

RESONANT SCATTERING OF LIGHT ATOMS — MEASURING METHODS AND APPLICATIONS

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The paper describes measuring methods of weak resonant scattering signals occurring in X-ray diffraction pattern of a crystal containing light atoms (C, N, O, F) in its unit cell. Difficulties resulting from the large distance on the energy scale between the *K*-absorption edges of light atoms and contemporary available energy range of X-ray sources for diffraction experiments may be overcome using mixed synchrotron radiation and sealed tube measurements. Techniques such as high resolution synchrotron radiation diffraction experiment, low resolution azimuthal scan, top reflection azimuthal scan are discussed and their applications are presented. Several enantiomer correctness indicators, evaluating the confidence level of absolute structure determination, are applied for crystals containing oxygen or nitrogen as anomalous scatterers. Resonant scattering of light atoms, which are fundamental constituents of organic molecules and polymers, carries the information about the absolute structure. Growing importance of stereospecific drugs which follows recent recognition of drug-receptor interaction mechanism increases the demand for determination of drug molecule handedness. Investigation of absolute structure for molecules in their original shape (without the introduction of heavy anomalous scatterers) is potentially a vast application field of the described methodology.

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1. Introduction

Atomic scattering factors f_0 for X-rays are calculated and tabulated [1] on the assumption that the frequency of the incident radiation is large compared with any absorption frequency of the atom. The assumption is made that all electrons scatter as free classical electrons interfering with each other due to spatial distribution. The scattering power of a bound electron in an atom as well as the phase of scattered X-rays may be different from that of a free electron. These effects are taken into account by representing the atomic scattering factor as a complex number

$$f = f_0 + \Delta f' + i\Delta f'' \quad (1)$$

TABLE I

Dispersion corrections for selected light atoms and wavelengths.

λ [Å]		0.5594	0.7093	1.5405	2.289	2.5
Element		Ag K_{α_1}	Mo K_{α_1}	Cu K_{α_1}	Cr K_{α_1}	(**)
C	$\Delta f'$	-0.005	-0.001	0.016	0.035	0.040
	$\Delta f''$	0.001	0.002	0.009	0.020	0.026
	μ/ρ	0.1	0.3	3.9	13.8	18.1
N	$\Delta f'$	-0.006	0.004	0.028	0.058	0.067
	$\Delta f''$	0.002	0.003	0.018	0.042	0.050
	μ/ρ	0.3	0.6	6.7	23.0	30.2
O	$\Delta f'$	-0.005	0.003	0.045	0.089	0.103
	$\Delta f''$	0.004	0.006	0.032	0.073	0.087
	μ/ρ	0.4	0.9	10.5	35.5	46.3
F	$\Delta f'$	-0.002	0.008	0.068	0.128	0.146
	$\Delta f''$	0.006	0.010	0.053	0.119	0.142
	μ/ρ	0.6	1.3	14.7	48.7	63.4
maximum(*)						
$\sin \theta/\lambda$ [Å ⁻¹]		1.68	1.32	0.61	0.41	0.37

(*) θ defined by the Bragg equation $n\lambda = 2d_{hkl} \sin \theta$; maximum usable value of θ angle is equal to 70° for most goniostats.

(**) maximum wavelength at 4-circle goniometer in HASYLAB.

where Δf and $\Delta f''$ are the real and imaginary dispersion corrections. The values of dispersion corrections depend on the difference in energy of the incident radiation and the absorption edge energies of a particular atom. They are less sensitive functions of the diffraction angle θ than f_0 , because the dispersion effect arises predominantly from the inner, tightly bound electrons located in a small volume near the nucleus. Calculation of the dispersion corrections are based on computation of photoelectric cross-sections [2, 3] as well as determined experimentally by X-ray interferometry, X-ray diffraction using sealed tube and SR sources [3]. For light atoms, such as O, N, C, K -absorption edges are quite far from the range of wavelength available for X-ray diffraction experiments: 0.3–4.3 Å for selected SR sources and 0.5–2.3 Å for sealed X-ray tubes (Ag to Cr anodes). For example: $\lambda(K)$ absorption for carbon equals 43.68 Å, for nitrogen 30.99 Å, oxygen 23.32 Å, and for fluorine 18.32 Å. With such a distance from the absorption edge all

dispersion terms are relatively small with respect to non-dispersive components of the atomic scattering factors. Comparative data are listed in Table I. It is worthwhile to point out that however an increase of wavelength seems necessary for recording any dispersion phenomena, it entails simultaneous an increase of systematic errors due to absorption (cf. line 3 of Table I — listed μ/ρ values) and decrease of the $\sin \theta/\lambda$ parameter, closely related to the resolution in diffraction experiments. Lower range of $\sin \theta/\lambda$, limited by the use of longer wavelengths, results also in a significant decrease of the dispersion component to the atomic factor f_0 ratio. For example, for F atom,

$$\text{for } \sin \theta/\lambda = 0 \quad \Delta f''/f_0 = 0.00086;$$

$$\text{for } \sin \theta/\lambda = 0.4 \quad \Delta f''/f_0 = 0.0025;$$

$$\text{for } \sin \theta/\lambda = 0.6 \quad \Delta f''/f_0 = 0.0035;$$

$$\text{for } \sin \theta/\lambda = 1.3 \quad \Delta f''/f_0 = 0.006;$$

$$\text{for } \sin \theta/\lambda = 1.7 \quad \Delta f''/f_0 = 0.0086.$$

2. Anomalous dispersion and crystal structure analysis

Now let us discuss briefly how the anomalous dispersion phenomena may affect a diffraction pattern from a crystal. The structure factor

$$F_H = \sum_j f_j \exp(2\pi i \mathbf{H} \cdot \mathbf{r}_j), \quad (2)$$

where

$\mathbf{H}(hkl)$ denotes a reciprocal lattice point $|\mathbf{H}| = 2 \sin \theta/\lambda$;

$\mathbf{r}_j(xyz)$ denotes a point in real space;

f_j is the atomic scattering factor for j -th atom

and summation is done over the whole unit cell contents. The intensity

$$I_H = K F_H F_H^*. \quad (3)$$

In case of no anomalous dispersion, i.e. negligible $\Delta f'$, $\Delta f''$ for all atoms $f = f_0$ is a real number and

$$F_H = F_H^* = F_{\bar{H}} \quad \text{and} \quad I_H = I_{\bar{H}}. \quad (4)$$

The above relationship, valid for non-centrosymmetric as well as for centrosymmetric crystals is called Friedel's law. For the above-mentioned case the scattered intensity is not affected by reversing the direction of the incident and that of the scattered beam. When atoms in a crystal have significant anomalous dispersion components, the atomic factors are complex and in general $f_j \neq f_j^*$. If a crystal has no centre of symmetry

$$F_{\bar{H}} F_H^* \neq F_H F_{\bar{H}}^* \quad \text{and} \quad I_H \neq I_{\bar{H}} \quad (5)$$

and Friedel's rule is no longer valid. It should be pointed out that the effect of anomalous dispersion results in the differences between \mathbf{H} and $\bar{\mathbf{H}}$ only through interference between an anomalous and other scatterers, i.e. it will not appear on a crystal composed of only one type of atom [4]. The latter conclusion has a

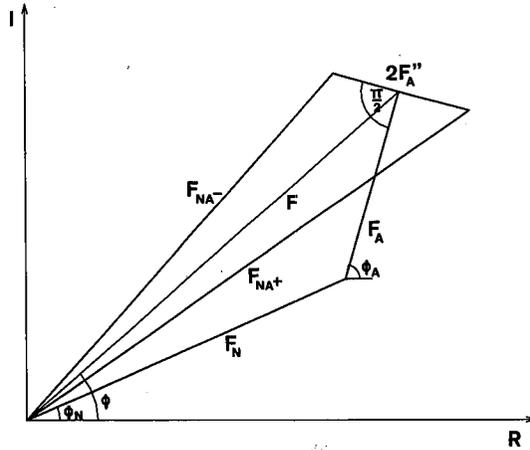


Fig. 1. Phase diagram: non-centrosymmetric group of anomalous (A) and normal (N) scatterers with anomalous contribution $F''_{A'}$, F_{NA-} and F_{NA+} are structure factors of the Friedel pair of reflections. R and I — real and imaginary parts of the structure factor.

limiting consequence for measurability of anomalous dispersion effects for crystals containing only two light atoms (like for example hydrocarbons). This also raises a question, how dispersion phenomena are distributed in a diffraction pattern, with a practical implication toward selection of the most dispersion sensitive reflections. Figure 1 presents a phase diagram for non-centrosymmetric group of atoms scattering anomalously (A) and without dispersion component (N). The intensity difference, the so-called Bijvoet difference [5], between two reflections with scattering vectors related by inversion (called the Friedel pair) is given by the expression

$$\Delta I = I_H - I_{\bar{H}} = K|F||F_A''| \cos \Delta\phi. \quad (6)$$

For a case when $\phi_A \rightarrow \phi$, $\Delta\phi \rightarrow \pi/2$ and consequently the Friedel pair will have equal intensities for $\phi_A = \phi - \pi/2$, the Bijvoet difference will approach a maximum. In the literature such a pair is often named the dispersion sensitive pair of reflections. For every point symmetry group higher than 1 a clear distinction should be made between the so-called Friedel pairs and the Bijvoet pairs of reflections. Reflections in a Friedel pair are related by inversion operation with respect to their scattering vectors, whereas in a Bijvoet pair reflections are related by any other symmetry operation allowed by point group of symmetry and the above distinction is significant for measuring procedures.

3. Measuring strategy

Starting from the first measurement of Coster, Knol and Prins [6] who determined the absolute polarity of zinc blende crystals using Au L_{α_2} radiation and the first application of anomalous dispersion method to determine the absolute configuration of organic compound Na-Rb-tartrate by Bijvoet, Peerdeman and van Bommel [5], there is constantly growing interest in anomalous dispersion methods and its wide application, originated in physics, now extended to chemistry, biology, materials science and medicine. Every improvement in the precision of detection and registration of X-rays pushes forward the "detection limits" of anomalous dispersion measurements. Particularly, the possibility to use synchrotron radiation resulted in opening new areas of application. The aim of our paper is limited to one specialized field of anomalous dispersion applications. In the course of crystal structure analysis based on single crystal diffraction experiment for substances crystallizing in one of 21 non-centrosymmetric point groups it is necessary to determine their absolute structure. The knowledge of absolute structure enables one to draw conclusions concerning chirality or polarity of crystals and to determine the enantiomer for the constituting molecules.

In organic chemistry, pharmacology, as well as in molecular biology there is a growing demand for determination of absolute configuration of molecules without the use of "inserted heavy atoms" as anomalous scatterers, as was the common practice previously. Particularly, modern drugs try to avoid the presence of heavy atoms. Several drugs containing a chiral centre in its molecule may exist as two enantiomers. For some receptors enantiomers show stereospecific activity ranging from opposite to synergetic action [7-9]. Since the late 1980's, the overlooked problem of enantiomeric purity in pharmacology is reinspected, causing strong demand for methods enabling the determination of chiral structure [10]. Most drugs of natural origin or recently synthesized often consist of only oxygen, nitrogen, carbon and hydrogen atoms. As it is indicated in Table I, within the available wavelength range dispersion terms for O, N, C, H are relatively small.

A general method of measuring weak anomalous dispersion should maximize the effect while eliminating potential sources of systematic errors. Further limitations are implied by the quality of organic crystals — often far from perfect.

3.1. Measured set of reflections is limited by $\sin \theta / \lambda$ only

Data collection covers all the symmetry independent part of the reciprocal space with the assumption of dispersion effects. During the process of refinement of the structure, the so-called global chirality (polarity) correctness indicators [11-13] are calculated or refined, taking into account all reflections, irrespectively of their dispersion components. In case of only weak anomalous scatterers, the results are often inconclusive. In order to improve the method, measurements are done for all symmetry dependent parts of the reciprocal lattice. Special precautions are focused on absorption and extinction corrections. The "size" of the experiment limited by the exposure time and the wavelength used requires careful consideration. For example, for a crystal containing oxygen as an anomalous scatterer in the

presence of C and H atoms, switching from copper to chromium anode increases $\Delta f''$ corrections from 0.032 to 0.087 (cf. Table I), but mass attenuation coefficient μ/ρ increases from 10.5 to 46.3. Simultaneously, the number of reflections available for measurements drops down from the side of higher order reflections, which decreases the $\Delta f''/f_0$ ratio. In sealed tube experiments, no advantage was reported of the use of Cr radiation over Cu anode during the determination of the absolute structure of (+)-tartaric acid [14]. For the strategy of measuring the whole set of reflections, the use of SR offers serious advantages: first of all, it significantly improves counting statistics due to higher intensity of the primary beam, it allows to use smaller single crystal samples, decreasing sample absorption and eliminating extinction. Their size is practically limited by the crystal mounting procedure. The use of SR improves markedly the peak-to-background ratio — owing to low vertical divergence and narrow wavelength bandpass $\Delta\lambda/\lambda$. It allows more precise measurements of high order reflections as well as weak high angle reflections [15].

High resolution data set collected at $\lambda = 0.5 \text{ \AA}$ (5-circle single crystal diffractometer, D-5 line, DORIS, HASYLAB) [16] indicates how the use of high resolution data could improve extremely weak dispersion signal from nitrogen. In spite of very low value of $\Delta f''$ for 0.5 \AA , polarity correctness indicators were more reliable than for the set collected for 0.98 \AA . However, highly collimated SR beam is very demanding with respect to crystal sample quality. For example, a single crystal of spirane [17], carefully chosen and examined under microfocus monochromated Cu K_α radiation (CAD-4 diffractometer) and successfully refined revealed under synchrotron radiation beam (DORIS II, single crystal 5-circle diffractometer $\lambda = 1.0 \text{ \AA}$ and 1.3 \AA , $\Delta E/E = 4 \times 10^{-4}$, vertical divergence $10 - 35''$) a rocking curve consisting of separate peaks with highly anisotropic width. Crystals affected by radiation damage doubly benefit from SR properties: high intensity, which decreases exposure time, and spectral purity.

3.2. Measurements of selected Friedel and Bijvoet pairs of reflections with a large dispersion component

The procedure described here is applied in two cases:

a) In order to save the global exposure time, only a subset of independent reflections of Laue symmetry of a crystal is measured (i.e. we do the measurements neglecting dispersion effects), which is subsequently used for solving crystal structure with 50% chance for the correct choice of handedness. Then most dispersion sensitive reflections are calculated basing on the structural model and adopted criteria like, e.g.

$$\Delta F_{\text{calc}, H, \bar{H}} > k F_{\text{calc}},$$

$$k' F_{\text{obs}} > \sigma(F_{\text{obs}}),$$

$$\text{or } \Delta F_{\text{calc}}/\sigma^2(F_{\text{obs}}) > D,$$

where k and k' are arbitrary numbers. The listed Friedel or Bijvoet pairs are remeasured and their intensities compared to the calculated pairs. If necessary, the structural model has to be reversed and refined. The described scheme [18],

however saving exposure time, might possibly lead to non-equivalent least-square minima for the structural model [19].

b) Anomalous dispersion is taken into consideration during data collection, but global chirality (polarity) indicators are inconclusive or we would like to increase their confidence level. Again, a list of most sensitive Bijvoet pairs is calculated and remeasured. The measuring procedure involves increased counting time in case of sealed X-ray tube or repeated measurements when using SR. All precautions are taken to eliminate systematic errors, particularly absorption, by analytical or empirical corrections. In case of a centrosymmetric shape of the crystal sample, measurements done for a Friedel pair with symmetrically distributed azimuthal angle ψ eliminates absorption errors in itself, under conditions described below. Comparing Friedel pairs intensities should be preceded by considering screening effect resulting from the crystal mount (glass fiber, glue, etc.). Pairs with significant difference in crystal illumination should be avoided. The procedure may be illustrated on an example of an organic crystal $C_{39}O_8H_{52}$ [17], where absolute chirality on three chiral centres (R, S) was determined using Cu K_α radiation diffraction experiment (see Table II, Fig. 2).

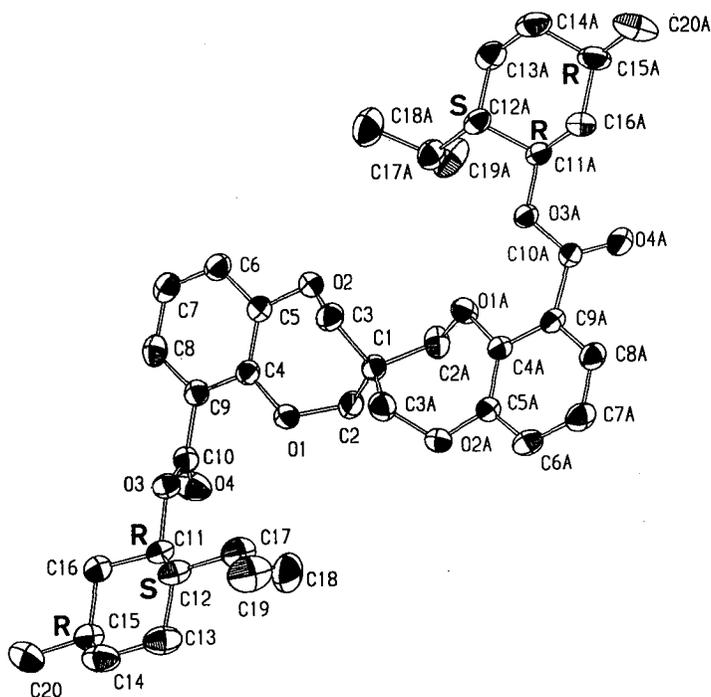


Fig. 2. A perspective drawing of the $C_{39}O_8H_{52}$ molecule. The absolute configuration as determined by anomalous dispersion is indicated (R or S) at the six chirality centres. Hydrogen atoms are omitted for clarity.

TABLE II

Values of the enantiomer correctness indicators [13, 20] for an organic crystal $C_{39}O_8H_{52}$ calculated for the original data set and for a set of remeasured most sensitive Bijvoet pairs.

	Original data		Remeasured Bijvoet pairs	
	Enantiomer		Enantiomer	
	Correct	Reversal	Correct	Reversal
R	0.048	0.049	0.056	0.056
R1	0.605	0.728	0.170	0.290
R2	1.019	1.023	1.016	1.026
R3	0.903	1.112	0.942	1.791
B	0.14		0.66	
$\sigma(B)$	0.04		0.26	

3.3. Analysis of low resolution azimuthal scan curves for selected Friedel pairs

This type of measurements requires a goniostat with at least three rotational degrees of freedom. A crystal is brought into reflection position for a chosen H scattering vector. The diffracted intensity is recorded vs. azimuthal angle ψ which describes rotation around vector H . Two curves $I_H(\psi)$ and $I_{\bar{H}}(-\psi)$ are recorded and their intensity curves compared [21]. Several precautions should be taken in order to avoid experimental errors:

- All n -beam interactions are examined for the used radiation range and the actual orientation matrix using PSILAM and PSIINT programs [22]; calculations allow to eliminate azimuthal regions with Renninger effect.

- Exact alignment of the diffractometer should be done using measured pairs of reflections; translational movements of the crystal sample are minimized during the alignment procedure; additional procedure should be applied to check the symmetry of the monochromated beam in the horizontal plane ($\omega/2\theta$) in order to eliminate the absorption differences for the measured Friedel pair [23].

- Errors occurring on a standard 4-circle diffractometer caused by combined ($\phi - \omega - \chi$) azimuthal rotation could be diminished by orienting the sample in the reflecting plane normal to ϕ (goniometer head) axis. It allows to mimic the 5- or 6-circle goniometer, where azimuthal scan could be done by a single axis rotation.

- In order to eliminate non-uniform illumination of the sample, a special low absorption capillary may be used enabling the fixing of the sample without glue. However, it decreases the usable range of ω and χ angle for measurements.

- As an "internal standard" of the method, azimuthal scan curves for a Friedel pair with zero dispersion components are recorded.

- Time-dependent errors are diminished using alternating H and \bar{H} measurements.

- In case of radiation decay or chemical instability, time corrections are calculated

basing on the absolute exposure time. Examples of azimuthal scan curves for hexamethylenetetramine $C_6H_{12}N_4$ are shown in Figs. 3 and 4, illustrating the determination of polarity using anomalous dispersion from nitrogen ($Cu K_\alpha$ radiation).

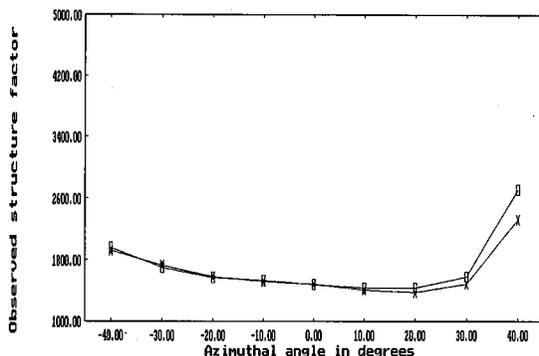


Fig. 3. Low resolution azimuthal scan of (020) and (0-20) reflections with zero dispersion components, for hexamethylenetetramine crystal $C_6H_{12}N_4$, $Cu K_\alpha$ radiation. Average e.s.d. of the structure factor is equal to 49.5.

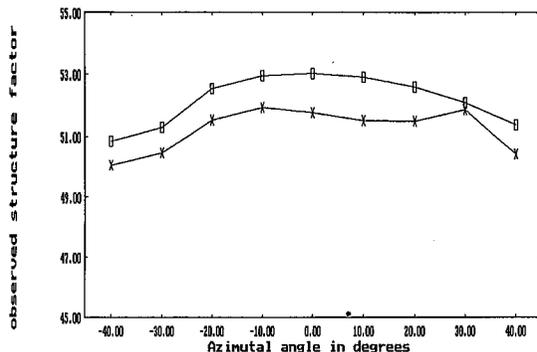


Fig. 4. Azimuthal scan of (2-62) (boxes) and (-26-2) (crosses) reflections. Experimental curves reflect the calculated Bijvoet difference. Average e.s.d. of the structure factor is equal to 0.19.

4. Conclusions

The determination of chirality using anomalous dispersion from oxygen atoms in the presence of carbon and hydrogen atoms is at present technically possible using copper radiation, even in case of a small atomic fraction of oxygen atoms. The determination of chirality using anomalous dispersion of nitrogen is on the edge of present diffraction technique in spite of previous predictions [24]. Primary reports indicate "chiral sensitivity with still too low confidence level" [21]. As a

promising but exclusive, due to unique instrumentation, alternative to the diffraction experiments described above, may be considered SR high resolution azimuthal scan [25].

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