Table 2. Relationship between scan range and aperture size

<table>
<thead>
<tr>
<th>Scan mode</th>
<th>Scan range</th>
<th>Minimum detector aperture</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\omega(s = 0)$</td>
<td>$a_s$</td>
<td>$2a_s \cos^2 \theta = a_s (\cos 2\theta + 1)$</td>
</tr>
<tr>
<td>$\omega/(2s = 1)$</td>
<td>$a_s$</td>
<td>$2a_s (\cos^2 \theta - 1/2) = a_s \cos 2\theta$</td>
</tr>
<tr>
<td>$\omega/2(s = 2)$</td>
<td>$a_s$</td>
<td>$-2a_s \sin^2 \theta = a_s (\cos 2\theta - 1)$</td>
</tr>
</tbody>
</table>

the main components, crystal mosaic distribution $\mu$, source distribution $\sigma$ and wavelength distribution $\lambda$. The orientation of the loci relative to the axes of the diagram indicates the functional dependence of the particular component (see Mathieson, 1983a, b). The length of the locus corresponds to the range of the component, the magnitude of the component varying along it.

In this diagram, Fig. 3(a), the locus of the crystal size component, $c$, is indicated by the thick line terminated by an arrow. $(\omega_0, \Delta 2\theta_{\text{eff}}) = (a_c, 2a_c \cos^2 \theta)$.

Since the $\omega/\theta$- and $\omega/2\theta$-scan-mode diagrams, Figs. 3(b) and (c), respectively, are related to the $\omega$-scan-mode diagram by a simple affine transformation (see Mathieson, 1983c), we can locate the locus of the $c$ component in these figures. Hence we readily deduce the relationship between scan range and aperture size given in Table 2. This derivation shows not only the magnitude of the required aperture but also the direction of the range relative to the origin of the diagram, this origin, in practice, being the centre of the main peak of the intensity distribution. In the right-hand side of column 3 of Table 2 are given alternative formulae which indicate more directly the relationship between the three scan modes. The results in column 3 also indicate that self-consistency is compatible with the $\cos^2 \theta/\sin^2 \theta$ relationship but not with the $\cos \theta/\sin \theta$ relationship recorded in Table 1.

References


The reduction of radiation damage in protein crystals by polyethylene glycol. By Duilio Casco, Roger Williams and Alexander McPherson,* Department of Biochemistry, University of California, Riverside, California 92521, USA

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Abstract
A simple technique has been developed to lessen radiation damage of protein crystals grown from low-ionic-strength solution. The technique consists of replacing the mother liquor with solutions containing 10–20% of polyethylene glycol 4000 or 20000.

Most protein crystals suffer substantially from radiation damage during the course of normal X-ray data collection. This is manifested both as fluctuations in individual intensities and as a general decline in average intensity. It is usually accelerated at higher values of $\sin \theta$. Indeed, radiation-induced degradation of the diffraction pattern often presents a major obstacle to the structure solution of some protein crystals. There have been several independent investigations showing the efficacy of low temperatures and cryogenic treatment in this regard (Matthews, 1977; Haas & Rossman, 1970) and also reports of agents diffused into the crystals that prolong their useful lifetime (Zaloga & Sarma, 1974). Few general methods have emerged, however, that are both practical and effective.

We have observed that the problem of decay is particular-ly prevalent in crystals grown at rather low ionic strength, from low concentrations of alcohol or methylpentanediol (MPD), or at low concentrations of polyethylene glycol (PEG), i.e. <10% w/w. This presumably occurs because the microenvironments of the protein molecules in the crystal lattice are inherently fragile and are provided little additional stability by the mother liquor.

We found it possible to lessen radiation damage to several protein crystals which were grown from low-ionic-strength solutions by slowly replacing the native mother liquor with comparable solutions but containing, in addition, approximately 20% by weight of PEG 4000 or PEG 20000.

In the first case we studied crystals of the protein $\alpha$-amylase from pig pancreas (McPherson & Rich, 1972) whose structure is being determined by us using both film and diffractometer methods. The normal mother liquor from which these crystals are grown contains only 10 mmol dm$^{-3}$ cacodylate at pH 6.75 and 4 mmol dm$^{-3}$ CaCl$_2$. The crystals are sensitive to radiation damage to the extent that 20 h of exposure to X-radiation generated by an Elliot GX-20 operating at 40 kV–40 mA with a 200 $\mu$m focal spot produced unacceptable alteration in the diffraction pattern; the decline in intensities ranging from 15% at low resolution, $\alpha$ to 50 Å, to as much as 90% for reflections with spacings in the range 3.0 to 2.5 Å. The intensities of some

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reflections with a spacing less than 5 Å in some instances decreased by nearly 90% of the initial values.

When crystals from the same sample and of comparable size were slowly transferred from the original mother liquor to a solution containing 10 mmol dm$^{-3}$ cacodylate at pH 6.75, 4 mmol dm$^{-3}$ CaCl$_2$, and 12% PEG 20000, crystals withstood exposure to the X-ray beam for 90 h before showing decay in the intensity pattern of no more than 10% as measured by scaling of initial and final photographs of the same reciprocal-lattice level. This procedure permits us to record three levels of reciprocal space on a Buerger precession camera or to collect an entire 5-Å-resolution data set on a CAD4 diffractometer from a single crystal.

In the second case we investigated crystals of canavalin from Jack bean whose structure has been determined in the rhombohedral crystal form (McPherson, 1980). These rhombohedral crystals, we wish to note, were analyzed by diffraction methods at all only because they could be stabilized by exchanging their mother liquor with an equivalent solution containing 20% MPD. Crystals of the orthorhombic form of canavalin, grown from 0.7% NaCl in H$_2$O, have a lifetime of 20 h or less in the X-ray beam showing declines in average intensity of greater than 50% over this period. After modifying the mother liquor by adding PEG 4000 to 15%, the crystals are stable for 80 h in the X-ray beam, generated by the Elliott rotating-anode, before suffering average decay of 10% or less.

In the third case, crystals of rabbit liver fructose 1,6-diphosphatase (FDPase) were grown from 8% PEG 4000 (Soloway & McPherson, 1978). These crystals were useful for no more than one precession photograph (approximately 24 h of exposure) on the Elliott rotating-anode generator. Beyond this period, these crystals, like canavalin crystals, declined in average intensity by more than 50%. When the mother liquor was slowly increased to 20% PEG 4000, the radiation damage to the crystals was diminished by nearly a factor of four.

In addition to the reduction in radiation damage, we observed that an increase in the PEG concentration, or its addition to the mother liquor, appeared to make the crystals less fragile during mounting and transfer. We would further point out that PEG-containing mother liquor acts as a cryogen, improving the capacity of crystals to withstand low temperatures. Fortunately, PEG is compatible with a great range of mother liquors and does not, in general, disrupt crystals already grown from other media upon substitution or addition.

References

Crystal Data

Crystal data for p-dichlorobenzene, p-dibromobenzene and their mixed crystals at 293 K.* By A. MAIGA, NGUYEN-BA-CHANH and Y. HAGET, Laboratoire de Cristallographie et de Physique Cristalline, LA 144, Université de Bordeaux I, 351, Cours de la Libération, 33405 Talence CEDEX, France and M. A. CUEVAS-DIARTE, Departamento de Cristallografia, Universitat de Barcelona, Gran Via 585, Barcelona, Spain

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Abstract
The powder data at 293 K of p-dichlorobenzene (pDCB), C$_6$H$_4$Cl$_2$, p-dibromobenzene (pDBB), C$_6$H$_4$Br$_2$, and of their mixed crystals [pDCB]$_x$ [pDBB]$_{1-x}$ are reported. Their thermal stability at 293 K is given. They are all isomorphous, monoclinic, $P2_1/a$ with $Z = 2$. $a = 14.792(6)$, $b = 5.839(2)$, $c = 4.036(2)$ Å, $\beta = 112.52(4)^\circ$, $V = 322.05(5)$ Å$^3$, $D_x = 1.516$ Mg m$^{-3}$ for pDCB; $a = 15.487(2)$, $b = 5.836(2)$, $c = 4.108(1)$ Å, $\beta = 112.74(1)$, $V = 342.5(3)$ Å$^3$, $D_x = 2.289$ Mg m$^{-3}$ for pDBB; $a = 14.893(10)$, $b = 5.837(7)$, $c = 4.046(3)$ Å, $\beta = 112.48(5)^\circ$, $V = 325.1(9)$ Å$^3$, $D_x = 1.592$ Mg m$^{-3}$ for [pDCB]$_{0.90}$[pDBB]$_{0.10}$; $a = 14.942(5)$, $b = 5.840(2)$, $c = 4.048(1)$ Å, $\beta = 112.53(3)^\circ$, $V = 326.3(4)$ Å$^3$, $D_x = 1.677$ Mg m$^{-3}$ for [pDCB]$_{0.80}$[pDBB]$_{0.20}$; $a = 15.018(4)$, $b = 5.846(1)$, $c = 4.061(1)$ Å, $\beta = 112.50(2)^\circ$, $V = 329.4(3)$ Å$^3$, $D_x = 1.751$ Mg m$^{-3}$ for [pDCB]$_{0.70}$[pDBB]$_{0.30}$; $a = 15.138(11)$, $b = 5.843(3)$, $c = 4.067(3)$ Å$^3$, $\beta = 112.57(8)^\circ$, $V = 332.2(8)$ Å$^3$.

*The powder data have been deposited with the British Library Lending Division as Supplementary Publication No. SUP 39012 (23 pp.). Copies may be obtained through The Executive Secretary, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.