

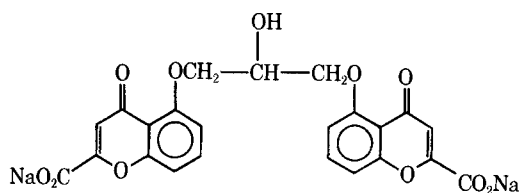
## Solid-State Chemistry of Cromolyn Sodium (Disodium Cromoglycate)

J. S. G. COX\*, G. D. WOODARD†, and W. C. McCRONE‡

**Abstract** □ A detailed crystallographic study was made to provide a better understanding and control of processing steps, purity control, and dosage formulations of cromolyn sodium. The compound, an odorless, white, hydrated crystalline powder, is the disodium salt of the dibasic acid: 1,3-bis(2-carboxychromon-5-yloxy)-2-hydroxypropane. No crystalline polymorphs were observed, but the crystals can quickly absorb (or lose) water as a continuous series of interstitial solid solutions. The amount of water absorbed or lost is proportional to the relative humidity of the environment of the crystals. At 90% R.H., the interstitial water approaches 24 wt. %, or nine molecules per molecule of cromolyn sodium. The absorbed water causes the lattice to expand reversibly, especially in the *b* crystallographic direction, and this results in remarkable changes in the X-ray diffraction pattern, density, and other physical properties. Above about 93% R.H., the compound absorbs more water than the crystals can hold. They then collapse to form one of two lyotropic mesophases, containing up to about 260 molecules of water per molecule of cromolyn sodium at 20° or about 10 wt. % cromolyn sodium.

**Keyphrases** □ Cromolyn sodium—crystallography □ Disodium cromoglycate—crystallography □ Aerosol dry powder—cromolyn sodium, crystallography □ Crystallography—cromolyn sodium (disodium cromoglycate)

Cromolyn sodium (I)<sup>1</sup> (also known as disodium cromoglycate) was recently introduced as a new pharmacological approach for the treatment of bronchial asthma (1). The compound, an odorless, white, hydrated crystalline powder, is the disodium salt of the dibasic acid: 1,3-bis(2-carboxychromon-5-yloxy)-2-hydroxypropane. Its crystals are, in a sense, an inter-



chemical structure of cromolyn sodium

I

stitial solid solution, with water the interstitial component. The unit cell expands to accommodate up to about nine molecules of water before collapsing to form liquid crystalline hydrates. Somewhat similar moisture pick-up was reported for zeolites (2), montmorillonite (3), rare earth oxalates (4, 5), zirconium oxalate (5), strychnine sulfate (5), hemoglobin (6), and another drug, cephaloridine (7).

Cromolyn sodium crystals are, however, unique in their extent of reversible water absorption without collapse of the lattice.

No solid polymorph of cromolyn sodium has been observed, although there are mesophases (liquid crystalline forms) of the hydrate. There is a peritectic reaction, however, between the solid solution and the mesophase at 73°; hence, they have different compositions and are not polymorphs.

The equilibrium between water and cromolyn sodium is very rapid and reversible in the vapor state or in aqueous solutions. Even though all of the physical properties (density, lattice parameters, refractive indexes, *etc.*) vary with water content, the changes are least pronounced in the relative humidity (R.H.) range of about 30–70%. Crystals of cromolyn sodium contain from five to six molecules of water over this relative humidity range, and the physical properties are reasonably constant. Relative humidity values either higher than 70% or lower than 30% cause larger changes in the physical properties. Above 93%, the mesophase forms on the surface of the crystals and, given time and sufficient water, the entire sample is converted to the mesophase.

The objective of this study was to characterize cromolyn sodium crystals as an aid to trouble-free processing and optimum formulation of dosages. It was also felt that any information on the shape of the molecule (planar, linear, spherical, *etc.*) might be useful in connection with separate studies on its mode of action. This information was particularly desirable because the drug is administered as a dry powder aerosol.

### EXPERIMENTAL

**Determination of Moisture Content of Solid Solution**—A constant-humidity chamber (Fig. 1) was designed and constructed to follow the weight of samples under known humidity conditions. The completely sealed system utilized a “quartz” spring balance in rapidly circulating air. Saturated aqueous solutions containing a solid excess of known inorganic salts were placed in the flask to maintain various constant, known, relative humidities.

The equipment was calibrated with balance weights. The extension of the spring was monitored by a traveling microscope fixed to the frame of the apparatus. Small marker flags on the wire suspending the aluminum foil pan were aligned with a cross-hair in the eyepiece of the microscope, and the height of the microscope was read on the scale. Changes in weight of 0.0001 g. could be measured with a sample weight of 0.1 g.

The sample was placed in the pan until the spring extension corresponded approximately to a weight of 0.1 g. The sample had been open to the laboratory atmosphere for a week before it was used. Simultaneously with the pan loading, an identical sample was

\* Intal, Fisons Ltd., Pharmaceuticals Division, Loughborough, Leicestershire, England.

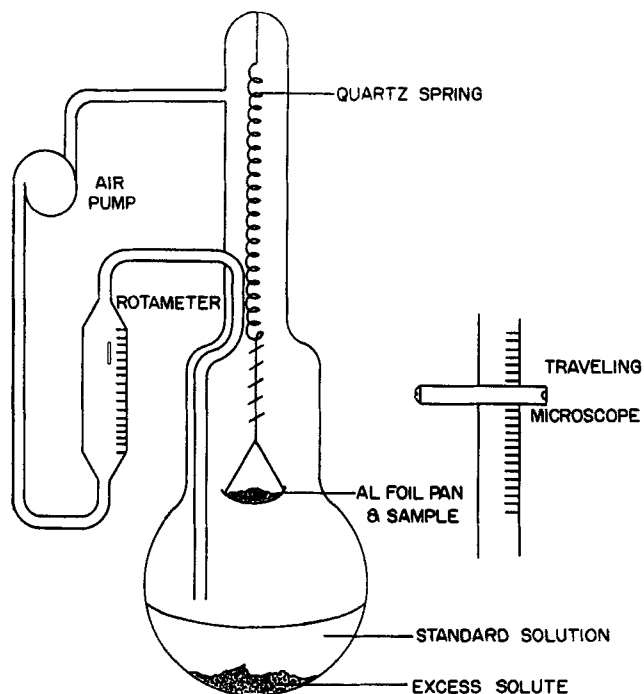


Figure 1—Experimental arrangement for "quartz" spring studies.

placed in a dry, tared, weighing bottle. This sample was heated at 150°, stoppered immediately on removal from the oven, and cooled over phosphorus pentoxide. This process was repeated until the sample showed constant weight. From these results, the moisture content was calculated for the sample on the quartz spring pan.

The aqueous solutions (containing excess solid solute) used to control the relative humidity in the apparatus are summarized in Table I. Phosphorus pentoxide was used to obtain 0% R.H.

After the proper aqueous solution was in position, the system was sealed and the air pump was started. Air blown at the surface of the solution caused ripples in the solution surface, thereby helping to keep it stirred. This method was preferred to bubbling the air through the solution because this procedure could give droplets of solution that might impinge on the pan and cause erroneous weight readings. The air was recirculated from the top of the column to the bottom flask at a rate of 3.6 l./min. To maintain the desired humidity, the apparatus was kept in a room thermostated at  $20 \pm 1^\circ$ . The atmosphere rapidly reached equilibrium, judged by the rapid weight changes. Often, 80% of the final weight change occurred in the 1st hr. after the solutions were changed.

The air flow was stopped while readings were taken to avoid small disturbances in the pan position. Weight readings were taken every hour immediately after the solution was changed but at longer intervals as the rate of change decreased. Moisture contents were monitored with both increasing and decreasing humidity.

**Melting-Point Data**—Melting points were taken using a Mettler hot stage fitted to a polarizing microscope (Swift; Dick model). The samples were mounted in silicone oil to improve visibility and to help prevent chemical decomposition in air at the high temperatures involved.

**Solubility Data**—Cromolyn sodium is generally soluble in polar solvents and insoluble in nonpolar solvents. It forms solid solutions or lyotropic mesophases with water and discrete solvates with methanol, ethanol, propanol, diethylene glycol, ethylene glycol monomethylether, glycerol, propylene glycol, dipropylene glycol, dimethylformamide, and dimethyl sulfoxide.

Quantitative solubility data for cromolyn sodium in water only were determined in this study. Crystals were added to water at constant temperature until no more dissolved. The precise saturation point was then found by careful temperature adjustment. An aliquot sample of the saturated solution was then taken for gravimetric analysis. To be certain the solid residue was anhydrous, the solution was evaporated in a weighing bottle and dried at 150° for 12 hr. and to constant weight. The bottles were sealed at 150° and kept sealed during cooling and weighing.

Table I—Aqueous Solutions Used for Humidity Control

Saturated barium chloride	88
Saturated sodium acetate	76
Saturated calcium nitrate	51
Saturated calcium chloride	31
Sulfuric acid, 59.0 wt. %	20
Sulfuric acid, 67.2 wt. %	10

**X-Ray Diffraction Data**—Samples of cromolyn sodium were stored over saturated solutions of each salt listed in Table I for several months. These were then opened to the air and quickly sealed for X-ray diffraction. Later, X-ray diffraction data were redetermined by X-raying each sample in a humidity-controlled camera. A focusing vacuum X-ray diffraction camera<sup>2</sup> was modified to permit circulation of controlled humidity air. The humidity was controlled by circulating air of known relative humidity just as in the quartz spring experiment. The same solutions (Table I) were used, but the flow rate through the camera was increased to 5 l./min.

The sample was pressed into a 4-mm. hole, 0.25 mm. deep, in the center of a metal disk. Once this disk was fixed in the camera, the sample was exposed to the controlled-humidity atmosphere on both sides. A single sample was used for X-ray diffraction over the full range of 0–88% R.H.

In the first experiment, with phosphorus pentoxide in the chamber, the sample was stored under a continuously pumped vacuum for 1 week before X-raying. This procedure was repeated with the solutions; but above 10% R.H., the equilibrium period was reduced to overnight. The quartz spring experiments had shown that this was sufficient time for equilibrium. The humidity was increased successively throughout this series of experiments.

To index the X-ray powder diffraction data, it was necessary to obtain single crystal data. This, in turn, required growth of large, well-formed, single, clear crystals. These were obtained by slow addition of ethanol to a saturated aqueous solution in an atmosphere saturated with ethanol. Slow absorption of ethanol vapors by the aqueous solution soon precipitated elongated crystals which grew slowly for several days, some to a length of 1–2 cm. The best of these crystals were selected for X-ray diffraction as well as crystal optics and morphology.

Single crystal X-ray diffraction data (precession camera), taken at near 50% R.H., gave cell dimensions permitting indexing of the appropriate powder data. A computer program was designed to index the powder data taken at 51% R.H. The powder data taken

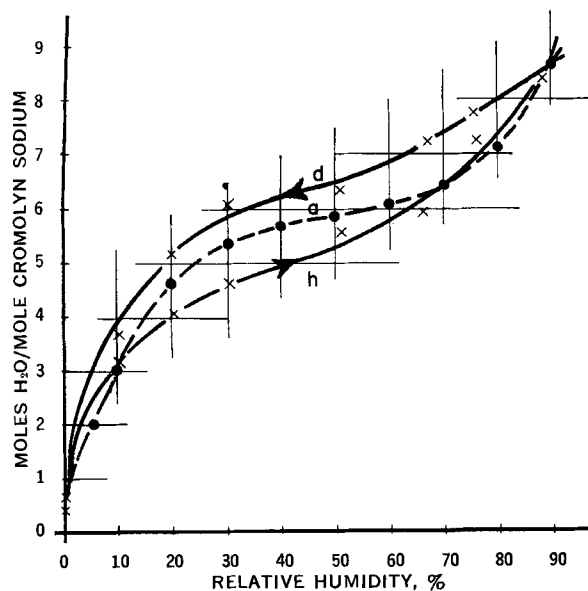


Figure 2—Water content as a function of relative humidity during hydration (h), dehydration (d), and an average curve for all data determined (a).

<sup>2</sup> Designed by Dr. James B. Nelson, McCrone Research Associates Ltd., London, England.

**Table II**—Powder X-Ray Diffraction Data as a Function of Relative Humidity ( $I/I_0$  in Parentheses)

Reflection hkl	Relative Humidity, %					
	0	10	20	31	76	88
S	27.0(10)	27.6(2)	27.8(1)	28.0(<1)	—	—
010	14.0(5)	15.0(3)	15.5(3)	15.8(4)	15.8(2)	15.8(2)
S	13.5(4)	13.8(1)	13.9(<1)	—	—	—
100	10.8(8)	11.1(6)	11.2(6)	11.2(2)	11.2(3)	11.2(4)
S	9.7(8)	9.8(10)	9.8(6)	9.9(4)	9.9(7)	9.9(8)
S	9.0(1)	9.2(6)	9.4(3)	9.4(3)	—	—
110	8.6(3)	8.9(6)	9.0(6)	9.0(3)	9.0(2)	9.0(2)
S	8.2(<1)	—	—	8.5(<1)	8.5(<1)	—
020	7.05(3)	7.55(8)	7.7(10)	7.90(5)	7.90(1)	7.90(1)
S	6.65(2)	6.60(1)	6.60(2)	6.90(<1)	—	7.15(2)
120	5.85(<1)	6.20(1)	6.35(3)	6.40(1)	6.40(1)	—
S	5.55(<1)	—	5.70(1)	5.80(1)	—	—
200	5.35(<1)	5.50(2)	5.50(1)	5.55(1)	5.60(<1)	—
S	5.15(<1)	5.25(<1)	5.25(4)	5.25(3)	5.30(3)	5.30(1)
S	4.84(<1)	4.90(1)	4.90(<1)	4.90(<1)	—	—
S	4.48(<1)	4.60(<1)	4.65(<1)	4.70(1)	—	—
220	—	4.50(1)	4.55(1)	4.60(1)	4.60(1)	4.60(1)
S	3.95(1)	3.98(<1)	3.98(1)	4.20(1)	4.25(<1)	—
S	3.82(3)	3.88(3)	—	—	—	—
S	3.68(1)	—	—	—	—	—
230	—	3.73(5)	—	3.80(1)	3.80(1)	3.80(6)
300	3.61(1)	3.73(5)	3.75(6)	—	3.70(10)	3.72(8)
101	3.61(1)	3.65(3)	3.65(6)	3.69(10)	3.70(10)	3.72(8)
040	3.54(<1)	3.73(5)	3.88(5)	3.90(5)	3.92(4)	3.94(4)
111	3.48(<1)	3.52(5)	3.55(6)	3.60(1)	3.60(1)	3.63(<1)
310	3.48(<1)	3.65(3)	3.65(6)	—	3.60(1)	3.63(<1)
201	3.44(<1)	3.52(5)	3.55(6)	—	3.60(1)	—
001	3.37(<1)	3.40(3)	—	3.42(5)	—	—
140	3.37(<1)	3.52(5)	3.65(6)	3.69(10)	3.70(10)	3.72(8)
011	3.31(1)	3.34(4)	3.34(6)	3.34(4)	—	—
211	3.31(1)	3.40(3)	—	3.42(5)	—	—
121	3.22(1)	3.23(4)	3.25(5)	3.34(4)	3.35(3)	3.35(3)
320	3.22(1)	3.34(4)	3.34(6)	3.34(4)	3.35(3)	3.35(3)
221	3.10(<1)	3.17(2)	3.17(<1)	3.25(2)	3.25(6)	3.25(6)
021	3.05(<1)	3.10(<1)	3.11(2)	3.17(2)	3.17(<1)	3.17(<1)
301	3.05(<1)	3.10(<1)	3.11(2)	3.12(2)	—	—
050	—	—	3.11(2)	3.17(2)	3.17(<1)	3.17(<1)
240	2.95(<1)	3.10(<1)	3.17(<1)	3.25(2)	3.25(6)	3.25(6)

over the full humidity range were then indexed, and the values of  $a$ ,  $b$ , and  $c$  were refined by successive iterations.

**RESULTS AND DISCUSSION**

**Physical Properties**—The properties of cromolyn sodium vary considerably with water content. The water content of the solid solution varies from zero to more than 24 wt. % corresponding to a range from zero to more than nine molecules of water per molecule of cromolyn sodium. The crystals exchange water freely with the atmosphere or with aqueous solutions. The rate-controlling step in the hydration-dehydration is diffusion of water through air (or aqueous solution) to the crystal. Crystals equilibrated in air at any given relative humidity show a corresponding water content. Undissolved crystals in excess, in any given aqueous solution, show an equilibrium water content corresponding to the relative humidity of air in equilibrium with that solution. If, however, the relative humidity exceeds 93%, the crystals collapse to form a mesophase.

Dehydration is easily effected by heat or desiccants. Rapid dehydration of the solid solution produces cloudy crystals, in contrast to the clearer crystals produced by slower changes in water content. Both clear and cloudy crystals show similar physical properties, although the clear crystals are able to hold somewhat more water before collapsing to the mesophase. Both clear and cloudy crystals are stable up to at least 93% R.H.

**Moisture Content of Solid Solution**—The importance of moisture content on structure and flow properties made it desirable to know the equilibrium moisture content for various relative humidities. It was also desirable to obtain a measure of the rate at which crystals gain and lose water when the humidity is changed. In early experiments, 1 week was allowed for equilibrium, but this was obviously unnecessary and was reduced to 24 hr. except at 0% R.H. over phosphorus pentoxide. The residual water came off slowly near 0%, and constant weight was not attained for 96 hr.

Figure 2 shows the change in water content as the relative humidity was increased and decreased for the same sample. This hysteresis curve would probably approach the average curve also shown in Fig. 2 if more time had been allowed for equilibrium at each relative humidity.

The shape of the hydration curve shows that, although there is a region of slow increase of moisture content with increasing humidity, there are no sharp plateaus corresponding to fixed hydrates. It is impossible to remove the final traces of water using phosphorus pentoxide. Oven-drying similar samples at 150° always removes slightly more water.

When the humidity of the air is altered, the rate of change of crystal moisture content is remarkably fast. Certainly the rate would be faster if the air humidity could be changed instantaneously, but the rate of moisture pick-up is still limited by the rate at which the air equilibrates.

The results of these and similar experiments permit plotting of an average equilibrium moisture content of cromolyn sodium crystals in air of known relative humidity (Fig. 2).

**Melting-Point Data**—Cromolyn sodium crystals are nearly always highly stressed, as indicated by the twisted and distorted crystals and by the behavior during dissolution in solvents on a microscope slide. Many needle crystals are distorted into C and S shapes, and many dissolve at greatly different rates in different portions of the same crystal. These indicate crystal lattice stress. In a given sample, some of the cromolyn sodium crystals, presumably somewhat stress relieved by physical cracking, melt at 264°, whereas the highly distorted and stressed crystals melt at 258°.

**Solubility Data**—The solubility data are incorporated in the composition diagram discussed later.

**X-Ray Diffraction Data**—The characterization of cromolyn sodium by X-ray diffraction would have been easier if the unusual behavior of these crystals with water vapor had been understood earlier. Early work in this laboratory indicated that crystals of cromolyn sodium only very slowly gained or lost water from the

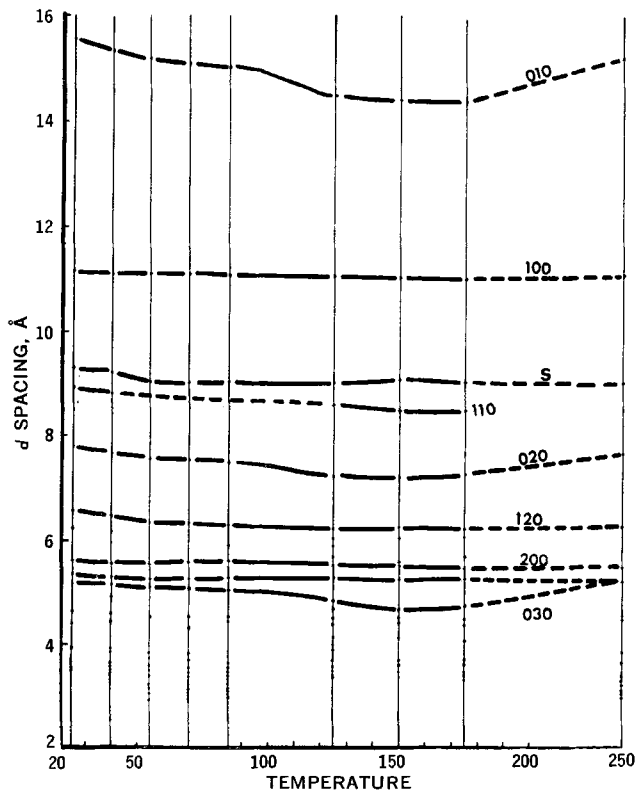
**Table III—Lattice Parameters for Cromolyn Sodium (Angstroms)**

Relative Humidity	Cell Edge		
	<i>a</i>	<i>b</i>	<i>c</i>
0	11.5	14.0	3.58
10	11.8	15.0	3.62
20	11.9	15.5	3.64
30	11.9	15.7	3.66
40	11.9	15.7	3.67
51	11.9	15.8	3.68
76	11.9	15.8	3.70
88	11.9	15.8	3.70

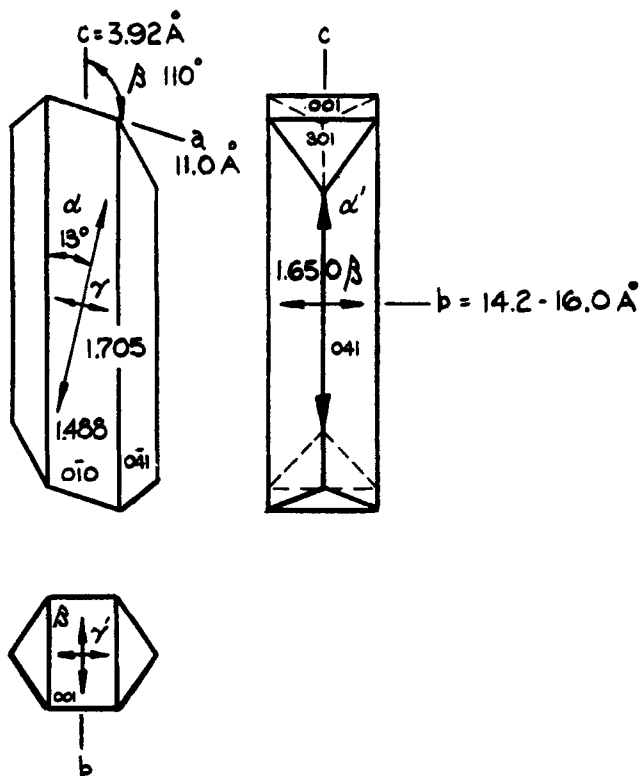
atmosphere. Thus, it was assumed that crystals equilibrated over a period of several weeks could be X-rayed at ambient relative humidities without significant change in water content.

Although the crystals were exposed to ambient humidities for less than 1 min., they changed remarkably rapidly in water content. Fortunately, differences were found and, at the time, they seemed large. Later, the quartz spring experiments showed that equilibration of cromolyn sodium crystals with the atmosphere was very rapid. Earlier experiments had measured, in a sense, the rate of diffusion of water molecules to the crystals. The crystals absorbed these molecules rapidly; the absorption rate was limited by the rate of diffusion or circulation of water molecules to the crystal surfaces.

It is necessary now to discuss the fact that the powder patterns show a large number of lines that cannot be indexed with the *a*, *b*, and *c* derived from the single crystal data. Some lines are also sometimes unaccountably broad and may even disappear when the relative humidity is changed. Furthermore, some lines have spacings larger than *a*, *b*, or *c*. It seemed clear that a larger unit cell must be related to moisture absorption by the lattice. To explain (and index these lines), an expanded lattice was postulated. It was assumed that the water molecules absorbed by the lattice in layers parallel to 010 (thereby expanding principally the *b* lattice dimension) did not add precisely the same number of water molecules between each pair of unit cells. The *b* dimensions of successive cells might vary as the number of associated water molecules varied. Yet the total number of molecules of water per 5, 7, or so molecules of drug might well be



**Figure 3—Powder X-ray diffraction data during successive stages of heating (and recooling) of cromolyn sodium.**



**Figure 4—Orthographic projection for crystals containing about five molecules of water per molecule of cromolyn sodium.**

constant. Then, in effect, the *b* dimension becomes 5, 7, or so times the value for the simplest cell containing one molecule of drug.

This expanded lattice hypothesis was tested by programming the computer to calculate all possible 0*k*0 diffraction lines for *k* = 0–36 and all values of *b* from *n*(14.0), *n*(14.2), *n*(14.4) . . . *n*(15.8), where *n* = 1–12. The lines, previously unindexed, were now easily indexed, usually with data for a new value of *b* equal to 7–9(14.0–15.8). The value of *b* was chosen in each case corresponding to the relative humidity during that diffraction run. These expanded lattice lines are marked S in Table II.

The expanded lattice lines tend to disappear for crystals equilibrated above 70% R.H. Small crystals show fewer expanded lattice lines than larger ones, presumably because diffusion of water molecules can be more easily equilibrated when distances are shorter. Small crystals, as a rule, show few, if any, expanded lattice lines, whereas large crystals often show more expanded lattice lines than parent lattice lines. One can conclude that large crystals must be hydrated or dehydrated slowly to avoid expanded *b*-axis spacings.

It is difficult to be sure of the indexing of all lines in the various powder patterns because of the multiple effects on spacings and intensities. Additional, though minor, variations occur in X-ray patterns for crystals of the same water content. This is probably due to the previous history of the sample and shows up predominantly in the expanded lattice lines.

The *b* crystallographic spacing expands continuously as the relative humidity increases (Table III). The change is most rapid below 20% R.H.; the unit cell dimensions are nearly constant above about 40% R.H. This is fortunate since the X-ray powder patterns

**Table IV—Refractive Indexes of Cromolyn Sodium as a Function of Relative Humidity**

Relative Humidity, %	Refractive Indexes, $n_D^{25^\circ}$			Optic Axial Angle, $2V$
	$\alpha$	$\beta$	$\gamma$	
4	1.488	1.68	1.705	40°
40	1.488	1.66	1.705	52°
90	1.488	1.615	1.705	76°

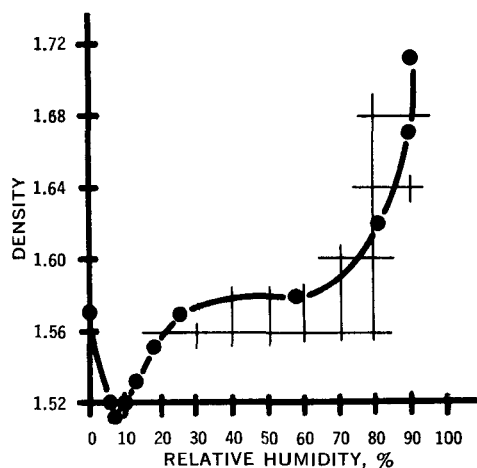


Figure 5—Density of cromolyn sodium versus relative humidity.

and densities are more nearly constant over the normal humidity range of 20–70%.

The effect of heat on the lattice dimensions was determined by installing a heating stage on an X-ray diffractometer. Powder diffraction data were taken at 45-min. intervals at successive temperatures of 25, 40, 55, 70, 85, 125, 175, and back to 25° (Fig. 3). It was difficult to index the shorter spacings because of the counterinfluence of lattice expansion on heating. The lines indexed show, however, that the crystals lose water gradually as the temperature rises, although more rapidly up to 40° and near 100°.

**Crystal Morphology**—Crystals of cromolyn sodium are not very well formed in laboratory or plant production. This is not, of course, unusual for a complex organic molecule. Most crystals are flat lath shapes, although a very few better formed rods can usually be found. Figure 4 is a somewhat idealized drawing, consistent with the observed X-ray data, optics, and morphology although, strictly speaking, the crystals must be hemisymmetric.

**Crystal Optics**—The optical properties vary considerably as a function of water content. The average crystal, containing about

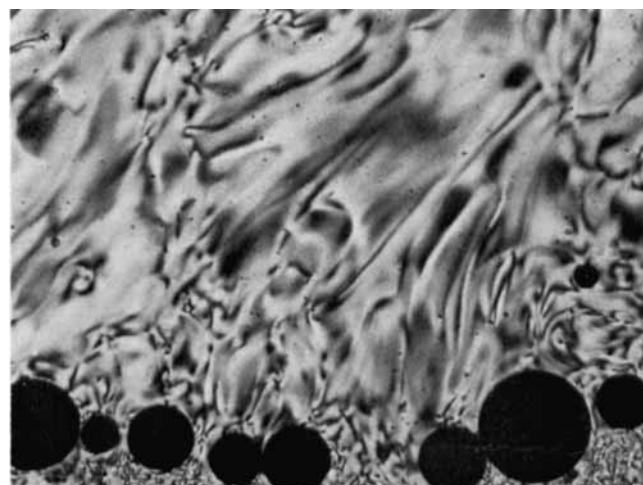


Figure 7—A 20:80 cromolyn sodium–water mixture at room temperature showing the nematic mesophase; crossed polars, 100×.

five molecules of water, is monoclinic with  $\beta$  parallel to  $b$  and  $\alpha$  inclined to  $c$ , the direction of elongation, by 13° and lying in the obtuse  $\beta$  angle. The refractive indexes  $\alpha$ ,  $\beta$ , and  $\gamma$  are 1.488, 1.650, and 1.705, respectively, with  $2V = 60^\circ$  (–) and little or no dispersion.

The refractive indexes, especially  $\beta$ , vary with the degree of hydration. To measure these variations, a glove box was provided with a polarizing microscope and provision for varying the relative humidity. Although it was impossible to achieve a relative humidity lower than about 4%, measurements were made at that level as well as 40 and 90%. A few crystals on a microscope slide were allowed to equilibrate for a few minutes within the glove box before each measurement of refractive index. These measurements (Table IV) were made on several individual crystals by immersion methods.

**Crystal Density**—The density can be calculated from the weight and volume of the unit cell. The unit cell varies, however, in both weight and volume as the water content of either air or aqueous

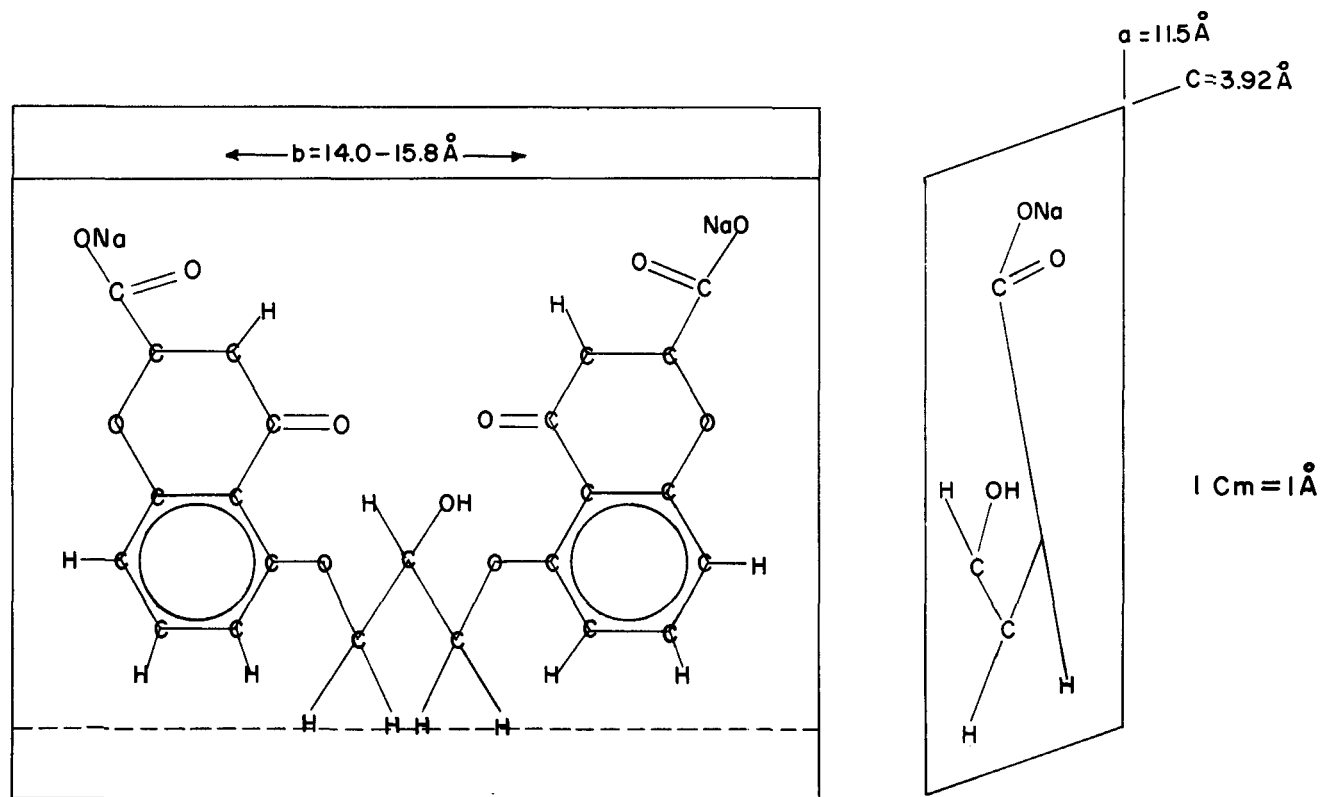
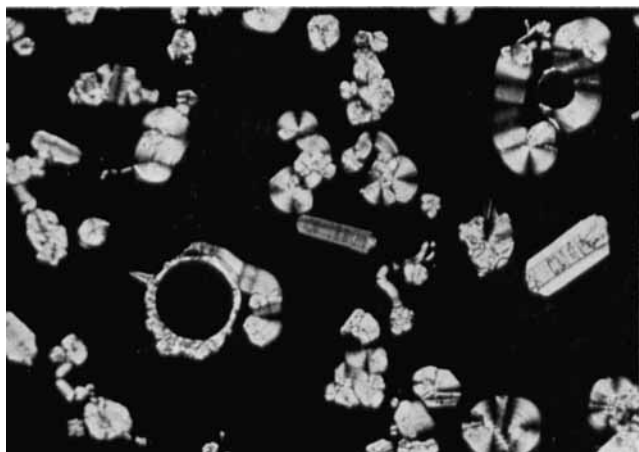


Figure 6—Probable approximate crystal structure for cromolyn sodium and its solid solution with water.



**Figure 8**—Spherulites and bâtonnets in a 20:80 cromolyn sodium-water mixture cooled to 50° from 55°; crossed polars, 100×.

suspensions varies. The experimental lattice dimensions and water content, as a function of relative humidity, are used for this calculation. Experimentally, on the other hand, one would not be sure the flotation or displacement medium was not affecting directly measured density values.

The information in Table III is used to calculate the density of any sample as a function of relative humidity. The appropriate formulas are:

$$\text{density} = \frac{\text{weight of unit cell}}{\text{volume of unit cell}} \quad (\text{Eq. 1})$$

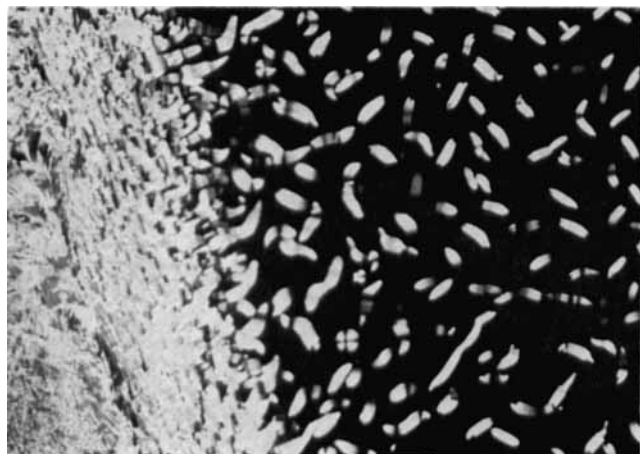
$$\text{density} = \frac{\text{mol. wt.} \times n \times 1.6604 \times 10^{-24}}{a \times b \times c \times \sin \beta} \quad (\text{Eq. 2})$$

where:

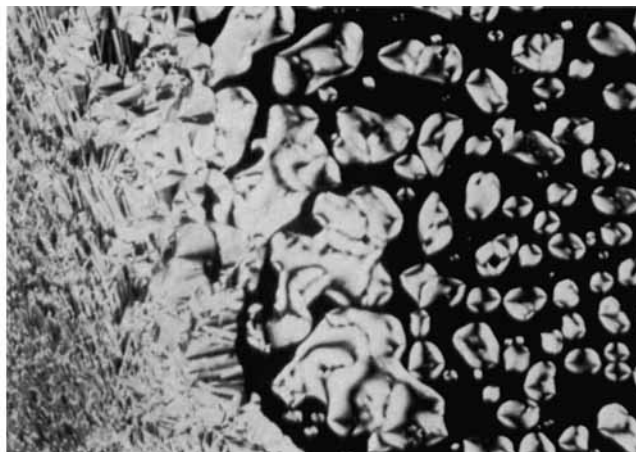
- $n$  = number of formula weights per unit cell
- $1.6604 \times 10^{-24}$  = mass in grams of 1 unit of atomic weight
- $a$ ,  $b$ , and  $c$  = dimensions of the unit cell expressed in cm.
- $\beta$  = crystallographic angle between the  $a$  and  $c$  axes

There is only one molecule per unit cell; hence,  $n = 1$ . This is apparent if only because densities higher than 1.5-1.7 by factors of 2, 4, or 8 (corresponding to  $n = 2, 4, \text{ or } 8$ ) are absurd for this compound. Several experimental density values, moreover, fall in the 1.5-1.6 range. The calculated density data are given in Fig. 5.

The unusual shape of the density-relative humidity relationship in Fig. 5 is a consequence of the relative rates of moisture pick-up and expansion of the lattice as the humidity increases. When the rate of expansion is high relative to the rate of moisture absorption, the density goes down (0-10%). When the rate of expansion de-



**Figure 9**—The middle mesophase of cromolyn sodium crystallizing from water on cooling to 45°; crossed polars, 100×.



**Figure 10**—Same field of view as Fig. 9 after transformation of the middle to the nematic mesophase. The temperature here is 41°; crossed polars, 100×.

creases and the rate of moisture absorption is high, the density rises rapidly (60-90%). From about 25 to 60% R.H., both rates are correspondingly slow and the density changes only slightly.

**Crystal Structure**—A fortunate set of circumstances made possible the prediction of a likely crystal structure for anhydrous cromolyn sodium. These circumstances are:

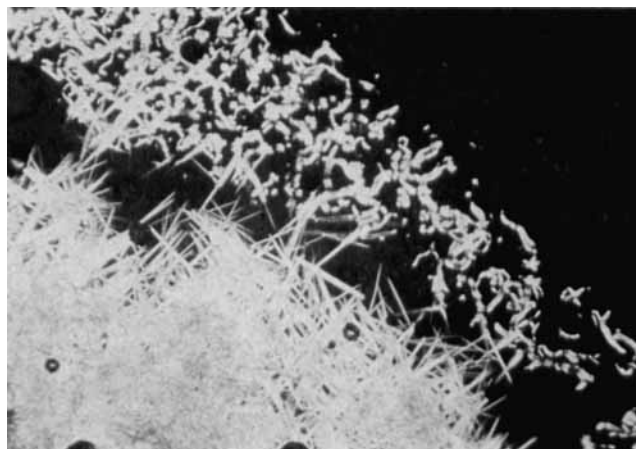
1. There is only one molecule per unit cell and every molecule must be oriented similarly within each unit cell.
2. One lattice spacing,  $c$ , is only 3.92 Å. Since the molecule must be able to fit entirely within a unit cell, it must lie in a planar configuration. Furthermore, for the same reason, the plane of all molecules in the crystal must lie more or less parallel to the  $a$ - $b$  plane and perpendicular to  $c$ .

3. The refractive indexes and extinction angles confirm this arrangement of the molecule and, in addition, show the plane of the molecule lying about 13° from  $c$  in the obtuse  $\beta$  angle.

4. Both morphology and optics of the crystals, taken with the fact that there is one molecule per unit cell, show that the molecule has a plane of symmetry and this plane lies in the  $a$ - $c$  crystallographic plane.

Study of molecular models suggests that there are only two possible structures, with the one shown in Fig. 6 most likely. The alternate possibility involves flipping the molecule over like a pancake. One of these two arrangements is most likely a close approximation to the crystal structure. Much more single crystal X-ray work under carefully controlled zero humidity conditions will be necessary to define precisely the position of each atom in the unit cell.

**Water-Cromolyn Sodium Composition Diagram**—Every known solid cromolyn sodium crystal can be characterized as an interstitial solid solution containing from zero to about nine molecules of water per molecule of drug, *i.e.*, 0-24 wt. % water.



**Figure 11**—The peritectic reaction between cromolyn sodium-water solid solution and the middle mesophase taking place at 73°. The needles at the top are the mesophase; crossed polars, 100×.

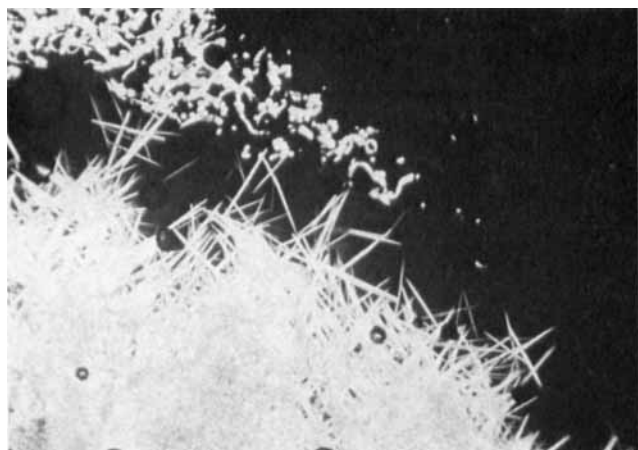


Figure 12—Same field of view as Fig. 11 as the peritectic reaction continues at 73°, giving more and more of the solid solution; crossed polars, 100×.

The water-cromolyn sodium composition diagram is based on microscopical observations and solubility data. The entire diagram was determined, at least semiquantitatively, by hot-stage microscopy. To complete the diagram, a solubility curve was determined and known mixtures were observed microscopically during heating.

If excess solid cromolyn sodium is placed in a drop of water between slide and coverslip at room temperature, a nematic lyotropic mesophase immediately forms. This mesophase shows a dynamic sinuous texture. Its appearance between crossed polars is shown in Fig. 7. On heating to about 42°, the appearance changes reversibly (the change is incongruent, *i.e.*, a peritectic). This change to a middle "M" phase is best observed in an aqueous mixture of 20–25 wt. % cromolyn sodium. If this mixture is heated above 42°, the "M" mesophase slowly dissolves. If the temperature is then slowly lowered (about 2°/min.) after holding for a minute or two at about 50°, excellent batonnets, spherulites, and fibers of "M" appear (Fig. 8). As the temperature continues to fall, the transition to the nematic mesophase is easily recognized at about 42°. At this temperature the more highly organized "M" mesophase collapses to the flowing sinuous nematic mesophase (Figs. 9 and 10).

Raising the temperature again reverses the transition at about 42°. Further increase in temperature to about 73° results in a second transition, this time to the solid solution, a crystalline phase (Figs. 11 and 12). This, too, is a reversible peritectic-type transition. A uniform mixture of about 15% cromolyn sodium shows the nematic phase (Fig. 11) at room temperature. At the edges of such a coverslip preparation, where water is evaporating at room temperature, one can observe the characteristic transition to the middle mesophase (Fig. 13). Careful study of this boundary between the nematic and

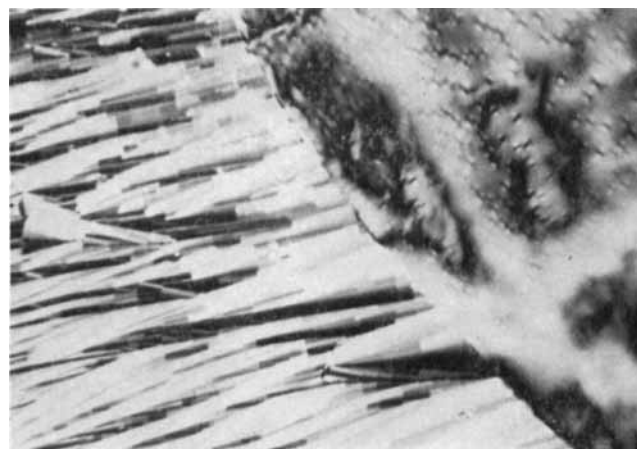


Figure 13—A coverslip preparation of the nematic mesophase (right) slowly drying at the edge of the preparation to give the middle mesophase (left); crossed polars, 100×.

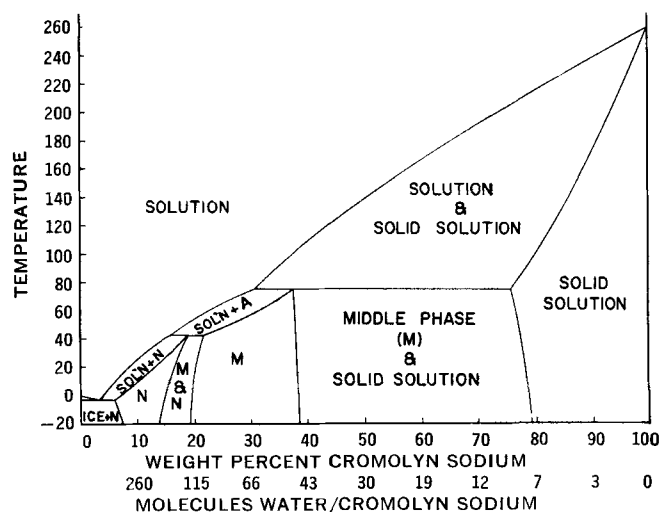


Figure 14—The cromolyn sodium-water composition diagram.

middle mesophases shows that the two mesophases are stable together over a short range of water content. This is best observed, as the stage is rotated, with crossed polars. The extinction pattern characteristic of the nematic mesophase extends into the region where the middle mesophase is growing.

These data define the composition diagram qualitatively. To make the diagram more quantitative, the boundary between aqueous solution and aqueous suspension of excess solute, the solubility curve, was determined. The eutectic temperature between water and the nematic mesophase was then measured using the Mettler hot stage. To measure this subambient temperature, the stage was chilled to below -20° using liquid nitrogen. The digital readout and programmed heating controls on the hot stage function from -20 to +300°; hence, temperatures can be measured over that range. A composition gradient preparation was made between slide and coverslip by placing a drop of water on one side and solid cromolyn sodium on the other. On contact, the nematic mesophase immediately formed. On placing this preparation in the precooled stage, all phases—water, eutectic, and cromolyn sodium—froze. Heating at 2°/min. gave a corrected melting point for the eutectic of -2.7°. The composition of the eutectic, 5 wt. % cromolyn sodium, is based on extrapolation of the solubility data to the eutectic temperature line.

To complete the diagram, a number of known mixtures were prepared. These were heated, thoroughly mixed, cooled, and kept sealed. A small portion of each was then placed between the slide and coverslip and observed carefully during heating in the Mettler hot stage. Any phase changes occurring during heating could be observed for each concentration. These changes included: (a) either mesophase giving a mixture of mesophase plus solution, (b) transition of nematic to middle mesophase, and (c) transition of middle mesophase to solid solution. The completed diagram (Fig. 14) is based on careful study of these prepared mixtures. Mixtures were prepared at 5 wt. % intervals, and the placing of the phase boundaries is believed accurate to within at least 2–3%.

The behavior of lyotropic mesophases is quite complex (8–10) and cromolyn sodium is no exception. More complete details will be published separately.

## CONCLUSION

Cromolyn sodium crystallizes with a single crystal lattice; no crystalline polymorphs were observed. These crystals can quickly absorb up to 24 wt. % water as a continuous series of interstitial solid solutions. The hydrated crystals containing up to nine molecules of water per molecule of cromolyn sodium can then quickly lose water, with no significant change in the molecular arrangement.

The percentage of water in the lattice is proportional to the relative humidity of the surrounding atmosphere. Similar changes in water content are induced by surrounding the crystals with an aqueous solution. The water content of the crystals is then related to the relative humidity of the solution in which the crystals are immersed.

This absorbed water, amounting to as much as 24 wt. % at about 90% R.H., results in reversible expansion of the lattice, especially along the *b* crystallographic direction. This, in turn, causes remarkable changes in X-ray diffraction patterns, density, and other physical properties.

A relative humidity higher than about 93% causes cromolyn sodium to absorb more water than the crystals can hold. They then collapse to form one of two lyotropic mesophases, containing from about 45 to as much as about 140 molecules of water per molecule of cromolyn sodium at 20°.

These data should be very helpful to plant processing and formulation experts. With this background, production and research personnel can be more sure that they have the best processing and storage procedures as well as the best dosage form. Problems arising in the plant or during storage and use are also more quickly solved. No important drug should be marketed without a careful study of its solid-state chemistry.

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*Note added in proof:* Recent X-ray work on the mesophases showed two important new results: (a) a single long spacing for the nematic mesophase, confirming a random arrangement of planar molecules, and (b) two shorter spacings as well for the middle mesophase related in the ratio  $1:1/\sqrt{3}:1/\sqrt{4}$ . The latter means, in turn, that the middle phase is properly named and consists of cylindrical assemblages of cromolyn sodium molecules about 25 Å in diameter and spaced from 25 to 37 Å apart, depending on water content.

## Quantitative Analysis of Microgram Quantities of Pilocarpine in Aqueous Solution

A. REPTA and T. HIGUCHI

**Abstract** □ Aqueous solutions containing microgram quantities of pilocarpine and pilocarpic acid were assayed quantitatively, using a kinetic method based on the catalytic activity of the imidazole portion of the alkaloids on the hydrolysis of 2,4-dinitrophenyl acetate. The results were accurate to better than  $\pm 5\%$  over a range of concentration of 6–60 mcg. of pilocarpine/ml. The effects of temperature, salt concentration, and pH were studied. Procedures for analysis of mixtures of pilocarpine and pilocarpic acid are given. Also a method is described for the analysis of small volume samples containing less than 1 mcg. of pilocarpine.

**Keyphrases** □ Pilocarpine and pilocarpic acid—kinetic analysis, hydrolysis of 2,4-dinitrophenyl acetate □ Pilocarpic acid and pilocarpine—kinetic analysis, hydrolysis of 2,4-dinitrophenyl acetate □ Kinetic analysis—pilocarpine and pilocarpic acid aqueous solutions

While numerous assay procedures are available for the determination of pilocarpine, including colorimetric, volumetric, polarimetric, and polarographic, (1, 2), all have some drawback. Among the drawbacks are a lack of sensitivity to small quantities of the alkaloid, the inability to distinguish between pilocarpine and its degradative products, and relatively high sensitivity to some inorganic and organic substances which may be present. The present investigation was concerned with the development of an assay procedure that would be: (a) sensitive to microgram quantities

of pilocarpine, (b) relatively unresponsive to many impurities, (c) capable of distinguishing between pilocarpine and its primary degradative product, pilocarpic acid, and (d) suitable for routine analysis of pilocarpine solutions.

The kinetic approach employed arose from the recognition of the substituted imidazole portion of the pilocarpine molecule as a potential catalyst for the hydrolysis of esters such as the nitrophenyl acetates. The strong catalytic effects of imidazole and some of its derivatives were shown previously (3–8).

While the employment of the kinetics of both catalyzed and uncatalyzed reactions in quantitative analysis is well known (9–11), most of the methods used involve inorganic species and/or enzymes. The procedure described here represents one of the first successful applications of kinetic analysis to relatively simple organic molecules.

#### EXPERIMENTAL

**Equipment and Chemicals**—An Orion model 801 pH meter was used for pH determinations. Cary models 14 and 15 spectrophotometers were used for absorption measurements. Titrations were carried out using a Scientific Industries model 200 ultra-buret. Small volumes of liquid were measured using Hamilton syringes. Graduated 5-ml., glass-stoppered, tapered-tip, Pyrex centrifuge