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An efficient method for mounting wet protein crystals for X-ray studies.*

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One of the problems in X-ray diffraction studies of protein crystals has been the mounting of wet crystals in equilibrium with their mother liquors. Generally the crystals have been sealed in capillary tubes with small portions of mother liquor, but the manipulation of the delicate crystals is often difficult, and the walls of the capillary are often covered with a film of mother liquor and amorphous protein, both of which absorb X-rays.

An efficient method for mounting wet protein crystals is described here. In this method, the crystal is sealed in a capillary having a water-repellent surface, in such a way that the mother liquor remaining in the capillary may be regulated in amount and kept out of the way of the X-ray beam. The minimum amount of mechanical manipulation is needed for positioning and orienting the crystal, so that the danger of deforming the crystal is small.

The glass capillaries used in this laboratory are produced by Paul Raebiger, Berlin-Spandau, Franzstrasse 43, Germany, and may be obtained from Caine Sales Co., 3020 N. Cicero Avenue, Chicago 41, Ill., U.S.A.; those of 1·5 mm. outer diameter were commonly used. These capillaries are made of highly alkaline soft glass, and must therefore be soaked overnight in concentrated acid to remove surface alkali. The capillaries are rinsed and dried, and then made water-repellent by filling them with Beckman Desicote, to which 5% of wet ether has been added. In our experience, the partly hydrolyzed alkylchlorosilane, produced when wet ether is added, gives much better water-repellent films than does fresh Desicote. The capillaries are rinsed with toluene, dried, filled with distilled water, and heated to 100° C. to remove the last traces of unhydrolyzed Desicote. The form of a capillary as used is shown in Fig. 1(*a*).

A short steel pin is cemented to the bottom of the capillary to serve as a handle. A small fragment of filter paper is now pushed down to the bottom of the capillary (Fig. 1(*b*)). Then the tube is filled with the mother liquor, except for an air space at the bottom. The size of this air space is adjusted by sucking up excess air, so that the meniscus comes at the level where the crystal is to be positioned (Fig. 1(*c*)). A selected crystal is dropped into the tube and allowed to fall down to the meniscus (Fig. 1(*d*)). The crystal may usually be oriented by suitably tilting the tube during the descent. The mother liquor is now sucked out of the tube (Fig. 1(*e*)); the crystal is now found clinging to the wall in approximately its final position, and may be manipulated, if necessary, by gentle pushing.

The amount of mother liquor remaining in the tube may be regulated by moistening the filter paper at the bottom with a small volume of the liquid (Fig. 1(*f*)), and swabbing out all clinging drops on the wall with a thin strip of filter paper (Fig. 1(*g*)). A volume of liquid somewhat smaller than the crystal is usually suitable. Too little liquid will increase the danger of dehydrating

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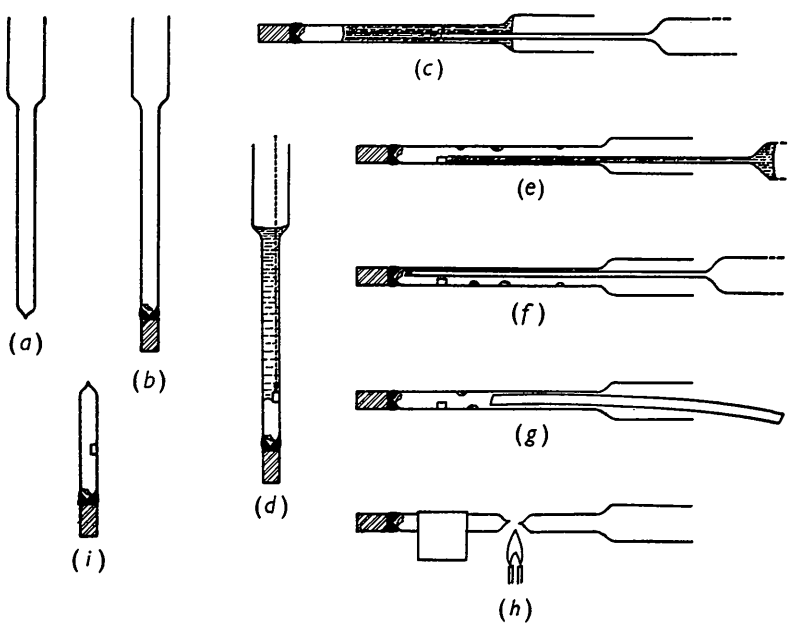

Fig. 1. Stages in mounting a crystal. (*a*) The prepared capillary tube. (*b*) The pin is cemented on and filter paper inserted. (*c*) The tube is filled with mother liquor and the air space adjusted. (*d*) The crystal is dropped in. (*e*) The mother liquor is removed. (*f*) The filter paper is moistened. (*g*) Drops of liquid are swabbed out. (*h*) The tube is sealed. (*i*) The completed mount.

the crystal, while too much may vaporize and condense around the crystal in large drops which will absorb X-rays.

Finally, the part of the capillary containing the crystal is wrapped with wet filter paper, and the tube is sealed in a micro flame (Fig. 1(*h*)). The only precautions which we have found necessary with the completed mount (Fig. 1(*i*)) are those against crushing, drafts (which may produce distillation of the mother liquor and surround the crystal with drops of condensate), and mechanical shocks (which may dislodge the crystal).

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Unit cell constants of ethanolamine hydrogen *d*-tartrate monohydrate.*

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Previous work by Steward (1952) indicated the unit cell constants of ethanolamine hydrogen *d*-tartrate as follows:

$a_0 = 8{\cdot}83 \pm 0{\cdot}03$, $b_0 = 7{\cdot}51 \pm 0{\cdot}03$, $c_0 = 7{\cdot}60 \pm 0{\cdot}03$ Å; $\beta = 92°$.
$N = 8$.
Density (calc.): 5·57 g.cm.$^{-3}$.
Density (obs.): 5·51 g.cm.$^{-3}$ (18° C.).
Space group: C_2^2–$P2_1$.

The unusually high density reported for this compound prompted us to check these results. The salt was prepared by evaporation of an equimolal solution of ethanolamine and *d*-tartaric acid in 50% water–ethanol. Crystals thus obtained were eight-sided prisms, of the monoclinic sphenoidal class elongated along the crystallographic *b* axis. They exhibited the pinacoids {100}, {101}, {001}, and {10$\bar{1}$}, and were terminated by sphenoids {110}, {1$\bar{1}$0} and {0$\bar{1}$1}. Goniometric measurements of a typical crystal are shown in Fig. 1.

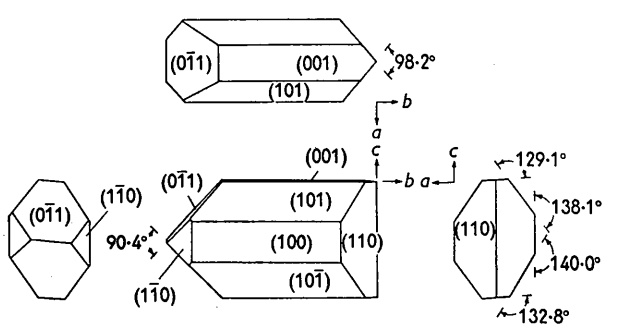

Fig. 1. Crystal habit.

The density of these crystals, as determined by the sink–float method in carbon tetrachloride–toluene, was 1·52 g.cm.$^{-3}$ at 25° C.

Single-crystal rotation and Weissenberg patterns were made using nickel-filtered Cu $K\alpha$ radiation. Measurements of these photographs yielded the following unit-cell data:

$a_0 = 8{\cdot}75 \pm 0{\cdot}03$, $b_0 = 7{\cdot}47 \pm 0{\cdot}02$, $c_0 = 7{\cdot}58 \pm 0{\cdot}02$ Å; $\beta = 92°\ 40' \pm 10'$.
$N = 2$.
Density (calc.): 1·538 g.cm.$^{-3}$.
Space group: C_2^2–$P2_1$.

The molecular weight calculated from these data is 229·2, which corresponds to the monohydrated salt. That this is actually the case was demonstrated by preparing the anhydrous material. Heating at 105° C. for 1 hr. resulted in a weight loss of 7·83% (theoretical, 7·86%), representing one mole of water. Powder patterns of the two compounds were run with nickel-filtered Cu $K\alpha$ radiation, using a recording diffractometer, and are summarized in Table 1. The anhydrous material slowly picked up moisture from the air on standing and reverted to the monohydrate.

Table 1. *Powder diagrams*

Ethanolamine hydrogen *d*-tartrate monohydrate		Ethanolamine hydrogen *d*-tartrate, anhydrous	
d (Å)	I/I_0	*d* (Å)	I/I_0
8·83	0·11	7·82	0·39
7·55	0·06	6·70	0·22
5·90	0·41	5·37	0·36
5·56	0·16	5·06	0·06
5·33	0·32	4·30	0·22
4·66	0·12	3·72	1·00
4·39	0·21	3·61	0·71
3·89	0·38	3·47	0·38
3·80	1·00	3·34	0·06
3·73	0·16	3·27	0·25
3·56	0·10	3·16	0·07
3·46	0·14	3·11	0·09
3·42	0·17	3·07	0·12
3·33	0·47	3·02	0·10
3·22	0·14	2·89	0·09
3·11	0·09	2·82	0·06
2·94	0·10	2·62	0·21
2·85	0·18	2·57	0·10
2·79	0·46	2·54	0·09
2·73	0·10	2·48	0·10
2·67	0·20	2·42	0·25
2·62	0·20	2·36	0·12
2·39	0·29	2·33	0·13
2·31	0·08	2·30	0·12

* This note is published with the approval of the Technical Director, U.S. Naval Ordnance Test Station.

Reference

Steward, E. G. (1952). *Acta Cryst.* **5**, 390.