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Electron Crystallography

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Abstract

The idea of solving unknown crystal structures from experimental electron-diffraction intensities and high-resolution electron micrographs has remained a controversial topic in the 60 year history of electron crystallography. In this review it will be shown that the application of modern direct phasing techniques, familiar to X-ray crystallographers, has decisively proven that such *ab initio* determinations are, in fact, possible. This statement does not, by any means, refute the existence of the several significant scattering perturbations identified by diffraction physicists. Rather, it does affirm that experimental parameters can be controlled to ensure that a 'quasi-kinematical' data set can be collected from many types of specimens. Numerous applications have been made to various types of specimens, ranging from small organics to proteins, and also some inorganic materials. While electron crystallography may not be the optimal means for determining accurate bonding parameters, it is often the method of choice when only microcrystalline specimens are available.

Doug Dorset was born in southeastern Pennsylvania, some 30 miles (50 km) east of where his great-grandfather (and the Army of Northern Virginia) had been an unwelcome visitor some threescore and 19 years before. After obtaining his baccalaureate degree in chemistry at a small college, he crossed the Mason–Dixon line to earn his PhD in biophysics at the University of Maryland. Cooler temperatures attracted him to upstate New York (where crystallization of ambient humidity can justify the ownership of cross-country skis). After two post-doctoral positions, he started his research career in electron crystallography at the Medical Foundation of Buffalo (now the Hauptman–Woodward Institute) in 1973, enhanced by a sabbatical at the University of Basel, Switzerland, in 1980. His work in this area, strongly influenced by Herb Hauptman and the direct methodists, has recently been summarized in a monograph entitled Structural Electron Crystallography. He is currently a member of the Commission on Electron Diffraction of the International Union of Crystallography and the US National Committee for Crystallography.

1. Introduction – Why electron crystallography?

Electron crystallography is a term used to describe the quantitative determination of crystal structures from electron scattering data. Since the instrument most used nowadays for data collection is the electron microscope, it is an optical technique, promising the direct visualization of atomic or molecular packing in crystals. Additionally, one may analyze diffraction intensities by means familiar to X-ray crystallographers. As will be shown in this review, there are cases when the combination of image and diffraction data will be useful for the determination of a crystal structure. In most cases, however, only the diffraction intensities will be analyzed.

Of the three radiation sources commonly used to resolve interatomic distances, electrons, compared with X-rays or neutrons, are scattered most efficiently by matter (Vainshtein, 1964*a*). Thus, a thin microcrystal can produce single-crystal diffraction patterns in the electron beam, where a random assembly of these would produce radially or axially disordered patterns with the other two radiation sources. Obviously, for determination of space-group symmetry and unit-cell dimensions, single-crystal information is preferred. For such qualitative work, electron-diffraction techniques have long served to facilitate the analysis of fiber or powder X-ray diffraction intensity data (Atkins, 1989). This can be very important when reflections with almost the same reciprocal spacing overlap.

The possibility to study microcrystalline objects as single crystals is very important for many applications. For example, there are cases where the properties of thin films must be specified and, indeed, many cases where two-dimensional layers are more favorably crystallized than the three-dimensional crystals themselves. Examples include numerous amphiphilic materials, such as detergents and polar lipids. The most convenient single-crystal form of a linear polymer is a chain-folded lamella, generally less than 100 Å thick. Integral membrane proteins can form true two-dimensional crystals in a phospholipid bilayer matrix. Surface monolayers of metal atoms on a bulk underlayer

are of current interest in the inorganic field. In these examples, and others, it could be very difficult to determine a structure using intensity data from the disordered bulk sample.

If it were also possible to determine crystal structures quantitatively from electron scattering data, ratios of atomic scattering factors different from those for X-rays could be yet another potential attraction of electron crystallography, similar to neutron diffraction techniques (Vainshtein, 1964*b*). For example, the favored detectability of H atoms in the presence of typical constituents of organic crystals motivated many of the early quantitative electron crystallographic analyses. (For example, the scattering factors of carbon, nitrogen and oxygen at the $\sin\theta/\lambda = 0$ limit actually decrease in value with increasing atomic number, compared with the *Z*-dependence of the X-ray form factors.) Also, if the low-angle region is sampled adequately by the reciprocal lattice, it is possible to detect charges on individual atoms (Vainshtein & Dvoryankin, 1956).

2. Electron crystallographic analyses – an historical perspective

2.1. *Early structure determinations*

Soon after the first Davisson & Germer (1927) low-voltage electron-diffraction experiment on a nickel crystal surface, so-called 'fast' electrons were used to investigate the structure of thin organic films – specifically those related to the boundary lubricant layer on a metal bearing (Thomson & Murison, 1933). While reflection (Bragg) diffraction experiments were extremely useful for finding the average chain tilt of such layers (Karle & Brockway, 1947), transmission (Laue) electron-diffraction intensity data from a thin alkane layer were used for one of the first quantitative electron crystallographic analyses of a crystal structure (Rigamonti, 1936). This resulted in a reasonably accurate estimate of the C—H bond distance as well as the chain 'setting angle' in the orthorhombic unit cell. Improvements were made on the *n*-paraffin analysis in Moscow, based on intensity data collected from polycrystalline 'textures', as well as from single crystals (Vainshtein & Pinsker, 1950; Vainshtein, Lobachev & Stasova, 1958). For a time, the resulting C—H bonding parameters were regarded by some lipid crystallographers to be the most accurate ones for a polymethylene chain (Larsson, 1964).

A series of other organic structures was subsequently published in the Soviet literature, including: retene (Karpov, 1941), urotropine (Lobachev, 1954), diketopiperazine (Vainshtein, 1955), urea (Lobachev & Vainshtein, 1961) and two polymorphs of thiourea (Dvoryankin & Vainshtein, 1960, 1962), as well as the copper salts of some amino acids (Vainshtein, D'yakon & Ablov, 1971; D'yakon, Kairyak, Ablov & Chapurina, 1977). Early linear polymer determinations included the analyses of the poly-γ-methyl-L-glutamate chain packing in either the α- or β-polymorph (Tatarinova & Vainshtein, 1962; Vainshtein & Tatarinova, 1967). Again, claims of accurate bonding parameters to hydrogen were made for a number of these molecules.

Analyses of inorganic structures were not uncommon. If the validity of the organic structures was not to be questioned, then it would also be logical to accept the results for boric acid (Cowley, 1953*a*), ammonium chloride (Kuwabara, 1959) and ammonium sulfate (Udalova & Pinsker, 1964), since these are also composed of light atoms. However, favorable structure analyses were also reported for heavier structures, including a number of layer silicates (Zvyagin, 1967), chlorides (Voronova & Vainshtein, 1958), the alloy $AlTlSe_2$ (Imamov & Pinsker, 1965) and a number of other examples (Pinsker, 1968).

2.2. *Are these results valid? A breakdown of confidence*

While light-atom structures seemed to yield reasonable results, difficulties were often experienced when selected-area electron-diffraction data from heavier-atom structures were utilized for structure determination, *e.g.* the case of λ-alumina, $3NiO{\cdot}5Al_2O_3$ (Cowley, 1953*b*), or lead carbonate (Cowley, 1956). There seemed to be no obvious correspondence between the observed diffraction amplitudes and those calculated from the packing model, as tested with structures solved earlier from X-ray diffraction data. X-ray crystallographers also had expressed some suspicion that the electron-diffraction results were not altogether trustworthy (Lipson & Cochran, 1966), especially since many of the structures reported in the literature had already been determined previously. Indeed, X-ray results were often employed as starting phasing models for the electron crystallographic determinations intending to locate lighter-atom positions in ensuing potential maps. [In defense of this work it should be pointed out that direct phasing techniques were only being developed during this period (Hauptman & Karle, 1953). It was no trivial matter to solve a crystal structure, especially with an incomplete set of intensity data.]

Further difficulties were being uncovered by diffraction physicists. It was found that the two-beam dynamical scattering model (often employed in X-ray crystallography) was insufficient to explain the specific variations of the observed selected-area diffraction intensities from their theoretical values (Cowley, 1967). Because of this, a more rigorous theoretical basis for electron scattering through thin crystals was developed. Essentially three major data perturbations were identified, which singly or in combination could produce a measured intensity set that could not be used for *ab initio* structure determination.

Since the wavelength of high-energy electrons is very small (*e.g.* 1/40 that of Cu $K\alpha$ X-rays for 100 kV electrons), the Ewald sampling sphere is often approximated by a plane. This means that, for any crystal orientation, many diffracted beams are simultaneously excited (resembling an X-ray precession photograph for major zonal projections), so that the two-beam diffraction theory cannot be accurate. The small number of defects actually present in a typical microcrystalline specimen (Cowley, 1967), furthermore, argues against the mosaic model sometimes proposed for such samples as a rationalization for the two-beam dynamical correction. A more accurate portrayal of the scattering from such objects was the multiple-beam dynamical theory, developed either from Bloch waves (*e.g.* Howie & Whelan, 1961) or from the Fresnel interference of electrons scattered from adjacent slices through the crystal (Cowley & Moodie, 1957). [These constructs have been shown to be equivalent (Goodman & Moodie, 1974).] It is useless to pretend that *n*-beam dynamical scattering has nothing to do with organic specimens since measurable perturbations have been observed from 50 Å thick *n*-paraffin layers, even for 1000 kV electrons (Dorset, 1976*a*).

If crystals have layer imperfections, yet another, incoherent, multiple-beam scattering effect can arise, in addition to the coherent multiple-beam dynamical perturbation just mentioned. Such secondary scattering can lead to the appearance of systematically absent reflections as well as a spurious hyper-resolution of the diffraction pattern. A convolutional model for this scattering (*via* intensities) was proposed by Cowley, Rees & Spink (1951).

Another problem can occur if a rather large unit-cell axis is projected parallel to the incident beam direction, especially if a beam with high spatial coherence is used for the diffraction experiment. Based on a perturbed Patterson function (Cowley, 1961), the resultant diffraction incoherence means that the pattern appears to originate from a smaller portion of the unit cell, especially if a substructure is present with a short repeat interval in the beam direction. In the conditions often used to form a selected-area diffraction pattern, the electron beam has a very small angular divergence, with a resultant spatial coherence that is much higher than most X-ray sources. Later, it was shown that this kinematical formulation could be regarded as a limiting case of dynamical scattering from a curved crystal (Moss & Dorset, 1983).

It was important that more accurate models for electron scattering could be formulated. However, there is also a depressing aspect of this improved theoretical framework. The packing of molecules or atom clusters in the unit cell must be known to explain the observed deviations from the kinematical limit, *i.e.* before the crystal structure is solved! [This is contrasted with the two-beam scattering theory, for which deviations from kinematical scattering can be estimated *a priori* by graphical techniques similar to a Wilson plot (*e.g.* Vainshtein & Lobachev, 1956; Li, 1963).] While this outcome does not rule out the possibility of trial and error techniques for structure determination, it is also well known that an educated guess of a crystal structure is suitably accurate only when the asymmetric unit is uncomplicated and/or when there are enough symmetry constraints to fix many of the atomic positions. Otherwise, for a diffraction pattern containing N unique reflections, there are 2^N possible phase combinations for an unknown centrosymmetric structure, only one of which is correct.

Difficulties arising from a rigorous electron scattering theory did not cause *ab initio* electron-diffraction structure analyses to be abandoned. (Nevertheless, the apparent quixotic nature of this endeavor continues to be argued passionately by some electron diffractionists.) Work on structure analysis was continued in Moscow, utilizing the intensity data from polycrystalline textures. As recognized by Cowley (1967), there was a case to be made for using such experimental data, since the distribution of crystallite orientations indeed could average out dynamical scattering effects, particularly when these were nonsystematic interactions. In fact, many researchers still fail to note the differences in the types of electron-diffraction geometries used to collect intensity data. For example, the results from a nanoprobe on a flat perfect crystal slab could be greatly different from those from an elastically bent micrometer-sized crystallite containing a few defects and different again from those obtained in a millimeter diameter area from a polycrystalline texture.

Needless to say, it soon became a matter of dogma to state that electron crystallographic determinations were impossible to carry out. There would be no new crystal structures determined from electron-diffraction data, period! In the case of organic specimens, in particular, there was yet another difficulty imposed by the inelastic interaction of the electron beam with the crystal, leading to radiation damage of the molecules and the destruction of the crystalline lattice itself. While some details of the molecular packing might be obtained from electron micrographs of relatively beam-stable molecules (*e.g.* those with extensive π-delocalized electron orbitals), the best expectation for an aliphatic model was thought to be an image resolution of perhaps 40–100 Å (Glaeser, 1975).

2.3. *The quasi-kinematical scattering concept*

Fortunately, strict adherence of experimental diffraction intensities to the single scattering, or kinematical, model is not an absolute precondition for the determination of a crystal structure. The relevant question is: how large are the actual deviations and how does one carry out and complete the analysis despite their

presence? Also, how does one manipulate experimental conditions to minimize their presence?

For a successful structure analysis one needs only to collect a 'quasi-kinematical' data set (Dorset, 1995*a*). This means that the Fourier transform of the experimental intensities, or Patterson function, is a sufficiently close match to the autocorrelation function of the actual crystal structure. By 'sufficient' is meant that there must be an extensive overlap of major intra- and intermolecular vectors in the corresponding maps. However, this condition does not presume that the observed intensity set is free of other perturbations.

A structure analysis is, of course, greatly facilitated when experimental data are as close as possible to the single scattering limit. This limit can be approached in a variety of ways. First of all, it is prudent to use the lowest electron wavelength possible. While an argument has been made for an 'optimal' electron wavelength to be used in diffraction experiments (Jap & Glaeser, 1980), the outcome for real crystals undergoing thermal vibration (defining the actual resolution limit) is always improved at the highest voltage (Tivol, Dorset, McCourt & Turner, 1993). Thin crystals containing light atoms favor this approximation. This explains why most of the development in electron crystallographic analyses has been made for organic specimens. It is always important to control the specimen thickness and to keep this at a useful minimum for data collection. For objects with heavy-atom constituents, it may be possible to identify subsets of intensity data that are close enough to the kinematical condition to permit *ab initio* analyses [*e.g.* the interesting use of higher-order Laue zone data for this purpose by a group at the University of Bristol (Vincent & Exelby, 1991, 1993; Vincent & Midgley, 1994)]. As mentioned above, the collection of intensity data from polycrystalline samples may be useful in some cases. Simulations of dynamical diffraction have shown that data collected within the bounds of realistic experiments can be used productively for structure determination by direct methods (Dorset, Jap, Ho & Glaeser, 1979). (More recently, similar simulations of electron micrographs in this laboratory have shown that sufficiently accurate crystallographic phases can be obtained from their Fourier transform after image averaging.)

While dynamical diffraction has been regarded as the chief impediment to structure analysis, there are actually cases where its presence may, in fact, facilitate the structure determination. As also found in X-ray crystallography (Marsh, 1995), one of the major reasons for inaccurate structure determinations is the misidentification of space-group symmetry. The effect of n-beam dynamical interactions on the extinctions of certain classes of reflections (Gjønnes & Moodie, 1965) can be used effectively for symmetry identification, especially in convergent-beam diffraction experiments (Buxton, Eades, Steeds & Rackham, 1970), where it is found that all space groups can be uniquely determined (contrasted to the uncertainties in an X-ray diffraction analysis). Furthermore, analysis of observed dynamical interactions can lead to very accurate crystallographic phases for low-angle reflections when phase invariant sums are experimentally excited (Spence & Zuo, 1992). These advantages, however, are best exploited for specimens that can tolerate rather large beam doses, *i.e.* organics are generally precluded.

The effect of other data perturbations can also be minimized. Secondary scattering also demands that the crystal thickness be limited when diffraction data are collected. Often its presence will be betrayed by the observation of measurable intensity for space-group forbidden reflections in some diffraction patterns (Cowley, Rees & Spink, 1951; Vainshtein, 1964*a*). (In some cases, its appearance is less obviously identified.) The effective diffraction incoherence imposed by crystal bending is minimized by either of two ways. First, and most obvious, the crystal must be forced to be sufficiently flat. For certain types of specimen, *e.g.* molecular organic crystals, this goal may be difficult to realize. Secondly, if a long unit-cell axis lies parallel to the electron beam (as it does for many solution-crystallized organics) an alternative crystallization technique, exploiting epitaxial orientation, may be beneficial (Dorset, 1995*b*).

In addition, the predicted limitations imposed by electron beam damage have been found to be overly pessimistic. By use of 'low-dose' techniques, the 'theoretical' resolution limits have been overcome in recent years. This is evidenced by 16 Å resolution images of an epitaxially crystallized paraffin, easily obtained at room temperature (Fryer, 1981), or 2.5 Å resolution images of a solution-crystallized paraffin obtained with a cryomicroscope (Zemlin, Reuber, Beckmann, Zeitler & Dorset, 1985) or 3.7 Å resolution images of polyethylene lamellae obtained at room temperature (Revol & Manley, 1986). Radiation damage is more important for high-resolution imaging experiments than in electron-diffraction work. Diffraction patterns can be recorded before discernable damage can be detected. Also, similar to the use of standards in X-ray data collection, phenomenological corrections can be made to the intensity data to find likely values at a null time (Perez & Chanzy, 1989).

After these precautions are taken, a further criterion must be applied for appraising any data set as being suitable for structure analysis: Are the data self-consistent? That is to say, for any given crystal orientation, is there a good agreement between independent data sets taken from several individual crystals? (This also presumes that the unit-cell symmetry, as expressed by equivalent intensities, is observed without significant deviations.) If these constraints are satisfied, then there is good hope for the

success of a structure determination. Otherwise, there is no point in beginning an analysis.

3. Crystal structure determination

3.1. *Solution of the phase problem*

The history of structure determination in electron crystallography mirrors similar developments in X-ray crystallography, except, of course, for the special case when electron micrographs can be used for visualization of atomic or molecular positions. So far, mostly the electron-diffraction intensities have been used for elucidation of structures at atomic resolution.

3.1.1. *Trial and error.* In initial work atomic positions from X-ray crystal structures were used to assign starting phase values for electron-diffraction amplitudes, in an attempt to find lighter atoms (*e.g.* hydrogen) during the ensuing refinement (Vainshtein, 1964*b*). There are numerous instances where a molecular geometry suggested from an X-ray structure is employed in a search for the best fit to the data, *e.g.* in the case of linear polymers, where the chain axis orientation can often be defined *a priori*. In this application crystal structures of oligomers or monomer units define the conformationally invariant part of the polymer repeat so that the structure search with electron-diffraction data actually involves conformational changes around so-called 'linkage bonds' to minimize both the crystallographic residual and an atom–atom nonbonded potential energy (Brisse, 1989; Perez & Chanzy, 1989). This technique, borrowed from fiber X-ray determinations (Campbell Smith & Arnott, 1978), has yielded numerous reasonable three-dimensional polymer chain structures, even when two-dimensional data from chain-folded lamellar crystals are used. More recently, three-dimensional intensity data have been consulted for such structure searches (Chanzy, Perez, Miller, Paradossi & Winter, 1987; Meille, Brückner & Lando, 1989; Mazeau, Winter & Chanzy, 1994).

Similar approaches have been successful for small molecules, even though the orientation of the molecule in the unit cell is not known initially. Voigt-Martin and her co-workers (Voigt-Martin, Yan, Yakimansky, Schollmeyer, Gilmore & Bricogne, 1995; Voigt-Martin, Yan, Wortmann & Elich, 1995) have solved the structures of flexible aromatics by this technique and have verified the molecular packing and conformation, found by energy minimization, by direct methods. When the number of recorded intensities is sparse, *ab initio* predictions of layer packing have been found to be very effective for structure analysis (Scaringe, 1992). For phospholipids, the molecular model suggested by the X-ray crystal structure of a near analog (with conformational geometry ascertained from a one-dimensional Patterson map) has been translated to find the best molecular position in the bilayer (Dorset, Massalski & Fryer, 1987).

3.1.2. *Patterson synthesis.* Patterson maps have also been frequently employed in electron crystallography, often in league with trial and error determinations (*i.e.* to provide important information about the molecular orientation). Because the relative range of electron scattering factor magnitudes is somewhat compressed, in comparison to the X-ray form factors, detectability of heavy-atom positions is not so favorable in electron crystallographic applications as it is in X-ray crystallography (Dorset, 1995*b*). Of course, the scattering from regular structural features, such as chains and rings, can be easily discerned with electron-diffraction data.

Patterson maps were interpreted in some of the earliest investigations, including location of the metal-atom sites in amino acid salts (Vainshtein, D'yakon & Ablov, 1971; D'yakon, Kairyak, Ablov & Chapurina, 1977). [However, the detectability of Cu in the copper (D,L-alaninate) structure, $\Sigma f^2_{\text{heavy}}/\Sigma f^2_{\text{light}}$, is only 0.47 for the electron-diffraction data where it would be 2.36 with an X-ray intensity set.] As will be described in detail elsewhere, the Cl-atom positions of copper perchlorophthalocyanine can be found in the Patterson maps calculated from 1200 kV electron-diffraction data and the remaining atomic positions found subsequently by Fourier refinement. Examples in inorganic electron crystallography include the analysis of orthorhombic silicon monophosphide (Wadsten, 1975).

For scattering from lighter atoms, these autocorrelation functions have been utilized for the determination of methylene chain packing in alkane derivatives, including lipids (Dorset, 1976*b*). They have also been useful, in one dimension, for determining the head group conformation of phospholipids, given lamellar diffraction data from epitaxially oriented specimens (Dorset, 1987*a*).

More recently, Patterson maps have been used in a highly innovative way to solve alloy crystal structures (Vincent & Exelby, 1991, 1993). Since reflections within the higher-order Laue zones have extinction distances approaching the kinematical approximation, their intensities can be used to solve the crystal structures. In this work partial Patterson syntheses based on separate higher-order Laue zones have been used to find atomic sites in various layers of the unit cell.

3.1.3. *Electron microscopy.* For all of the improvements made in instrumental optics in recent years (including optimization of the objective lens transfer function), the electron microscope actually has done very little to permit the visualization of separate atomic positions in a crystal, so that the image can be interpreted directly without simulations based on multiple-scattering theory (Spence, 1981). Nevertheless, in inorganic applications there are already some

cases of oxides where heavy-metal sites have been identified after image averaging, with positions agreeing very closely with those found in X-ray crystal structures of similar materials (Hovmöller, Sjögren, Farrants, Sundberg & Marinder, 1984; Li & Hovmöller, 1988). A high-resolution three-dimensional study of the silicate staurolite, carried out with high-voltage electrons, has also yielded very impressive results, locating all atoms in the unit cell (Wenk, Downing, Hu & O'Keefe, 1992). For organics, the greatest achievement so far has been the 2.0 Å resolution images of copper perchlorophthalocyanine (Uyeda, Kobayashi, Ishizuka & Fujiyoshi, 1978/1979), in which the heavier copper and chlorine sites are found at their correct locations, but the positions of the lighter carbons and nitrogens were not resolved.

Direct images of crystals need not be obtained at atomic resolution to be useful as an independent source of crystallographic phases. Even 2.3 Å images of copper perchlorophthalocyanine, where only the molecular quatrefoil outline is seen (O'Keefe, Fryer & Smith, 1983), have been found useful as a starting point for phase extension *via* the Sayre equation or the tangent formula (Dorset, McCourt, Fryer, Tivol & Turner, 1994; Dorset, Kopp, Fryer & Tivol, 1995). Images of other organics, including linear polymers and layer structures, have been used in a similar fashion (Dorset, 1995*b*). There is also sufficient evidence to suggest that certain inorganic materials, such as the zeolites, can give *e.g.* 2.0 Å images of sufficient quality to provide a sufficient basis set for phase extension into the electron-diffraction resolution (Pan & Crozier, 1993).

High-resolution electron microscopy has been the most important source of crystallographic phases in the study of thin protein microcrystals. Three-dimensional determinations of three quite different integral membrane proteins, bacteriorhodopsin (Henderson, Baldwin, Ceska, Zemlin, Beckmann & Downing, 1990), a bacterial outer membrane porin (Jap, Walian & Gehring, 1991), and the light-harvesting complex from pea chloroplast (Kühlbrandt, Wang & Fujiyoshi, 1994) at approximately 3.5 Å diffraction resolution, have permitted fitting the derived potential maps with polypeptide sequences and the location of nonprotein cofactors in two cases.

A major utility of high-resolution electron microscopy is the visualization of disorder in crystalline lattices. For example, edge dislocations have been observed in polymer crystals (Isoda, Tsuji, Ohara, Kawaguchi & Katayama, 1983) and thin organic crystals (Zhang, Kuo, Dorset, Hou & Ni, 1989; Kobayashi & Isoda, 1993) in terms of molecular packing sites. Screw dislocations and grain boundaries have also been visualized at similar resolution (Fryer, 1980; Kobayashi, Fujiyoshi, Iwatsu & Uyeda, 1981; Van Tendeloo, Amelinckx, Muto, Verheijen, Loosdrecht & Meijer, 1993). Aided by computations, there have been numerous descriptions of disorder for inorganic materials (Busek & Veblen, 1988; Eyring, 1988; Smith & Barry, 1988).

3.1.4. *Direct methods.* The determination of crystal structures from electron-diffraction intensities by probabilistic or algebraic direct methods was not attempted until 1975. Two methylene subcell structures were solved from experimental data by symbolic addition (Dorset & Hauptman, 1976), a result that was regarded only as an interesting curiosity at the time. Later, tests were made with simulated data from representative organic structures to evaluate how data perturbations due to *n*-beam dynamical scattering (Dorset, Jap, Ho & Glaeser, 1979) or elastic crystal bending (Moss & Dorset, 1982) would influence the outcome of these direct determinations. From these studies it was apparent that experimental conditions (specimen preparation, electron accelerating voltage) could be manipulated so that the quasi-kinematical approximation necessary for *ab initio* structure determination could be realized.

Direct methods for electron crystallographic analyses have been extensively tested within the past decade, largely by three research groups. In this laboratory (Dorset, 1995*b*) extensive use has been made of symbolic addition to solve centrosymmetric structures of various kinds. One important outcome of this initial work was to establish that early texture diffraction data from organics could be used to solve crystal structures *ab initio* without foreknowledge of X-ray results. This (hopefully!) has quietened much criticism of the important Russian pioneering effort in electron-diffraction structure analyses. Automated techniques have also been explored. For example, the tangent formula (Karle & Hauptman, 1956) has been shown to be useful for many noncentrosymmetric data sets, even if more suitable figures of merit be found [*e.g.* the minimal principle (Hauptman, 1993)] that are least sensitive to multiple-scattering perturbations. The Sayre (1952) equation, in a multi-solution form, has also been shown to be a powerful tool for many problems. (In general, various utilizations of the Σ_2 structure invariant seem to be very effective for electron-diffraction structure analyses, whereas other phase invariants, such as the negative quartet, may be much less reliable. The latter problem arises because the negative quartets require an accurate measure of weak reflections. These can easily have larger than expected values due to the 'convolutional smearing' effect of multiple electron scattering.) When the Cochran (1952) condition of 'peakiness' is imposed, it has been useful for finding solutions from atomic resolution data sets and when the opposite criterion of density flatness is imposed (Luzzati, Mariani & Delacroix, 1988), it has been suitable for determining membrane protein structures to 6 Å resolution, either by phase extension from a lower-resolution image-derived data set (Dorset, Kopp, Fryer & Tivol,

1995; Dorset, 1996) or, to a limited extent, in true *ab initio* determinations, including phase annealing steps (Dorset, 1995*c*, 1996). [The 'peakiness' criterion, developed as a figure of merit by Stanley (1985), merely states that the volume integral of scattering density in the unit cell should have a maximum positive value for the correct crystal structure. This presumes atomic resolution data are measured. At the other end of resolution, it has been proposed that the flatness or smoothness of density distribution in the unit cell corresponds to the correct crystal structure.] With the development of other automated methods such as 'Shake-and-Bake' (Miller, DeTitta, Jones, Langs, Weeks & Hauptman, 1993), based on the minimal principle, other applications have been demonstrated more recently.

In Glasgow, much effort has gone into the application of maximum entropy and likelihood procedures (Bricogne & Gilmore, 1990) to structure determination (Gilmore, Shankland & Bricogne, 1993). A number of small molecule structures have been reported, in collaboration with J. R. Fryer or I. G. Voigt-Martin (see below). Independent determinations of many structures determined in Buffalo by other techniques have been carried out, thus providing an important verification of this overall approach. Most impressively, the idea of phase extension of a membrane protein phase set from 15 to 3.5 Å originated in this laboratory (Gilmore, Shankland & Fryer, 1993), pioneering an important alternative to (an often problematic) reliance on just electron micrographs for the derivation of high-resolution crystallographic phases.

In Beijing much effort has gone into the determination of phases from electron micrographs (Li, 1991) and their extension by the Sayre equation (Fan, 1991; Woolfson & Fan, 1995). Significant results were demonstrated initially with data from copper perchlorophthalocyanine, in a collaboration with the Kyoto laboratory (Fan, Xiang, Li, Pan, Uyeda & Fujiyoshi, 1991). However, many important advances by this laboratory also have been made in the inorganic area. One of the most intriguing problems has been the analysis of incommensurately modulated superstructures (Xiang, Fan, Wu, Li & Pan, 1990; Mo, Cheng, Fan, Li, Sha, Zheng, Li & Zhao, 1992).

3.2. *Structure refinement*

3.2.1. *Fourier refinement.* With a partial set of phases obtained by direct methods it is often profitable to use Fourier refinement techniques to complete the structure model (if only a fragment is found in the initial map) and to improve the positioning of the identified atomic sites. The efficacy of this approach has been demonstrated with a number of structures (see Dorset, 1995*b*). If the presence of heavy atoms (or the crystal thickness or the nature of the crystalline layering) results in a large mutiple-scattering component in the observed intensity data, then there may be a practical limit for the use of the kinematical crystallographic residual as a figure of merit for identifying an improved structural model. When the data are significantly perturbed, there are cases where a false R-factor minimum will be found, corresponding to a geometrically deformed model, *i.e.* yielding chemically unreasonable bond distances and angles. On the other hand, if the quasi-kinematical criterion is satisfied, Fourier refinement will often locate all atoms in the structure, *e.g.* even for the perhalogenated copper phthalocyanines (Dorset, Tivol & Turner, 1991, 1992).

The best approach to Fourier refinement is to constrain the improvement of the R factor to a local minimum, while preserving the optimal bonding geometry of the molecule itself. Ideally, some sort of correction of the calculated structure factor magnitudes for multiple scattering should be built into the refinement to give a better figure of merit (Dorset, Tivol & Turner, 1992; Sha, Fan & Li, 1993), but it is sometimes difficult to ascertain which correction, *i.e.* for dynamical or secondary scattering, or a bit of both, is most appropriate.

3.2.2. *Least-squares refinement.* Least-squares refinement procedures, at least in a constrained form, have often been used in electron-diffraction analyses, particularly for the determination of linear polymer structures (Brisse, 1989; Perez & Chanzy, 1989). As outlined above, bond distances and angles are held at idealized values and a search is made around various linkage bonds to minimize both the atom–atom nonbonded potential of the unit cell and the crystallographic R factor. There are no particular limitations due to the restricted size of the data set (Campbell Smith & Arnott, 1978).

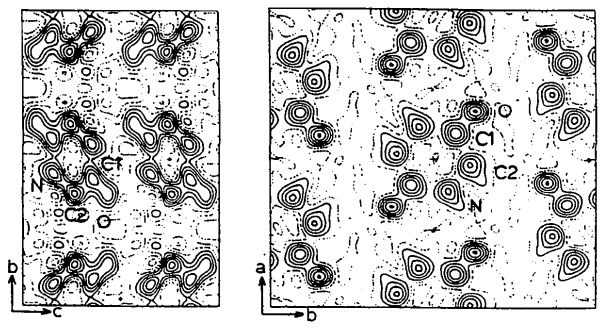
Less experience has been gained with unconstrained refinements. As in X-ray crystallography, there is, of course, a requirement that there are sufficient recorded data for each refinable parameter before such a procedure be undertaken. In the refinement of the diketopiperazine structure (Dorset & McCourt, 1994*a*), based on the texture diffraction data originally collected in Moscow (Vainshtein, 1955), it was found that shifting the atomic positions should be uncoupled from the variation of the thermal parameters. Also, the magnitude of the shifts had to be restricted so that a false minimum could be avoided (a similar situation to that observed before in Fourier refinements). Nevertheless, an improved model could be found, starting from atomic positions found after direct phasing, matching closely the solution given separately by an X-ray crystal structure analysis. Another least-squares refinement of polyethylene yielded a similarly improved structure (Dorset, 1995*b*).

3.3. *Accuracy of crystal structures*

Although there is no question that true *ab initio* analyses can be carried out with experimental electron crystallographic data and also that previously unsolved structures can be determined, one has to be realistic about the accuracy of the final results, in comparison with typical modern X-ray crystal structures. In a sense, electron crystallography has advanced to the point where X-ray crystallography was in the early 1960's (even though many of the phasing methods did not exist in this time period). Intensity data are still collected mostly on film and the reciprocal lattice often is severely undersampled during data collection. One is often forced to work with zonal data, hopefully in a view onto the most meaningful projection of the unit cell (aided, in no small part, by appropriate crystallization techniques). Hence, for a typical light-atom structure, the final crystallographic R factor, before corrections, may be in the low 20% range, if the determination is good. Derived bond distances and angles are not highly accurate, perhaps within 5–10% of their accepted values for individual ones (although the average over equivalents should be better). It is often thought, therefore, that electron crystallographic determinations are best-suited for determination of packing motifs and conformation.

4. Representative structure analyses

There are numerous examples of actual *ab initio* structure determinations carried out with electron crystallographic data. In the following, an attempt at an overview of this progress will be made. Specific descriptions of structure analyses have been given in the cited papers as well as in a recent monograph that surveys the field in some detail (Dorset, 1995*b*).



(*a*) (*b*)

Fig. 1. Crystal structure of diketopiperazine (two projections) determined from texture electron-diffraction data (Vainshtein, 1955). Phases were assigned by the tangent formula.

4.1. *Small organic structures*

It was not until some 35 years after the initial electron-diffraction analysis of the diketopiperazine structure (Vainshtein, 1955) that its solution by direct methods (Fig. 1) was reported (Dorset, 1991*a*). Not only has symbolic addition proved useful for solving this structure, but also automated techniques including the tangent formula (Dorset & McCourt, 1994*a*), as well as maximum entropy and likelihood, have been equally successful. Similar successes were realized with other data sets from Moscow, including urea (Dorset, 1991*b*) and two polymorphic forms of thiourea (Dorset, 1991*b*, 1992*a*). For the latter two structures, an automated phasing procedure was very effective, even though multiple-scattering perturbations to the intensities did not permit the structures to be refined.

Using selected-area diffraction intensities other small molecule structures have been determined. The structure of graphite (Ogawa, Moriguchi, Isoda & Kobayashi, 1994), the parent of all aromatic structures, has been reported as well as other fullerite forms of carbon, including C_{60} buckminsterfullerene (Dorset & McCourt, 1994*b*) and the C_{60}/C_{70} solid solution in the fullerite soot (Dorset, 1995*d*). Two perhalogenated derivatives of copper phthalocyanine have been characterized. The perchloro derivative was the source of the best high-resolution micrographs of an organic reported so far (Uyeda, Kobayashi, Ishizuka & Fujiyoshi, 1978/1979) and its structure was solved, either using the image Fourier transform as the basis set for phase extension with the Sayre equation or the tangent formula (Fan, Xiang, Li, Pan, Uyeda & Fujiyoshi, 1991; Dorset, McCourt, Fryer, Tivol & Turner, 1994; Dorset, Kopp, Fryer & Tivol, 1995) or in a true *ab initio* phase determination (Dorset, Tivol & Turner, 1991) with just electron-diffraction amplitudes. Despite increased dynamical scattering interactions, the perbromo derivative structure (Fig. 2) could also be

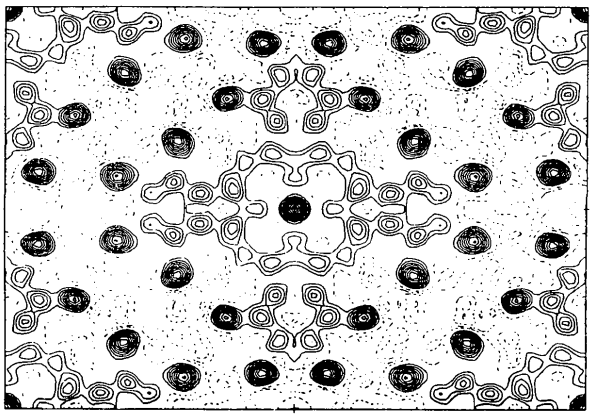


Fig. 2. Crystal structure of copper perbromophthalocyanine determined from 1200 kV electron-diffraction intensity data.

determined from 1200 kV electron-diffraction data (Dorset, Tivol & Turner, 1992), but the final carbon–halogen bonding distances were *ca* 0.1 Å too short.

Other small aromatic structures determined from electron crystallographic data, by maximum entropy and likelihood, include perchlorocoronene (Dong, Baird, Fryer, Gilmore, MacNicol, Bricogne, Smith, O'Keefe & Hovmöller, 1992), a pyrazolidine derivative (Voigt-Martin, Yan, Gilmore, Shankland & Bricogne, 1994) and a bianthryl derivative (Voigt-Martin, Yan, Yakimansky, Schollmeyer, Gilmore & Bricogne, 1995).

4.2. *Small linear molecules*

One of the first uses of the electron-diffraction technique in organic crystallography was the study of polymethylene chain derivatives, particularly those responsible for the boundary lubricant films of bearings. The extensive history of this work has been treated in a recent review (Dorset, 1990*a*). Some of the older techniques (*e.g.* RHEED) have been revived in recent years to study Langmuir–Blodgett layers of similar materials that can be chemically cross-linked into conductive devices. (One notes, however, that for the hundreds of thousands of dollars spent on the apparatus for carrying out these experiments, many of the diffraction results are quite inferior to those obtained in the 1930's and 1940's with hand-made equipment!)

Following the initial use of texture electron-diffraction data from *n*-paraffins for structure analysis, reviewed above, the suitability of selected-area diffraction intensities was evaluated (Dorset, 1976*c*). It was soon apparent that the total unit-cell contents could not be observed in diffraction from solution-crystallized multilayers (Dorset, 1980). To circumvent the effects of crystal bending (Dorset, 1980; Moss & Dorset, 1983), epitaxial crystallization (Wittmann & Lotz, 1990; Dorset, 1995*b*) was employed to position a shorter unit-cell edge parallel to the electron beam. As demonstrated by the crystal structure analysis of representative even- and odd-chain *n*-paraffins (Dorset & Zemlin, 1990; Dorset & Zhang, 1991), the intensities then correspond to the entire unit-cell contents.

Electron diffraction has played a significant role in the study of disordered chain packing, since the data can be obtained almost uniquely from single microcrystals. For example, the structure analysis of oriented paraffin single crystals heated near the 'rotator' phase demonstrated that cooperative chain rotation could not be taking place (Dorset, Moss, Wittmann & Lotz, 1984), in agreement with vibrational spectroscopic results. The study of binary solids has been equally important. The first quantitative crystal structure analysis of a *n*-paraffin binary solid solution was based on electron-diffraction intensity data (Dorset, 1990*b*). Such characterizations have been extended to polydisperse solid solutions, including the structure of a synthetic wax and even that of an actual paraffin wax from a child's birthday candle (Dorset, 1995*e*). [Both diffract to very high resolution, an interesting observation, given that the nucleation of successive layers is made at the most disordered region of the crystal structure. Molecular-resolution AFM measurements attest that crystalline order is preserved at these surfaces (Dorset & Annis, 1996).] The structure of the superlattice solid obtained when metastable solid solutions are held within a binary phase boundary has also just been determined recently from electron-diffraction data (Dorset & Snyder, 1996). While quantitative studies have not been made yet of the eutectic nature of solid solutions or the totally phase-separated binary solids, electron diffraction and high-resolution electron microscopy have demonstrated that 'mechanical mixtures' of components do not exist (Zhang & Dorset, 1989). Rather, there is always a very tight crystallographic contact between domains, minimizing any possible 'void' spaces.

With epitaxial orientation on salt crystals, it is also possible to study perfluoroalkanes in the same way. The room-temperature layer packing of such molecules has already been reported in two orthogonal projections, based on electron-diffraction intensity data (Dorset, 1977; Zhang & Dorset, 1990). The crystal structure of the more ordered phase, nucleated at low temperature, is now being investigated.

Extensive studies have also been carried out on fatty acids and their salts, including the quantitative determination of methylene subcell packing and chain orientation (Dorset, 1995*b*). A promising analysis of a lead soap has been reported recently from Moscow and utilizes intensities collected from texture diffraction patterns (Vainshtein & Klechkovskaya, 1993). Other *n*-alkane derivatives studied include wax esters (with the beginning of a quantitative crystal structure analysis) as well as ketones (Dorset, 1995*b*).

Interest has been shown in the study of glycerolipids in the electron microscope. In the food and dairy industry, for example, the polymorphic behavior of triglycerides is important for the formulation and storage of foodstuffs, hence prompting extensive quantitative and semi-quantitative electron-diffraction investigation of chain packing (Buchheim, 1970; Dorset, 1983). Diglycerides have also been examined in this manner, leading to fresh insights into the nature of the solid-state acyl shift for these compounds (Dorset & Pangborn, 1982; Dorset, 1987*b*).

The phospholipid component of cell membranes has also received considerable attention. It is again easy to elucidate the chain orientation and methylene 'subcell' packing with electron-diffraction experiments from solution-crystallized samples (Dorset, 1995*b*). One can also investigate layers formed on a Langmuir trough (Hui, 1992), especially when the hydration is maintained in an environmental chamber for the electron

microscope. With epitaxial orientation, one can also find the characteristic bilayer profile, either by model searches or by direct methods (Dorset, 1991*c*). Most recently the layer profile of a phospholipid binary solid solution was described (Dorset, 1994*a*). On the other hand, paracrystalline disorder of epitaxially oriented samples has frustrated the collection of true three-dimensional intensity data so that the complete structure analyses envisioned by Parsons & Nyburg (1966) are not currently possible.

4.3. *Linear polymers*

The history of polymer electron crystallography has been frequently reviewed (Dorset, 1995*b*), including citations to structure determinations, based on the linked-atom least-squares search with an assumed chain model (Campbell Smith & Arnott, 1978). Because much of this early work was based on two-dimensional diffraction data collected from untilted lamellar crystals, a test of direct phasing methods was made (Dorset, 1992*b*) to ascertain if these could be used to derive an equivalent phase set, assuming nothing about the underlying structure. In many cases, independent determination of the projected molecular envelope for fitting the chain model is surprisingly successful. To establish that such phasing procedures were not biased by the foreknowledge of the structure, an unknown *hk*0 intensity data set from the polysaccharide chitosan was kindly sent by colleagues in Grenoble (Mazeau, Winter & Chanzy, 1994). Symbolic addition (including one algebraic unknown) arrived at two possible solutions, one of which was a close match to the model determined by the model search (Dorset, 1995*b*). The correct map could be discriminated using the Cochran (1952) condition of 'peakiness' (see above).

It is also possible, occasionally, to project the electron beam onto the chain axis for data collection (and, in fact, epitaxial growth is well-suited for the purpose, as will be discussed below). For example, cast films of the polypeptide poly-γ-methyl-L-glutamate (Vainshtein & Tatarinova, 1967) were stretched and electron-diffraction data were collected for chains packing in the β-polymorph, plane group p.g. Despite the noncentrosymmetry of the projection, it was possible to solve the crystal structure by symbolic addition, permuting the values of algebraic unknowns (Dorset, 1995*b*). A similar noncentrosymmetric projection was grown recently by polymerization in dilute solution to form whiskers of poly(*p*-oxybenzoate) and its structure was solved using the Sayre equation *via* algebraic unknowns, resembling a model found by energy minimization (Liu, Yuan, Geil & Dorset, 1996).

Far more interesting is the *ab initio* elucidation of actual three-dimensional chain architecture based on three-dimensional sampling of the reciprocal lattice using goniometric tilts of the specimen. If only one crystal orientation is experimentally available, there is the unfortunate loss of a 'missing cone' of data left by the practical tilt limit (*ca* $\pm 60^\circ$) of the goniometer stage. For polymer crystals grown by self-seeding, information about the chain repeat will be blurred. Nevertheless, an experimental three-dimensional potential map, found by direct phasing, can be fit with a monomer model to find the structure, *e.g.* demonstrated with data from the polysaccharide mannan in its form I polymorph (Dorset & McCourt, 1993). The fit can be more constrained if the amplitudes and phases in the 'missing cone' are predicted (*e.g.* with the Sayre equation). Earlier analyses of such data utilized model-based conformational searches, where the three-dimensional intensities provided a further constraint over the two-dimensional sets employed previously. Notable examples based on single-crystal data include: poly(*trans*-1,4-cyclohexanediyl dimethylene succinate) (Brisse, Remillard & Chanzy, 1984), mannan, form I (Chanzy, Perez, Miller, Paradossi & Winter, 1987), γ-poly(pivalolactone) (Meille, Brückner & Lando, 1989) and chitosan (Mazeau, Winter & Chanzy, 1994).

Another option for overcoming the goniometer tilt limitation is to supplement intensity data from single lamellar crystals with those from fibers. Of course, the same polymorphic form of the chain packing needs to be expressed in both preparations, but at least a view onto the chain axis is being sampled experimentally from the disordered fiber sample. An example of a structure determined from such combined data (again using the Sayre equation to generate multiple solutions) is poly(ethylene sulfide) (Dorset & McCourt, 1996). By analogy to X-ray analyses, sometimes just electron-diffraction fiber patterns have been used to determine structures, again *via* conformational searches, *e.g.* the study of Valonia cellulose by Claffey & Blackwell (1976).

The optimal case is one where an orthogonally oriented single-crystal preparation can be nucleated by epitaxial methods [*e.g.* on a suitable organic diluent crystal face (Wittmann & Lotz, 1990)]. Using these data to supplement those from chain-folded lamellae, the entire three-dimensional reciprocal lattice can be sampled. Examples where data sets of this kind were phased by direct methods include: polyethylene (Dorset, 1991*d*), poly(ε-caprolactone) (Dorset, 1991*e*) and the form III polymorph of poly(1-butene) (Dorset, McCourt, Kopp, Wittmann & Lotz, 1994). In each example, the atomic positions were visible in the three-dimensional potential maps (Fig. 3).

4.4. *Membrane proteins*

After the application of crystallographic principles to the analysis of electron micrographs from periodic

macromolecular arrays, work resulting in a Nobel Prize (DeRosier & Klug, 1968), extensive studies were made on a variety of assemblies to the resolution limit allowed by negative stain (as well as the actual radiation dose given the specimen). This limit was often somewhere near 20 Å. The next revolutionary development was the visualization of detail from unstained specimens, incorporating nonvolatile substitutes for the native specimen hydration or preserving quickly frozen specimens in an amorphous ice layer. 'Low-dose' imaging techniques were developed for such preparations as well as improved image averaging methods. The landmark paper in this field was the three-dimensional structure analysis of the integral membrane protein, bacteriorhodopsin, from a halophilic bacterium, reported to an in-plane resolution of ~7 Å (Henderson & Unwin, 1975).

With the improvement of cryomicroscopes and techniques for specimen crystallization, resolution was increased for this preparation until, most recently, a structure below 3 Å has been reported, permitting the fitting of a polypeptide backbone to the density in the three-dimensional potential map (Henderson, Baldwin, Ceska, Zemlin, Beckmann & Downing, 1990). Conformational differences occurring within the protein photocycle have also been detected (Glaeser, Han, Hendrickson & Vonck, 1995). Protein structures carried out to similar resolution limits also include transmembrane porins from the outer membrane of *E. coli* (Sass, Büldt, Beckmann, Zemlin, Van Heel, Zeitler, Rosenbusch, Dorset & Massalski, 1989; Jap, Walian & Gehring, 1991). The electron crystallographic results strongly resemble the X-ray structure (Cowan, Schirmer, Rummel, Steiert, Ghosh, Pauptit, Jansonius & Rosenbusch, 1992), but the former structures were determined before the latter was solved. The light-harvesting complex from pea chloroplast membranes (Kühlbrandt, Wang & Fujiyoshi, 1994) was also investigated, possibly identifying charged atoms on amino acid side chains due to the characteristic scattering of electrons. Recently (Jap & Li, 1995), structural results from erythrocyte aquaporin have been reported to a similar resolution. In addition, structural proteins, such as tubulin, have been investigated at similar detail (Downing, Wolf & Nogales, 1995).

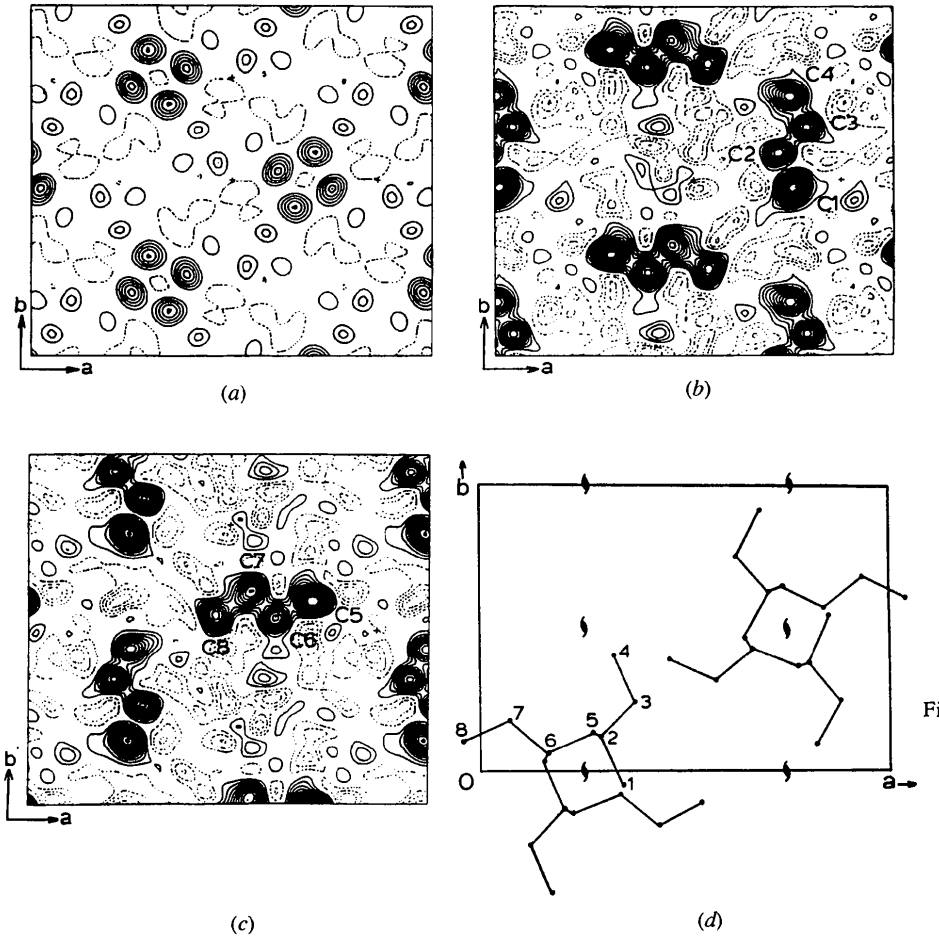


Fig. 3. Direct determination of the poly(1-butene) structure in its form III polymorph from three-dimensional electron-diffraction data: (*a*) potential map for [001] projection; (*b*) potential map on a slice at $z/c = 0.12$; (*c*) potential map on a slice at $z/c = 0.35$; (*d*) packing model projecting down the chain axis.

In all of this work the Fourier transform of electron micrographs has been used as the sole source of crystallographic phases for the electron-diffraction intensities. This procedure becomes more difficult as resolution is increased because of the increased radiation dose that is required to distinguish details (and, hence, the increased likelihood of radiation damage to the specimen), not to mention the difficulties of defining the actual phase contrast transfer function envelope at high spatial frequencies for the microscope objective lens. In addition, the samples themselves often contain paracrystalline disorder (since a membrane protein array is embedded in a phosopholipid bilayer) so that the calculated transform contains peaks out to 10 to 6 Å, whereas the electron-diffraction resolution may extend beyond 3 Å. This restriction has required the use of cross-correlation averaging techniques to 'unbend' the lattice distortion (Henderson, Baldwin, Downing, Lepault & Zemlin, 1986) in order to find high-resolution crystallographic phases. As mentioned above, were it possible to use the information from a lower-resolution image to extend to the readily obtained electron-diffraction resolution by some direct phasing technique, then much of the difficulties encountered in the search for a higher-resolution map might be easily overcome (Gilmore, Shankland & Fryer, 1993; Dorset, Kopp, Fryer & Tivol, 1995; Dorset, 1996). There are also some prospects for carrying out true *ab initio* phase determinations with just the diffraction data, with sufficient accuracy to visualize many details of the secondary structure (Dorset, 1996; Gilmore, Nicholson & Dorset, 1996). Alternatively, with accurate enough intensity data, there might be some utility of Patterson syntheses for the determination of protein structures (Burmester, Holmes & Schröder, 1995).

4.5. *Inorganic structures*

There are numerous reports of inorganic structure determinations, often based on the use of very low electron wavelengths to approximate the single-scattering condition. It has been useful to reevaluate earlier

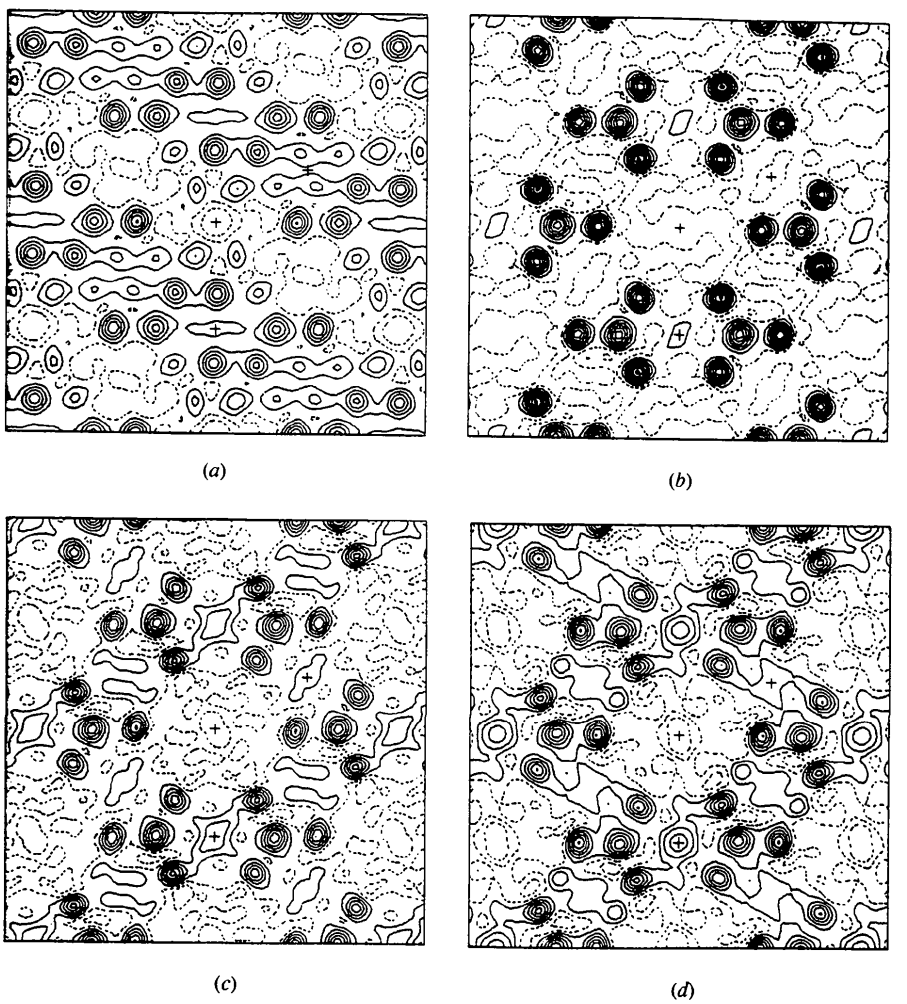


Fig. 4. Crystal structure analysis of boric acid using room-temperature electron-diffraction data of Cowley (1953*a*): (*a*) initial solution by direct methods; (*b*) potential map calculated from all phases $|F_o|$; (*c*) difference potential map calculated from $2|F_o| - |F_c|$; (*d*) potential map calculated only with reflections where $|E_h| \geq 0.85$. In the latter two maps, likely H-atom positions are found.

published data sets from inorganics. Light-atom structures, such as boric acid (Cowley, 1953*a*), are easily elucidated by direct methods and, indeed, a new determination has been carried out with selected-area diffraction data collected at low temperature (Dorset, 1992*c*). Reasonable positions have been found for the H atoms (Fig. 4). Surprisingly, texture electron-diffraction data from layer silicates (Zvyagin, 1967), collected at ~50 kV, have also been found to be suitable for direct analysis (Dorset, 1992*d*). Similarly, three-dimensional intensities from basic copper chloride (Voronova & Vainshtein, 1958) have been successfully assigned phase values (Dorset, 1994*b*). These observations justify the argument that data collection from disordered textures can effectively minimize *n*-beam dynamical interactions, especially if they are nonsystematic.

Greater difficulties have been experienced in the analysis of selected-area diffraction data from inorganic crystals, collected at conventional electron accelerating voltages. For example, a reanalysis of the data set from λ-alumina, collected by Cowley (1953*b*), was only partially successful (Dorset, 1995*b*). However, at greater accelerating voltages there is a good chance that the intensities will be sufficient for a structure determination. An overview of such possibilities is reviewed in the following paragraphs.

Silicate structures have attracted considerable interest. For example, there have been very good 2 Å resolution electron micrographs taken from zeolite microcrystals at high voltage in which essential details of the structural cage can be discerned (Pan & Crozier, 1993). A true *ab initio* direct phase determination of a zeolite was carried out from the electron-diffraction intensities, yielding an atomic resolution view of the structure (Nicolopoulos, Gonzalez-Calbet, Vallet-Regi, Corma, Corell, Guil & Perez-Pariente, 1995). High-resolution micrographs have been obtained at 800 kV from staurolite in various projections to determine its structure, which is in good agreement with the X-ray crystal structure (Wenk, Downing, Hu & O'Keefe, 1992). [An attempt to solve the structure by direct methods (Dorset, 1995*b*) was only partially successful, limited by the large experimental standard deviation of amplitudes obtained from the image transform – reliable electron-diffraction data had not been collected, unfortunately.]

Using high-resolution microscopy and image analysis, two oxide structures were solved to yield metal-atom sites directly, in good agreement with previously determined X-ray structures of similar minerals (Hovmöller, Sjögren, Farrants, Sundberg & Marinder, 1984; Li & Hovmöller, 1988). Most recently, a perovskite-related structure has been determined (Zou, Hovmöller, Parras, Gonzalez-Calbet, Vallet-Rigi & Grenier, 1993). The use of image transforms as a basis set for phase extension to the electron-diffraction resolution limit has been exploited successfully by workers in Beijing, *e.g.* for potassium niobium oxide (Hu, Li & Fan, 1992) and a high T_c superconductor (Mo, Cheng, Fan, Li, Sha, Zheng, Li & Zhao, 1992). The latter determination is very interesting since it uses the average lattice structure, found in electron micrographs, to provide the basis for phase extension into superstructure reflections to solve the incommensurate crystal structure. Analysis of the ankangite structure was the first incommensurate solid solved from electron crystallographic data by this technique (Xiang, Fan, Wu, Li & Pan, 1990). This laboratory has also worked out innovative techniques for finding the actual phase-contrast transfer function of the electron microscope objective lens for any particular experimental image (Li, 1991) so that experimental crystallographic phases can be estimated more accurately at high resolution.

There are other strategies for the collection of intensity data near the single-scattering limit aside from the use of high-voltage electron microscopes. It is well known that the reflections in higher-order Laue zones often have an extinction distance longer than the actual crystal thickness. Such data from alloys (Vincent & Exelby, 1991, 1993) and an oxide (Vincent & Midgley, 1994) have been used to solve their crystal structures, first employing the convergent-beam dynamical scattering information in the zero-order Laue zone to determine the space-group symmetries.

Most recently transmission electron-diffraction data from surface layers (*e.g.* gold on silicon) have been used to determine their two-dimensional crystal structures (Plass, Marks & Dorset, 1996). Intensities from just the top layer can be isolated from those of the bulk underlayer and, by use of the Sayre equation or the tangent formula in a multisolution approach, reasonable representations of the average layer can be obtained, agreeing well with results obtained by other approaches.

5. Conclusions

Electron crystallography is best exploited for its ability to visualize the microcrystalline state when the very nature of the sample itself resists the growth of large three-dimensional crystals. In some cases, electron diffraction (or microscopy) is the only hope for an undistorted view of reciprocal (or real) space, *e.g.* in the case of linear polymers or the two-dimensional crystalline arrays of integral biomembrane proteins in a lipid bilayer. Disordered specimens often can be grown as thin single microcrystals when larger crystalline samples are not available and the electron beam will allow details to be discerned that would be obscured when a powder or fiber specimen is examined by X-rays. In fact, there is a good case in the future for utilizing the results of single-crystal electron-diffraction determination as the basis for a later Rietveld refinement with powder X-ray data.

From the examples given above, it is clear that quantitative crystal structure determinations, based on data collected in electron microscopes (or in electron-diffraction cameras), are not the stuff of fantasy. Although the success of numerous determinations based on direct methods is quite gratifying, there is still much to be done for the improvement of the technique. Often, the major difficulty for obtaining good data resides with the sample preparation and not the instrument. On the other hand, collection of intensity data on some other medium than film will be helpful – already promising results have been reported with imaging plates (Ogawa, Moriguchi, Isoda & Kobayashi, 1994) and with slow-scan charge-coupled device (c.c.d.) cameras (Brink & Tam, 1996).

The paucity of observed diffraction data remains a significant problem. The relatively sharper falloff of normalized electron scattering factors, compared with X-ray form factors, indicates why it is possible to collect a larger X-ray data set, given crystals of equal quality for either technique (Dorset, 1995*b*). Furthermore, three-dimensional data collection in the electron microscope is tomographic, with blades of goniometer stages typically limited in their tilt range (*e.g.* $\pm 60°$) by the physical dimensions of the objective lens pole piece gap. An equivalent of screened diffraction methods only exists (and only for higher angles) when extremely flat specimens are examined that diffract to very high resolution. For some compounds, specimen orientation techniques have been devised to provide two orthogonal orientations of the crystal packing, so that nearly all the reciprocal space can be sampled when tilt data from both kinds of specimen are combined. There seems to be an advantage to resurrecting some of the early texture diffraction methods used in early Russian work (but at higher electron accelerating voltages than used before), since relatively numerous three-dimensional intensities had been collected by this means.

A number of different phasing methods have already been tested in electron crystallography – yielding an overview of what approaches are the most effective for intensities that are perturbed significantly by multiple scattering. There is need for further development in this area, especially when the number of recorded data is sparse compared with the complexity of the asymmetric unit. For example, there is also a case to be made for translation and rotation functions based on a known structural fragment. Unfortunately, with sparsely sampled reciprocal lattices, highly automated X-ray crystallographic packages based on this idea are not very useful. A somewhat more constrained search with available projections may be required instead. As will be demonstrated in a future communication, such an approach also can be effective for cases when heavy-atom components prevent the accurate determination of an initial structural model by more conventional procedures (*e.g.* the case of copper perbromophthalocyanine cited above).

Refinement is another area where extensive work is needed for improvement. Fourier methods have been shown to be very effective in many cases, but there often appears to be a limiting value of the kinematical crystallographic residual, beyond which any further 'improvement' is actually a structural distortion. The incorporation of dynamical scattering corrections in the refinement procedure has been proposed, but it is, first of all, important to establish that this, and not secondary scattering, is the major perturbation to the intensity data. Somewhat unconstrained least-squares refinements have also been carried out, with the same limitations experienced with Fourier methods, in addition to the requirement of a relatively large data set. Improvement of this approach may be found when further, chemically realistic, geometrical constraints to structural fragments are imposed.

Crystallography, therefore, has three major radiation sources for structure determination and the data sets from all three can be used for quantitative structure analysis. X-ray crystallography will remain the workhorse for deriving accurate structural geometries, while electrons and neutrons will continue to provide the basis for specialized views of important details that are not easily discerned by the X-ray crystallographer.

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