

PRACTICAL APPLICATION OF THREE BEAM DIFFRACTION EXPERIMENTS

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INTRODUCTION

It has been shown that triplet phase invariants $\Phi = -\varphi(\mathbf{h}) + \varphi(\mathbf{g}) + \varphi(\mathbf{h}-\mathbf{g})$ (φ 's represent the phase angles of structure factors; \mathbf{h} , \mathbf{g} are reciprocal lattice vectors) can experimentally be determined by a three beam interference experiment (1,2). The phase information is deduced from the intensity change during an azimuthal scan around a primary reflection \mathbf{h} (ψ scan) due to the influence of a second reflection \mathbf{g} . In figure 1 the arrangement of the different reciprocal lattice vectors relative to the Ewald sphere is shown. The wave field inside the crystal due to the primary reciprocal lattice vector \mathbf{h} serves as a kind of reference wave with which the so called 'Umweg' wave, that is successively diffracted by the reciprocal lattice vectors \mathbf{g} and $\mathbf{h}-\mathbf{g}$, interferes. During the azimuthal scan around \mathbf{h} of \mathbf{g} through the Ewald sphere the amplitude of the 'Umweg' wave behaves approximately like a Lorentzian. Its phase is $\varphi(\mathbf{g}) + \varphi(\mathbf{h}-\mathbf{g})$ as long as \mathbf{g} is inside the Ewald sphere, it is $\varphi(\mathbf{g}) + \varphi(\mathbf{h}-\mathbf{g}) + \pi/2$ at the exact Bragg position of \mathbf{g} and it is $\varphi(\mathbf{g}) + \varphi(\mathbf{h}-\mathbf{g}) + \pi$ for \mathbf{g} outside the Ewald sphere. The additional phase shift is due to a resonance term that changes from 0 to π when \mathbf{g} moves from the inner side through the Ewald sphere. As the phase of the wave field due to \mathbf{h} is $\varphi(\mathbf{h})$, the difference of the phases of the two wave fields $\Phi = -\varphi(\mathbf{h}) + \varphi(\mathbf{g}) + \varphi(\mathbf{h}-\mathbf{g}) + \delta$ (δ is the resonance term) is the phase that governs the interference pattern.

Here one of the main experimental problems becomes immediately obvious: the value of the wave field due to \mathbf{h} as a reference wave is only as good as \mathbf{h} remains in the exact Bragg position during the azimuthal scan, otherwise one gets a reference that is not constant. This led to a special designed diffractometer as will be seen below.

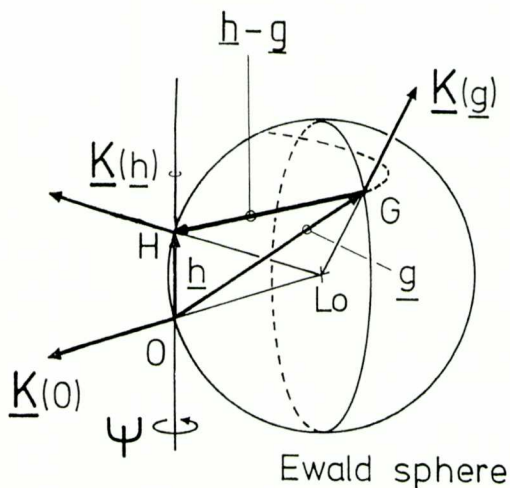


Fig. 1. Schematic representation of a three beam case in reciprocal space.

The measured intensity change during a ψ scan in general depends on two quantities. Firstly, an interference term as already discussed above and, secondly, a term that is independent of the phase. This second one is due to a phase independent energy transfer between the reflections involved. It shows up symmetric in the profiles relative to the exact three-beam position. Because of this term for every triplet phase two ψ -scan profiles have to be recorded, one of the three-beam case \mathbf{h} , \mathbf{g} , $\mathbf{h}-\mathbf{g}$ and one of the centrosymmetric correlated one $-\mathbf{h}$, $-\mathbf{g}$, $\mathbf{g}-\mathbf{h}$. By comparison of the two profiles the symmetric part can be separated to obtain the pure interference effect to evaluate the phase.

At present the accuracy of the interpretation of the interference profiles is about 45° . Therefore, it is possible to distinguish between triplet phases of $+45^\circ$ and -45° or $+90^\circ$ and -90° . That means for a non-centrosymmetric compound by measurement of one triplet phase with $45^\circ < |\Phi| < 90^\circ$ (two profiles) one is able to decide whether the structure model with the coordinates \mathbf{x} and the phase Φ or the model with the coordinates $-\mathbf{x}$ and the phases $-\Phi$ is the true one. Therefore, the absolute structure can be fixed by means of experimental measured triplet phases without the use of any anomalous dispersion effects (3). Some examples will be discussed below.

Given the above mentioned accuracy there should be a good chance to make the solution of difficult or large structures easier if enough triplet phases of a structure can be obtained experimentally.

EXPERIMENTAL

A. The ψ -circle diffractometer

It is known that the angular width of the ψ -scan profiles is of the order of some arc minutes. Moreover, the intensity change due to the three-beam interference is only a few percent, when the moduli of the structure factors involved have about the same magnitude. Therefore, the measurement of ψ -scan profiles requires high precision in the angular resolution and in the ψ -scan accuracy of the diffractometer. In any case, one must avoid any staggering motion of the primary scattering vector during the ψ -scan. That means, the scattering vector of the primary reflection must always lie exactly on the Ewald sphere for the reasons mentioned above.

In our experimental experience exact ψ scans are difficult to perform with a conventional four-circle diffractometer. Therefore, a special ψ -scan diffractometer has been constructed (figure 2). This instrument has two perpendicular circles denoted by ϑ and ν to move the detector. Four circles are available to move the crystal. The first crystal axis ω is parallel to the first detector axis ϑ ($\omega - 2\vartheta$ relation). Perpendicular to the ω axis a second axis for the ψ rotation is installed. This ψ axis bears an Eulerian cradle with motions χ and φ . Thus an arbitrary scattering vector \mathbf{h} can be aligned with the axis χ and φ into the ψ axis and a ψ scan can be performed by moving only the ψ circle.

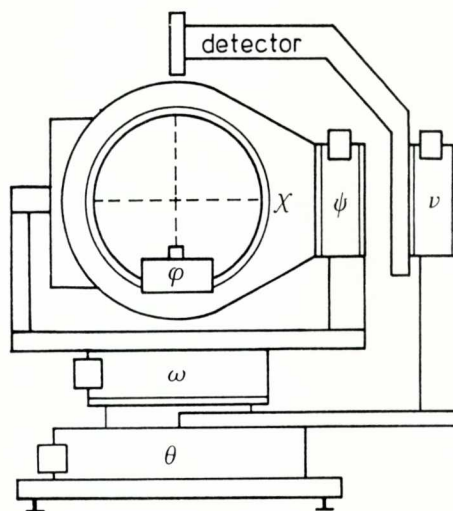


Fig. 2. Schematical drawing of the ψ -circle diffractometer. During the measurement of the three-beam ψ -scan profile the detector is set in the horizontal plane.

With the ν circle the detector can be moved above or below the horizontal plane defined by the incident beam and the ψ axis. In this way the ψ angle of the exact three beam position can be controlled by measurement of the second Bragg reflection by means of a ψ scan. All circles are computer controlled and driven by stepper motors. The angular resolution is at least 0.001° .

B. X-ray sources

The measured intensity change due to the interference of two wavefields inside the crystal is a convolution of an intrinsic profile, that depends on the geometry of the three-beam case, the polarization state of the incident radiation and the moduli of the structure factors involved, and experimental parameters as divergence and wavelength spread. Due to this convolution, the intensity change obtained in an experiment is more pronounced for smaller divergence.

On the other hand, there are already for a small molecule structure quite a lot of secondary reflections for an azimuthal scan around a primary reflection. Figure 3 shows the dependence of ψ positions of multiple beam points on wavelength for L-asparagine monohydrate. The thick line represents the three-beam case of interest. All thin lines represent other three- or multiple-beam cases that may overlap with the main one if their ψ positions get very close. For a smaller divergence overlap is less likely as the interference profiles are less wide. It is also evident from figure 3 that overlap can be avoided by selection of a suitable wavelength.

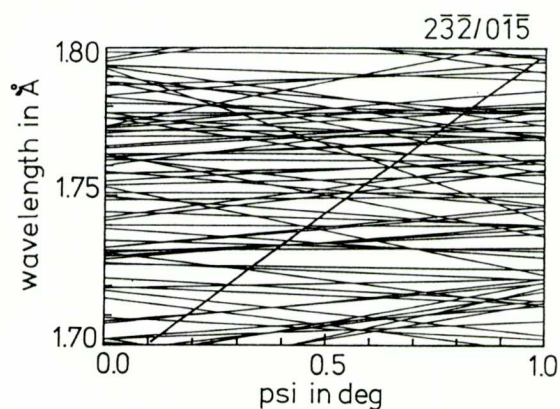


Fig. 3. Dependence of ψ positions of multiple-beam points on wavelength for L-asparagine monohydrate.

B.1. Rotating Anode

For our in house experiments a Rigaku high brilliance rotating-anode generator is available. We use the effective $0.3 \times 0.3 \text{ mm}^2$ focus with 5.4 kW and a copper target. The radiation is filtered by a $100 \text{ }\mu\text{m}$ nickel foil (β filter), hence the spectrum consists mainly of the $K\alpha$ emission lines. An appropriate divergence ($\sim 0.025^\circ$) is achieved by a focus to crystal distance of 1.2 m. Absorption in air between focus and crystal is reduced by an evacuated pipe.

The advantages of the rotating anode is a nearly unlimited availability and good beam stability. Disadvantages are the fixed wavelength and the relative low intensity due to the long distance needed to get a low divergence.

B.2. Synchrotron Radiation

Many of our experiments were performed at HASYLAB in Hamburg using synchrotron radiation from a bending magnet of storage ring DO-RIS running at an electron energy of 3.7 to 4.5 GeV. A computer controlled double crystal Ge(111) monochromator is used. The wavelengths used so far range from 0.7 to 3.0 Å. The whole beam path including the monochromator is kept in He atmosphere up to the final beam conditioning slits close to the diffractometer. For wavelengths longer than 2.1 Å the whole diffractometer is kept in He atmosphere. The synchrotron provides a divergence of about 0.008° at 8 keV. The intensity is about 50 times the intensity of the rotating anode setup described above.

The greatest advantage of the synchrotron is the tuneability of the wavelength and high intensity. One disadvantage are beam instabilities. The primary intensity changes with time, as the current in the storage ring decreases slowly. The orbit of the electrons is not totally stable, thus the source point is slightly changing what affects the experiment seriously. Another disadvantage is the limited amount of beam time at a synchrotron source that is available.

C. Crystals

The size of the crystals we normally use for experimental phase determination is about 200 to 500 μm in diameter. The size of the primary beam is suitable adjusted, that the crystals are totally bathed in the beam.

One major requirement to obtain reliable phase information by means of an interference experiment is a small mosaic spread. Figure 4 shows the profiles of the same three-beam case of two enantiomorphic crystals. The (+)Butafenon crystal was of good quality and therefore a relatively large and narrow interference effect could be measured. For the broader mosaic spread of (-)Butafenon, the measured intensity change is much smaller and wide. A crystal with a very broad mosaic spread can still be used for experimental phase determination if a single mosaic block is isolated enough, so it can be selected and excited alone due to the very low divergence used for this kind of experiment.

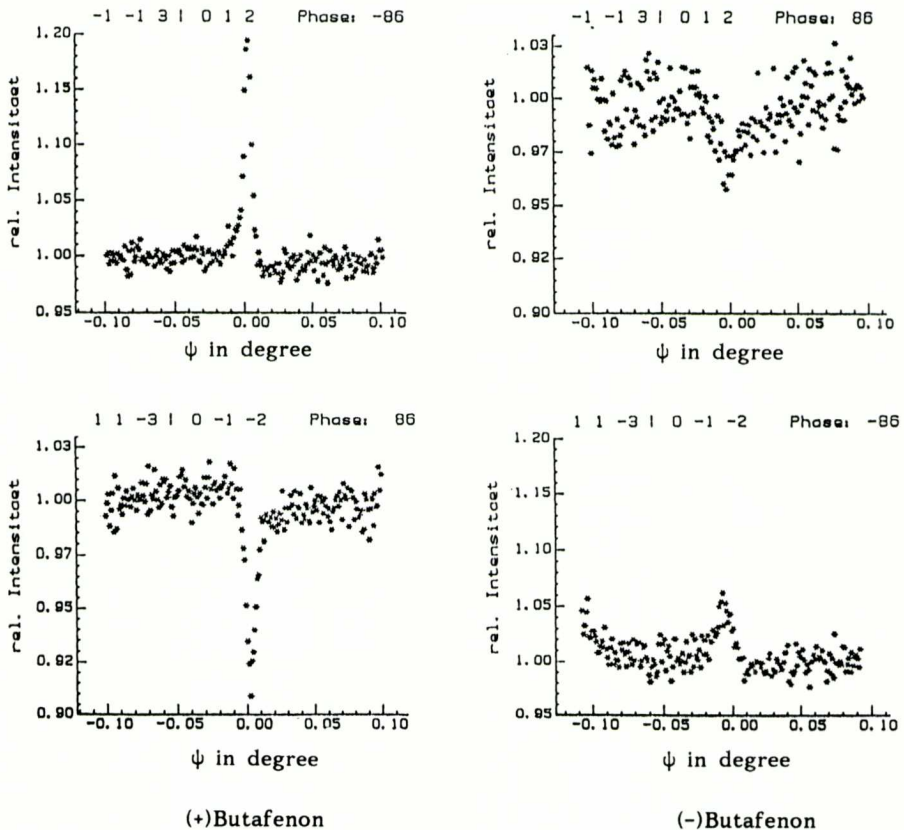


Fig. 4a. ψ -scan profiles of three-beam case $\bar{1}\bar{1}3|012|\bar{1}\bar{2}1$ of (+) and (-)Butafenon measured with synchrotron radiation at $\lambda = 1.8000 \text{ \AA}$

(+)/(−)Butafenon: P2₁, $a = 7.51 \text{ \AA}$, $b = 9.16 \text{ \AA}$, $c = 20.85 \text{ \AA}$, $\beta = 95.2^\circ$, $Z = 2$



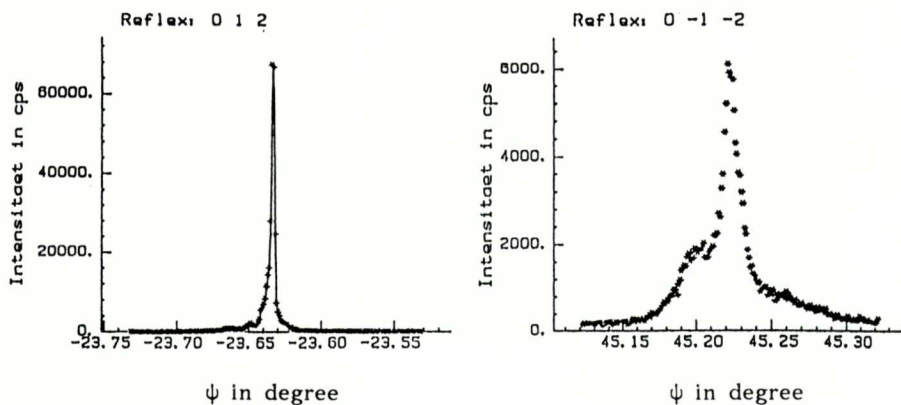


Fig. 4b. ψ -scan profiles of the secondary reflections of the three-beam case shown in Fig. 4a. The mosaic spread of the (-)Butafenon (right) crystal was much higher than the one of the (+)Butafenon crystal (left).

DETERMINATION OF ABSOLUTE STRUCTURE

The term 'absolute structure' (4, 5) has different meanings dependent on the point group symmetry. For enantiomorphic groups with a chiral compound, the determination of the absolute structure is equivalent to the determination of the absolute configuration of the molecule. For the same groups and an achiral compound the absolute conformation of the molecule is obtained.

For hemimorphic point groups the structure can be fixed with respect to a polar direction. And for the last case, namely the roto inverse point groups, an absolute assignment of the axis is obtained by the determination of the absolute structure.

The usual way to fix the absolute structure is the exploitation of anomalous dispersion effects. This can be done by refining an enantiomorph sensitive parameter, e.g. (6) or by measuring the intensity of selected Bijvoet pairs very carefully. Difficulties arise on one hand if there is no or only a few weak anomalous scattering atoms in the structure (e.g. light atom structures) and on the other hand, if the crystals can only be grown to very small size what makes it difficult to obtain a good statistic for the reflection intensities necessary to exploit the very small anomalous dispersion effects. Some examples will be discussed now:

In figures 4 and 5 two examples are given for which we had both enantiomorphic forms of the molecule available. The details about the compounds are given in the figure captions. All of them are light atom structures where a determination of the absolute structure by anomalous

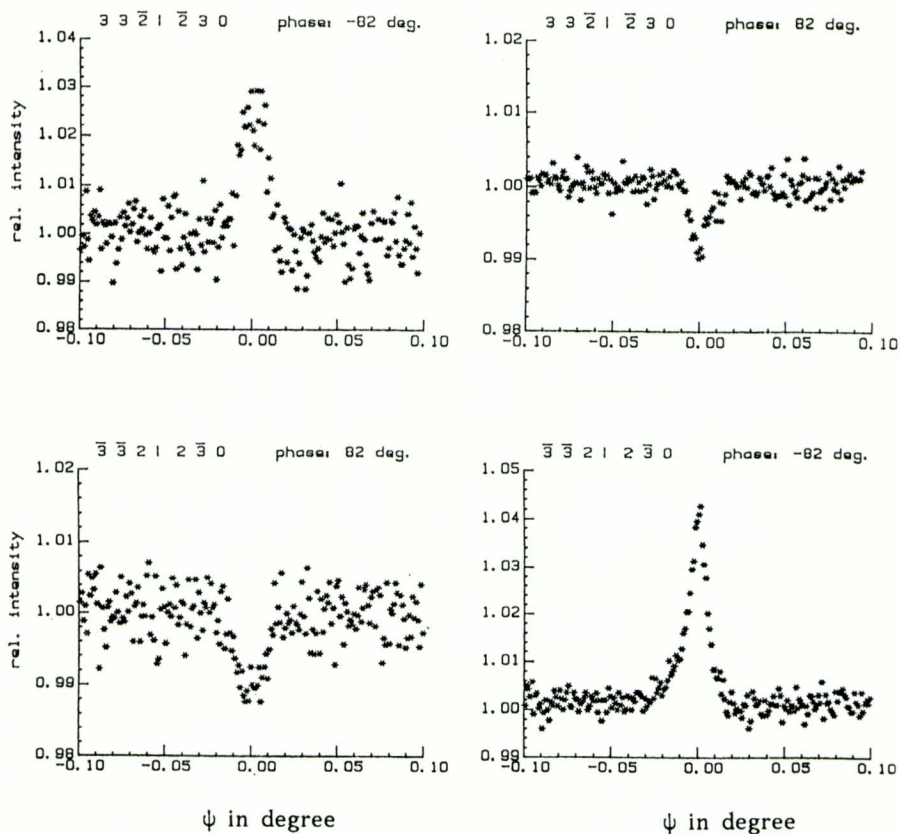
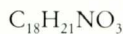


Fig. 5. ψ -scan profile of three-beam case $3\ 3\ \bar{2}\ |\ \bar{2}\ 3\ 0\ | 5\ 0\ \bar{2}$ of (-)Rolipram(left) and (+)Rolipram (right) measured with synchrotron radiation at $\lambda = 2.3900\ \text{\AA}$.

(+)(-)Rolipram: $P2_1$, $a = 18.55\ \text{\AA}$, $b = 7.38\ \text{\AA}$, $c = 22.63\ \text{\AA}$, $\beta = 104.7^\circ$, $Z = 8$



dispersion effects would be difficult. It is obvious from the figures, that the same three-beam case for two different enantiomorphous molecules gives exactly the negative triplet phase what is to be expected. The noise in the profiles is mainly due to primary beam instabilities and not statistics.

In figure 6 an example is given, where it was not possible to determine the absolute structure by means of anomalous dispersion effects using copper radiation as the crystals were only available as very small needles. The mosaic spread of these crystals was rather small, so experimental phase determination worked well.

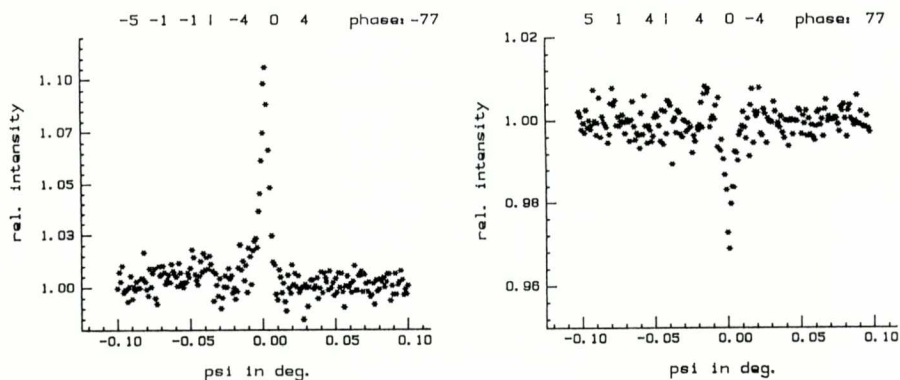


Fig. 6. ψ -scan profile of three-beam case $\overline{511}|\overline{404}|\overline{115}$ of $C_{22}H_{23}O_7N$ measured with synchrotron radiation at $\lambda = 1.6527 \text{ \AA}$;

$$C_2, a = 23.29 \text{ \AA}, b = 6.47 \text{ \AA}, c = 14.97 \text{ \AA}, \beta = 116.4^\circ$$

FUTURE ASPECTS: STRUCTURE SOLUTION AND APPLICATION TO LARGE STRUCTURES

To test the reliability of experimental phase determination about 65 triplet phases were measured from the compound rolipram before the structure was solved. The structure had 80 non-H atoms in the asymmetric unit and could be solved by direct methods. Not all direct method programs have been able to solve this structure. By comparison of the calculated triplet phases with the ones measured before the structure was solved, we got a mean phase error of 13° , the maximum deviation was 51° .

Meanwhile structures with 100 to 150 or even more independent non-H atoms can be solved by direct methods, so we were looking for bigger structures. Finally we chose sperm-whale myoglobin as a test compound (7). For crystal structures of that size an overlap of neighbouring three-beam cases can no longer be avoided. In preliminary experiments using small-molecule structures we investigated the way in which ψ -scan profiles of three-beam cases with strong structure factors are influenced by overlapping three-beam reflections with weak structure factors. The structure factor moduli of the main three-beam cases were chosen to fulfil the following condition: $2 \leq Q(\mathbf{hg}) \leq 6$ with $Q(\mathbf{hg}) = F'(\mathbf{g})F'(\mathbf{h-g})/F'(\mathbf{h})^2$ (F' are structure factor moduli corrected for polarization). It was found that in spite of an overlap of weak reflections the triplet phase of the 'main' three-beam case can be exploited provided that $Q(\mathbf{hg})_{\text{weak}} \leq 0.1 \cdot Q(\mathbf{hg})_{\text{strong}}$. Thus, one can be confident that experimental phase determination is also possible for large structures.

In figure 7 and 8 the first triplet phases measured in a small protein are shown. In each of the diagrams about 200 to 300 small three-beam cases occur in a ψ -angular range of $\pm 0.1^\circ$ with respect to the three-beam position of the main three-beam case. In figure 9 the profile of a secondary reflection during a ψ scan is shown. The crystal quality was rather bad, but due to the small divergence at the synchrotron, we were able to select just one of the big mosaic blocks to perform the experiment. As the structure factor moduli of the main three-beam case have to be very strong, only the phases of low and medium resolution reflections are accessible by means of experimental phase determination in large structures.

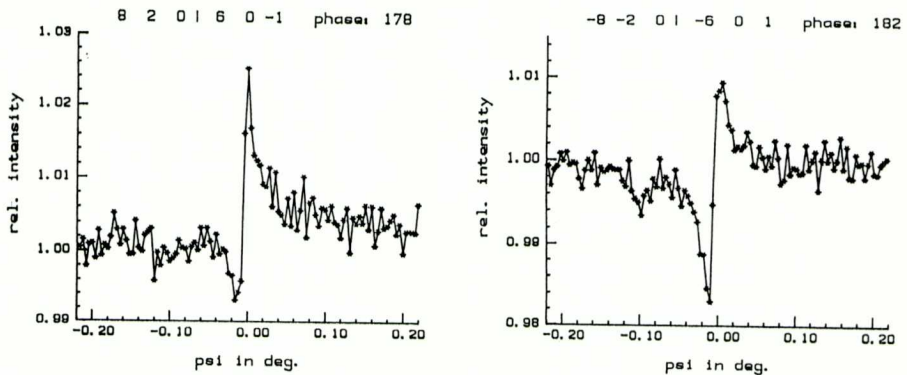


Fig. 7. ψ -scan profile of three-beam case $8\ 2\ 0\ |\ 6\ 0\ 1\ |\ 2\ 2\ 1$ of Myoglobin, $\lambda = 1.54\ \text{\AA}$,
Myoglobin: $P2_1$, $a = 64.51\ \text{\AA}$, $b = 30.91\ \text{\AA}$, $c = 34.86\ \text{\AA}$, $\beta = 105.8^\circ$

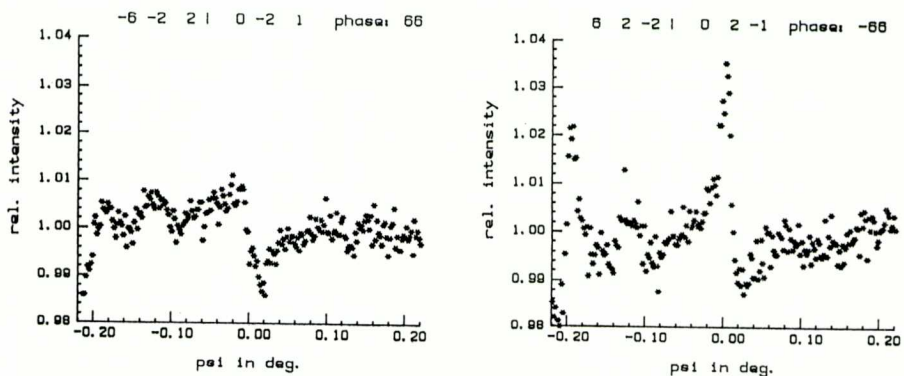


Fig. 8. ψ -scan profile of three-beam case $6\ 2\ 2\ |\ 0\ 2\ 1\ |\ 6\ 0\ 1$ of Myoglobin, $\lambda = 1.9349\ \text{\AA}$.

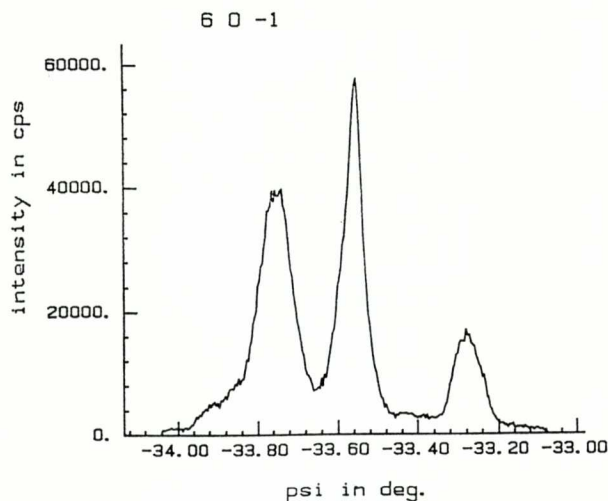


Fig. 9. Profile of the secondary reflection $60\bar{1}$ of the ψ -scan of Fig. 7. The interference experiments were performed with the mosaic block in the middle.

SUMMARY

It is shown that experimental phase determination can be a helpful tool to determine the absolute structure of non-centrosymmetric light atom structures if the exploitation of anomalous dispersion effects becomes difficult.

For large organic structures the three-beam method may also become interesting, if one is able to measure enough phases to boost the structure solution process. It has been shown that even for small proteins phases for a certain class of triplets can be measured if precaution is taken that overlapping neighbour three-beam cases are not too strong.

ABSTRACT

The experimental setup for the measurement of triplet phases by means of three beam interference experiments is discussed. Attention is drawn to the influence of crystal quality, size of the unit cell, different radiation sources and diffractometer design.

Further on, the application of the three beam method to determine the absolute structure of non-centrosymmetric organic light atom structures is discussed. Also first experiments to exploit experimental phase information in a structure solution process are presented.

LITERATURE

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