

## Disorder in Cyclodextrin Clathrates: X-ray Diffuse Scattering Analysis of $\alpha$ -Cyclodextrin Cyclopentanone Hydrate

G. LE BAS<sup>a\*</sup> AND J. DOUCET<sup>b</sup>

<sup>a</sup>Laboratoire de Physique, Centre Pharmaceutique, Université Paris-Sud, F-92290 Chatenay-Malabry, France, and  
<sup>b</sup>LURE, Batiment 209 D, Université Paris-Sud, F-91405 Orsay, France. E-mail: lebas@psisun.u-psud.fr

(Received 7 August 1996; accepted 10 January 1997)

### Abstract

Most of the cyclodextrin clathrates display disorder, consequently the structure determination is often difficult to carry out. Previous studies by X-ray and neutron diffraction have shown that the  $\alpha$ -cyclodextrin cyclopentanone hydrate crystallizes in a channel-like structure with hexagonal *P6* symmetry. Two inclusion complexes were found in the asymmetric unit; there is an orientational disorder of the guest with six positions found in one host cavity, three in the other. In addition, certain host hydroxyl groups and water molecules were found to be disordered and their disorder was correlated with the guest disorder. In this paper we investigate X-ray diffuse scattering phenomena in order to go further in the interpretation of disorder. Complicated diffuse scattering patterns revealed a variety of disorder phenomena. Some were not shown by the average structure determination. The interpretation of the results (structure in domains, supercell and vacancies) helps to explain the rotational disorder of the guest and some observed strains of the averaged structure. The description of different microstructures may be of interest for crystal growth and inclusion chemistry.

### 1. Introduction

Cyclodextrins, consisting of linked D-(+)-glucopyranose units, have a toroidal shape with hydrophilic upper and lower rims. A hydrophobic cavity is found in the interior of the cyclodextrin molecule. Cyclodextrins are able to form inclusion complexes with different types of guests of suitable size.

Inclusion phenomena may modify the physicochemical properties of the guest molecules. For this reason, cyclodextrins are used in many fields as pharmaceutical preparations, solubilizing agents or even as an enzymatic catalysis model (Szejtli, 1988).

Inclusion also modifies the host conformation in order to obtain better complementarity between host and guest (Saenger, 1976).

Solvent molecules, usually water, play a great part in the inclusion phenomenon. When the guest is polar,

the water molecules may influence the specificity of the interaction by competing for hydrogen bonds. The water molecules may also modify the host conformation in interacting *via* hydrogen bonds and in cooperating to build assemblies of different molecules such as dimers or columns *etc.*

Cyclodextrin complex crystals have a reasonable number of atoms, which provide crystallographers with a reliable model system for studying molecular interactions. The interactions being governed by weak binding forces and spatial complementarity as in biological complexes, the crystal structure analyses are often impaired by disorder phenomena. The most frequent disorder is an orientational disorder of the guest, *i.e.* the guest molecule can occupy different sites in the host cavity. As in biological complexes, the solvent plays a critical role in the occurrence of disorder. Water molecules occupy disordered positions; these different positions produce specific interactions with the cyclodextrin molecules and can modify in different ways the host–guest interaction. In these cases different frozen pictures of the hydrated host–guest complex are shown in the same crystal. These disorder phenomena are worthy of study as results of different states of equilibrium in the solid state and help to describe the deformability of the host molecule, and the role of the solvent in the inclusion phenomenon and also in the dynamics of the process.

Whereas X-ray and neutron diffraction analyses see the averaged structure of crystalline inclusion complexes, and in some cases of disorder allow at best to observe different sites for one atom or a chain of atoms, diffuse scattering studies are able to go further in providing valuable information on a large variety of disorder phenomena. This is possible because, in contrast to Bragg diffraction, diffuse scattering is sensitive to correlations between atomic displacements (Guinier, 1963; Epstein & Welberry, 1983).

In this paper we report the diffuse scattering analysis of the hydrated complex of  $\alpha$ -cyclodextrin cyclopentanone. The  $\alpha$ -cyclodextrin cyclopentanone hydrate crystallizes in a channel-like structure. This structure has been studied by X-ray diffraction at room

temperature and neutron diffraction at 20 K (Le Bas & Mason, 1994). The symmetry is hexagonal  $P6$  with two types of channels along the  $c$  direction; one with sixfold symmetry going up and a second with threefold symmetry going down. The  $\alpha$ -cyclodextrin molecule has potentially two-, three- and sixfold symmetries. The average structure analysis showed that the guest molecules are statistically distributed over three or six equivalent orientations in the channels, as expected, the cyclopentanone molecule not allowing three- or sixfold symmetry. In addition, host hydroxyl groups and water molecules were found disordered and their disorder was correlated with the guest disorder.

Alternative intermolecular hydrogen-bonded networks involving host-guest and solvent molecules were described. The alternate conformations of this inclusion compound clearly show that the  $\alpha$ -cyclodextrin flexibility is correlated to the hydration pattern.

An X-ray diffuse scattering study was undertaken in order to further our interpretation of the disorder.

## 2. Experimental and computational procedures

Single-crystals were grown by slow cooling techniques. They were sealed in glass capillaries with a drop of mother liquor.

X-ray pictures were taken on at least seven crystals at room temperature using synchrotron radiation on the D43 station at Lure (Paris-Sud University). A monochromatic beam ( $\lambda = 1.49 \text{ \AA}$ ) was selected and focused by a single Si(111) crystal. The diffraction and diffuse scattering features were recorded on a precession camera, either in the normal Buerger mode or in still geometry. The size of the beam was limited to 0.5 mm by a collimator. Two types of two-dimensional detectors were used: photographic films or image plates (Molecular Dynamics scanner) with pixel size  $0.176 \times 0.176 \text{ mm}^2$ .

Diffuse scattering data analysis was carried out using a three-step procedure: hypothesis of disorder (model), calculation of the corresponding scattering pattern (simulation) and comparison between calculated and experimental diffuse scattering.

The first step is a careful investigation of the diffuse scattering features and the corresponding disorder phenomena. The analysis of the localization of the diffuse scattering and the overall intensity distribution provides the correlation volumes and the direction of the atomic displacements.

For the simulation step, calculations were performed using a treatment by isolated substructures. The diffraction pattern of an inhomogeneous structure with limited ordered domains or groups of molecules or groups of atoms, having no correlations between them, may be described by the difference

$$\langle |F|^2 \rangle - |\langle F \rangle|^2,$$

where  $\langle |F|^2 \rangle$  is the average intensity scattered by all the domains composing the crystal and  $|\langle F \rangle|^2$  the intensity from the averaged arrangement of all the units. In the case of displacement  $u$ , this difference becomes

$$|F|^2 [1 - \exp(-4\pi^2(s.u)^2)],$$

where  $s.u$  is the scalar product of the atomic displacement  $u$  and the scattering vector  $s = 2 \sin \theta / \lambda$ .

The third step consists of a comparison between simulated films and the experimental films to determine the quality of the simulations.

These calculations were carried out with the software package developed by Doucet & co-workers, which has been used to explain diffuse scattering features in protein crystals, in particular orthorhombic lysozyme crystals (Doucet & Benoit, 1987).

## 3. Results

### 3.1. Description of diffuse scattering features

Different diffuse scattering features were observed. They are described in the following:

(a) All the pictures exhibit a broad diffuse ring located at  $0.3 \text{ \AA}^{-1}$ . This ring is known to correspond both to scattering from bulk water (solvent) and also to scattering due to uncorrelated or poorly correlated motions of atoms. These features were not studied here.

(b) The diffraction pattern of Fig. 1 reveals sharp layer lines perpendicular to the  $[001]$  direction and coinciding with the Bragg planes. These layers correspond to diffuse planes as shown by analysis of different pictures obtained by rotation around the  $c$  axis. The width of these layers,  $< 0.001 \text{ \AA}^{-1}$ , corresponds to the experimental resolution. Layer intensities are modulated, *i.e.* they present maxima and minima, and obey sixfold symmetry. Interestingly, the zero-order layer is weaker in comparison with others and especially with the layer of the order  $l = 1$ , which is the strongest. These layers tend to disappear after several days of exposure to the synchrotron beam, even though the Bragg diffraction pattern does not deteriorate.

(c) There are also weak diffuse peaks corresponding to the supercell period:  $c' = 3c = 24.00 \text{ \AA}$ . They are centred at positions  $h,k,l/3$ , where  $h$ ,  $k$  and  $l$  are integers, as was seen on precession photographs  $h,k,1/3$  and  $h,k,2/3$ . These weak peaks are observable only for  $s = 2 \sin \theta / \lambda > 0.25 \text{ \AA}^{-1}$ . The width of these superstructure reflections corresponds approximately to  $0.013 \text{ \AA}^{-1}$  in the plane  $(h,k,0)$  and along the  $[001]$  direction (Figs. 1–2). For some samples, these reflections are like streaks along the  $[001]$  direction.

(d) Finally, clearly visible on Figs. 1 and 2 are three other periodic diffuse layer systems parallel to the  $[001]$  direction which are, respectively, perpendicular to directions  $[h00]$ ,  $[0k0]$  and  $[hh0]$ . They correspond to  $h$  and  $k$  integers and as superstructure reflections they

are observable for  $s > 0.25 \text{ \AA}^{-1}$ . These layers are much thicker than the layers described above; their width is around  $0.013 \text{ \AA}^{-1}$ , *i.e.* about the same as the superstructure reflection width. Recording of different patterns corresponding to different orientations has confirmed

that these layers correspond to diffuse planes and not only to streaks.

### 3.2. Interpretation of diffuse scattering

Previous studies of the highly symmetrical averaged structure of  $\alpha$ -cyclodextrin cyclopentanone hydrate revealed a rotational disorder of the guest molecules and consequently of their associated host molecule, of which one primary hydroxyl group exhibits a modified conformation resulting from a guest interaction. Solvent molecules were also found to be disordered, each position being specifically hydrogen bonded with different positions of the disordered hydroxyl groups. Views of the averaged structure are shown in Figs. 3 and 4. It was not surprising that there are superstructure reflections: disordered atoms in the average cell may display order in a supercell.

**3.2.1. Superstructure.** We could expect that the superstructure would not obey the same symmetry as the average structure, but all recorded pictures display sixfold symmetry. Therefore, we used a supercell model with lower symmetry rotated by a sixfold twinning axis.

The periods of the superstructure cell are  $a$ ,  $b$  and  $3c$ . The asymmetric unit of the supercell was formed by one unit cell,  $a$ ,  $b$ ,  $c$ , with one position of the guest molecule in each channel, with the corresponding induced conformation of the associated host hydroxyl group and with correlated bonded water molecule positions. The model of the asymmetric unit was deduced using all the possible alternative arrangements of solvent molecules found in the neutron study (Le Bas & Mason, 1994). The  $P3_1$  supercell consists of three such asymmetric units. This symmetry has been considered from several structural considerations as the most likely to give rise to the same overall repetition in the two guest columns. The superposition due to the twinning pattern described below makes it impossible to deduce the exact symmetry of the supercell.

The superstructure is limited to short-range ordered domains ( $\sim 70 \text{ \AA}$ ), as indicated by the width of the peaks. Therefore, we considered a domain built with three supercells in the  $ab$  plane and a height of two supercells along  $c$  (Fig. 5).

The disappearance of the reflections surrounding the centre can be explained by small displacements of the domains in the  $ab$  plane. Displacements of  $0.05 \text{ \AA}$  were introduced in the calculations. These displacements may be either in the  $a$  or  $b$  or  $(a + b)$  directions (depending on the domains) and the correlation length corresponds to the extent of the domain. In short, the superstructure reflections correspond to the supercell  $a$ ,  $b$  and  $3c$ . This crystal structure is built up from small domains which are displaced in different directions,  $a$ ,  $b$  and  $(a + b)$ , and rotated around a sixfold twinning axis. The superposition of the scattering from all these domains gives rise to the superstructure diffuse scattering.

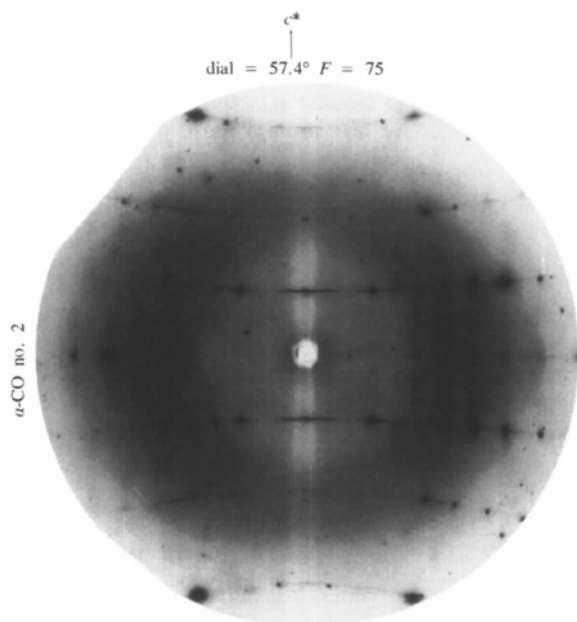


Fig. 1. X-ray experimental still photograph of  $\alpha$ -cyclodextrin cyclopentanone hydrate parallel to the  $c$  axis (vertical on the figure). Sharp diffuse layers perpendicular to the  $[00l]$  direction are shown, as well as broad diffuse layers parallel to the  $[00l]$  direction and superstructure reflections along these broad diffuse layers.

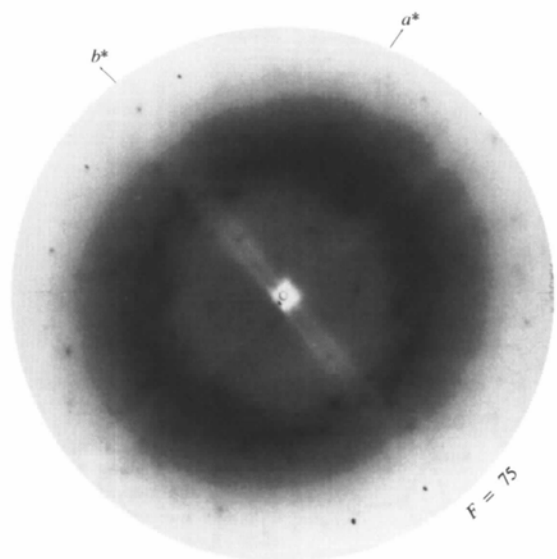


Fig. 2. X-ray experimental still pattern perpendicular to the  $c$  axis showing superstructure reflections as well as broad diffuse layers perpendicular to  $[h00]$ ,  $[0k0]$  and  $[hh0]$  directions (these directions are parallel to the figure).

The corresponding simulations are shown in Figs. 7 and 8. Although there are slight differences between some calculated and experimental intensities, the overall calculated picture is close to the experimental features.

**3.2.2. Broad diffuse planes.** To treat the diffuse layer system parallel to the  $c$  direction containing Bragg reflections as well as superstructure reflections, we used a model of rows of rigid host molecules perpendicular to the  $c$  axis and parallel to the  $a$  and  $b$  axes. These rows are periodic of periods  $a$ ,  $b$  and  $(a + b)$ , which also are the periods of the supercell. In each row host molecules

are strongly linked by hydrogen bonds (Fig. 6). For a given row, for example a row of period  $a$ , all the atoms are given the same mean displacement  $0.05 \text{ \AA}$  (in the  $a$  direction) from the mean position. In our calculations we assumed that the displacement correlation is extended in the  $a$  direction over 3 units cells (according to the width of the corresponding diffuse scattering planes,  $\sim 70 \text{ \AA}$ ), the displacements being uncorrelated between different rows.

Again we had to assume six equivalent rows rotated from each other by  $60^\circ$  around the  $c$  axis in order to

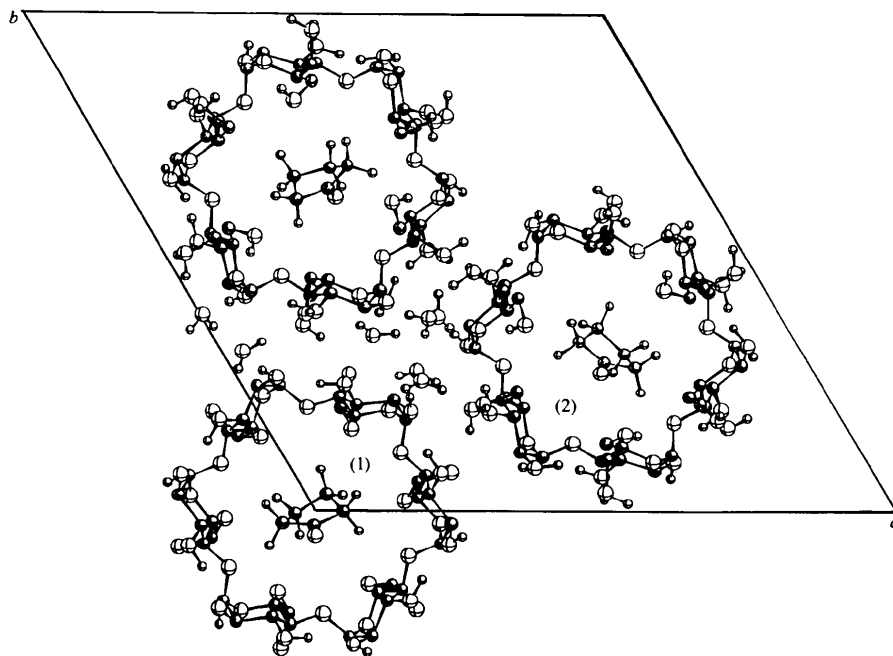


Fig. 3. Projection of the unit cell on the  $ab$  plane. For cyclodextrin molecules, only H atoms involved in hydrogen bonds are shown. Atoms are shown using three sizes of balls; large for O atoms, medium for C and small for H. There are two independent cyclodextrin molecules in the cell, molecule (1) lying along the crystallographic sixfold axis and molecule (2) lying along the crystallographic threefold axis. Only one position is shown for the guest molecules.

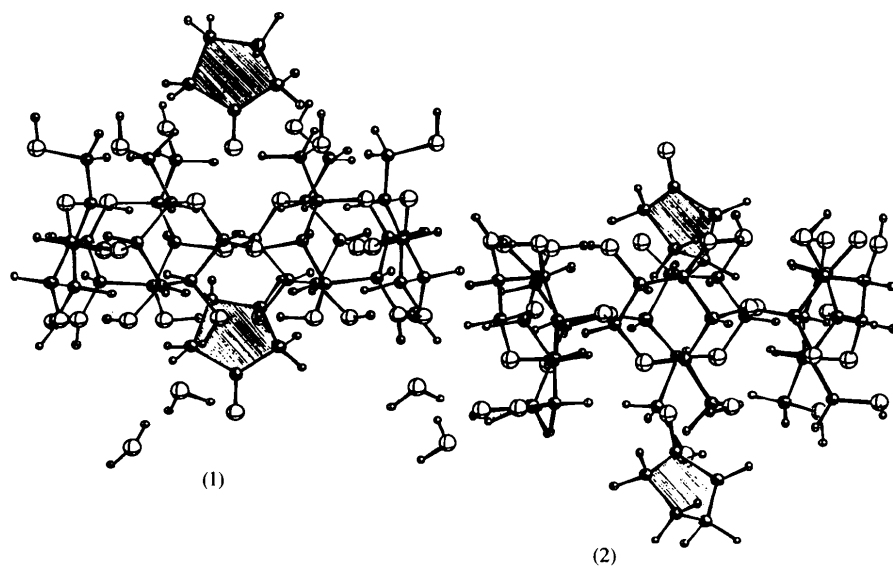


Fig. 4. A view of the structure approximately along the  $a$  axis showing the cyclodextrin molecule (1) and molecule (2). For each molecule two translated guest cyclopentanone molecules are shown (shaded). This figure and Fig. 3 were drawn using *Ball & Stick* (Muller & Falk, 1986) software.

obtain the experimental sixfold symmetry. The simulation using this model gives the diffuse scattering shown in Fig. 7, to be compared with Fig. 2.

**3.2.3. Sharp diffuse layers.** In a first attempt to account for the system of very sharp layers perpendicular to the  $c^*$  axis (with correlation distances in the range of the experimental resolution), different models were built assuming a rotational disorder of the guest molecules within the host frame. The results did not correspond to the observed data. Finally, the intensities of these sharp layers were shown to correspond to the Fourier transform of the cyclodextrin molecule lying around the threefold axis, while a calculation with the cyclodextrin molecule around the sixfold axis does not produce the correct distribution of intensity in the corresponding region of reciprocal space.

A model was built with columns of one hundred correlated molecules along the threefold axis. In this model

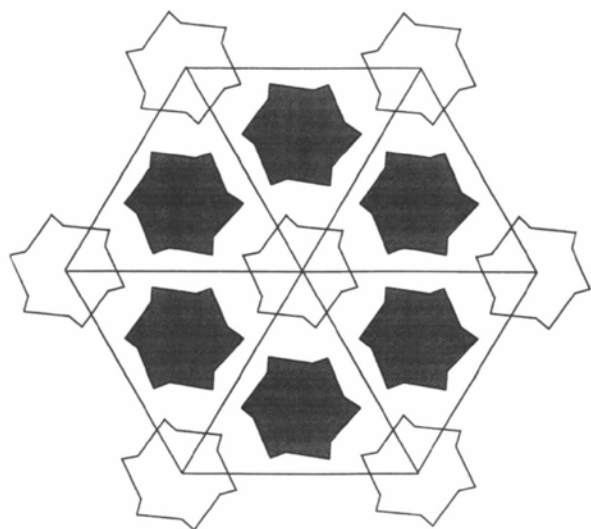


Fig. 5. One domain layer with three cells in the  $ab$  plane;  $3_1$  axes pass through the centre of each cyclodextrin molecule. The period along the  $3_1$  axis is  $3c$ . The first three layers are generated by the  $3_1$  axis. The fourth layer is identical to the first layer. Shaded molecules are cyclodextrin molecules (2) of the averaged structure; they are on the opposite side (with respect to the  $c$  axis) of molecules (1), not shaded.

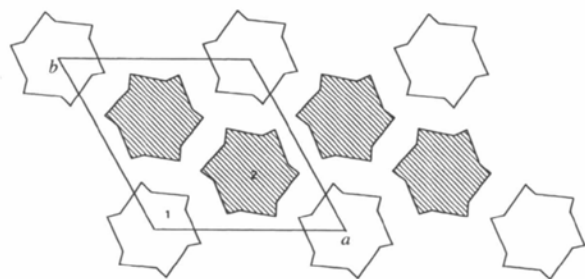


Fig. 6. Row of molecules ( $12\bar{1}2$ ) subject to displacement parallel to the  $a$  translation.

the columns are limited by vacancies filled with solvent molecules. An experimental fact supports this hypothesis; this system of diffuse layers tends to fade away as the crystals dry out and is obviously time-dependent. The solvent molecules are able to diffuse easily and probably induce a lateral diffusion of cyclodextrin molecules.

As the zero-order layer displays a considerably reduced intensity, we used a model with a combination

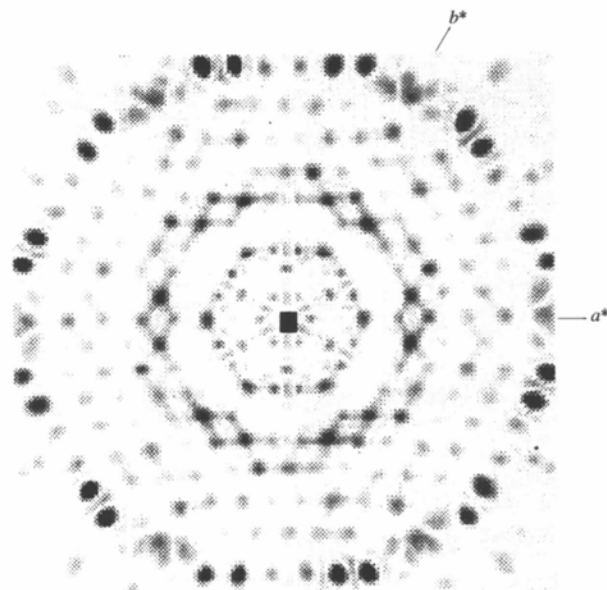


Fig. 7. Simulated pattern corresponding to Fig. 1.

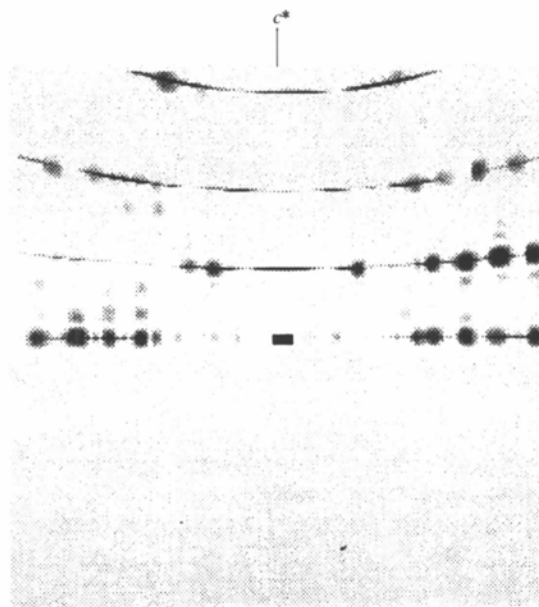


Fig. 8. Simulated pattern corresponding to Fig. 2. On the periphery, the circle of intense reflections corresponds to the  $(hk1)$  plane.

of translation and substitution of molecules. As a first step the columns of molecules were slightly translated along the  $c$  axis,  $u = 0.1 \text{ \AA}$ . With this hypothesis the zero-order layer disappears totally. Consequently, we assume that these columns with all host molecules in the same direction along  $c$ , as shown by the averaged structure, might be replaced by columns of cyclodextrin molecules in the inverse order along  $c$  (*i.e.* with the inverse polarity) and if  $c$  is set vertical, with the primary hydroxyl up instead of down. With this model the calculated intensity of the zero-order layer is in agreement with the observed data. The best fit was obtained by one inverted column for 15 normal ones. This proportion explains why this disorder was not observable in the average structure. Again, six orientations rotated by  $60^\circ$  from each other around the  $c$  axis were assumed in order to restore the observed sixfold symmetry. The simulated films are seen in Fig. 8. This model is in agreement with the previous model of microstructures and accounts for the hexagonal packing of the host molecules.

#### 4. Discussion

##### 4.1. Hexagonal packing

The model described above may be explained by the symmetry of the packing of the host structure. In the plane perpendicular to the  $c$  axis, the distance between the centre of two cyclodextrin molecules with the same orientation is equal to the distance between two molecules with different orientations ( $d = 13.77 \text{ \AA}$ , at room temperature).

In the average structure described previously there is one column in one direction, along the crystallographic sixfold axis, and two columns in the opposite direction, along the threefold axis. If one of the latter two is reversed, the structure becomes the inverse structure with the inverse polarity, but still with hexagonal packing. This hypothesis is consistent with the experimental observation of twin crystals with  $P622$  symmetry, the twinning twofold axis being perpendicular to the sixfold axis. This twin pattern might always be present to some extent.

##### 4.2. Two different types of columns

Although the hexagonal packing is the same for the two types of columns, there is, however, a distinction between the two types of columns as shown by the averaged structure. The hydrogen bonds along the columns or between two columns are different in each case. The columns of cyclodextrin molecules lying along the sixfold axis display direct hydrogen bonds between the secondary O3H hydroxyl of one molecule and the primary O6H hydroxyl of another cyclodextrin molecule of the same column, as well as hydrogen bonds involving water molecules. In contrast, for the columns

of cyclodextrin molecules lying along the threefold axis, the cyclodextrin molecules are bonded only to water molecules or to hydroxyl groups of adjacent columns. There are no direct hydrogen bonds between two cyclodextrin molecules along the column; all observed hydrogen bonds along these columns involve water molecules. Finally, the cohesion of the sixfold columns is greater than in the case of the threefold columns. This fact supports the hypothesis of vacancies filled with solvent molecules along the threefold column.

##### 4.3. Intermolecular contacts

Another argument is in favour of the proposed model. On the same level parallel to the  $ab$  plane, as previously explained, there are two contiguous molecules lying along the crystallographic threefold axis with the same orientations. Unexpectedly they have the same separation as two molecules in opposite orientations. In other  $\alpha$ -cyclodextrin complexes, this contact is very frequently observed for two molecules with opposite orientations. However, when this distance is observed for two translated cyclodextrin molecules, the two molecular sixfold axes are tilted from the perpendicular to the line between the centre of the two molecules (Harata, 1981). This is not the case here. In this crystal the average structure analysis has shown that the two O6H primary hydroxyl groups of the two contiguous cyclodextrin molecules, which are related by a twofold crystallographic axis, are disordered, as are the corresponding glucose residues (Fig. 3). The most likely structure would have one hydroxyl with a conformation away from the cavity and the other contiguous hydroxyl with a conformation toward the cavity. However, the occupation factor ( $> 0.5$ ) of this hydroxyl, with a conformation away from the cavity, shows that the two contiguous hydroxyls would be both, in this conformation away from the cavity, at a distance which is too short for non-covalently bonded atoms. Two positions for this hydroxyl with a conformation away from the cavity were found. The main position corresponds to the most probable solution, *i.e.* the contiguous hydroxyl being in the conformation toward the cavity, the other position observed with unusual geometry, but with correct intermolecular distances, occurs when the two contiguous hydroxyls have both conformations away from the cavity. This might introduce deformation or displacement of the cyclodextrin molecules. The observed longitudinal displacements of rows of molecules along  $a$  and  $b$  and the occurrence of columns of molecules containing vacancies filled with solvent and slightly translated support this argument. These vacancies allow relaxation of the strains.

##### 4.4. Short-range domains and twin patterns

It is not surprising that the domains in our model with perfect  $P3_1$  symmetry are not large. In this model

each position of the guest molecule is correlated with the conformation of the host molecule, *i.e.* the guest hydrogen bonded to one primary O6H hydroxyl with a conformation toward the cavity and the five others of the host molecule with conformations away from the cavity and hydrogen bonded to water molecules. As shown in the average structure analysis, the modified conformation of the glucose induced by the alternate conformation of the corresponding primary O6H hydroxyl may break the usual intramolecular hydrogen bonds between the secondary hydroxyl groups which are replaced by intermolecular hydrogen bonds between water molecules and secondary hydroxyl groups. These correlations are limited to small domains (with dimensions  $10^2$  or  $10^3$  Å) as a consequence of the occurrence of defects as previously discussed.

These domains have different orientations in the crystal, but with very similar hexagonal close packing. The apparent sixfold symmetry is given by the average structure of these microstructures subjected to the hexagonal twinning axis. The crystal is built up from ordered domains by rotation around the *c* direction. The high symmetry is achieved by averaging individual units of lower symmetry.

These microstructures may depend on crystal growth. This crystal structure suggests that this complex of cyclodextrin tends to form finite columns with one guest molecule attached by a hydrogen bond to one molecule of cyclodextrin and enclosed in the cavity of another. In a second step of crystal growth the columns may interact in forming hexagonal close packing: the most probable way to form close packing is when one column is surrounded by columns in opposite polarities.

Another step would be further coalescing these starting aggregates to form crystals.

## 5. Conclusions

Diffuse scattering analysis has shown how strains observed in the averaged structure may be relaxed by the occurrence of vacancies filled with solvent molecules and displacement of rows of cyclodextrin molecules. In addition, this analysis has shown different twin patterns present in the crystals; the sizes of different microstructures have been estimated. The occurrence of different twin patterns and the extent of static disorder phenomena due to the averaging of different units hide the dynamic disorder. We are pursuing these studies on several other cyclodextrin hydrates with very similar host packing, with different guests and cosolvents. The new results show that the diffuse scattering features are very sensitive to the solvent. Their analysis will provide more information about motions involving the solvent.

## References

- Doucet, J. & Benoit, J. P. (1987). *Nature*, **325**, 643–646.
- Epstein, J. & Welberry, T. R. (1983). *Acta Cryst.* **A39**, 882–892.
- Guinier, A. (1963). *X-ray Diffraction*. San Francisco: Freeman.
- Harata, K. (1981). *Bull. Chem. Soc. Jpn*, **54**, 1954–1959.
- Le Bas, G. & Mason, S. A. (1994). *Acta Cryst.* **B50**, 717–724.
- Muller, N. & Falk, A. (1986). *Ball & Stick*, 1.78. *Three Dimensional Visualization and Manipulation of Molecular Structure*. Johannes Kepler University, Linz, Austria.
- Saenger, W. (1976). *Bioorg. Chem.* **5**, 187.
- Szejtli, J. (1988). *Cyclodextrin Technology*. Dordrecht: Kluwer Academic Publishers.