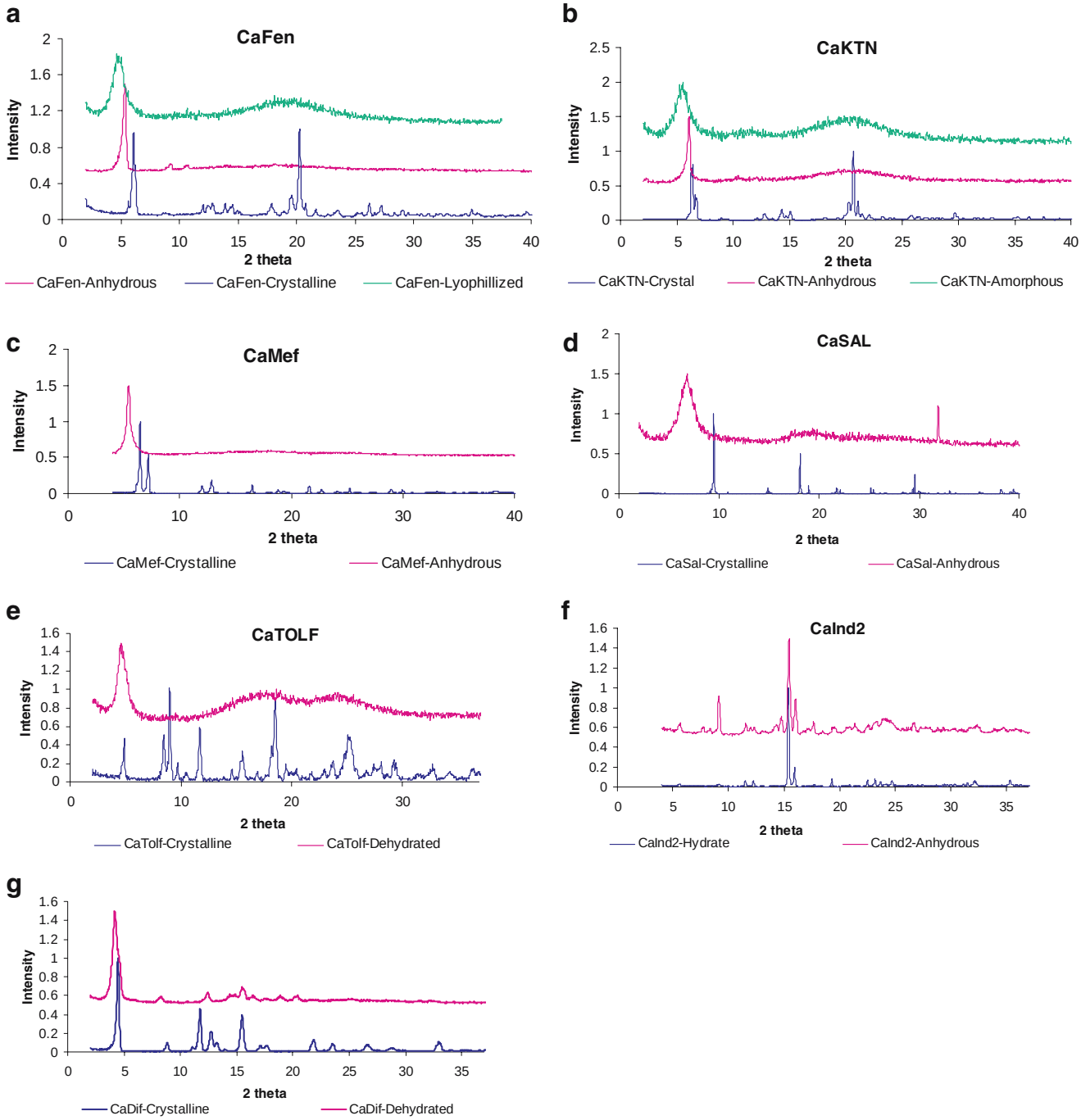


Fig. 1. XRPD patterns of (a) CaFEN, (b) CaKTN, (c) CaMEF, (d) CaSAL, (e) CaTOLF (f) CaInd2. The *top patterns* are of the desolvated salts and the *bottom patterns* are of the crystalline salts and (g) XRPD of crystalline and anhydrous CaDIF. The intensity units are arbitrary units and all X-ray intensities are normalized.

reported that liquid crystals of fenoprofen calcium have higher solubility than the crystalline form of this salt (4). Moreover, Lechuga–Bellesteros *et al.* showed that liquid crystalline state can have a profound influence on physical stability and that liquid crystalline tobramycin freebase/sulfate was more stable and less sensitive to moisture than its amorphous counterpart (9). This suggests that liquid crystalline calcium salts can be used to enhance the solubility of those carboxylic acid salts without sacrificing stability. Put another way, this suggests that liquid crystalline calcium salts can be used as part of an overall strategy to utilize the solid state to control the release rate of a biologically active molecule from a formulation.

In this study, several pharmaceutically relevant organic calcium salts were investigated. The liquid crystalline (mesophase) behavior of the desolvated calcium salts was characterized using conventional methods, like XRPD and polarized light microscopy, and the solubilities of both parent hydrated Ca salts and the dehydrated materials were measured and compared to those of their crystalline counterparts.

Table III. Summary of the Crystal Structures of CaFEN, CaKTN, CaMEF, CaTOLF, CaIndo, and CaInd2

Name	Space group	Number of calcium–oxygen coordinates	Channels	Hydrate/solvate	Becomes a mesophase upon dehydration
Fenoprofen calcium (CaFEN)	$P2_1/n$ (#14)	8	No	Dihydrate	Yes
Calcium ketoprofen (CaKTN)	$P2_1/n$ (#14)	8	No	Dihydrate	Yes
Calcium mefenamate (CaMEF)	$P2_1/c$ (#14)	8	No	Dihydrate	Yes
Calcium tolfenamate (CaTOLF)	P1 (#2)	8	Yes	Trihydrate	Yes
Calcium indomethacin (CaIndo)	C2/c (#15)	8	Yes	Diethanolate	No
Calcium indomethacin (CaInd2)	Pbca (#61)	8	Yes	Pentahydrate	No
Calcium salicylate (CaSAL)(15)	C2/c (#15)	8	No	Dihydrate	Yes
Calcium diflunisal (CaDIF)	Structure not solved				No

EXPERIMENTAL AND METHODS

Materials

Fenoprofen calcium, ketoprofen, indomethacin, tolfenamic acid, diflunisal, and mefenamic acid were obtained from Sigma (St. Louis, MO), calcium salicylate was obtained from Wako (Japan, Lot# CER1965).

Calcium ketoprofen, calcium mefenamate, calcium tolfenamate, calcium diflunisal and calcium indomethacin were synthesized by dissolving aproximately 0.01 mol of sodium hydroxide in 50 ml of 10% solution of ethanol in water; then a slightly larger molar quantity of the acid (about 0.015 mol) was added. The suspension was stirred for 1 h at 45–50°C then cooled to room temperature and the excess of acid was filtered. Then, 10 ml of 1.0 M aqueous calcium chloride solution was added at once and the precipitate was stirred for one to two hours at room temperature. The solids were then filtered and washed several times with water.

The salts were crystallized as follows:

Fenoprofen calcium (CaFEN): crystallized from H_2O/EtOH 50/50 w/w saturated solution.
Calcium ketoprofen (CaKTN): crystallized from H_2O/EtOH 50/50 w/w saturated solution.
Calcium mefenamate (CaMEF): crystallized from H_2O/PrOH 70/30 w/w saturated solution.
Calcium tolfenamate (CaTOLF): crystallized from H_2O/EtOH 50/50 w/w saturated solution.
Calcium salicylate (CaSAL): used as is.
Calcium Indomethacin (CaIndo): crystallized from H_2O/EtOH 50/50 w/w saturated solution.
Calcium Indomethacin (CaInd2): crystallized from saturated aqueous solution.
Calcium Diflunisal (CaDIF): crystallized from H_2O/EtOH 80/20 w/w saturated solution.

The purity of all synthesized salts was determined by elemental analysis (see Table I).

Mesophases were generated by drying the crystalline salts in an oven at 120°C for 1 h (CaKTN and CaFEN) or in vacuum oven at 100°C for 3 to 72 h.

Preparation of the Amorphous Samples

Aqueous solution of fenoprofen calcium (2.3 mg/ml) was sonicated for 2 min then it was lyophilized to obtain the amorphous form of this salt using Dura Stop freeze dryer (FTS Systems, Inc., Stone Ridge, NY). Aliquots of 1 ml of aqueous solution of fenoprofen calcium were placed into 10 ml vials and were frozen at −40°C for 10 h and dried (primary drying) at −40°C for 48 h at 70 mTorr pressure. The secondary drying was conducted at 25°C for 12 h under 10 mTorr pressure. Amorphous calcium ketoprofen was prepared by quench cooling of the melt in liquid nitrogen.

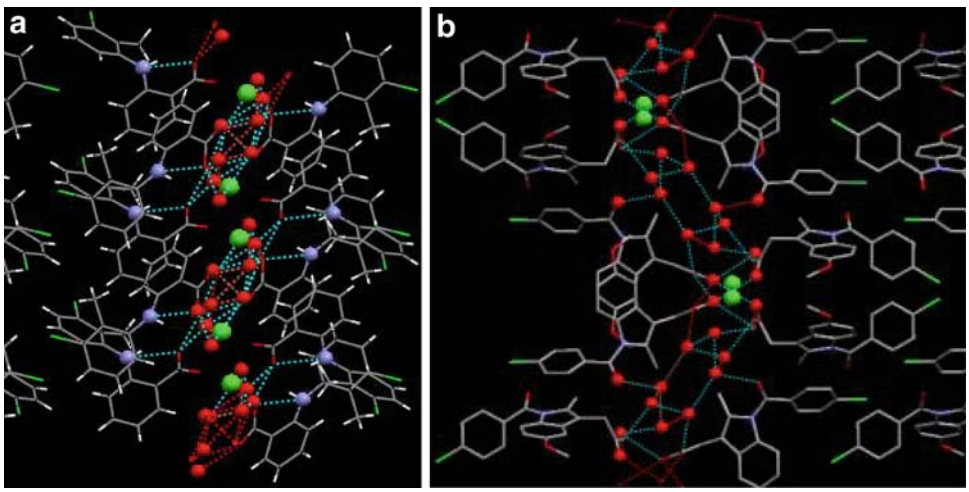


Fig. 2. (a) Crystal packing and hydrogen bondings of calcium tolfenamate seen along *axis b*. (b) Crystal packing and hydrogen bondings of calcium indomethacin pentahydrate seen along *axis b*.

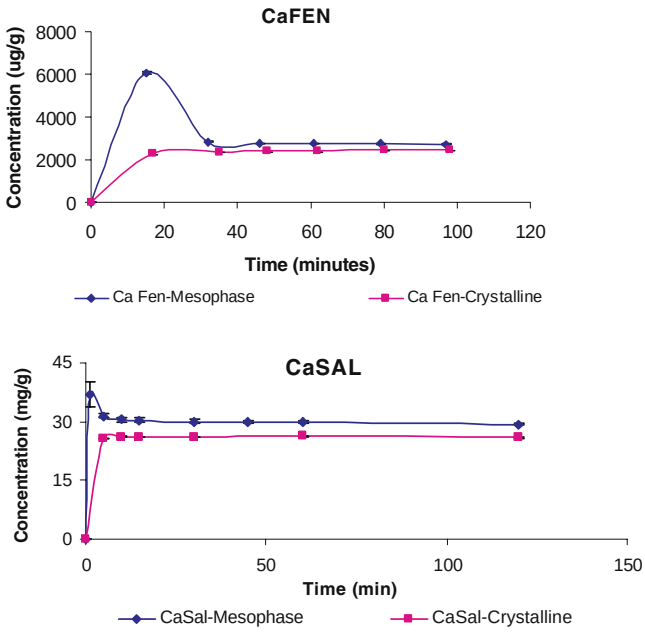


Fig. 3. Solubility profiles of crystalline and mesomorphous CaFEN and CaSAL.

HPLC Analysis

An Agilent 1100 Series HPLC system from Agilent Technologies, Inc. (Waldbronn, Germany), was used. The column was 5 μ Zorbax SB-C18 4.6×250 mm. The column was kept at 30°C.

The mobile phase consisted of acetonitrile, water, and glacial acetic acid (0.58:0.38:0.04).

The flow rate was 1 ml/min, the injection volume was 10 μl, and the UV detection wavelength was 280 nm.

Quantitative analysis was based on the peak area using external standards.

Solubility Studies

The solubilities of CaKTN, CaFEN and CaSAL were determined at 25 ± 0.1°C. Suspensions of the calcium salts in water were stirred using a magnetic stirrer. At approximately 10 to 15 min intervals about 1 ml of the sample was withdrawn using a syringe and filtered using a 0.45 μm filter and diluted and analyzed by the above mentioned HPLC method. Three samples (n = 3) were measured for each point of the solubility profile.

Single Crystal X-ray

Data collection of the Calcium salts was performed with Mo Kα radiation (λ = 0.71073) on a Nonius Kappa CCD equipped with graphite crystal, incident beam monocromator.

Cell constants for data collection were obtained from least-square refinement.

Elemental Analysis

The elemental analysis experiments were performed by the Microanalytical Laboratory at the Chemistry Department, Purdue University, West Lafayette, Indiana.

XRPD

XRPD was performed on a Siemens D500 diffractometer. It was operated at ambient temperature in the step scan mode using a 2θ step size of 0.04 and a count time of 4 s. The instrument was equipped with a graphite diffracted beam monochromator and copper radiation source. The XRPD pattern was collected by measuring the scintillation response to CuKα radiation versus 2θ value over a 2θ range of 3–40°.

VTI

Vapor sorption isotherms of CaKTN, CaFEN and CaSAL were generated using a Symmetrical Gravimetric Analyzer (SGA-100) (VTI Corporation, Hialeah, FL) at 25°C.

For all samples the total weight was in the range of 20–25 mg and samples were not dried in the analyzer before the start of the experiment. The isotherm equilibrium criterion was 0.01% wt.% in 5 min with a maximum equilibrium time of 180 min.

Humidity Studies

The humidity studies were performed by placing vials containing about 0.5 g of the salt in desiccators over saturated solution of different salts to provide controlled relative humidities. Then, the desiccators were placed in an oven at 40 ± 0.5°C and the samples were analyzed by PXRD to evaluate the degree of crystallinity of each sample at different time intervals.

RESULTS AND DISCUSSION

There are few articles in the pharmaceutical literature that discuss thermotropic mesophases (12,13), as well as few articles that are devoted to the study of calcium salts.

Our study of organic calcium salts explored the behavior of calcium salts upon desolvation and showed that significant number of calcium salts became mesophases upon dehydration. Table II shows the structures of the parent compounds of all the salts investigated in this study. The calcium salts of ketoprofen, fenoprofen, mefenamic acid, tolfenamic acid, and salicylic acid were hydrates and became mesophases upon dehydration. Besides, in this study we investigated the effect of desolvation on different organic calcium salts (CaIndo,

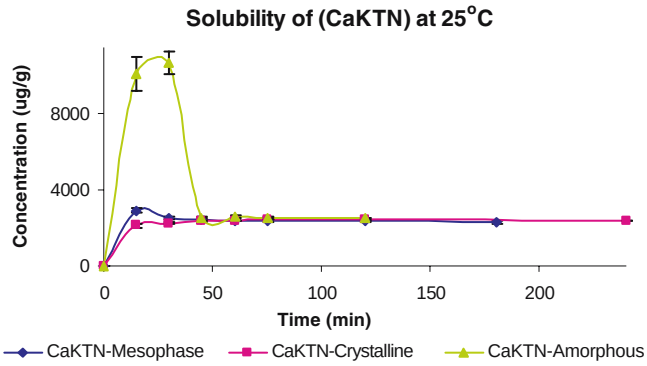


Fig. 4. Solubility profiles of amorphous, mesomorphous, and crystalline CaKTN.

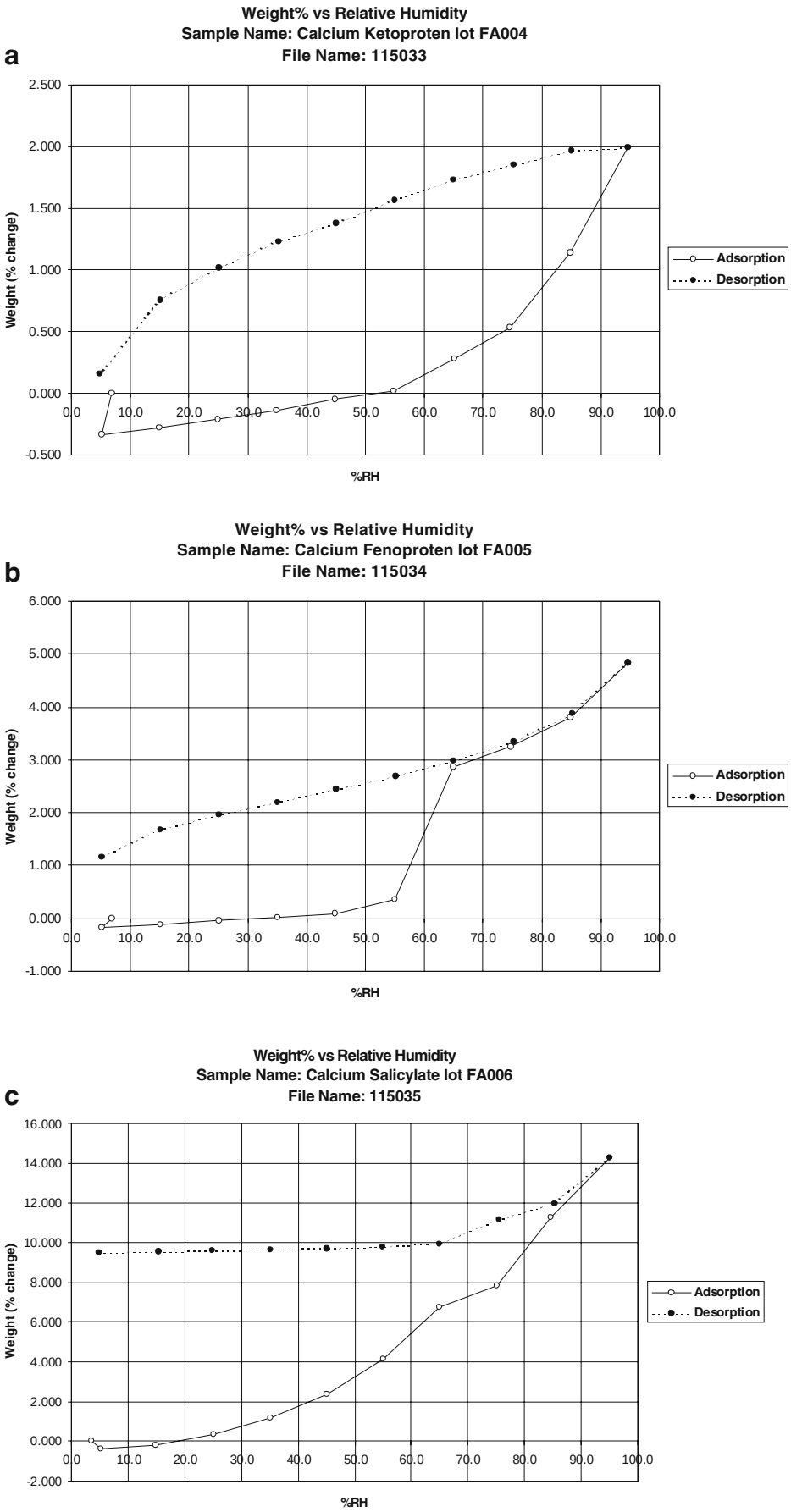


Fig. 5. Moisture sorption/desorption data for (a) CaKTN, (b) CaFEN, and (c) CaSAL.

CaInd2 and CaDIF) and the effect of dehydration on the solubility of CaSAL, CaKTN and CaFEN.

Elemental Analysis

The crystalline samples all gave elemental analysis values which are quite close to the calculated values (Table I). This, along with the fact that a well known starting material (the free acid), was used establishes the structure. Three of the anhydrous forms (CaKTN, CaTOLF, and CaDIF) showed somewhat large discrepancies between the calculated and observed carbon percentages. Two of these materials showed a difference of more than 1% between the calculated and observed. These differences were always to the low side; that is, the actual value was always lower than the observed value. Such discrepancies are common when the anhydrate form is not fully dehydrated. In such cases, residual water, which is difficult to remove, causes the %C to be low. In conclusion, these data establish that the materials of interest are authentic Ca salts of the structures shown.

X-Ray Data

Figure 1 shows the XRPD of the crystalline and the desolvated fenoprofen calcium, calcium ketoprofen, calcium salicylate, calcium mefenamate, calcium tolfenamate, calcium diflunisal and calcium indomethacin pentahydrate. The X-ray powder diffraction patterns of desolvated CaKTN, CaFEN, CaTOLF and CaMEF have Bragg peaks at low two theta angles indicating the existence of two-dimensional structures (13). This conclusion was backed by polarized light microscopy (PLM) study where all the above mentioned dehydrated salts were birefringent. We hypothesize that the removal of crystalline water forces the carboxylic acids to reorganize around the calcium ions creating two-dimensional lamellar structures. This is consistent with Density Functional Theory calculations which show that each successive O–Ca interaction is very high energy up to a Ca coordination of at least 6 (14).

We believe that the mobility of the two benzene rings in the studied systems plays an important role in their dehydration behavior. CaKTN where the two benzene rings are linked by a ketone (C=O), CaFEN where the two rings are linked by an ether (–O–), and CaMEF and CaTOLF where the benzene rings are linked by an amine all became mesomorphous upon dehydration. On the other hand, calcium diflunisal (the benzene rings are directly attached) became partially disordered upon heating regardless of the heating and dehydration methods used (Fig. 1g).

Dehydrated calcium salicylate (CaSAL) was birefringent under polarized light however, its X-ray powder diffraction showed a slightly diffused Bragg peak in the low two theta field and had a diffused peak around 19 two theta and a small sharp Bragg peak around 32 two theta suggesting that the salt has maintained some short range order and lost its long range order.

The XRPD patterns of amorphous CaFEN and CaKTN were not birefirngent and had no Bragg peaks except one diffuse peak at around 5 two theta indicating that there was a very little order and that the systems were not completely isotropic.

Table III summarizes the single crystal X-ray data and the thermal behavior of several calcium salts. In general, it appears that crystals that do not have water channels are more likely to become mesophases upon dehydration than crystals with water channels. Thus, the crystal structures of CaFEN, CaKTN, and CaMEF do not have water channels and become mesophases upon dehydration. On the other hand, calcium indomethacin ethanolate and calcium indomethacin pentahydrate have water channels and do not become mesomorphous upon desolvation. Calcium tolfenamate trihydrate is an exception to this trend and contains a water channel yet becomes a mesophase upon dehydration. This shows that the existence of water channels is not essential for a calcium salt to become mesomorphous upon desolvation.

We believe that the networks of hydrogen bonds are very important in dictating the desolvation behavior of these materials. For example, for calcium tolfenamate trihydrate (see Fig. 2a) all of the water molecules form hydrogen-bond bridges between the nitrogen atoms on the opposite sides of the calcium plane. As suggested above, we believe that these hydrogen-bond bridges will collapse upon the removal of water molecules causing the molecules to rearrange in a relatively random manner creating a two dimensional lamellar structure.

On the other hand, CaInd2 which has water channels and does not become a mesophase shows a different hydrogen bonding pattern (see Fig. 2b). The hydrogen bonds in CaInd2 have a zigzag pattern and beside the carboxylic acid oxygens only the ketone oxygen atoms are involved in hydrogen bonding with the water molecules. However, the close packing of the molecule allows very little movement of the benzene ring and thus the removal of water and the breakage of the hydrogen bonding system do not significantly impact the crystal structure of the salt.

Solubility Studies

The solubilities of anhydrous CaFEN, CaKTN, and CaSAL were higher than their crystalline counterparts (Figs. 3 and 4). All the solubility profiles peaked before they decreased due to the crystallization (this was confirmed by analyzing the precipitated powder by XRPD). As for amorphous samples, only the solubility of the amorphous CaKTN was studied (Fig. 4) due the difficulties we encountered obtaining sufficient amounts of amourphous CaFEN and CaSAL. As expected, the solubility of amorphous CaKTN was higher than that of liquid crystalline CaKTN because of the existing molecular order of the mesomorphous phase and the higher energy state of the amorphous phase.

Stability Studies

The mesomorphous CaFEN and CaSAL salts, as evident by XRPD, recrystallized when placed at 75% relative humidity and 40°C in less than 24 h. However, CaKTN remained a mesophase for more than a month due, probably, to the structure collapse when the crystalline water was removed, causing the hydrophilic groups (the oxygen atoms) to move inward closer to the calcium ion meanwhile the hydrophobic groups (the benzene rings) formed a shell that prevented water from reaching the calcium ion.

The moisture sorption and desorption data were consistent with the results of the stability studies. Figure 5 shows a hysteresis in the sorption/desorption profiles for all salts. However, the end point of CaFEN and CaSAL was consistent with the formation of crystalline hydrates; meanwhile the CaKTN profile was not consistent with the formation of the crystalline hydrate. The sample gained a maximum of about 2% water (the sorption curve) and gave most of this water up at lower relative humidity (the desorption curve).

CONCLUSIONS

The flexibility of the benzene rings in CaKTN, CaFEN, CaMEF, and CaTOLF was important for these products to become mesomorphous when they loose their crystalline water.

Besides, the existence of water channels and the hydrogen bonding networks in the crystals played an important role in the desolvation behavior of these calcium salts.

On the other hand, mesomorphous calcium salts have higher solubility than their crystalline counterparts and mesomorphous CaKTN has less solubility than its amorphous form.

This work suggests a strategy for classifying and predicting which calcium salts are likely to form mesomorphic phases upon thermal dehydration. This strategy would involve: (1) Determining the crystal structure of the hydrated material, and (2) analyzing the crystal packing. If water tunnels are present it is possible to form dehydrated materials that are not liquid crystals. If water tunnels are not present, then mesomorphic phases are likely to form upon thermal dehydration.

ACKNOWLEDGMENTS

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