

## The Accurate Electron Crystallographic Refinement of Organic Structures Containing Heavy Atoms

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### Abstract

Prospects for the accurate structure determination of heavy-atom-containing organic crystals were evaluated with electron diffraction data from perchloro and perbromo derivatives of copper phthalocyanine. While the extensive overlap of experimental Patterson maps (from 1200 kV intensities) with respective crystal autocorrelation functions explains the success of previous direct structure analyses, it is clear that multiple-scattering perturbations still evident at high voltage will frustrate the determination of accurate bond distances and angles. If, however, the result obtained after direct structure analysis and Fourier refinement is used to position an idealized molecular model (*i.e.* with chemically reasonable bonding parameters), the correct structure can then be justified by a rotational search coupled with a multislice dynamical calculation. Even though dynamical scattering is not the only major perturbation to such data sets, the resolution-limited correction is still sufficient to identify the correct molecular orientation in the unit cell. Alternatively, an acceptable unconstrained structure refinement can be carried out *via* a procedure proposed by Huang, Liu, Gu, Xiong, Fan & Li [*Acta Cryst.* (1996), A52, 152–157]. A phenomenological adjustment of observed intensities, based initially on the heavy-atom positions found in a high-resolution electron micrograph, will permit all light atoms to be observed near their ideal positions in the ensuing Fourier refinement.

### 1. Introduction

When microcrystals are the only samples available for data collection, electron crystallographic techniques (Dorset, 1995a) are particularly useful for structure determination. Experimental variables such as specimen thickness and electron wavelength can optimize the 'quasi-kinematical' approximation for observed diffraction intensities so that the underlying crystal structure is still represented by the experimental Patterson map (Dorset, 1995b). Furthermore, direct phasing methods have been found to be favorable for carrying out these analyses. For example, probabilistic estimates based on

the  $\Sigma_2$  invariant (Hauptman, 1972) are particularly robust, even with appreciable multiple-scattering perturbations to the observed intensities. In fact, the most serious effect of multiple-beam dynamical scattering and/or secondary scattering is often found at the refinement step (Dorset, 1995a).

Although the determination of light-atom structures generally encounters few problems, inclusion of heavier atomic components in an organic specimen can impose significant barriers to a structure analysis, as predicted in the early theoretical work of Cowley & Moodie (1959). Redeterminations of sulfur-containing structures from published 50 kV texture electron diffraction data (Dvoryankin & Vainshtein, 1960, 1962), for example, have demonstrated that the direct phase determination itself is not significantly impeded (Dorset, 1992). However, visualization of a chemically meaningful molecular geometry is almost impossible when a Fourier refinement is attempted. Similar observations were made during the recent analysis of a sulfur-containing polymer (Dorset & McCourt, 1997).

Perhaps the greatest challenge to *ab initio* electron crystallographic analyses of organic molecules experienced so far has been for the perhalogenated copper phthalocyanines. When the halogen is chlorine, the structure determination is least restricted, as long as the intensity data are collected from thin crystals at a sufficiently high electron accelerating voltage, *e.g.* 1200 kV (Tivol, Dorset, McCourt & Turner, 1993). Phase determination by symbolic addition was sufficient to generate a map with a few identifiable atomic positions and, eventually, after Fourier refinement, the complete structure could be visualised (Dorset, Tivol & Turner, 1991). Other starting phase sets derived from the Fourier transform of electron micrographs (Uyeda, Kobayashi, Ishizuka & Fujiyoshi, 1978–1979; O'Keefe, Fryer & Smith, 1983) were also useful for the direct analysis after phase extension *via* the tangent formula or the Sayre equation (Fan, Xiang, Li, Pan, Uyeda & Fujiyoshi, 1991; Dorset, McCourt, Fryer, Tivol & Turner, 1994; Dorset, Kopp, Fryer & Tivol, 1995). When constraints were imposed on the refinements, chemical bonding parameters in the final model were in good agreement with those found in

X-ray determinations of other metal-containing phthalocyanines (Brown, 1968), even though the final kinematical  $R$  factor was somewhat higher than expected for typical electron crystallographic determinations.

The outcome of the determination was less favorable when the bromine was the substituted halogen, even when diffraction patterns again were recorded from thin crystals at 1200 kV. Direct phase determinations could not be said to produce results that were quite as useful as found for the perchloro compound, although Fourier refinement located all but one atomic position after a few starting positions were found (Dorset, Tivol & Turner, 1992). However, the visualization of a 'cosmetically pleasing' molecular representation in the final potential map (corresponding to the lowest kinematical  $R$  value) did not at all coincide with a model that was *chemically* correct, since the bond distances and angles were significantly distorted. A model with a geometrically reasonable organic moiety resulted in a larger crystallographic residual. Moreover, there was no local minimum of this  $R$  factor that would allow, just by Fourier summation, a correction of carbon-halogen bond lengths that were more than 0.1 Å too short.

By now it is well known that bonding geometry is a property of many organic molecules that can be safely predicted *a priori*, at least at the accuracy of most electron crystallographic determinations. After the spatial relationships between individual atoms are suggested in an initial electron crystallographic determination, it may be possible to optimize the bonding geometry to accepted values at some stage of this analysis and then to demonstrate the validity of this optimization by a suitable data correction. In this paper, the feasibility of this approach is demonstrated for the perhalogenated copper phthalocyanines.

## 2. Materials and methods

### 2.1. Crystallization and data collection

The two perhalogenated copper phthalocyanines used in this work were gifts of Dr John R. Fryer. Under his supervision, they were crystallized by vapor deposition onto cleaved KCl (001) plates, using a procedure similar to the one described by Uyeda, Kobayashi, Suito, Harada & Watanabe (1972). After depositing carbon films over the epitaxially oriented organic layers, the films were floated away from the salt substrate on a clean water surface and then picked up with 400-mesh copper electron-microscope grids and allowed to dry in air.

Electron diffraction experiments on preparations tilted 26° would find the [001] orientation of the monoclinic  $C2/m$  unit cell for these isostructural materials. As stated earlier (Dorset, Tivol & Turner,

Table 1. *Idealized molecular models for copper perhalophthalocyanine in their correct orientation in the unit cell (projected coordinates)*

	X = Cl		X = Br	
Cu	0.000	0.000	0.000	0.000
X1	0.075	0.312	0.079	0.316
X2	0.158	0.204	0.166	0.201
X3	0.268	0.120	0.263	0.126
X4	0.414	0.056	0.418	0.059
C1	0.141	0.041	0.139	0.040
C2	0.212	0.027	0.209	0.027
C3	0.274	0.054	0.269	0.053
C4	0.335	0.027	0.330	0.027
C5	0.057	0.106	0.056	0.105
C6	0.036	0.160	0.036	0.157
C7	0.070	0.207	0.069	0.204
C8	0.036	0.254	0.036	0.250
N1	0.097	0.000	0.095	0.000
N2	0.121	0.090	0.120	0.089
N3	0.000	0.072	0.000	0.072

1991, 1992), the rectangular spacings determined in the  $cm\bar{m}$  projection were  $d_{100} = 17.56$ ,  $b = 26.08$  Å for the perchloro derivative and  $d_{100} = 17.88$ ,  $b = 26.46$  Å for the perbromo derivative. The projected  $c$  spacing was assumed to be 3.76 Å for both compounds (Uyeda, Kobayashi, Suito, Harada & Watanabe, 1972). Selected-area experiments at 100 kV were carried out with a JEOL JEM-100B7 electron microscope. High-voltage diffraction patterns from the same samples were collected at 1200 kV on an AEI EM-7 electron microscope by W. F. Tivol, as described previously (Tivol, Dorset, McCourt & Turner, 1993). Intensity data were obtained from these patterns by integrating peaks of scans of the films made by a Joyce-Loebl Mk IIIC flat-bed microdensitometer. Experimental Wilson plots indicated that  $B \simeq 0.0 \text{ \AA}^2$ , often a sign that significant multiple-scattering perturbations are present (Dorset, 1995a).

Intensity data at the extremes of voltage used in the data collection were used to compute Patterson functions. Those representative of 100 kV intensities from both materials are shown in Fig. 1.

### 2.2. Model construction and structure search

Chemically reasonable skeletal models for both compounds (Fig. 2) were constructed using the average bond distances and angles in the copper phthalocyanine X-ray crystal structure (Brown, 1968) for the organic residue. The carbon-halogen distances were also set to standard values (Sutton, 1958). The models (see also Table 1) were then placed in symmetry-constrained positions of the unit cell (obviously with Cu placed at an origin) and tilted around the crystallographic  $b$  axis. With  $mm$  molecular symmetry, the  $b$  axis could bisect the two outmost carbon-carbon bonds of benzene rings or,



Multislice dynamical scattering calculations were carried out with a group of programs obtained from Arizona State University (see Cowley, 1981). The geometry for the molecular packing to be used in the calculation was suggested earlier (Uyeda, Kobayashi, Suito, Harada & Watanabe, 1970). An initial structure-factor calculation repeated the input parameters used in the tilt experiments described above but allowing zonal data to be generated to  $3.0 \text{ \AA}^{-1}$  resolution. The ensuing phase-grating calculation was limited to produce values for 800 unique beams at 1200 kV and these were then incorporated into the multislice calculation to generate dynamical data through 30 slices of the structure ( $t = 112.8 \text{ \AA}$ ), in good agreement with the observed crystal thickness for experiments on both materials. For this multislice calculation, carried out to a resolution of  $0.96 \text{ \AA}$ , 797 symmetry-related beams were used. At the final thickness, 99.79% of the total beam energy was retained within this limiting aperture for the perchloro compound and 99.27% for the perbromo derivative.  $R$  factors calculated after the dynamical correction substituted  $|F_{\text{dyn}}|$  for  $|F_{\text{calc}}|$  in the above expression.

### 2.3. Interpretation of Patterson maps or electron micrographs, followed by Fourier refinement

In an attempt to find alternative ways to solve these crystal structures, heavy-atom positions, derived either by the interpretation of Patterson maps or visualized directly in averaged high-resolution electron micrographs [taken from the work of Uyeda, Kobayashi, Ishizuka & Fujiyoshi (1978–1979)], were employed to begin Fourier refinements. In neither case were geometrical constraints imposed on the interatomic bonding parameters. For the refinement starting from Patterson positions, the observed 1200 kV amplitudes listed by Dorset (1995a) were used without correction. Positions found from electron micrographs, on the other hand, were used to initiate a refinement procedure described by Huang, Liu, Gu, Xiong, Fan & Li (1996). Approximate structure factors  $|F_h^{\text{calc}}|$ ,  $\alpha_{\text{calc}}$  were calculated from the heavy-atom positions and the corresponding intensities  $I_h^{\text{calc}}$  were compared to the observed intensities  $I_h^{\text{obs}}$  in successive resolution shells,  $h_i \pm \Delta h_i$ . For each interval, a scale factor  $k_h = (I_h^{\text{calc}})/(I_h^{\text{obs}})$  was calculated so that the observed amplitudes used in the Fourier refinement could be scaled, *i.e.*  $|F_h^{\text{obs,corr}}| = (k_h I_h^{\text{obs}})^{1/2}$ , as a new map was calculated from  $\alpha_{\text{calc}}$ . When new atomic positions were identified in successive maps, they were used to calculate new approximate structure factors, their magnitudes providing new scaling terms for intervals of observed intensity and their phases used for calculation of the next potential map after combination with the rescaled observed magnitudes *etc.*

## 3. Results

### 3.1. Copper perchlorophthalocyanine

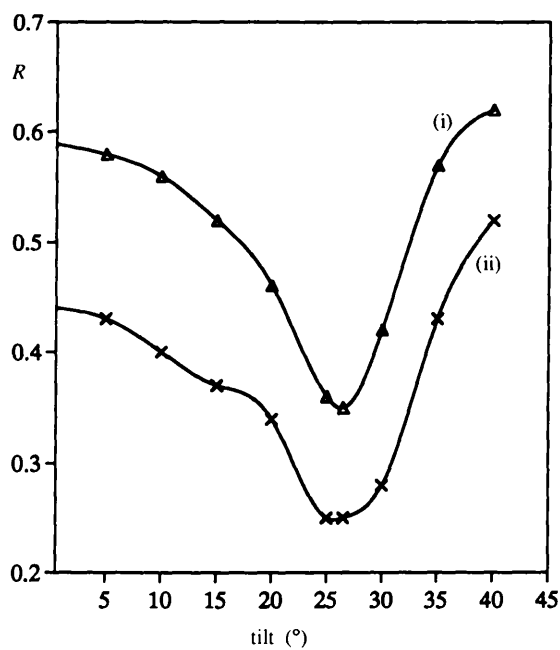
#### 3.1.1. Orientation of geometrically optimized model.

The orientation of the molecule in the unit cell was suggested by the *ab initio* crystal structure analyses carried out previously. If a flat molecule, with ideal bonding geometry, was superimposed on the unit-cell projection, to match the molecular *mm* symmetry to that of the unit cell, and then tilted around the *b* axis, the best value of the kinematical  $R$  factor was found at the correct inclination value for the molecular plane at  $26.5^\circ$  [Fig. 3(a), curve (i)], the value required by the crystal structure (Uyeda, Kobayashi, Suito, Harada & Watanabe, 1972). In the resultant potential map, calculated with correct kinematical phases but observed amplitudes, all atom peaks were well resolved (Fig. 4a). The model atomic positions, input to the structure-factor calculation, closely matched the peak centers found in the potential map.

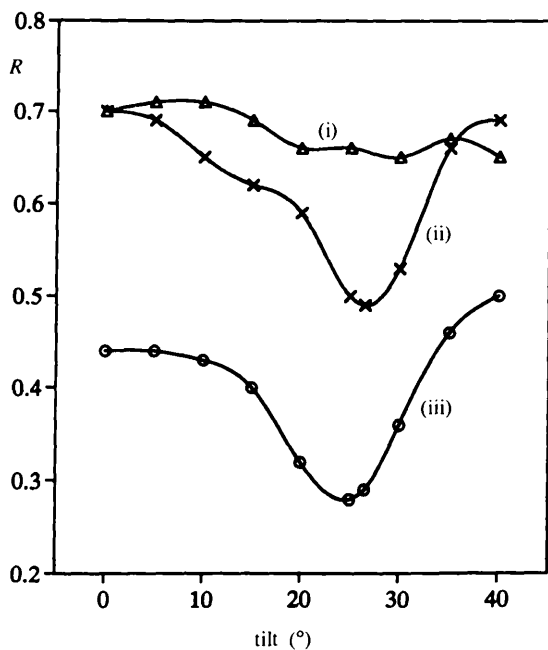
If a subset of the data, limited in resolution to  $d^* = 0.54 \text{ \AA}^{-1}$ , was used to monitor the tilt search for the structure solution, then the  $R$  factors were very near to values calculated for the complete data set. Within the limited data resolution, dynamical amplitudes (at  $t = 112.8 \text{ \AA}$ ) were compared to the observed values for each tilt value. Again, the minimum of the crystallographic residual [Fig. 3(a), curve (ii)] was found for the correct structure and the  $R$  value was reduced to 0.25 at the optimal molecular tilt. A comparison of the model atomic positions in Table 1 with those published previously (Dorset, Tivol & Turner, 1991) reveals that the halogen positions differ by an average shift of  $0.14 \text{ \AA}$ , while the light-atom positions differ, on average, by  $0.11 \text{ \AA}$ .

#### 3.1.2. Patterson map interpretation and Fourier refinement.

Structural information contained in the experimental diffraction pattern is also portrayed by the Patterson function. The experimental 1200 kV Patterson, compared with the autocorrelation function of the best model (Fig. 5a,b), shows that there is considerable overlap of major peaks. Note also that there is also a great similarity to the Patterson function calculated from 100 kV data (Fig. 1a). Given the similarity between Figs. 5(a) and (b), the structure has been determined from the 1200 kV Patterson map. Positions of two halogen atoms could be inferred from the major axial vector terminations in the map ( $2x,0; 0,2y$ ), in addition to the Cu position, fixed by symmetry, to produce a starting point shown in Fig. 6(a), generated with phases from a structure-factor calculation using these positions. A third Cl-atom position and three N-atom positions then could be identified and these were added by the next structure-factor calculation. As before (Dorset, Tivol & Turner, 1991; Dorset, McCourt, Fryer, Tivol & Turner, 1994), Fourier refinement continued to find new peaks until the



(a)



(b)

Fig. 3. (a) Variation of crystallographic  $R$  factor vs model of copper perchlorophthalocyanine tilted around the unit-cell  $b$  axis. (i) Kinematical  $R$  factor; (ii) dynamical  $R$  factor after a multislice calculation based on model coordinates at each tilt value. (b) Structure searches for copper perbromophthalocyanine. (i) Tilt of incorrect molecular orientation around  $b$ . (ii) Tilt of correct molecular orientation around  $b$  (kinematical factor). (c) Correction of models for dynamical scattering. [The slight improvement of the residual at  $25^\circ$ , compared to  $26.5^\circ$  tilt ( $R = 0.28, 0.29$ , respectively), may not be statistically significant.]

structure in Fig. 6(b) was observed. (During the nine refinement cycles, all but two C-atom positions appeared in potential maps; the remaining two positions were added, based on approximate knowledge of the chemical structure and these positions were reinforced in subsequent maps.) However, the final model is inferior to the one found by a search with a geometrically correct model. On average, the halogen positions differ from those in Table 1 by  $0.31 \text{ \AA}$ , whereas the C- and N-atom positions differ by  $0.37 \text{ \AA}$ .

3.1.3. *Enhanced Fourier refinement.* Contrasting with this rather poor result, the refinement procedure starting from the heavy-atom positions located in the averaged 500 kV electron micrographs of the molecular packing (Uyeda, Kobayashi, Ishizuka & Fujiyoshi, 1978–1979) quickly resulted in an accurate structure when the data adjustment of Huang, Liu, Gu, Xiong, Fan & Li (1996) was employed. (It was, again, assumed in the structure-factor calculations that  $B_{\text{iso}} = 3.0 \text{ \AA}^2$  for all atoms.) All atoms were found within five cycles (Figs. 6c,d) with very little ambiguity in their placement. The average halogen or light-atom sites were within  $0.15 \text{ \AA}$  of the positions given in Table 1.

### 3.2. Copper perbromophthalocyanine

3.2.1. *Orientation of a geometrically optimized model.* Direct methods were useful for finding a few starting atom positions in the original analysis (Dorset, Tivol & Turner, 1992) and these led to a nearly complete model after Fourier refinement (requiring the insertion of one C atom). For the search with a model, it was assumed that only the molecular symmetry could be used to start the search for an optimal solution by tilting. If information from any previous analysis were lacking, two orientations of the molecular model (depicted by Fig. 2b) in the unit cell could satisfy the  $cm\bar{m}$  symmetry of the plane group, as stated above, *i.e.* the one resembling that used above for the perchloro analog and the position rotated by  $45^\circ$ . If the latter model (where the  $b$  axis intersects the linkage N atoms) was used for tilting in the unit cell, the  $R$  factor remained large and did not vary much with tilt [Fig. 3(b), curve (i)]. In the proper orientation, there was an identifiable minimum at the correct inclination of the molecular plane [Fig. 3(b), curve (ii)], *i.e.* near  $26^\circ$  tilt. When a multislice calculation was used to monitor this oriented change (again at the restricted resolution mentioned above), a minimum value [Fig. 3(b), curve (iii)] is found at nearly the same inclination of the molecular plane, corresponding to  $R = 0.28$ .

When kinematical phases from the best structural model were combined with experimental amplitudes, the potential map shown in Fig. 4(b) was obtained. Many of the atomic positions were resolved in this map but the density of light-atom positions near the Cu atom on the origin were found to be significantly distorted.

For atoms distant from the central peak, the maximum-peak positions were nearly coincident with the coordinates of the idealized model, although the deviation of heavy-atom sites was somewhat greater than found for

the perchloro analog. Because of the general distortion of potential profiles near the Cu-atom position, the light-atom peak positions were also found to differ significantly from the ideal model coordinates.

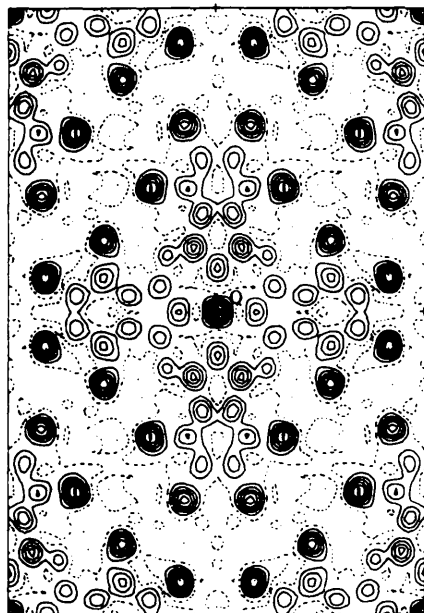
Although the experimental diffraction data are clearly perturbed by multiple-scattering effects, there is a reasonable match of peak positions in observed and calculated Patterson functions (Figs. 5*c,d*). [Note, however, that there is a less favorable comparison with the 100 kV Patterson map in Fig. 1(*b*).] This means that the intensity data at 1200 kV still contain the essential features of the underlying crystal structure. Although a complete structure analysis was not attempted from an interpreted Patterson map, a starting point similar to the one found for the perchloro derivative could be identified from the major peak positions.

#### 4. Discussion

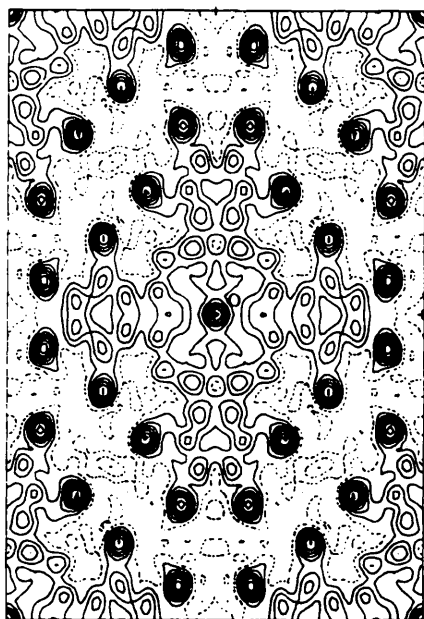
Increasing experience in the analysis of crystal structures with experimental electron diffraction intensities continues to raise an important question: at what amount of data perturbation is the 'quasi-kinematical' approximation no longer useful? Certainly, the limits imposed by  $n$ -beam dynamical scattering and/or secondary scattering are quite real. Thus, these perturbations can so distort the recorded diffraction amplitudes that no *ab initio* analysis is possible when a poor data set is collected. For the perhalogenated copper phthalocyanines considered in this study and in earlier work, it has been clear that crystallization (to limit the sample thickness) and electron-microscope accelerating voltage (to shorten the wavelength) were important factors for recording data sets that could be analyzed. Data from 100 kV electrons, diffracting from the samples, were not useful for direct structure analysis, on the other hand, since details of the unit-cell transform could not be discerned nearly so clearly in the intensity distribution of the diffraction pattern as was possible with the higher-voltage data. Yet there is an obvious correspondence of many peaks in the Patterson maps of Fig. 1(*a*) (100 kV) and Fig. 5(*a*) (1200 kV).

When chlorine was replaced by bromine, the possibility for structure analysis was pushed further toward a limit, even when high-voltage electrons were used to obtain the diffraction pattern. There are, nevertheless, many correlations to be found between the Patterson maps shown in Figs. 5(*c*) and (*d*), certainly enough to explain the progress of a Fourier refinement after direct phase determination (Dorset, Tivol & Turner, 1992). Even though there is a different weighting of axial peaks in the 100 kV maps, some information about heavy-atom positions is still preserved in the lower-voltage data.

From the above observations, it is clear that the limit to *ab initio* structure analysis does not necessarily



(a)



(b)

Fig. 4. (*a*) Experimental potential map of copper perchlorophthalocyanine calculated from observed  $|F_{\text{obs}}|$  and kinematical phase values (from model coordinates) from the best model in Fig. 3(*a*). (*b*) Potential map for copper perbromophthalocyanine based on experimental amplitudes and kinematical phases from the best model in Fig. 3(*b*).

require strict adherence to kinematical scattering, else very few electron crystallographic analyses could have been reported to date. Obviously, the number of structures reviewed in a recent monograph (Dorset, 1995a) is an experimental justification of this methodology. The influence of multiple scattering on crystallographic phase determination already has been

discussed in various experimental papers (Dorset, 1992; Dorset, Tivol & Turner, 1992; Dorset & McCourt, 1994). The overall 'smearing' of low-intensity regions in the molecular transform, sampled by the reciprocal lattice, is caused by the convolutional nature of these perturbations (whether coherent or incoherent). Such perturbations have been shown to

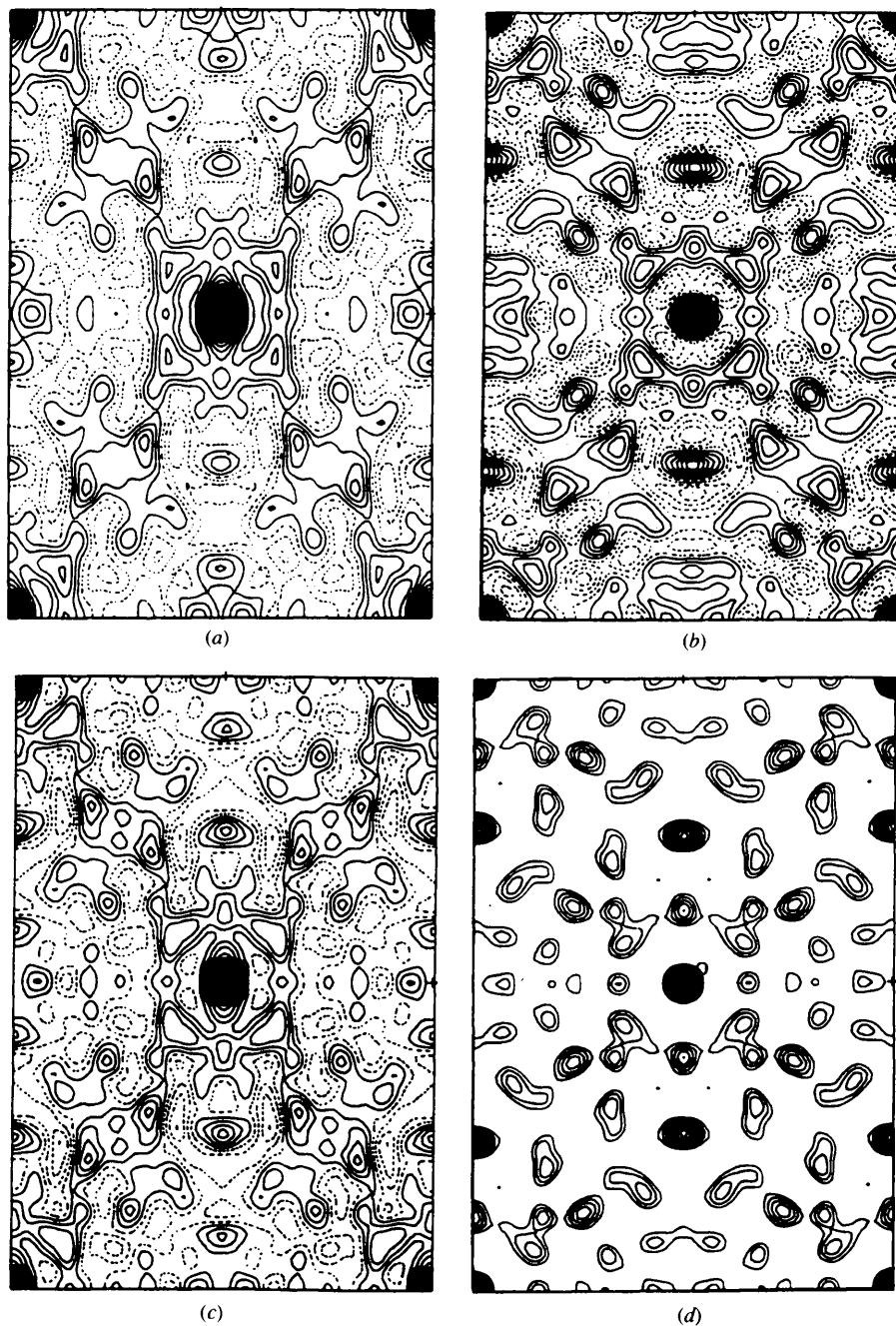


Fig. 5. Comparison of Patterson functions for copper perchlorophthalocyanine: (a) calculated from  $I_{\text{obs}}$  and (b) calculated from  $|F_{\text{calc}}|^2$  for the best structure model. Comparison of Patterson maps for copper perbromophthalocyanine (c) calculated from  $I_{\text{obs}}$  and (d) calculated from  $|F_{\text{calc}}|^2$  for the best structure model.

seriously affect those phase-invariant relationships that rely on the accuracy of weak structure-factor amplitudes for prediction of new phase terms and have been shown to create a problem when the correct structural solution is to be identified from the tangent formula, *i.e.* when a

figure of merit based on the so-called negative quartets, such as NQUEST (DeTitta, Edmunds, Langs & Hauptman, 1975), is employed to choose the best answer in a multisolution set. This does not say, however, that the tangent formula itself generates incorrect phase rela-

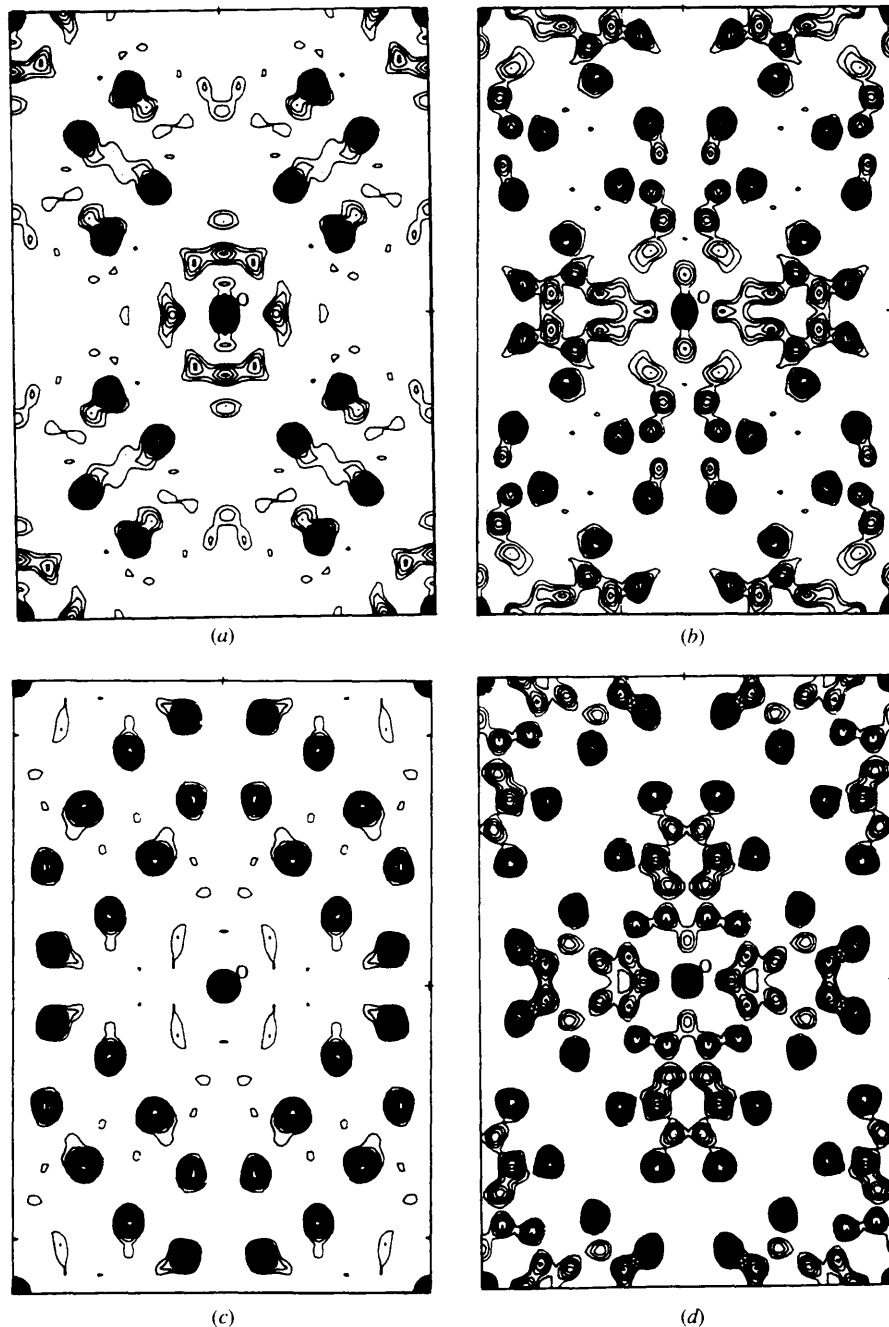


Fig. 6. Determination of the copper perchlorophthalocyanine crystal structure from the 1200 kV Patterson map in Fig. 5(a). (a) Atomic positions after the first structure-factor calculation (based on identified Cl and Cu positions). (b) Final map after Fourier refinement. The Fourier refinement started with atomic positions measured from a 2 Å resolution electron micrograph and observed diffraction amplitudes and was adjusted iteratively in resolution shells with approximate calculated intensities: (c) Initial potential map with some light-atom positions suggested. (d) Final potential map with all atomic sites.



tionships. In fact, this automated procedure, or any other one that relies on the so-called  $\Sigma_2$  invariants [including the Sayre (1952) equation], is found to be extremely reliable, even in the face of the scattering perturbations. When a figure of merit is chosen that also depends on this invariant, *e.g.* the minimal principle (Hauptman, 1993), there is no difficulty in finding the structure by completely automated procedures. This means that invariants that depend mostly on large amplitudes (also positive quartets) will remain reliable for predicting new phases.

Strictest limitations to the quasi-kinematical approximation are to be found when a completely refined structure is sought, *i.e.* if the determination of chemically meaningful bonding parameters is also required. Even when completely accurate kinematical phases are applied to the observed structure-factor amplitudes, refinement can be completely frustrated because the Fourier summation yielding the potential map is made with falsely weighted phase terms. Similar effects have been noted before when random amplitudes were applied to correct phase terms, for example (Ramachandran & Srinivasan, 1970). For the perchlorophthalocyanine, these amplitude deviations are not serious enough to restrict the determination of a correct structure (Dorset, Tivol & Turner, 1991), provided that adequate constraints are placed on the bond distances and angles during Fourier refinement. The outlook for the perbromo analog is less auspicious, however. It has already been shown (Dorset, Tivol & Turner, 1992), for example, that any potential map found after Fourier refinement, with nicely defined atomic peaks, may not represent a structure with acceptable bond distances and angles. The crystallographic residual for this erroneous structure, moreover, might be lower than that found for a chemically reasonable bonding geometry.

It appears, therefore, that there are electron diffraction data sets that will not yield geometrically correct structures after direct phasing and refinement but may, nevertheless, still contain sufficient information in the intensity distribution to allow the correct model to be obtained by another means. In this context, there is a place for structure searches with geometrically invariant molecular fragments, a procedure already practised routinely by polymer electron crystallographers (Brisse, 1989; Perez & Chanzy, 1989). Although the symmetry constraints on the molecular packing imposed by the unit cell for the examples in this paper are unusually specific for organics, a rotational and translational search for the type used by the program *DIRDIF* (Buerskens & Smykalla, 1991), for example, may have general applicability, also for examples where the symmetry constraints are less specific. The major criterion for such searches depends on how well the experimental Patterson function indicates the correct model orientation. With copper perbromophthalocya-

nine, for example, the starting position of the molecule could also be identified before tilting of the molecular plane by comparing the Patterson maps of two possible models to that calculated from the experimental intensities. As the tilt search is made around the long unit-cell axis, there would be a point where the overlap of experimental and calculated autocorrelation functions would be maximized and this would give a good approximation to the structure solution. In either case, however, direct methods and Fourier refinement are sufficient to find the starting position of a model to be refined by rigid-body tilt/translation.

As an alternative approach, it has been suggested earlier that *n*-beam dynamical corrections, incorporated into the structure refinement (Sha, Fan & Li, 1993), would allow the unconstrained optimization of atomic positions and thermal vibrations. There are problems with this suggestion, however, especially if more than one structure projection is used to record a three-dimensional data set. For a known sample thickness (or a representative mean thickness), separate calculations would have to be made for each crystal orientation and the values for reflections appearing in several zones would have to be averaged (Turner & Cowley, 1969). Also, it is not clear, necessarily, that multiple-beam dynamical scattering is the only significant perturbation to the observed intensity data. For the data set from copper perchlorophthalocyanine (Dorset, 1995a), it was found that a correction for secondary scattering was more useful for accounting for the complete zonal data set and, in fact, this incoherent multiple-scattering effect seems to be observed in data from many organics, even when they are composed only of light atoms. The correction for secondary scattering (Cowley, Rees & Spink, 1951), however, is less specific than the one for dynamical diffraction because it is not clear *a priori* how much of a weighted contribution from the convolution of intensities should be applied. Yet, as shown in this paper, a multislice correction for a data set somewhat restricted in resolution may be a fair approximation for both perturbations, at least to demonstrate that a geometrically plausible model can account for the observed data.

The empirical scaling procedure suggested by Huang, Lui, Gu, Xiong, Fan & Li (1996) may be a very practical way to overcome the dilemma of incorporating very complicated corrections into a refinement. If a high-resolution image of a suitably thin crystal can be obtained, *e.g.* to 2 Å resolution, then accurate heavy-atom positions can be found experimentally. As shown above, a partial structure-factor calculation based on these positions permits a rescaling of the observed electron diffraction intensities to be carried out iteratively, leading to a surprisingly accurate structure without the necessity to apply any other constraints. Although it was fortunate that suitable high-resolution images of this structure were available (Uyeda,

Kobayashi, Ishizuka & Fujiyoshi, 1978-1979) to permit the correction to be carried out as described, such images also may not be an absolute necessity in all cases. Therefore, if this approach were generally applicable to other heavy-atom-containing organic structures, then rescaling of intensities could be adapted easily to least-squares refinement in the manner of existing weighting schemes used in X-ray crystallography.

Although this data adjustment is somewhat related to primary-extinction procedures (Vainshtein & Lobachev, 1956; Li, 1963), it should not be construed as an intensity *correction* in a rigorous sense. What is implied by the success of two applications of this methodology, so far, is that the adjustment of the intensity data fall-off itself is sufficient to permit the successful completion of the analysis. Actual corrections (*i.e.* justifications of the model based on observed data) after the analysis must still rely on the known multiple-beam theories. Also, it should be noted that, although the atomic scattering factor fall-off is known, the actual thermal parameters is not. Since  $B \simeq 0 \text{ \AA}^2$ , this estimate cannot be found initially from the Wilson plot for data sets perturbed by multiple scattering. Thus, estimated values, based on those typically observed in X-ray crystallography, may have to suffice for the initial application of the scaling procedure.

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