Comments on the Validity of the Direct Phasing and Fourier Methods in Electron Crystallography

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Abstract

A recent simulation has attempted to evaluate the validity of direct phasing and Fourier techniques in electron crystallography. In response to this study, experimental electron diffraction data from copper perchlorophthalocyanine collected at 1200 kV were re-assessed to determine the most important deviation of these intensities from the single-scattering approximation. While n-beam dynamical scattering has indeed been observed for these electron diffraction intensities (in agreement with the simulation) and has been shown to be important for selection of data suitable for *ab initio* structure analysis, it is, however, not the major perturbation to data obtained at very high voltages. Rather, a simple correction for secondary scattering provides the best fit to the experimental data, an observation consistent with the analyses of other organic structures. Thus, in order to justify the use of electron diffraction intensities from any substance for an *ab initio* structure determination, it is, first of all, requisite that the actual conditions used for the diffraction experiment be closely modeled.

Introduction

Recently, interest in the use of electron crystallographic techniques for quantitative structure analysis has been revived for various reasons. Firstly, many substances are difficult to crystallize to sample sizes suitable for single-crystal X-ray data collection. While powder diffraction techniques are a powerful alternative, problems with unit-cell and space-group identification persist and it is often difficult to separate intensity data that arise from reciprocal-lattice planes with nearly the same spacing. Given the enhanced scattering cross section of matter for electrons, compared to X-rays or neutrons, electron diffraction readily obtains information about the undistorted reciprocal lattice from individual thin crystalline microareas. Secondly, electron crystallography also includes high-resolution electron microscopy. Hence, there is always the possibility to use so-called 'lattice images' as an independent source of low-resolution crystallographic phases, a possibility not available to any other branch of crystallography. Thirdly, more recent work with convergent-beam techniques has provided a basis for determining unequivocally the space group of a single crystal (Buxton, Eades, Steeds & Rackham, 1976; Spence & Zuo, 1992) – again, a prospect not available to any other crystallographic technique, where ambiguities in space-group identification often exist. (Unfortunately, however, this methodology is only useful for materials that are not easily damaged by the incident electron beam.)

Although the results of pioneering structural determinations based on electron diffraction intensity data (Vainshtein, 1964; Zvyagin, 1967) had often been met with a certain degree of skepticism, a more optimistic outlook has emerged in the organic field owing to extensive experimental studies of linear polymer chain-folded lamellae and two-dimensional protein crystals. In the former case, the results of conformational searches, with models based on X-ray crystal structures of the monomer or oligomer units (Brisse, 1989; Perez & Chanzy, 1989), could be compared favorably to the results of independent fiber or powder X-ray studies. In the latter case, low- or high-resolution structures of proteins derived from electron-microscope images have recently been shown (Engel, Hoenger, Henn, Hefti & Zulauf, 1992; Earnest, Walian, Gehring & Jap, 1992) to agree well with independent X-ray crystal structures that had been carried out after the electron crystallographic determination. Even the earlier electron diffraction structure analyses, which had often employed contemporary X-ray structural results for starting crystallographic phase information, have been vindicated by direct phase determinations, which assumed nothing *a priori* about the crystal structure (Dorset, 1994a). As shown mainly by three laboratories (Dorset, 1994b; Fan, 1993; Gilmore, Shankland & Bricogne, 1993), a variety of organic and inorganic structures have been successfully elucidated, yielding results that are entirely consistent with crystallographic analyses based on X-ray or neutron diffraction data.

While the results of direct analyses of light-atom structures, based on phase information derived from electron micrographs and/or the probabilistic techniques adapted from X-ray crystallography (Hauptman & Karle, 1953; Hauptman, 1972; Schenk, 1991), seem now to be accepted by many researchers, some disquiet about the

validity of results from heavy-atom-containing compounds seems to persist. A recent attempt to rationalize successful structural analyses with the underlying dynamical diffraction theory has been published by Peng & Wang (1994). To quote this work: 'The success of the direct phasing and Fourier method is clearly not a result of the validity of the kinematic approximation of electron diffraction in any rigorous sense.' Thus, in their paper (hereafter cited as P&W), multiple-beam dynamical scattering calculations for copper perchlorophthalocyanine were used to test the variation of diffracted-beam amplitude and phase with crystal thickness and then to assess its consequence on the interpretability of theoretical holographic images and/or potential maps produced from data obtained at different resolutions and crystal thicknesses. In these assessments, questions arose about the uniqueness of the structure determination based on experimental data as well as the appropriateness of the n-beam dynamical scattering for simulating the diffraction from actual crystals of this heavy-atom-containing organic compound. It is the intent of this paper to evaluate the conclusions of this study by examining the *experimental* electron crystallographic data that had actually been used to determine its crystal structure.

Experimental data and structure analyses

Following earlier methodology (Uyeda, Kobayashi, Suito, Harada & Watanabe, 1972), thin crystals (*ca* 100 Å thick) of copper perchlorophthalocyanine, $C_{32}Cl_{16}N_8Cu$, were epitaxically oriented on the (001) faces of freshly cleaved KCl crystal plates by sublimation of the material onto this surface *in vacuo*. When the goniometer stage in the electron microscope was tilted by 26.5°, the *hk*0 electron diffraction pattern could be found when the monoclinic *b* axis was parallel to the stage tilt axis. The measured unit-cell constants were $a = 19.62$, $b = 26.08$, $c = 3.76$ Å, $\beta = 116.5°$, space group $C2/m$. The rectangular projection is then $d_{100} = a\sin\beta = 17.56$, $b = 26.08$ Å, plane group *cmm*. Electron micrographs of *ca* 100 Å thick crystalline plates have also been published at 2.0 Å resolution (Uyeda, Kobayashi, Ishizuka & Fujiyoshi, 1978–1979) and at 2.3 Å resolution (O'Keefe, Fryer & Smith, 1983), both images obtained on 500 kV instruments. Electron diffraction intensity data have been collected in 100 kV increments of accelerating voltage from 200 to 1200 kV on a high-voltage electron microscope (Tivol, Dorset, McCourt & Turner, 1993).

Diffracted intensities obtained at 1200 kV (Dorset, Tivol & Turner, 1991), also from *ca* 100 Å thick crystals, will be the major subject of this paper. Their accuracy and near approximation by the single-scattering theory are really what ensures the direct phase determinations will succeed. These data were quantitatively measured after scanning the electron diffraction patterns on the films with a Joyce Loebl Mark IIIC flat-bed

microdensitometer and integrating under the peaks. There was no phenomenological Lorentz factor applied to these 198 unique data collected to a resolution limit $\sin\theta/\lambda = 0.57$ Å^{-1}, consistent with the procedure for many selected-area diffraction data sets (Dorset, 1994b). The significance of this assumption will become apparent later.

It is important to realize that this crystal structure had not been solved previously from X-ray diffraction data. *Ab initio* structure determinations, based solely on electron crystallographic observations, have been carried out in a number of ways:

(*a*) *High-resolution images.* After averaging of low-dose 2 Å (diffraction) resolution electron micrographs (Uyeda, Kobayashi, Ishizuka & Fujiyoshi, 1978–1979), it was possible to show that the heavy-atom locations of the molecules could be visualised at their correct positions. The organic residue of the molecule was not interpretable in terms of atomic positions. However, the Fourier transform of this averaging image, *i.e.* of the partial structure, has provided a basis set for phase extension into higher-resolution electron diffraction magnitudes (see below).

(*b*) *Symbolic addition followed by Fourier refinement.* If only the electron diffraction intensity data were used to generate structure-invariant phase relationships, ranking these in order of decreasing reliability, using the Cochran (1955) distribution, with an argument calculated from the normalized structure-factor amplitudes, *i.e.* $A = (2/N^{1/2})|E_{h_1}E_{h_2}E_{h_3}|$, then the phases of 27 reflections could be found by symbolic addition (Dorset, Tivol & Turner, 1991). (Here, N is the number of atoms in the unit cell, an approximation strictly valid only for a structure with just one atomic species. The normalized structure factors are defined by $E_h^2 = F_h^2/\varepsilon \sum_i f_i^2$, compensating $|F_h|$ for the fall-off of scattering factors f_i.) A potential map generated from the combined electron diffraction amplitudes and phases could be used to find the positions of some of the heavy atoms. When these were used to calculate a set of complete phases, a Fourier refinement could then be begun to locate, eventually, all of the remaining atoms in the structure, including the lighter-C- and -N-atom positions of the organic residue. Bond distances and angles in the final model were entirely consistent with values obtained for similar compounds (Brown, 1968).

(*c*) *Tangent formula.* Various applications have been made of the tangent formula (Karle & Hauptman, 1956). In its *QTAN* form (Langs & DeTitta, 1975), basis sets comprised of either the 27 reflections found by symbolic addition or from the Fourier transform of the 2.3 Å electron micrograph (Dorset, McCourt, Fryer, Tivol & Turner, 1994) were sufficient to access, for example, 137 phase values. When the former basis set was used, the best phase set corresponded to the lowest value of NQEST (DeTitta, Edmonds, Langs & Hauptman, 1975). This was not true when the image-derived phases were

used (even though an acceptable phase set could also be found in the multiple-solution list). Nevertheless, an initial structure could be improved by Fourier refinement to reproduce the structure obtained by the procedure outlined in (b). Earlier, phases from the 2 Å resolution micrograph had been extended to 1 Å electron diffraction resolution (Fan, Xiang, Li, Pan, Uyeda & Fujiyoshi, 1991) by *RANTAN* (Yao, 1981), yielding similar results.

(d) *Sayre equation.* The convolution of phased structure factors expressed by the Sayre (1952) equation has been a powerful means for phase extension. Basis sets derived from the symbolic addition set or the Fourier transform of high-resolution micrographs (after compensation for the objective-lens phase-contrast transfer function) have been successfully utilized for this purpose in two laboratories. The initial structure model could then be improved by Fourier refinement as before. Starting phase sets could be obtained from the Fourier transform of the 2.0 Å average image, as shown with simulated data (Liu, Fan & Zheng, 1988), or the one obtained at 2.3 Å, as shown with experimental data (Dorset, Kopp, Fryer & Tivol, 1995).

Although the match of the kinematical model to the observed diffraction amplitudes did not yield a very low crystallographic R value, an improvement could be demonstrated after carrying out a multislice dynamical calculation (Cowley & Moodie, 1957). At 1200 kV, for example, $R = 0.31$ if data are scaled so that $\sum |F_o|^2 = k \sum |F_c|^2$ or 0.36 if $\sum |F_o| = k \sum |F_c|$. At the limit of $\sin\theta/\lambda = 0.27\,\text{Å}^{-1}$, $R = 0.21$, where all atoms are assumed to have an isotropic temperature factor of $3.0\,\text{Å}^2$. The geometrically most reasonable kinematical model did not represent the global minimum of the crystallographic R factor (Dorset, McCourt, Fryer, Tivol & Turner, 1994). For the first scaling criterion mentioned above, this was $R = 0.28$. Such a global minimum, although representing a cosmetically acceptable representation of the atoms, corresponded to a geometrically deformed structure with chemically unreasonable bond distances and angles.

Evaluation of the structure determination

Uniqueness

Although any of the *ab initio* determinations described above would arrive at a refined crystal structure of copper perchlorophthalocyanine (Dorset, Kopp, Fryer & Tivol, 1995) that is consistent with X-ray structures of similar compounds (*e.g.* Robertson, 1953; Brown, 1968), there seems to be a concern about the *uniqueness* of the derived result by many investigators. To quote P&W: 'The solution returned by using the direct methods is therefore not unique. It sometimes takes the crystallographer's chemical intuition to know which of these may be correct'. Since the kinematical R factor is somewhat inadequate as a figure of merit for recognizing

the correct model, this suspicion is understandable. On the other hand, there may also be an implication in this statement that direct phase determinations themselves will not necessarily recognize which atomic model best corresponds to the diffraction data, even if the data are very close to kinematical limit.

The problem of uniqueness is an old nightmare of crystallographers. A basic statement of this problem is: can the Patterson function of a crystal, calculated from the Fourier transform of a measured intensity data set, be shown to be satisfied by only one chemically reasonable structural model? The concept of homomorphic structures, *i.e.* different structures that produce the same Patterson function, has been discussed extensively by Hosemann & Bagchi (1962). The simplest example of homomorphism is the Babinet phase solution, where the inverse phase set is found instead of the true set. However, imposition of atomicity and positivity in X-ray crystallography [a pre-condition for many direct-phasing procedures (Harker & Kasper, 1947; Hauptman & Karle, 1953)] seems to minimize severely the likelihood of finding possible homomorphs, since the negative of an atomic structure has no meaning. The same constraints are often useful in electron crystallography. Although ions can lead to negative values for scattering factors, this affects mainly the very low angle region of the diffraction pattern (Vainshtein, 1964) and, anyway, is of little consequence to typical organic structures. Other valid and rigorous examples of homomorphs are enantiomeric structures. Even in X-ray crystallography, an enantiomorph is very often arbitrarily selected in the phase determination (Rogers, 1980) without knowing whether the choice is absolutely correct.

Also, there may be accidental near-homomorphs that may give nearly the same Patterson function, especially if only a projection data set is being considered. This is particularly problematic for highly symmetrical molecules. A typical illustration of this could be the initial X-ray structure analysis of triphenylene (Klug, 1950), based on two zonal data sets. (This example is chosen from a period when the accuracy of X-ray analyses was about at the same point as current electron crystallographic determinations.) Solution of the structure by an optical transform approach seemed to arrive at a reasonable model where every atom was found in the electron density map and the resulting bond distances and angles appeared to be consistent with the known values for aromatic hydrocarbons. However, there were some unusual features of intermolecular contacts that were noted (Klug, 1950; Robertson, 1953) – on one side, the distances were large and, on the other, the contacts were rather close. A redetermination of the crystal structure by Pinnock, Taylor & Lipson (1956) discovered the discrepancy in the original analysis. Essentially, although the density features of the Patterson function nearest to the origin (*i.e.* the intramolecular vectors) were satisfied quite well, features at longer distance (*i.e.* the

intermolecular vectors) were not. In the discussion of these results, Pinnock, Taylor & Lipson (1956) pointed out that the 'reasonable' appearance of the electron density map itself was not an ultimate proof of a correct structure, since features imposed by the choice of phases often recur in the map. On the other hand, there were variations of atom peak densities that should have indicated that the map was not entirely correct.
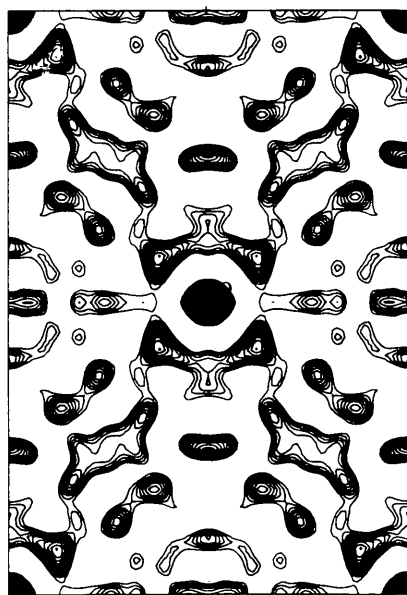
(a)
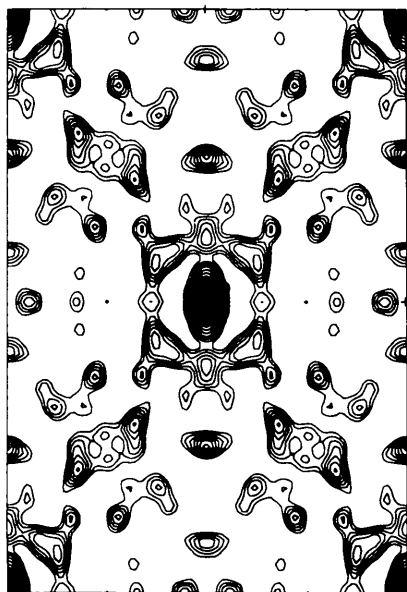
(b)

Fig. 1. Patterson functions of copper perchlorophthalocyanine calculated from 198 hk0 data: (a) based on kinematical intensities calculated from the refined structural model; (b) based on observed electron diffraction intensities obtained at 1200 kV.

Another typical way of finding a false structure is the misidentification of unit-cell symmetry. There are many examples of such errors in X-ray crystallography, especially for structures where a regular motif in the molecule (e.g. a sublattice) dominates the scattering, so that an incorrect model will still lead to an 'acceptable' crystallographic residual (although the correct structure will result in a better fit to the observed data). One example is the X-ray structure of ethyl stearate (Aleby, 1962, 1968). Aside from the ambiguity of identifying space groups from systematic absences (Stout & Jensen, 1968), secondary scattering (Cowley, Rees & Spink, 1951) can be a major factor in causing space groups to be incorrectly determined in electron crystallography, since even these absences are violated.

What, then, can be expected from electron crystallographic data, where various perturbations can be demonstrated to exist, leading to significant deviations from ideal kinematical conditions? How is it possible to justify an *ab initio* determination of the kind described in previous work? A comparison of experimental and model Patterson functions is useful. As shown in Fig. 1, although differences between the autocorrelation maps exist for calculated and observed data, there is still a good correspondence of major peaks. This means that enough information about the structure remains in the observed intensity data so that direct methods will arrive at a useful starting set of atomic positions and that Fourier techniques can be used (with constraints) to find atomic positions during a refinement. That is to say, density features that are close to actual fractional atomic positions in the molecule should appear in potential maps during the phase determination and refinement. The structure analysis, moreover, must end up with chemically meaningful bond distances and angles and the intermolecular contacts must be those expected for repulsive van der Waals molecules. These considerations were kept in mind as the crystal structure of copper perchlorophthalocyanine was determined and are anything but a subjective constraint. Nevertheless, it is important to find the major cause for the observed deviation between the two Patterson functions in Fig. 1. Obviously, as has often been discussed (e.g. Cowley & Moodie, 1959), if these perturbations are too severe, then the data no longer represent the underlying structure in a simple way and an *ab initio* structure analysis would be impossible. As cited also by P&W, this was the original motivation for attempting to retrieve such lost information with modified Patterson functions (Cowley, 1956) for cases where the diffraction intensities were not entirely distorted by multiple scattering.

Accuracy of the phase determination

Even in the X-ray crystallographic structure analysis of organic molecules, difficulties experienced in determining structures by direct methods in no way imply that a

unique solution of the Patterson function cannot be found. Reasons for the failure of direct methods are frequently discussed (e.g. Lessinger, 1976) – e.g. in the applications of MULTAN (Germain, Main & Woolfson, 1970), one of the 'workhorse' computer versions of the multisolution tangent formula. [Also, see a lengthy discussion of this topic by Ladd & Palmer (1980) under the heading Success is not guaranteed.] Often, restrictions can be imposed by the limited number of normalized structure factors used to generate phase-invariant relationships. Depending on the number of atoms in the unit cell (Fan, Hao & Woolfson, 1991), the accuracy of predicting the phase of an invariant can also be compromised, since the associated distributions (Cochran, 1955) become very flat. For multisolution techniques, the quality of the figures of merit used to identify the best phase set can also lead to difficulties (Cascarano, Giacovazzo & Viterbo, 1987). In other instances, the (relative) size of the starting phase set is very small so that the resultant set of phased structure factors will produce an initial map where only a few atoms (or a small molecular fragment) will appear. It is just here where the chemical knowledge of the investigator can be of great assistance to find a reasonable starting model that will lead to a complete solution after Fourier refinement.

Even with the limited data accuracy in electron crystallography, the experience of applying the direct methods commonly employed in X-ray crystallography still seems to furnish useful guidelines for many applications. This statement is based on the solution of nearly 50 structures of various kinds from electron diffraction data in this laboratory. For example, use of the tangent formula to solve the 3D structure of 2,5-piperazinedione (Dorset & McCourt, 1994a) and thiourea (Dorset, 1992a) directly yielded accurate phase sets. In the initial potential maps, the atomic resolution structures were immediately recognized, although there were problems with later refinement. More recently, similar results have been experienced in the direct analysis of the 3D structure of a poly(1-butene) polymorph (Dorset, McCourt, Kopp, Wittmann & Lotz, 1994).

Admittedly, the ab initio determination of the copper perchlorophthalocyanine structure (reviewed above) was somewhat difficult. Symbolic addition required the use of three algebraic unknowns to find 27 phase terms. (The restriction to a single allowed zonal phase term for origin definition in the centred plane group was partially responsible for this difficulty.) Thus, $2^3 = 8$ initial potential maps were required to find one with likely positions of some of the heavy atoms to begin the Fourier refinement (Dorset, Tivol & Turner, 1991). There were also two phase errors in this basis set, probably because of inaccurately defined triple invariant sums, incorrectly ranked in probability because of data perturbations. If the tangent formula was used (Dorset, McCourt, Fryer, Tivol

& Turner, 1994) to expand these 27 phases (including errors) to 137 values, there were 35 phase discrepancies to the refined model. The resulting map, nevertheless, strongly resembled the final structure but contained some distorted bond distances and angles. (An ab initio phase determination by the tangent formula was not possible.) When the Sayre equation was used to extend the 27-phase basis set, 63 of the 174 phases accessed were in error but the map still contained most of the atomic positions. Both phase sets could be refined by Fourier methods to the structure found before. Expansions based on the Fourier transform of the 2.3 Å image via the Sayre equation (Dorset, Kopp, Fryer & Tivol, 1995) were also quite successful, even though uncompensated lens astigmatism (details given in paper) resulted in 10 phase errors in a basis set of 39 terms. After expansion, there were 45 errors for the 147 reflections. Again, the resulting model could be refined to the previously determined structure, even though use of the kinematical R factor again could not be pushed to a global minimum. This experience, therefore, contradicts the assumption made by P&W that 'the restored phases from the direct method (should be) perfect'. Such is rarely the case, even in X-ray crystallography. An accurate model of a structure is found only after sufficient refinement.

How do we evaluate the results outlined above in terms of the arguments given by Peng & Wang (1994)? Unfortunately, their paper provides very few insights into the accuracy of such ab initio phase determinations. It is incorrect, for example, to compare directly the phase values derived from a dynamical scattering calculation with crystallographic phases. Even though the two types of phase term certainly are related to one another, the dynamical set deviates from the symmetry constraints of the unit cell, even violating Friedel's law as the electron wave front propagates through successive slices of a crystal. Thus, a centrosymmetric crystal yields dynamical phases that are non-centrosymmetric in nature. On the other hand, when a (dynamical) image of the crystal is recorded as an intensity distribution on a film to be used for image analysis (but still assuming that the observed data nearly obey the weak-phase-object approximation), symmetry constraints are re-imposed and an origin is defined to conform to the choices allowed by the plane group. Thus a (somewhat distorted) set of crystallographic phases is obtained from this analysis and not the actual dynamical phases for beams propagating in the microscope column. After deconvolution of the phase contrast transfer function, the accuracy of the derived crystallographic phases will depend on the nature and importance of multiple-beam interactions but it is difficult to say a priori how much dynamical scattering will degrade these values to the point where sufficiently accurate crystallographic phases cannot be recovered after image analysis. We have already indicated that a small number of phase errors in the resultant basis set may not adversely affect the further extension or refinement.

Suppose we imagine the dynamical scattering from a 112.8 Å thick crystal (see below) in a hypothetical ultra-high-resolution electron microscope similar to the one installed in Kyoto (Uyeda, 1991). The relativistic electron wavelength is $\lambda = 0.00759$ Å ($W = 1200$ kV) and $C_s = 1.6$ mm. At Scherzer focus (-402 Å), the image in Fig. 2 is seen. Fractional positions can be found for all atoms and, if these are used to calculate kinematical structure factors, there are 47 or 192 crystallographic phases to a 1.05 Å^{-1} resolution limit that disagree with the best refined model found for the structure. This corresponds to average atomic shifts of 0.36 Å for Cl, 0.10 Å for N and 0.15 Å for C, i.e. within easy reach of refinement.

It was proposed by P&W that dynamical phases applied to dynamically distorted amplitudes would be superior to the kinematical phases being applied to the altered amplitudes if the former were used to generate an 'electron density map' via a holographic procedure. That distorted amplitudes combined with correct kinematical phases will create some distortions to an otherwise reasonable map has been known since the early description of Ramachandran & Srinivasan (1970). Phase errors can be more serious, however. Nevertheless, these authors have demonstrated that electron density maps and structure maps have similar appearance for 107 Å thick crystals while the former contain more details of light atoms for thicker crystals. However, crystallography is not a just matter of obtaining molecular images. Rather, it is most important to find accurate atomic coordinates for calculation of bond distances and angles, conformational angles and intermolecular contacts. At 107 Å, the quality of the structural coordinates measured from their figures is actually superior for the structure maps generated with kinematical phases. While some claim might still be made for the use of holography with thicker crystals, there are other practical reasons, to be given below, for avoiding such samples.

Data perturbations

P&W imply that multiple-beam dynamical scattering is the most significant perturbation to diffracted beams from copper perchlorophthalocyanine microcrystals. To test this proposition, another multislice calculation was carried out for this structure (as already implied by Fig. 2) using the atomic coordinates obtained from the original direct structure analysis (Dorset, Tivol & Turner, 1991). The geometric arrangement of the molecular stacks has been described by Uyeda, Kobayashi, Suito, Harada & Watanabe (1970). From this and the resulting coordinates from our refined structure, a slice model for the dynamical calculation was constructed with planar dimensions 17.56 by 26.80 Å and an orthogonal projected interval of 3.76 Å down the molecular columns, in fundamental agreement with the earlier calculation carried out by Ishizuka & Uyeda (1977). Doyle & Turner (1968) X-ray scattering factors were converted to electron form factors (neutral atoms assumed) via the Mott formula. Thermal parameters applied to the atoms were $B = 3.0$ Å^2. Although the initial structure-factor calculation was carried out to a resolution $d^* = 3.0$ Å^{-1}, it was soon found that most of the scattering power from the molecule is significantly lost beyond 1.0 Å^{-1} (see Fig. 3) because of the atomic temperature factors used. This is to be contrasted with the very high resolution limits considered by P&W, which might be justified if there was no thermal motion (Fig. 3). For the multislice calculation, a resolution limit $d^* = 1.05$ Å^{-1} was imposed, even though there are six reflections observed beyond this limit. This boundary was applied to maintain an approximate circular cross section to the diffraction limit. Even with this cut-off, 99.80% of the incident intensity is retained in the diffraction pattern after the beam passes through $t = 112.8$ Å crystal thickness (30 slices).

At the $d = 1.05$ Å^{-1} resolution of the calculation, the dynamical correction improved the fit to the data to $R = 0.27$, compared to 0.36 for the kinematical model. While this is a crystallographic residual within the range accepted for many ab initio electron crystallographic determinations, it is still rather higher than would be expected if multiple-beam scattering were the only perturbative influence on the measured intensities. The fit is improved somewhat ($R = 0.21$) if only a lower-resolution domain, within $h_{max} = 10$ and $k_{max} = 8$, is
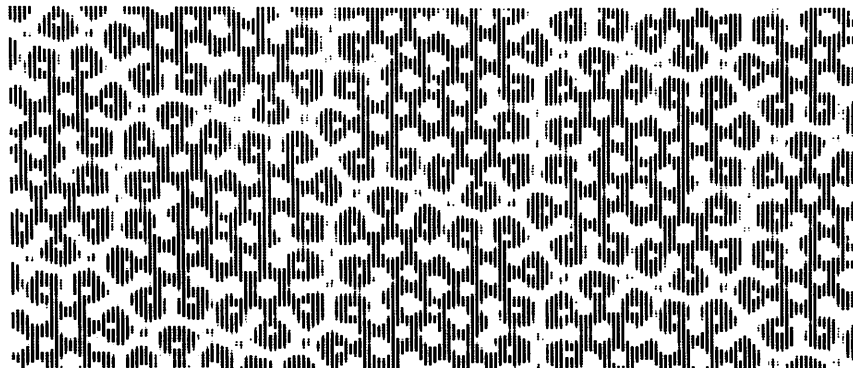

Fig. 2. Theoretical image of a 113 Å thick crystal of copper perchlorophthalocyanine obtained at Scherzer focus ($\Delta f = -402$ Å). A high-voltage electron microscope is modeled with a spherical aberration constant $C_s = 1.6$ mm and operating at 1200 kV. Note that the centered rectangular lattice is transformed to a primitive rhombic one with cell edges $a = 15.72$, $b = 15.72$ Å, $\gamma = 112.1°$.

considered. In addition, it cannot be said that there is a consistent increase in R factor with data taken at higher shells of resolution, despite the claim by P&W that higher-order structure factors actually should be 'more dynamical'. For the first five increments of resolution in shells, where the mean value of d^* is increased by $0.1\,\text{Å}^{-1}$, one calculates $R = 0.11, 0.23, 0.21, 0.12, 0.24$, respectively, starting at the lowest-resolution shell. Comparison of the dynamical calculation, carried out to the stated resolution limit, to calculated kinematical structure factors, incorporating the amplitude fall off owing to the deviation parameter of the Ewald sphere from the reflection center (Self & O'Keefe, 1988; Cowley, 1988), results in $R = 0.19$. In other words, at 1200 kV, the diffracted amplitudes from a 113 Å thick perfectly flat crystal slab of copper perchlorophthalocyanine should not deviate as greatly from the kinematical condition as has been actually observed.

What other scattering perturbations can significantly affect the observed intensity data? The projected unit-cell repeat is very small: hence, the diffraction incoherence due to elastic crystal bending (Cowley, 1961) will not play a major role for this structure. The other possible perturbation could be secondary scattering, *i.e.* incoherent multiple scattering (Cowley, Rees & Spink, 1951), when strong beams from upper crystal layers, which are uncoupled (owing to defects) from the lower crystalline regions, act as ancillary primary sources for the lower layers. The result is a summation of weighted convolutions in intensity. To a first approximation,

$$I'_{h,k} = I_{h,k} + mI_{h,k} * I_{h,k} + \dots$$

The presence of secondary scattering is relatively easy to detect in structures where glide elements of symmetry should lead to systematic absences along reciprocal-lattice rows. As shown by Cowley, Rees & Spink (1951) and Vainshtein (1964), the space-group-forbidden reflec-

tions can be found instead to have appreciable values. This is because the convolution of intensity does not follow the extinction rule for the axial reflections. An example where such violations are often observed is for solution-crystallized n-paraffin crystals (see previous references) that are thick. The perturbation can also cause a spurious increase of data resolution and severely restricts the finding of the R-factor minimum when attempting to solve this structure in searches with a chain model (Dorset & Moss, 1983). For plane groups where such extinctions do not occur, the secondary scattering influence is even more difficult to identify. For example, in cmm, *i.e.* the projection of copper perchlorophthalocyanine, only reflections where $h + k = 2n$ are permitted; thus, in the convolution of intensities, only reflections of this index class can be used in the vectoral interactions so that no forbidden reflections will be generated. Secondary scattering has been shown to occur in a number of organic structures, *e.g.* paraffins epitaxically oriented on a substrate (Hu, Dorset & Moss, 1989) and C_{60} buckminsterfullerene (Van Tendeloo, Van Heurck, Van Landuyt, Amelinckx, Verheijen, van Loosdrecht & Meijer, 1992), where its perturbation to electron diffraction intensities plays a major role in permitting the determination of a correct crystal structure (Dorset & McCourt, 1994*b*).

Because it is a very easy calculation to carry out, a secondary scattering model was used for copper perchlorophthalocyanine, starting with the kinematical intensities and for the complete data set. Although no attempt was made to find the ultimate value of m to give the best agreement to the observed data set (nor to expand to terms containing multiple convolutions), a model was easily found that lowered the match from $R = 0.36$ to 0.21 for the entire $hk0$ data set. Hence, this simple correction is a lot more successful than the multiple-beam dynamical calculation for accounting for the perturbation of the high-voltage electron diffraction intensities.

Discussion

Much of the controversy about using observed electron diffraction intensity data and/or electron micrographs for *ab initio* structure determinations arises from a very fundamental question: What amount of data perturbation can be allowed before this endeavour will fail or, even worse, lead to an attractive but false structure? Experience in the determination of crystal structures from electron diffraction intensity data certainly has appeared to be anything but consistent so far. Why, for example, would difficulties occur in determining the structure of copper perchlorophthalocyanine from *e.g.* 400 kV (or even 1200 kV) single-crystal electron diffraction intensities when 50 kV data from copper chloride hydroxide crystalline textures (Voronova & Vainshtein, 1958) are sufficiently good for this purpose (Dorset,
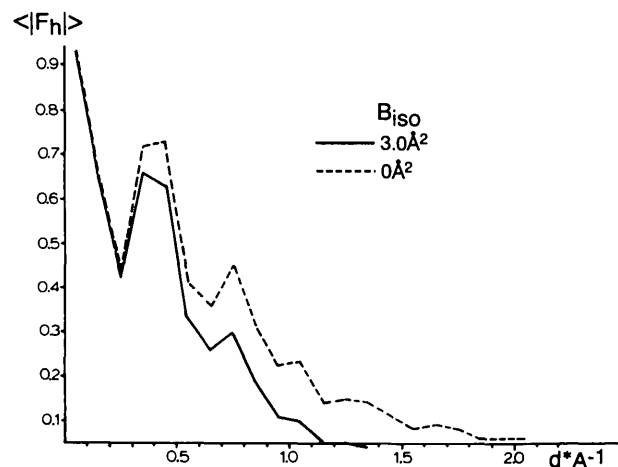

Fig. 3. Plot of $\overline{F_h^{calc}}$ *vs* d^* for copper perchlorophthalocyanine comparing data when $B = 3.0$ and $0.0\,\text{Å}^2$. The former isotropic thermal parameter most closely matches the observed data.

1994c)? This apparent contradiction to theory needs to be addressed, since the two materials have similar atomic contents (substitute oxygen in the inorganic for carbon and nitrogen in the organic). Moreover, from unit-cell size alone ($a = 5.73$, $b = 6.12$, $c = 5.63$ Å, $\beta = 93.75°$), one would expect the latter data set, not the former, to be completely unusable, even though the latter structure was easily obtained by direct methods. All atomic positions were visible in the initial potential maps and the kinematical model ($R = 0.24$) was in good agreement with an X-ray structure of a related halide. Although it is also possible to solve the former structure *ab initio* at 1200 kV (with data from *ca* 100 Å thick crystals, as discussed), diffraction data collected at 400 kV can lead to difficulties (Tivol, Dorset, McCourt & Turner, 1993).

Possible reasons for this apparent contradiction can be anticipated by studying Fig. 4, which is a schematic representation of a crystalline preparation on a grid surface. In historical context, early Russian efforts made use of electron diffraction cameras (Pinsker, 1953), not electron microscopes, in which *millimeter* diameters of specimen could be irradiated to produce a texture diffraction pattern. Besides the azimuthal distribution of plate-like crystals on the grid, there is also a considerable angular distribution of crystallites along the beam path. In addition, crystal thickness is not constant. The resultant distribution of crystallites leads to the phenomenological Lorentz corrections proposed by Vainshtein (1956). Any marked deviations from strictly kinematical scattering had been conveniently treated by a two-beam dynamical model (Vainshtein & Lobachev, 1956; Li, 1963), since this correction could be made without any prior knowledge of the unknown crystal structure. (This, of course, contrasts with the *n*-bond theories that require foreknowledge of the crystal structure.) In 1967, Cowley had pointed out that the data observed from textures and powders often seemed to be well corrected by this simple procedure, although deviations from the two-beam model could be noted: "For most of the structure analyses
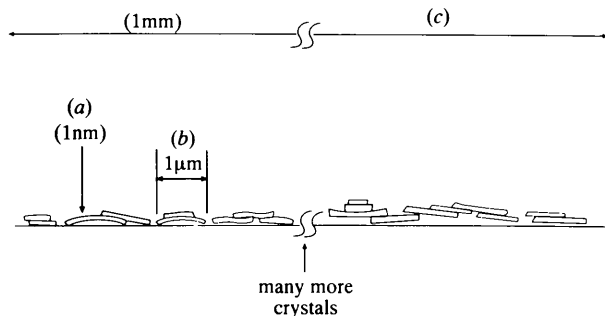

Fig. 4. Schema of illumination sizes for nanodiffraction, microdiffraction (selected area) and millimeter diameter for obtaining texture diffraction patterns. In the former, a flat crystalline area devoid of defects can often be isolated. For selected-area diffraction, crystal curvature and some defects can be problematic, even though a single crystal is being examined. In the latter condition, there is an additional azimuthal random orientation.

which have been made on the basis of arc or ring patterns, the interpretation of the intensities would appear to be justified on the basis that the deviations from the kinematical approximation are not greater than, for example, the 'extinction effects' present in the data for many contemporary X-ray diffraction structure analyses. Also, particularly for light-atom structures where some correction has been made for dynamical effects on a two-beam basis, the number of reflections seriously affected by *n*-beam systematic interactions has not been high.... In light of the above discussions of dynamical effects, it seems clear that the most serious doubts must be reserved for the structure analyses of crystals containing heavy atoms for which large two-beam dynamical corrections have been applied." Nevertheless, many of the early structural results from Moscow have been rejected by electron crystallographers in the West. However, most recently, direct phasing procedures have been shown to solve these structures *ab initio* from the published data (Dorset, 1994a,b), even if automated procedures, involving minimal interaction by the investigator, were used. This statement does not refute the existence of multiple-beam dynamical scattering by any means but only demonstrates that a complete model for the crystalline array could be quite complicated (see Turner & Cowley, 1969). Not only is dynamical scattering important but also the diffraction of electrons from a coherent source by bent crystals or the effect of secondary scattering. Even for heavy-atom materials, the more accurate formulation was found to be required for the refinement procedure but after the initial structural model was determined from the observed data, *e.g.* by the interpretation of Patterson maps (Turner & Cowley, 1969).

The other extreme of data collection is at the nanometer scale used in convergent-beam experiments for example. Here (Fig. 4), a perfectly flat crystalline slab with a constant thickness can be isolated for the experiment. In the electron diffraction patterns, one observes the high-order Laue-zone (HOLZ) as well as the zero-order Laue-zone (ZOLZ) reflections because the effective 'Lorentz' factor for the diffraction pattern is determined by combining the Ewald-sphere curvature and the shape transform of the crystal thickness. As shown by extensive studies (*e.g.* Spence & Zuo, 1992), the multiple-beam dynamical theory is a rigorous description of the diffraction from such perfect-crystal slabs and can even be used to advantage for space-group determination as well as the elucidation of some crystallographic phases (when wavelength is used as a variable). Nevertheless, methods have been found to use the HOLZ diffraction intensities for *ab initio* structure analyses, mainly because they have a large enough extinction distance that the kinematical approximation will lead to a favorable result (Vincent & Exelby, 1991, 1993; Vincent & Midgley, 1994). Nanobeam experiments cannot be used conveniently to investigate organic

specimens because of their susceptibility to radiation damage – even for the phthalocyanines considered here (Fryer, McConnell, Zemlin & Dorset, 1992). Even if the samples were radiation stable, there would be less likelihood of obtaining good data from, for example, 300 Å thick crystals owing to the presence of layer defects and the resulting secondary scattering.

Typical selected-area diffraction experiments of the kind often used to collect data from organic specimens fall somewhere between the two extremes mentioned above. As shown schematically (Fig. 4), a nearly perfect crystalline area of several micrometers diameter can be isolated to produce a single-crystal diffraction pattern. However, with this selected area, the crystal typically is not flat (especially for aromatic compounds). The distribution of orientations permits one to neglect the phenomenological Lorentz factor (Dorset, 1976a.b) because the Gaussian elongation of diffracted beams ensures that the Ewald sphere still falls close enough to the peak center to be an acceptable measure of intensity, even at 20 kV (Dorset, 1992b)! The selected area may also be large enough to include a few crystal defects, including a layering of coherent crystallite layers that can lead to significant secondary scattering. For selected-area diffraction data from such samples, the existence of n-beam dynamical scattering can be verified even for the lightest-atom structures (Dorset, 1976a,b; 1980), even at 1000 kV. At 'conventional' voltages, the best fit to the intensities is given by the phase-grating approximation, not the rigorous multislice model. The former n-beam model is one where the Ewald-sphere curvature is not considered and is effective only because the sampled crystal areas are not flat. Other perturbations can be just as important as dynamical scattering when data are collected from such micrometer-diameter areas. That is to say, bending and crystal imperfections result in the need for a more complicated model to describe rigorously the diffraction from such microareas. An attempt to approach this complexity in a more rigorous way was made in dynamical calculation for elastically curved crystals (Moss & Dorset, 1983) but secondary scattering was not included. In general, it must often suffice just to treat the most important of several perturbations.

Thus, when deciding on the validity of direct phasing and Fourier methods in electron crystallography, it is important to know the details of the actual diffraction experiment [as originally recommended by Turner & Cowley (1969)] so that the resulting model physically matches the conditions used to collect the intensity data. The model of Peng & Wang (1994) implies a perfect crystal that is flat and probably held near absolute zero – conditions mimicking a nanobeam experiment on an instrument with a liquid-helium specimen stage. Unfortunately, these conditions do not correspond to our electron diffraction experiments, where data were collected at room temperature from non-flat (i.e. elastically deformed) crystals (typical for organics) that

contained enough defects to permit secondary scattering to occur. At high voltage, secondary scattering has a greater effect on the intensities than n-beam dynamical interactions, even though the dynamical interactions are also present.

In electron crystallography, a valid theoretical framework, e.g. Cowley (1981), must be exploited to establish experimental conditions for measurement of 'quasi-kinematical' intensities. This means that experimental intensities still contain enough information about the unit-cell transform that the structure can be solved directly by conventional phase-determination techniques. For selected-area diffraction experiments, this means that the crystal thickness and accelerating voltage must be controlled to favor this outcome, even if it requires the use of a high-voltage electron microscope for some materials containing heavy atoms. Crystallite distributions producing texture diffraction patterns may be beneficial for data collection, especially if higher-voltage sources are used (Popov & Zvyagin, 1958). The success of our ab initio analyses of eight early data sets from Moscow underscores the importance of this possibility, which merits further study.

In the same breath, it must be understood that the actual deviations from kinematical scattering may be significant enough to affect phase determination or structure refinement. This point should never be disputed. We have already indicated instances where such difficulties have occurred in some direct phase determinations or later refinements. Nevertheless, for certain phase-invariant sums, it seems sufficient just to preserve experimental $|E_h|$ values in domains of large and small quantities [but not necessarily just the values of I_h as indicated by Peng & Wang (1994)] and not to worry about their absolute kinematical ranking. For one data set from thiourea (Dorset, 1992a), dynamical scattering is enough of a problem that good bonding parameters cannot be determined unless the higher-angle data are emphasized (!), even though the phases found by direct methods are not especially compromised. Even so, the structure cannot be refined. In other applications with the tangent formula, multiple scattering precludes use of the NQEST figure of merit (DeTitta, Edmonds, Langs & Hauptman, 1975) because this requires an accurate estimate of low normalized structure-factor magnitudes (Dorset & McCourt, 1994a; Dorset, McCourt, Kopp, Wittmann & Lotz, 1994; Dorset, McCourt, Fryer, Tivol & Turner, 1994). Other figures of merit such as the minimal function (Hauptman, 1993) are successful, on the other hand, when they rely on the estimate of Σ_2 three-phase invariants (based on large $|E_h|$ magnitudes). In some cases, the use of maximum entropy and likelihood seems to be beneficial (Gilmore, Shankland & Bricogne, 1993) for recognizing correct structures. These considerations are merely benchmarks on the way to developing optimal procedures for electron crystallographic structure analysis. Suggestions have been made

to incorporate dynamical scattering calculations as part of the refinement (Sha, Fan & Li, 1993) but this is only useful if it is the principal data perturbation. It is important to stress that *ab initio* structure analysis allows the initial model to be obtained so that a suitable correction can be made afterward; the point is to obtain this model! Otherwise, the 'indirect' approach advocated by others is liable to fail just because of the extreme difficulties involved with trial-and-error techniques for solving crystal structures.

Summary

The points made in this paper, supported by the analysis of an experimental electron diffraction intensity data set from epitaxically oriented copper perchlorophthalocyanine, can be summarized as follows:

Accuracy of diffraction intensity data

There are at least three major perturbations to electron diffraction intensity data that can adversely affect an *ab initio* crystal structure analysis (neglecting radiation damage). These are: *n*-beam dynamical scattering, secondary scattering and the effective diffraction incoherence from elastically bent crystals. Often, experimental variables (low electron wavelength, minimal crystal thickness) are manipulated to reduce the effect of dynamical scattering. Even so, it is desirable that these observed intensities are also self-consistent, *i.e.* that there is a good agreement between any two independent diffraction patterns recorded from the same projection of different crystals. In this paper, it was shown that secondary scattering can also be a significant source of error, even at high voltage. Nevertheless, intensities close enough to 'quasi-kinematical' conditions can be recorded to permit a structure to be determined directly from them, even if the fit to the observed data is not appreciably improved by an *n*-beam dynamical correction.

Uniqueness of a structure solution

The suitability of a 'quasi-kinematical' intensity data set for direct structure determination depends on the match of the experimental Patterson function to the autocorrelation function of the actual crystal structure. While the uniqueness of the structure analysis has often been discussed, it is generally assumed that conditions of positivity (often as good an approximation in electron crystallography as it is in X-ray crystallography) and atomicity will constrain the structure solution to the correct answer. On the other hand, the more data there are, the better the outcome will be.

Accuracy of the initial phase set

It is not required that the initial set of crystallographic phases, *e.g.* that obtained by direct methods, be entirely correct. A few errors are often tolerated but it is better, of course, that they be associated with weak reflections rather than intense ones. In electron crystallography, as in X-ray crystallography, the object of the structure analysis is just to arrive at a good starting model. Knowing the physical theory of electron scattering will permit appropriate corrections to be made during the refinement stage, providing, of course, that the most significant perturbation to the diffraction intensities can be accurately identified.

Simulations and corrections

Whenever a correction is to be made to electron diffraction intensity data, it is important and necessary that the model parameters used for a scattering simulation accurately match those used to collect the data. Otherwise, the desired simulation may merely be an academic exercise that says nothing about the validity of an analysis. Moreover, because of the resultant complexity of selected-area diffraction experiments, a rigorous correction may not be easily made so that just a consideration of the most important perturbation may have to suffice.

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