Solid-State Behavior of Cromolyn Sodium Hydrates

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Abstract □ Cromolyn sodium (CS, disodium cromoglycate) is an antiasthmatic and antiallergenic drug. The solid-state behavior of CS is still not completely understood. CS forms nonstoichiometric hydrates and sorbs and liberates water in a continuous manner, although with hysteresis. The reported continuous changes in crystal lattice parameters of CS, which are associated with the changes in water stoichiometry, renders CS physically variable, which may complicate formulation and processing. In addition, controversies still remain as to whether CS exists as different stoichiometric hydrates, mainly because of its variable powder X-ray diffraction (PXRD) patterns (Cox, J. S. G. et al. *J. Pharm. Sci.* **1971**, *60*, 1458–65), which indicates a variable crystal structure. The objectives of this study are (a) to understand this unusual water uptake in the light of the molecular and crystal structures of CS, (b) to understand the relationship between the crystal structure and the PXRD patterns using Rietveld analysis, and (c) to investigate whether CS exists as different stoichiometric hydrates. The crystal structure of CS containing 6.44 molecules of water per molecule of CS was determined at 295 and 173 K. The packing arrangements in these structures (space group $P\bar{1}$) are similar to those in a previous report, in which the water stoichiometry is 5 to 6, but the bond lengths, bond angles, and lattice parameters are different, reflecting the different water stoichiometries. In the crystal structure solved at 295 K, the position of only one of the two sodium ions could be determined. In the crystal structure solved at 173 K, the previously undetermined sodium ion is disordered over three sites, while four of eight water positions are partially occupied. The 2-hydroxy-propane chain that links the two cyclic moieties of CS was found to be flexible, perhaps allowing the CS crystal to accommodate variable amounts of water. The lack of a fixed coordination site for the second sodium ion may contribute to the disorder of the water molecules. The nonstoichiometric water content of CS is mainly attributed to the water molecules that are associated with the two unoccupied sodium sites. From the PXRD patterns of CS powder, equilibrated at various relative humidities, the various lattice parameters, including previously unreported α, β, and γ values, were calculated using Rietveld analysis. The peak shifts in these PXRD patterns are quantitatively explained by slight changes in the unit cell parameters. The recently described solid forms of CS were prepared and were found to correspond to the original crystalline CS, described by Cox et al. (1971), but with contamination by the known M mesophase in various proportions. The present results support a variable crystal structure and not the existence of different stoichiometric hydrates of CS.

Introduction

Background—Cromolyn sodium (CS, the disodium salt of 1,3-bis(2-carboxychromon-5-yloxy)-2-hydroxypropane, also

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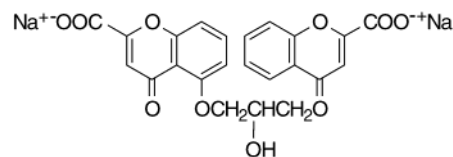‖ Inhale Therapeutics Systems.

Figure 1—Molecular structure of cromolyn sodium.

known as disodium cromoglycate, Figure 1), was introduced as an antiasthmatic and antiallergenic drug in 1967.[1] Despite a history of more than 30 years, the solid-state behavior of CS is still not completely understood.

In 1971, Cox et al.[2] reported that CS exists as two liquid crystalline phases (M and N) and a crystalline hydrate phase that sorbs and liberates water continuously and reversibly (although with a hysteresis loop) to give an infinite series of nonstoichiometric hydrates with a limiting composition of about 9 molecules of water per molecule of CS. The nonstoichiometric hydrate is physically unstable, in the sense that its composition changes readily as a function of the environmental relative humidity (RH). This potential for change may complicate formulation and processing. Understanding the reasons why cromolyn sodium forms nonstoichiometric hydrates, from the perspective of molecular and crystal structures, may help future preformulation and formulation of nonstoichiometric hydrates.

No polymorph of the crystalline hydrate phase was found by Cox et al.[2] The PXRD pattern of this phase was observed to change considerably with the surrounding RH, which the authors attributed to lattice expansion upon water sorption. In the same work, the lattice parameters, *a*, *b*, and *c*, of CS, were successfully calculated for RH values from 0 to 88%. However, only the changes in these lattice parameters *a*, *b*, and *c* were used to account for the shift in the PXRD peak positions. These changes alone could neither account for the peaks at small 2θ angle that correspond to *d*-spacings much larger than *a*, *b*, or *c*, nor for the observed appearance and disappearance of some PXRD peaks, because all six parameters, *a*, *b*, *c*, α, β, and γ, are actually influential. Further study is therefore needed to understand how the properties of the crystalline hydrate phase of CS change in response to the surrounding RH and to explain fully the changes in the PXRD patterns in the light of the molecular and crystal structures.

The crystal structure of cromolyn sodium with 5 or 6 water molecules per CS molecule was partially solved at room temperature by Hamodrakas et al. in 1973.[3] Unfortunately, the three-dimensional coordinates of the crystal structure were not reported or registered in any structural database. Hamodrakas et al.[3] located the positions of only one sodium ion and two water molecules and found that the other sodium ion and water molecules were disordered. Therefore, to further understand the crystal structure and to correlate it with the physical properties, redetermination of the crystal structure is necessary.

The detailed analysis of the two liquid crystalline phases, M and N, of CS, was provided by Hartshorne and Woo-

Table 1—Unit Cell Parameters[a] (space group P1) of Cromolyn Sodium Hydrate Equilibrated at Various Relative Humidities (RH)[8]

RH (%)	moles of water[b]	aqueous solution[8]	a (Å)	b (Å)	c (Å)	α (deg)	β (deg)	γ (deg)	V (Å³)	R_p[c] (%)	R_{wp}[d] (%)
0	0	pure P_2O_5		∼11.08	∼14.6	∼92.4					
6.4	2–3	LiBr	3.79	11.07	15.21	90.11	97.41	94.21	631	13.32	19.25
11.3	3–4	LiCl	3.84	11.10	15.31	90.30	97.25	94.31	646	14.67	21.89
21.6	4–5	CH_3COOK	3.91	11.17	15.41	90.05	97.60	94.37	665	10.00	12.14
32.8	4.5–6	$MgCl_2$	3.92	11.18	15.42	90.11	97.60	94.55	668	14.35	18.43
57.5	5.5–6.5	NaBr	3.93	11.19	15.55	90.07	97.22	94.63	676	7.12	9.02
75.3	6.5–7.5	NaCl	3.96	11.19	15.69	92.53	97.33	94.53	686	7.83	9.74
80.9	7–8	KBr	3.96	11.21	15.94	92.41	97.21	94.40	699	7.12	10.54

[a] The setting of the unit cell follows Hamodrakas et al.,[3] who interchanged b and c of Cox et al.[2] [b] Number of moles of water associated with 1 mol of cromolyn sodium in sorption (lower number) and desorption (higher number) from Cox et al.[2] [c] Profile residual: $R_p = \sum |y_i(o) - y_i(c)|/\sum y_i(o) \times 100\%$, where $y_i(o)$ is the observed signal and $y_i(c)$ is the calculated signal. [d] The weighted profile residual. $R_{wp} = [\sum w_i[y_i(o) - y_i(c)]^2/\sum w_i[y_i(o)]^2]^{0.5} \times 100\%$, where $y_i(o)$ is the observed signal and $y_i(c)$ is the calculated signal.

dard.[4] Their X-ray diffraction study showed that the M phase has one intense sharp peak and two faint but sharp peaks at 2.4° to 6.0° 2θ, and a broad peak at 25.5° to 26.0° 2θ. The positions of the sharp peaks were reported to vary with the water composition, while the position of the broad peak remained invariant. This X-ray diffraction study also showed that the N phase, which exists at high water compositions, gives only the broad line at 25.5 to 26.0° 2θ. The CS crystalline hydrate phase readily converts to the M liquid crystalline phase and does so reversibly under certain circumstances.[4] The conversion from the M to the N liquid crystalline phase is also rapid and reversible.[4] Mesophase N is a nematic liquid crystal, whereas M is a "middle" phase in which the molecules are arranged as rods of indefinite length, parallel to the optic axis, and with the molecular planes oriented at 90°, on average, to the axes of the rods.[4]

In 1996 and 1997, Oguchi et al.[5,6] reported two new crystalline forms of CS, termed forms B and C, and applied designation form A to the original crystalline form of CS reported by Cox et al.[2] Oguchi et al.[5,6] reported differences between these forms of CS in their powder X-ray diffraction (PXRD) patterns, moisture sorption diagrams, and differential scanning calorimetric (DSC) traces. However, doubts remain as to whether B and C are truly new crystalline forms of CS. First of all, the hysteresis in water sorption and desorption of B and C is still not well explained. Second, the sharp diffraction peaks at small 2θ angles correspond to the sharp diffraction peaks of large d-spacings for the M liquid crystalline phase.[4] Other differences in the PXRD patterns may be attributed to the lattice expansion caused by the uptake of nonstoichiometric water, as reported by Cox et al.[2] On the other hand, the moisture sorption diagrams of B and C show stepwise water uptake, which is a characteristic of stoichiometric hydrates. Therefore, further investigations are needed to prove or to disprove the existence of the new crystalline forms of CS. In view of these conflicting interpretations, this report seeks to clarify the solid-state behavior of CS hydrates. Because the exact nature of the reported new forms A, B, and C is not clear, they are termed materials A, B, and C in this report.

Hypotheses To Be Tested—(1) Contamination of solid cromolyn sodium hydrate phases by liquid crystalline phases can occur under certain conditions, which will complicate the PXRD patterns, the moisture sorption isotherms, and the thermal analytical data. (2) The unusual nonstoichiometric water uptake of CS can be explained in light of its particular molecular and solid-state structures.

Specific Aims of the Work—(1) To redetermine the crystal structure of CS and to locate the second sodium ion and all the water molecules, and hence (2) to explain why cromolyn sodium forms nonstoichiometric hydrates and sorbs and liberates water continuously, (3) to understand the influence of relative humidity on the PXRD patterns of CS crystalline hydrate, and (4) to investigate whether CS exists as different stoichiometric crystalline hydrates.

Experimental Section

Materials—Cromolyn sodium, in the form of hydrated white crystalline powder, was a gift from Fisons, plc, Pharmaceutical Division (now Astra Charnwood and Rhône-Poulenc Rorer, Loughborough, UK). The glass capillaries, 0.5 and 1.0 mm in diameter and 0.01 mm in thickness, used to house single crystals for structure determination and small crystallites for obtaining PXRD patterns of CS at various defined RH values, were purchased from the Charles Supper Company (Natick, MA). The silicon powder, 60 mesh, used as a peak position reference for the PXRD experiments, was obtained from Aldrich Chemical Co. (Milwaukee, WI). Methanol was obtained from Fisher Scientific (Fair Lawn, NJ). Water was deionized and glass distilled in-house.

The single crystal used for the structural determination was crystallized from a slightly supersaturated solution of CS in water + methanol mixtures (v:v = 10:9, water activity = 0.76).[7] The elemental analysis of the crystals was carried out to ascertain their sodium stoichiometry.

Elemental Analysis—The prepared single crystals were dried at 170 °C for 30 min prior to elemental analysis by Quantitative Technology (Whitehouse, NJ). The carbon and hydrogen weight percentages were calculated from the evolved carbon dioxide and water vapor upon combustion. The sodium weight percentage was determined from atomic absorption.

Preparation of the Reported Crystalline Materials of Cromolyn Sodium—The three reported[5] materials of CS were prepared here by the methods outlined[5,6] for further characterization. Material A, the original CS crystal form reported by Cox et al.,[2] was crystallized from ethanol + water mixture (v:v = 4:7, water activity = 0.88).[7] Material B was crystallized by slow diffusion of n-hexane + 2-propanol (v:v = 1:1) into a 10% w/v aqueous solution of CS. Material C was crystallized from ethanol + water mixture (v:v = 6:9, water activity = 0.87).[7]

Optical Microscopy—Crystals of materials A, B, and C were observed under a video-enhanced microscope (Nikon Optiphot-Pol, Tokyo, Japan) equipped with the Metamorph Imaging System software (Universal Imaging Co., West Chester, PA).

Powder X-ray Diffraction (PXRD)—The PXRD patterns of materials A, B, and C were determined under ambient condition using a powder X-ray diffractometer (D-5005, Siemens, Munich, Germany) with Cu Kα radiation at 40 mA and 45 kV. The sample was packed in a quartz holder and scanned at room temperature (23 °C) at a step size of 0.05° with a counting time of 1 s per step.

The PXRD patterns of the original CS powder[2] were determined after equilibration at defined RH values. For this purpose the CS powder was sealed in glass capillaries together with saturated salt solutions (Table 1) of known relative humidity values[8,9] and allowed to equilibrate at 23 °C for more than one week. After equilibration, the PXRD patterns were obtained using a microdiffractometer (Bruker AXS, Siemens, Munich, Germany), equipped with an area detector (HI−STAR, Siemens, Munich, Germany) and a data analysis software (General Area Detector Diffraction System, GADDS, Siemens, Munich, Germany). Cu Kα radiation at 40 mA and 45 kV was used. The diffraction data were collected

Table 2—Isotropic Temperature Factors and Occupancies of the Sodium Ions and Oxygen Atoms in the Water Molecules in the Cromolyn Sodium Structure Solved at 173 K

atom	isotropic temperature factor	occupancy[a]
Na(1)	0.0384	1
Na(2)	0.0316	0.316
Na(3)	0.0419	0.319
Na(4)	0.0518	0.365
O(12)	0.1049	1
O(13)	0.0325	1
O(14)	0.0922	0.684
O(15)	0.0940	0.681
O(16)	0.1239	0.635
O(17)	0.0623	0.439
O(18)	0.7954	1
O(19)	0.1773	1

[a] The occupancies of Na(2), Na(3), and Na(4) add up to 1. The occupancies of O(12)–O(19) add up to 6.44.

for 3000 s in a frame of 30° 2θ and a resolution of 0.02° 2θ. The sample was spun at 1 cycle per 5 s to minimize the effect of preferred orientation. At a given RH value, the same PXRD pattern was obtained when the original CS crystals were recrystallized from methanol + water mixtures (v:v = 2:1, water activity = 0.60)[7] and gently ground in an agate mortar.

Crystal Structure Determination and Refinement—An elongated crystal of CS, 0.40 × 0.20 × 0.05 mm, was covered with a thin layer of heavy oil to prevent moisture exchange with the environment, was attached to a glass fiber with epoxy resin, and was mounted on a goniometer (SMART system, Siemens, Munich, Germany) for data collection at 173 and 295 K. A cryogenic nitrogen stream was used to obtain the low temperature, 173 K. To determine the crystal structure of CS with low water content, a crystal was equilibrated at 23 °C and at 0, 11.6, or 21.6% RH for more than one month in a sealed glass capillary 0.5 mm in diameter,[10] which was then mounted on the diffractometer. An initial set of cell constants and an orientation matrix were calculated from reflections collected from three sets of 20 frames which were oriented such that the orthogonal wedges of reciprocal space were surveyed. This procedure produced orientation matrixes determined from 54 reflections. Final cell constants were calculated from a set of 3593 strong reflections of the actual data collection.

The data collection technique used was hemisphere collection. A randomly oriented reciprocal space was surveyed to the extent of 1.3 hemispheres to a resolution of 0.84 Å. Three major swaths of frames were collected with 0.30° steps in ω. Because the lattice is triclinic, some additional sets of frames were collected to provide a more accurate absorption correction.

A successful direct-methods solution (SHELX-V5.0, Siemens Industrial Automation, Inc., Madison, WI) was calculated and revealed most non-hydrogen atoms from the E-map. Several full-matrix least squares/difference Fourier cycles were performed which located the remainder of the non-hydrogen atoms. All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were placed in ideal positions and refined as riding atoms with individual (or group, if appropriate) isotropic displacement parameters.

Lattice Parameter Determination—The unit cell parameters of the original CS crystal form equilibrated at various RH values were refined using a Rietveld module (DBWS, Cerius 2, San Diego, CA), which interfaces with the DBWS[11] program for Rietveld refinement. Because CS gives complex diffraction patterns with severe peak overlap, it is difficult to index and to measure accurately the positions of each diffraction peak, as required for conventional unit cell parameter least squares refinements. The Rietveld method, which obtains a global least-squares fit between the calculated and the observed powder patterns, is a well-accepted method for refining accurate unit cell parameters.[12] The crystal structure of the original CS crystal form, solved at 295 K, was used as the starting trial structure. The diffraction intensities, peak shape (modeled by pseudo-Voigt profile shape function), systematic instrumental error in powder pattern measurement, and the unit cell parameters were refined.

Calculation of Theoretical Powder X-ray Patterns—The theoretical PXRD pattern of a given structure was calculated from

Table 3—Crystallographic Data, Data Collection, Solution and Refinement Parameters for the Crystal Structure of Cromolyn Sodium Prepared from Methanol + Water Mixtures (v:v = 9:10, water activity = 0.76) and Solved at 173 K

Crystallographic Data	
empirical formula	$C_{23}H_{26.88}Na_2O_{17.44}$
crystal habit, color	plate, colorless
crystal size	0.40 × 0.20 × 0.05 mm
crystal system	triclinic
space group	$P1$
	$a = 3.8715\ (3)$ Å, $\alpha = 92.868\ (1)°$
	$b = 11.0771\ (8)$ Å, $\beta = 95.864\ (2)°$
	$c = 15.6930\ (12)$ Å, $\gamma = 94.400\ (2)°$
volume	666.36 (9) Å^3
Z	1
formula weight	628.34
density (calcd)	1.566 Mg/m^3
absorption coefficient	0.162 mm^{-1}
$F(000)$	326

Data Collection	
diffractometer	Siemens SMART Platform CCD
wavelength	0.71073 Å
temperature	173 (2) K
θ range for data collection	1.31 to 25.05°
index ranges	$-4 \le h \le 4, -13 \le k \le 13, 0 \le l \le 18$
reflections collected	5034
independent reflections	2329 ($R_{int} = 0.0382$)

| Solution and Refinement	
system used	SHELXTL-V5.0
solution	direct methods
refinement method	full-matrix least-squares on F^2
weighting scheme	$w = [\sigma^2(F_o^2) + (AP)^2 + (BP)]^{-1}$, where $P = (F_o^2 + 2F_c^2)/3$, $A = 0.1658$, and $B = 0.3236$
absorption correction	SADABS (Blessing, 1995)[14]
max. and min transmission	1.000 and 0.581
absolute structure parameter	0.5 (12)
data/restraints/parameters	2327/4/419
R indices ($I > 2\sigma(I) = 1976$)	$R1 = 0.0825$, $wR2 = 0.2133$
R indices (all data)	$R1 = 0.0934$, $wR2 = 0.2280$
goodness-of-fit on F^2	1.036
largest diff peak and hole	0.991 and -0.425 eÅ^{-3}
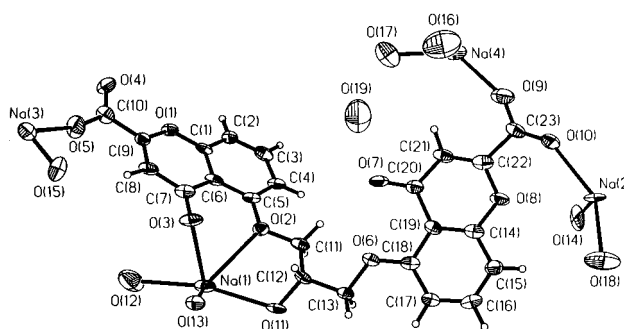
Figure 2—Thermal ellipsoid diagram, showing the atomic labeling scheme, of cromolyn sodium crystallized from methanol + water mixtures (v:v = 9:10, water activity = 0.76) and solved at 173 K.

the crystal structure using suitable software (Diffraction Simulation Module, Cerius2, San Diego, CA).

Results and Discussion

Crystal Structure of the Original Crystalline Phase of Cromolyn Sodium—The CS structure was initially determined at 295 K and is similar to the structure previously reported.[3] The space group was determined as $P1$, a chiral space group, even though the molecule itself
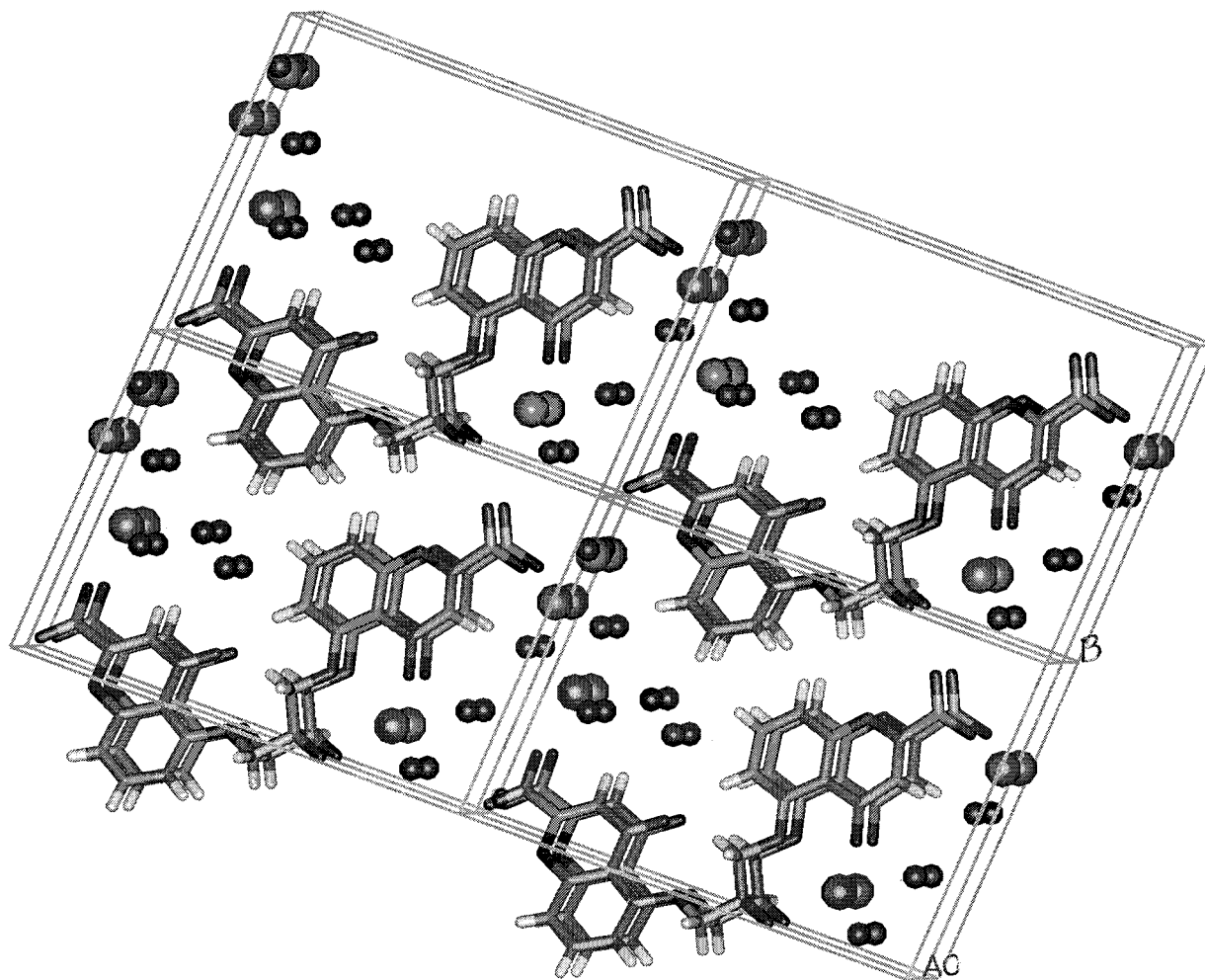
Figure 3—Crystal packing diagram, looking down the *a*-axis and showing 2*2*2 unit cells, for cromolyn sodium crystallized from methanol + water mixtures (v:v = 9:10, water activity = 0.76) and solved at 173 K. The smaller balls represent the oxygen atoms of the water molecules that have been located crystallographically, while the larger balls represent the located sodium ions.

is achiral. Examination of the molecular structure shows that the carbon atom bonded to the hydroxyl group can act as a chiral center in the crystal because the two cyclic moieties attached to this carbon are inequivalent in three-dimensional space. The CS crystallized as a racemic conglomerate of the two packing enantiomers. The structure solved at 173 K was a mirror image of the structure solved at 295 K and of that reported in the literature. Inversion was necessary for direct visual comparison of the three structures. The structures solved at 295 and 173 K were essentially identical. The intramolecular bond lengths and bond angles differed by less than 0.04 Å or 2°.

The structure was determined at 173 K in an attempt to locate all the water molecules and sodium ions. The smaller thermal motions at the lower temperature allowed better refinements of the water and sodium positions. The sodium positions are differentiated from the water positions by their coordination environment and smaller displacements (Table 2). Table 3 shows the crystallographic data and refinement parameters. Figure 2 is the thermal ellipsoid diagram with the atomic labeling scheme, while Figure 3 displays the crystal packing of 2*2*2 unit cells. Both Figures 2 and 3 show the ordered sodium ions, Na(1), and the disordered sodium ions, Na(2), Na(3), and Na(4), at 173 K, discussed in the next paragraph. Table 4 compares the intramolecular bond lengths and angles of the structure solved at 173 K with those of the structures solved at 295 K. Table 5 gives the sodium coordination distances in the structure solved at 173 K.

For the structure solved at 173 K, one sodium cation, Na(1), is tightly held in coordination with five neutral oxygen atoms of one ketone, one alcohol, and one ether group and two water molecules (Figure 4a). The second sodium ion is disordered over three partially occupied sites, Na(2), Na(3), and Na(4), in a maze of Na–O contacts (Figure 4b, c, and d, respectively). Four out of eight water positions are partially occupied. The occupancies of the sodium ions and water molecules are provided in Table 2. The three disordered sodium positions, Na(2), Na(3), and Na(4), all appear to have inferior coordination when compared to Na(1). All three disordered sodium positions have at least one water oxygen that must be displaced, due to unrealistically short contacts, if they are to occupy these sites. These contacts are Na(2)/O(14), Na(3)/O(15), and Na(4)/O(16). A restraint was used to force the sum occupancy of the three partially occupied sodium positions to unity. The occupancies of the unrealistic oxygen contacts were forced to have residual occupancy with respect to the sodium sites. Finally, O(17) appears to be partially occupied and not paired with a specific sodium ion. The total count of water molecules was 6.44 per cromoglycate anion.

Elemental analysis was deemed necessary because only one sodium ion could be located. Elemental analysis, in duplicate, afforded as follows: C 53.11, 52.97; H 2.91, 3.02; Na 8.32, 8.39%. These results agree well with those calculated from the molecular formula of cromolyn sodium: C 53.92; H 2.75; Na 8.97%. The observed differences between the experimental and the calculated weight per-

Table 4—Comparison of the Intramolecular Bond Angles and Bond Lengths of the Cromolyn Sodium Structures Reported by Hamodrakas et al.[3] at 298 K with Those Solved at 295 K and at 173 K in the Present Work

angle[a]	ref 3	295 K	173 K	bond[a]	ref 3	295 K	173 K
C6–C1–O1	119.5 (1.6)	122.1	122.3 (6)	O1–C1	1.42 (2)	1.359	1.355 (10)
C6–C1–C2	122.6 (1.8)	122.1	120.5 (7)	C1C2	1.41 (3)	1.389	1.406 (11)
O1–C1–C2	117.9 (1.4)	115.8	117.2 (6)	C2–C3	1.37 (3)	1.350	1.335 (12)
C1–C2–C3	119.6 (1.7)	118.9	120.0 (7)	C3–C4	1.35 (3)	1.395	1.416 (12)
C2–C3–C4	118.8 (1.8)	121.5	122.5 (7)	C4–C5	1.42 (3)	1.369	1.404 (11)
C3–C4–C5	124.4 (1.8)	120.0	118.4 (8)	C5–C6	1.42 (3)	1.422	1.420 (12)
C4–C5–C6	118.5 (1.6)	120.6	120.3 (70	C6–C7	1.53 (3)	1.443	1.474 (11)
C4–C5–O2	128.0 (1.6)	124.0	122.8 (7)	C6–C1	1.44 (2)	1.406	1.418 (10)
C6–C5–O2	113.5 (1.5)	115.4	116.8 (2)	C7–O3	1.26 (2)	1.224	1.249 (10)
C5–C6–C1	115.8 (1.8)	116.7	118.2 (6)	C7–C8	1.44 (3)	1.438	1.432 (13)
C5–C6–C7	125.8 (1.5)	124.0	124.2 (7)	C8–C9	1.33 (3)	1.332	1.339 (11)
C1–C6–C7	118.3 (1.7)	119.2	117.6 (7)	C9–O1	1.37 (2)	1.345	1.345 (2)
C6–C7–C8	116.2 (1.5)	114.3	114.6 (7)	C9–C10	1.55 (4)	1.506	1.506 (13)
C6–C7–O3	124.5 (1.6)	124.9	122.7 (8)	C10–O4	1.25 (3)	1.226	1.229 (11)
C8–C7–O3	119.2 (1.7)	120.8	122.6 (7)	C10–O5	1.28 (3)	1.233	1.257 (11)
C7–C8–C9	120.5 (1.9)	122.7	123.6 (7)	C5–O2	1.34 (2)	1.335	1.345 (9)
C8–C9–O1	125.4 (1.8)	122.7	121.3 (7)	O2–C11	1.44 (2)	1.427	1.432 (10)
C8–C9–C10	121.0 (1.9	123.5	123.4 (7)	C11–C12	1.56 93)	1.503	1.500 (10
C10–C9–O1	113.7 (1.6)	113.8	115.3 (7)	C12–O11	1.40 (2)	1.420	1.440 (8)
C9–C10–O4	115.7 (1.9)	117.7	117.4 (7)	C12–C13	1.59 (3)	1.477	1.515 (12)
C9–C10–O5	117.9 (1.8)	115.3	115.2 (8)	C13–O6	1.40 (2)	1.432	1.443 (9)
O4–C10–O5	126.3 (2.0)	127.0	127.4 (9)	O6–C18	1.38 (3)	1.332	1.316 (10)
O2–C11–C12	102.1 (1.5)	106.7	105.7 (6)	C18–C17	1.31 (3)	1.367	1.405 (12)
C11–C12–O11	108.6 (1.5)	108.6	109.4 (6)	C17–C16	1.40 (3)	1.371	1.365 (13)
C11–C12–C13	108.6 (1.5)	111.3	111.6 (6)	C16–C15	1.34 (3)	1.371	1.363 (13)
O11–C12–C13	105.9 (1.4)	110.4	109.7 (6)	C15–C14	1.36 (3)	1.357	1.389 (13)
C12–C13–O6	99.9 (2.4)	106.9	106.0 (6)	C14–C19	1.36 (3)	1.380	1.372 (12)
C15–C14–C19	126.8 (1.7)	123.7	123.8 (8)	C14–O8	1.37 (2)	1.380	1.389 (10)
C15–C14–C8	112.7 (1.6)	115.2	113.6 (8)	O8–C22	1.31 (3)	1.326	1.367 (11)
O8–C14–C19	120.3 (1.7)	121.1	122.6 (7)	C22–C23	1.49 (3)	1.542	1.543 (11)
C14–C15–C16	117.6 (1.8)	119.0	118.3 (8)	C23–O9	1.24 (3)	1.224	1.231 (12)
C15–C16–C17	121.1 (1.8)	120.5	120.7 (8)	C23–O10	1.25 (3)	1.217	1.228 (12)
C16–C17–C18	118.7 (1.7)	120.7	121.8 (7)	C22–C21	1.28 (3)	1.320	1.326 (13)
C17–C18–O6	124.4 (1.6)	124.5	124.5 (7)	C21–C20	1.43 (3)	1.442	1.436 (11)
C17–C18–C19	124.0 (1.8)	120.1	118.1 (7)	C20–O7	1.25 (3)	1.216	1.215 (11)
O6–C18–C19	111.6 (1.7)	115.4	117.4 (7)	C20–C19	1.43 (3)	1.461	1.493 (12)
C14–C19–C18	111.5 (1.8)	115.9	117.3 (7)	C19–C18	1.45 (3)	1.435	1.430 (11)
C14–C19–C20	121.7 (1.7)	120.3	119.5 (7)				
C18–C19–C20	126.7 (1.8)	123.7	123.2 (7)				
C19–C20–O7	125.2 (1.7)	125.9	123.5 (7)				
C19–C20–C21	113.3 (1.8)	113.3	113.8 (7)				
O7–C20–C21	121.3 (1.7)	120.8	122.7 (8)				
C20–C21–C22	122.3 (1.9)	122.4	122.7 (8)				
C21–C22–O8	124.9 (1.7)	123.7	123.6 (7)				
C21–C22–C23	122.5 (1.8)	124.7	125.8 (8)				
O8–C22–C23	112.1 (1.6)	111.5	110.6 (7)				
C22–C23–O9	117.1 (1.8)	115.7	114.5 (8)				
C22–C23–O10	114.8 (1.7)	116.8	117.4 (8)				
O9–C23–O10	127.8 (1.8)	127.5	128.0 (8)				
C1–O1–C9	120.0 (1.3)	119.0	120.5 (6)				

[a] The atom parentheses are omitted for clarity.

centages were ascribed to residual moisture, which the samples may have sorbed from the ambient environment during transfer from the drying oven to the analyzer. Nevertheless, these results indicate that the sodium stoichiometry of the prepared single crystals is indeed two (not one) sodium cation per cromoglycate anion, in accordance with the requirement of charge balance. Thus, one of the sodium ions, and some of the associated water molecules, are disordered in the CS crystal structure, explaining why the location of the sodium ion cannot be refined.

In the packing diagram (Figure 3), large sodium and water channels, which occupy approximately 50% of the cell volume, can be observed parallel to the *a*-axis and perpendicular to the plane of cromoglycate anions. The presence of relatively large sodium and water channels allows easy passage of water molecules with minimum energy expenditure and minimum disruption to the packing arrangements.

The entire structure of CS, equilibrated at 295 K and at RH values of 0, 11.4, and 21.6%, could not be solved by single crystal X-ray diffraction, suggesting significant reductions in crystallinity and emphasizing that the water molecules are structural elements necessary for holding together the crystal structure. On reducing the RH, both the water content and the crystallinity decreased gradually, suggesting that the transition between the crystalline form A and the amorphous form cannot be precisely defined.

Comparison of the Unit Cell Parameters of Cromolyn Sodium Structures with Different Water Contents—The lattice parameters, expressed as a function of RH and water content (Table 1), were derived from the PXRD patterns (Figure 5) using the Rietveld method. Figure 6 compares the observed PXRD pattern with that calculated after Rietveld refinement.

As the RH in the environment decreased, the unit cell parameters, *a, b, c,* α, and the volume, *V*, also decreased

Table 5—Sodium Coordination Distances of the Cromolyn Sodium Structure Solved at 173 K. The Structure Corresponds to 6.44 Water Molecules per Cromoglycate Anion

sodium ion position	occupancy	sodium ion–oxygen[a] notation[b]	distance (Å)
Na1	1	Na1 (0,0,0)–O2 (0,0,0)	2.699
		Na1 (0,0,0)–O3 (−1,0,0)	2.592
		Na1 (0,0,0)–O3 (0,0,0)	2.495
		Na1 (0,0,0)–O11 (0,0,0)	2.488
		Na1 (0,0,0)–O12 (0,0,0)	2.508
		Na1 (0,0,0)–O13 (0,0,0)	2.430
		Na1 (0,0,0)–O13 (0,0,0)	2.380
Na2	0.316	Na2 (0,0,0)–O5 (0,1,−1)	2.358
		Na2 (0,0,0)–O10 (0,0,0)	2.446
		Na2 (0,0,0)–O14 (0,0,0)	2.316
		Na2 (0,0,0)–O14 (1,0,0)	1.937[c]
		Na2 (0,0,0)–O18 (0,0,0)	2.316
Na3	0.319	Na3 (0,0,0)–O5 (0,0,0)	2.506
		Na3 (0,0,0)–O5 (1,0,0)	2.696
		Na3 (0,0,0)–O14 (0,0,0)	1.990[c]
		Na3 (0,0,0)–O18 (0,0,0)	2.316
		Na3 (0,0,0)–O15 (0,0,0)	1.694[c]
		Na3 (0,0,0)–O18 (0,0,0)	2.409
Na4	0.365	Na4 (0,0,0)–O9 (0,0,0)	2.388
		Na4 (0,0,0)–O15 (−1,0,−1)	2.484
		Na4 (0,0,0)–O16 (0,0,0)	1.762[c]
		Na4 (0,0,0)–O16 (−1,0,0)	2.589
		Na4 (0,0,0)–O17 (0,0,0)	2.464
		Na4 (0,0,0)–O18 (0,−1,0)	2.271

[a] These atoms are shown in Figure 4a–d. The atom parentheses are omitted for clarity. [b] (0,0,0) refers to the original unit cell. The digit 1 indicates forward transition by one unit cell. The digit −1 indicates backward transition by one unit cell. [c] This sodium ion–oxygen distance is unreasonably small, so the water molecule will be displaced when the sodium ion occupies this position.

(Table 1). The general trend of the unit cell dimensions as a function of RH is consistent with Cox et al.,[2] but the cell lengths differed slightly (<1%). The α angle was approximately 90° at RH ≤ 58.5%, but was approximately 92.5° at RH ≥ 75.4%. The β and γ angles were found to be relatively constant, at approximately 97.5° and 94.5°, respectively, over the entire RH values examined. At 0% RH, CS is mainly amorphous, as indicated by the broad amorphous halo, with only four distinguishable PXRD peaks for the (001), (010), (01−1), and (011) planes (Figure 5). From the 2θ values of these four peaks and with the assumption that the values of the lattice parameters, a, $β$ and $γ$, at 6.5% RH are approximately equal to those at 0% RH, the lattice parameters b, c, and α of the CS structure at 0% RH were estimated (Table 1).

Molecular Conformation of the Cromolyn Anion— The structure of cromolyn sodium reported by Hamodrakas et al.[3] contains 5 to 6 (∼5.5) water molecules per cromoglycate anion, while that solved here contains 6.44 water molecules per cromoglycate anion. Comparison between two structures can provide insight into the influence of water content on the molecular conformation of the cromolyn anion.

The two structures show similarities in packing arrangements and in most intramolecular bond lengths and bond angles. The major differences occur in the torsional angles of the two carboxylate groups and in the torsional and bond angles of the 2-hydroxypropane linking chain. The part of the 2-hydroxypropane chain that is coordinated to Na(1) is more rigid than the part that is not (Figure 7a). The largest changes in bond angle occur in C(12)−C(13)−O(6) and C(13)−O(6)−C(18), and are approximately 6° and 5°, respectively. As the water content increases from ∼5.5 to 6.44, the two carboxylate groups rotate away from each other (Figure 7a), while the angle formed by the planes of
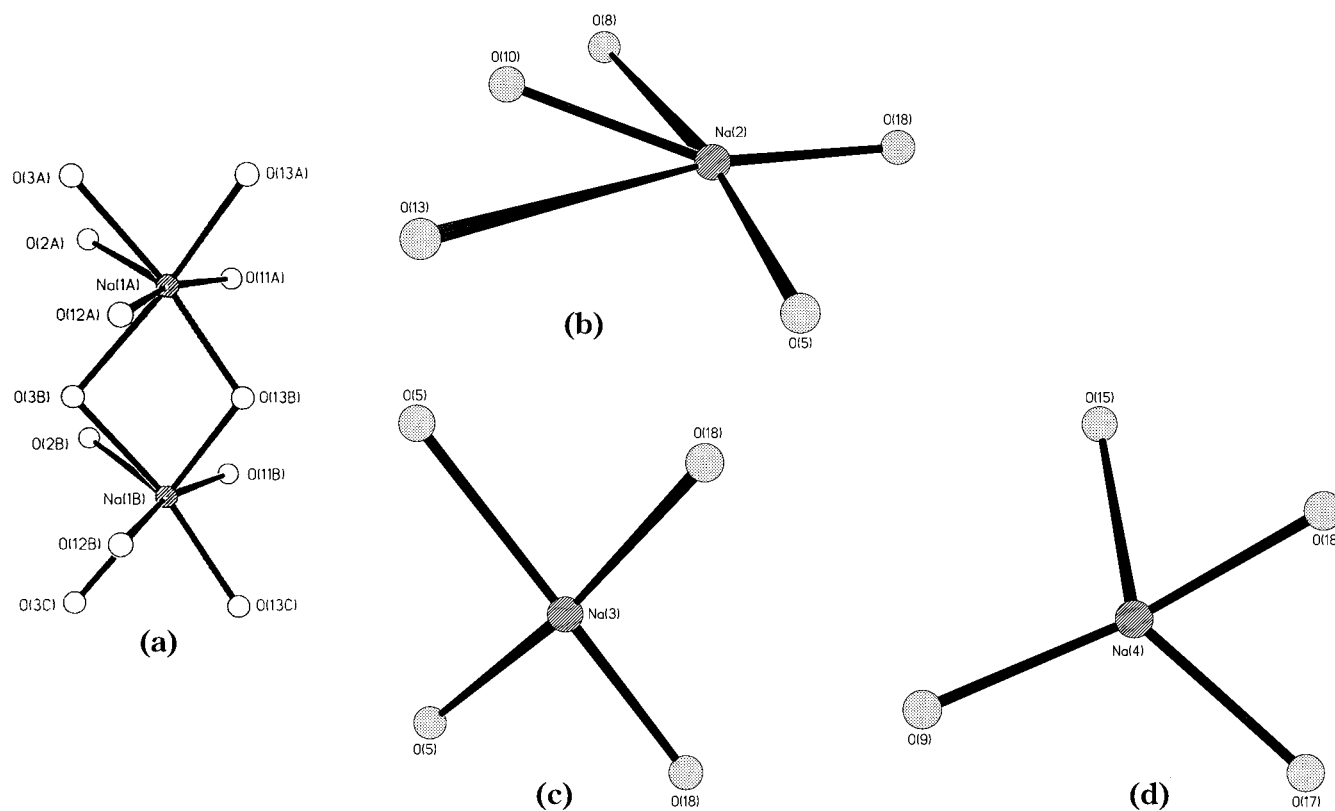
Figure 4—Coordination environment of: (a) the first (ordered) sodium ion, Na(1), shown in two neighboring unit cells (A and B); and the second (disordered) sodium ion at the three partially occupied sites, (b) Na(2), (c) Na(3), and (d) Na(4). The striped circles represent the sodium sites. The open circles represent the oxygen atoms coordinated to Na(1). The dotted (gray) circles represent the oxygen atoms coordinated to Na(2), Na(3), or Na(4).
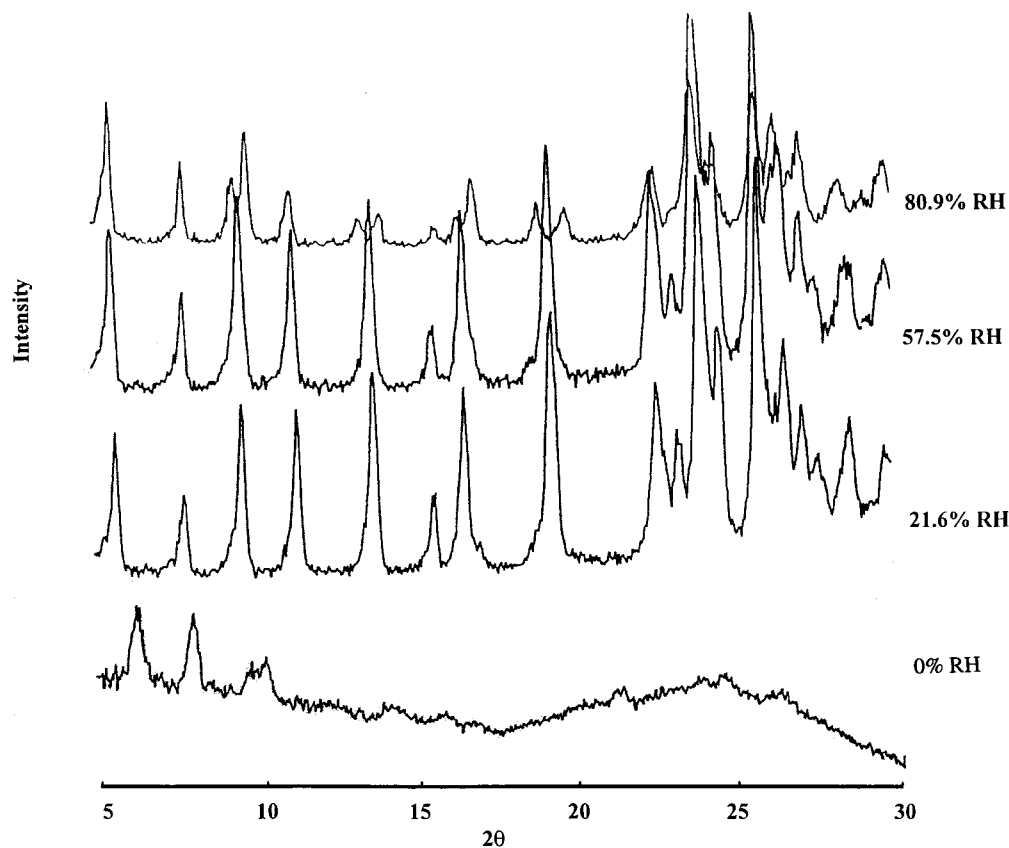
Figure 5—Powder X-ray diffraction patterns of cromolyn sodium crystallites equilibrated at four relative humidities: 80.9, 57.5, 21.6, and 0% at 23 °C.
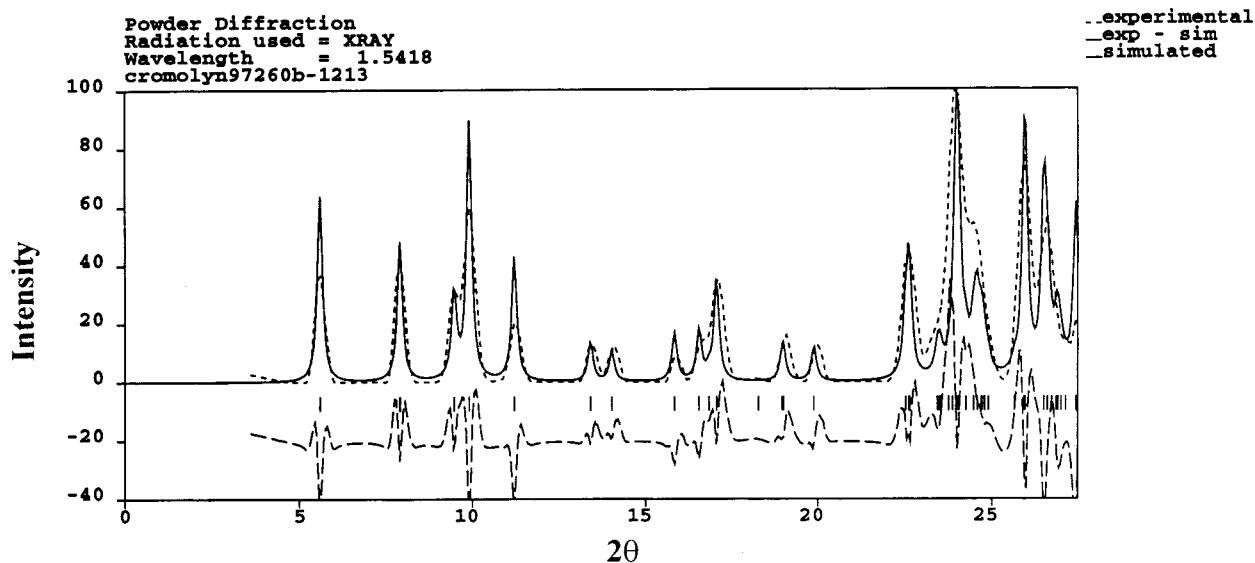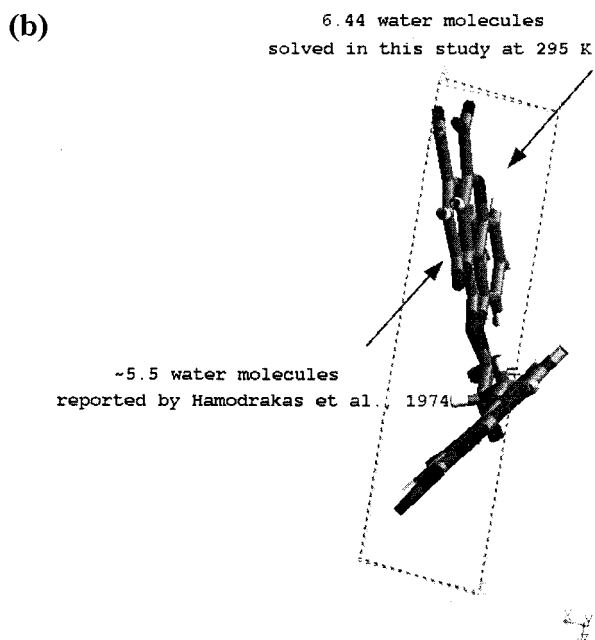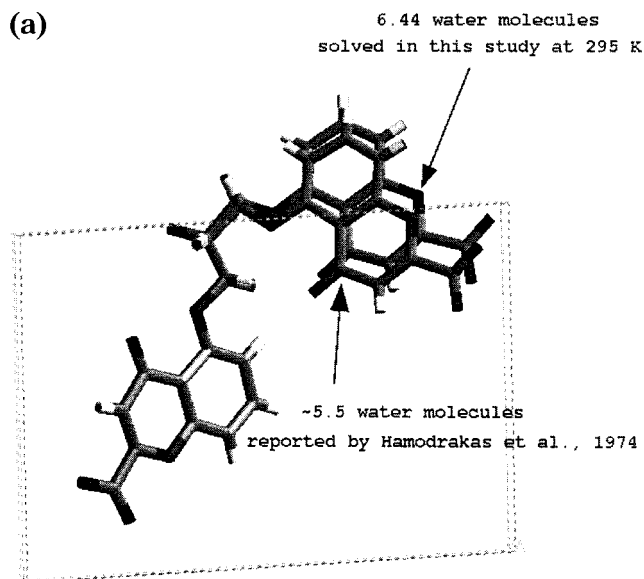

Figure 6—Powder X-ray diffraction patterns of cromolyn sodium: observed after equilibration at 80.9% relative humidity at 23 °C (top dashed line); calculated from the Rietveld-refined crystal structure at 173 K (top full line); difference between the calculated and the observed patterns (bottom dashed line). The vertical sticks mark the positions of the calculated diffraction peaks.

the two cyclic moieties decreases (Figure 7b) to form a larger water channel and stronger hydrophobic interactions between the cromoglycate anions in the *a* and *b* crystallographic directions. Because the carboxylate groups are coordinated to the second sodium ion, Na(2), Na(3), and Na(4), the change in torsional angles, C(8)−C(9)−C(10)−O(5) and C(21)−C(22)−C(23)−O(9), most likely reflect the change in its relative position.

In symmetrical molecules containing two planar moieties, such as CS or biphenyl,[13] the question arises whether the planar groups are in the same plane. For biphenyl in the crystalline state, Bushing[13] found that, above the transition temperature, the molecules have an average coplanar conformation, whereas below the transition temperature one phenyl ring is twisted relative to the other by as much as 10° at 22 K. While the two phenyl rings in biphenyl are linked by a covalent bond, the two cyclic moieties in CS are linked by a flexible 2-hydroxypropane chain, as discussed above. In the solution state, the cyclic moieties of CS are coplanar and the molecule (divalent anion) is symmetric. However, CS crystallizes in the space group, *P*1, corresponding to a complete lack of symmetry.

(a)

6.44 water molecules
solved in this study at 295 K

~5.5 water molecules
reported by Hamodrakas et al., 1974

(b)

6.44 water molecules
solved in this study at 295 K

~5.5 water molecules
reported by Hamodrakas et al., 1974

Figure 7—Overlay of the unit cells of the following two cromolyn sodium structures solved at 295 K: the structure with ~5.5 molecules of water per molecule of cromolyn sodium (reported by Hamodrakas et al., 1974);[3] the structure with 6.44 molecules of water per molecule of cromolyn sodium (solved in the present study); looking down (a) the *a*-axis and (b) the *b*-axis. The sodium ions and water molecules are omitted.

Moreover, the crystal contains a channel parallel to the short *a*-axis in which the disordered array of sodium ions and water molecules is found. It is possible that the crystal might achieve an ordered state with a certain water content at a certain temperature. However, this behavior has not been observed and would require further study.

Water Uptake of Cromolyn Sodium and Its Relationship to Structural Features—The high affinity for water and the reversible water sorption−desorption behavior of CS (Cox et al.,[2] also summarized in Table 1) can be understood from its molecular and crystal structures.

The hygroscopicity of CS is due to its ionic nature and the various polar functional groups: carboxylate, carbonyl, ether, and hydroxyl. The large sodium and water channels
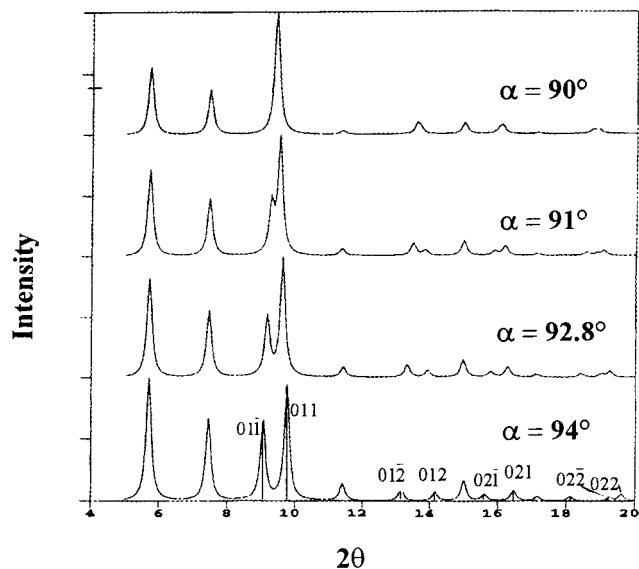
Figure 8—Theoretical powder X-ray diffraction patterns calculated by varying the α angle of the crystal structure of cromolyn sodium crystallized from methanol + water mixtures (v:v = 9:10, water activity = 0.76) and solved at 295 K.

along the *a*-axis, seen from the crystal packing diagram (Figure 3), allow easy passage of water molecules with little energy expenditure and minimal disruption to the crystal packing. This feature may explain the reversible water uptake of CS. Several factors contribute to the continuous and nonstoichiometric uptake of water by CS. First and foremost, the flexible 2-hydroxypropane chain linking the two cyclic moieties can relax readily to accommodate continuous lattice expansion caused by the continuous uptake of water. Second, the disordered sodium ion and the variable size of the large water channel cause the water to be disordered and allow for variable nonstoichiometric water contents that depend on the occupancies of the second sodium ion and the size of the water channel.

Powder X-ray Diffraction Pattern of Cromolyn Sodium as a Function of Relative Humidity—As stated above, the crystal structure of CS equilibrated at RH < 22% could not be solved by single-crystal X-ray diffraction, suggesting significant reductions in crystallinity. As the immediate environmental RH decreases, the PXRD pattern of CS (Figure 5) shows peak shifts toward smaller *d*-spacing (larger 2θ). Some PXRD peaks disappear and some appear as the RH changes. These appearances and disappearances can be explained by the change in the α angle of the crystal structure. The α angle is approximately 92.5° at RH $\geq$ 75.4% and is approximately 90° at RH $\leq$ 58.5%. As the α angle changed from 92.5° to 90°, the (100) and (004) peaks separated and the following pairs of peaks merged: (01−1) and (011); (01−2) and (012); (02−1) and (021); (02−2) and (022) (Figure 8).

Studies of the Different Crystalline Forms of Cromolyn Sodium Previously Reported[5,6]—The crystalline materials A, B, and C, prepared here following the procedures outlined by Oguchi et al.,[5,6] are all elongated transparent slabs (Figure 9); their PXRD patterns (Figure 10) correspond to those of the original crystal form of CS reported by Cox et al.,[2] with the exception of a diffraction peak below 5° 2θ, attributed to the formation of M liquid crystalline phase.[4] Close examination of the PXRD patterns of materials A, B, and C reported by Oguchi et al.,[5,6] reveals peaks corresponding to the M liquid crystalline phase.[4] Excluding the peaks corresponding to the liquid crystalline phase, the PXRD pattern of material A is identical to that of the original crystal form of CS equilibrated at RH $\leq$ 58%.
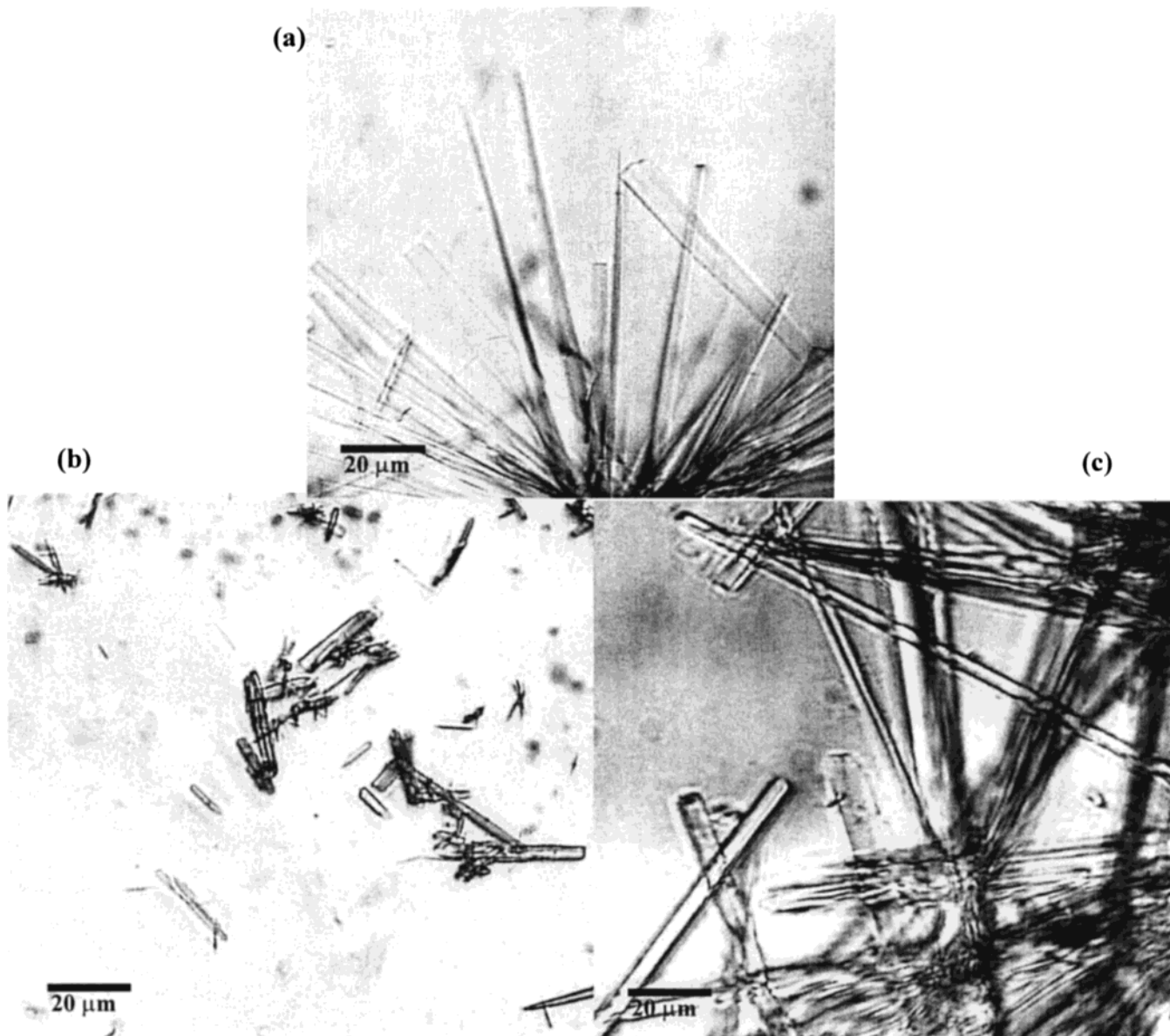
Figure 9—Photomicrographs of (a) material A, (b) material B, and (c) material C, prepared in our laboratory, according to procedures described by Oguchi et al.,[5–6] and submerged in silicone oil.

Similarly, the PXRD pattern of material B can be superimposed on the PXRD pattern of the original crystal form of CS equilibrated at RH $\geq$ 75.4%, with the exception of some small shoulder peaks, which can be attributed to fluctuations in the lattice parameters.[2] The sharp diffraction peaks in the PXRD pattern of material C can also be superimposed on the PXRD pattern of the original crystal form of CS equilibrated at RH $\geq$ 75.4%. When damp freshly harvested materials A, B, and C were observed under the polarized microscope, the crystals changed to a birefringent gel within 5 min. PXRD analysis showed that this gel is the M mesophase of CS.[4] The preferential evaporation of the alkanol used to prepare materials A, B, and C increased the water concentration and caused the observed formation of the M mesophase. These results indicate that materials A, B, and C are the original crystalline hydrate form[2,3] of CS contaminated by varying proportions of M, and hence explain the moisture sorption diagrams and the DSC curves of Oguchi et al.[5,6] The reported[6] DSC curve of material C appears identical to that of the M mesophase in the present work. The steps observed in the reported[5,6] moisture sorption diagrams are probably a reflection of the
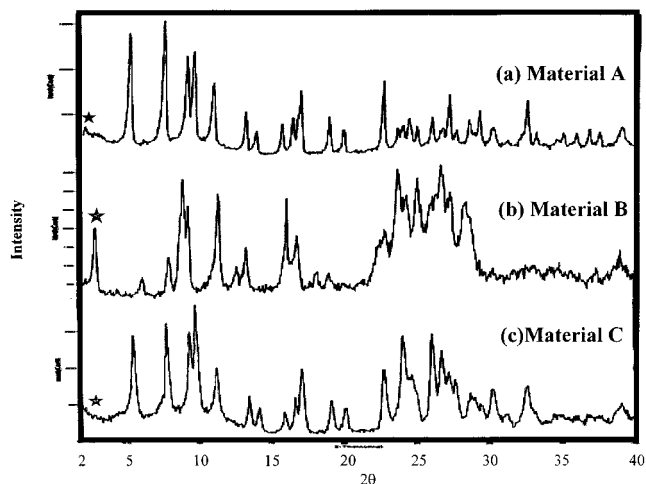

Figure 10—Powder X-ray diffraction patterns of (a) material A, (b) material B, and (c) material C prepared in our laboratory, according to the procedures described by Oguchi et al.[5,6]

reduction in the number of degrees of freedom caused by contamination with the liquid crystalline phase, M, in accordance with Gibbs phase rule and are not evidence for the formation of stoichiometric hydrates.

Conclusions

1. The "new forms" of CS reported by Oguchi et al.[5,6] have been prepared, characterized, and shown to be contaminated by the liquid crystalline phase, M, which complicated the PXRD pattern, moisture sorption, and the thermal analytical data.

2. Only one crystalline hydrate form of CS is observed, is nonstoichiometric, and corresponds to that reported by Cox et al.[2]

3. The crystal structure of CS with 6.44 water molecules per cromoglycate anion has been solved at 295 and 173 K. At 0% RH, loss of crystallinity accompanies loss of water, suggesting that the water molecules are necessary for holding together the crystalline structure. The second sodium ion of the CS occupies three partial crystallographic sites.

4. The solved crystal structure of CS is compared with that of Hamodrakas et al.,[3] which contains 5 to 6 water molecules per cromoglycate anion. The CS crystal structure is found to be flexible. The bond and torsional angles in the 2-hydroxypropane linking chain can change the relative orientations of the two cyclic moieties to accommodate lattice expansion or contraction resulting from water adsorption and desorption. The torsional angles of the two carboxylate groups can also change with changing water content.

5. The high water affinity and reversible water sorption–desorption of CS can be explained in terms of its molecular and crystal structure. The ionic nature and the polar groups of CS explain its hygroscopicity. The reversible and nonstoichiometric water sorption and desorption can be explained by the presence of large water channels, the flexible 2-hydroxypropane linking chain, and the disordered second sodium ion and the surrounding water molecules.

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Acknowledgments