

Prospects for kinematical least-squares refinement in polymer electron crystallography

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Least-squares refinement is unusual in the context of electron crystallography because of the sparsity of the measured intensity data set and the problems of systematic errors due to multiple dynamical scattering. With 120 unique hkl electron diffraction intensities measured from polymorphic form III of isotactic poly(1-butene), conditions for improving an existing structural model derived from initial direct structure analysis have been evaluated. The polymer crystallizes in space group $P2_12_12_1$ with $a = 12.38$, $b = 8.88$, $c = 7.56$ Å and there are 8 unique atoms in the asymmetric unit. Starting with atomic positions resulting from Fourier refinement, four cycles of least-squares refinement, where the positional shifts of atomic positions were constrained, produced better bonding parameters than found before while lowering the conventional crystallographic residual, based on $|F|$, from an overall value of $R = 0.26$ to $R = 0.185$ for the 58 most intense reflections where $|F_h^{\text{obs}}| \geq 4\sigma(F_h^{\text{obs}})$ or 0.216 for the complete data set of 120 reflections. The weighted residuals based on $|F|^2$ fell from 0.50 to 0.41 for the complete data set. This refinement was not improved however when attempts were made to fill in very weak intensities by default values. Also, effects of multiple-scattering perturbations were found in the irregularity of the final isotropic thermal parameters.

1. Introduction

Of all of the organic materials applications for quantitative structure analysis with electron diffraction data, those involving linear polymers are most commonly found in the crystallographic literature. Thin chain-folded lamellae are most easily grown from dilute solution by the self-seeding method so that the readily available $hk0$ electron diffraction intensities obtained from them have been utilized for a linked-atom least-squares refinement (*LALS*) (Campbell Smith & Arnott, 1978) of a starting-chain conformer (Brisse, 1989; Perez & Chanzy, 1989), much as the constrained refinement of a similar model would be carried out against fiber X-ray diffraction data (Tadokoro, 1979). Dual criteria for locating the best structural solution would identify the simultaneously reached minimum values of a crystallographic residual and a nonbonded potential function.

Recently, the prospects of true *ab initio* structure investigations have been evaluated, particularly since the goniometer stage of the electron microscope can be used to collect three-dimensional selected-area electron diffraction data. Problems with the missing cone imposed by a single orientation of lamellar chains can be solved many times by epitaxial orientation techniques to obtain a view orthogonal to the one obtained by solution growth (Wittmann & Lotz, 1990). Therefore, within the tilt limits of the goniometer stage, the

full three-dimensional reciprocal lattice can be sampled completely. Without the second single-crystal orientation, it might also be possible to combine diffraction intensities from microfibrils, where the chains lie along the fiber axis, with the single-crystal diffraction from the tilted chain-folded lamellae (Hasegawa *et al.*, 1977; Dorset & McCourt, 1997).

Direct phasing methods have also been shown to be useful for determining the initial atomic model, particularly when three-dimensional intensity data are available (Dorset, 1994). The remaining problem, therefore, is one of refinement. Generally, in electron crystallography, the number of intensity data collected compared to the number of variable parameters does not seem to favor the type of least-squares refinement routinely carried out in X-ray crystallography. For this reason, Fourier methods have been more often employed (Dorset, 1995), following a tradition begun in the early years of organic electron crystallography (Vainshtein, 1964).

In some instances, however, the number of data collected may satisfy the 4 or 5 measurements per refineable parameter needed [see, for example, Watkin (1994) and Cruickshank (1999), both of which contain up-to-date reviews of the current state-of-the-art of the least-squares technique] to justify the least-squares refinement. Even though there are intrinsic errors in recorded electron diffraction (Cowley, 1995) due to multiple scattering (coherent or incoherent), it is not yet known if these data are of sufficient quality to justify a full-

matrix minimization. Furthermore, it is difficult to obtain reliable variances for the intensities and these are important in a least-squares calculation. There has been an initial favorable trial on the data collected from polyethylene (Dorset, 1995) but this structure only requires refinement of a single heavy-atom position, so that the result may be nonrepresentative of other more practical examples. For this reason, the least-squares refinement of poly(1-butene) in its form III polymorph (Dorset *et al.*, 1994) is discussed here.

2. Material and methods

2.1. Polymer crystallization and data collection

Two orthogonal views of the form III polymorph of isotactic poly(1-butene) were crystallized by S. Kopp (Kopp *et al.*, 1994). Selected-area electron diffraction experiments were carried out at 120 kV with a Philips CM-12 electron microscope, following usual low-dose procedures for recording diffraction intensities, including the use of a screenless X-ray film (Kodak DEF-5). Typically, organic crystals (generally <10 nm thick, estimated by transmission of the electron beam) are elastically bent so that an approximation to an integrated intensity is obtained when equivalent reflections from multiple measurements are averaged (Dorset *et al.*, 1998). The crystals also are not perfect; polymer crystals will contain a number of defects (Geil, 1963). In principle, with goniometric tilts in the electron microscope, a complete reciprocal lattice could be sampled because the two separate orthogonal crystallizations leave no 'missing cone' region within the tomographic data collection. Practically speaking, however, 13 highly visible crystallographic projections were sampled by tilting solution- or epitaxially oriented samples for assembling the three-dimensional data set. The measured unit-cell constants were found to be consistent with the values given in an earlier powder X-ray study (Cojazzi *et al.*, 1976): $a = 12.38$, $b = 8.88$, $c = 7.56$ Å, the latter being the direction of the polymer chain axis. Systematic absences along the reciprocal axes indicated the space group to be $P2_12_12_1$.

Diffraction films from the various zonal projections were scanned on a flat-bed microdensitometer (Joyce Loebel Mk IIIC) and intensities of the individual reflections were obtained as a triangular estimate of the peak areas. Each pattern was from a separate crystal. There were generally several independent tests of a single projection to determine the reproducibility of a given pattern. A typical value for R_{merge} was 0.16, similar to values found in other work on organics (Dorset *et al.*, 1998). For tilts of the chain-folded lamellae, where diffraction peaks were observed to be sharp, no Lorentz correction was applied to the raw intensity values. However, the patterns from the orthogonal projection, nucleated on a substrate, were observed to be somewhat arced so that a phenomenological correction, similar to the one discussed by Vainshtein (1964), was applied. In all, 120 unique reflections had been recorded in the original experiments (Dorset *et al.*, 1994) and this corresponds to 30% of the theoretically available data to a resolution of 1.1 Å (here

defined as the d^* value of the highest-resolution reflection observed in recorded diffraction patterns). Although the number of diffraction films from any particular projection was not as large as often employed for electron crystallographic data collection from representative organic structures, the R_{sym} (typical value was 0.08 for $hk0$ data) found for symmetry-equivalent reflections indicated that the patterns were at least internally consistent (*i.e.* no great intensity deviations due to irregular elastic crystal bend, controlled by a conscious effort to find the most symmetric pattern before recording it). In a systematic study of data collection from organic samples (Dorset *et al.*, 1998), it was found that the observed standard deviation of the intensity measurement, averaged over a number of equivalent diffraction patterns, was well correlated to the counting statistics assumed in X-ray crystallography ($r = 0.92$) although the value of k in $\sigma I = kI^{1/2}$ was more like 0.14 than 1.00. In the refinement of the crystal structure of this polymer, an approximation of the standard deviation to the structure-factor magnitude was made that was somewhat higher than this estimate, *i.e.* $\sigma(F_{\text{obs}}) \approx 10\%$. (A test of recorded $hk0$ data revealed that this value was, on average, 11% with no obvious correlation to the magnitudes of the reflections. The estimate was made from the distribution of values for symmetry-related reflections.)

In any data collection from diffraction films, there is also a problem with the occurrence of weak intensities that cannot be detected above the background intensity. A number of suggestions have been made to estimate these data. In X-ray crystallography (Buerger, 1960), it has been suggested that a value

$$\langle |F_{\text{unobserved}}| \rangle = 0.5|F_{\text{min}}|$$

should be used for centrosymmetric zones and

$$\langle |F_{\text{unobserved}}| \rangle = 0.667|F_{\text{min}}|$$

for noncentrosymmetric data, where $|F_{\text{min}}|$ is the minimum observed $|F|$ for the relevant zone. In polymer electron crystallography, the unobserved intensity has been assigned (Perez *et al.*, 1979; Chanzy *et al.*, 1987) values from 0.333 to 0.50 that of an identified I_{min} . In this paper, we use an overall estimate, where $\langle |F_{\text{unobserved}}| \rangle = 0.333|F_{\text{min}}|$, without any theoretical justification but just to provide a default intensity value for a seemingly missing reflection within a recorded diffraction pattern.

2.2. Structure analysis

The crystal structure of this polymer was originally solved using the tangent formula, aided by a symbolic addition phasing of the $hk0$ reflections (Dorset *et al.*, 1994). Subsequently, the structure has been solved directly *via* the Sayre equation, by SnB (Dorset, 1995) and by use of maximum-entropy and likelihood procedures (C. J. Gilmore, unpublished results). These all produce the same starting chain model and some improvement of the model can be found subsequently when the atom positions are refined by Fourier techniques. The starting atomic coordinates for this study, therefore, are derived from this model or from the linked-atoms least-

Table 1
Original and refined models for poly(1-butene).

	Original e. d. model			Least-squares model			U_{iso} (Å ²)	Shift (Å)
	x	y	z	x	y	z		
C1	0.346	−0.038	0.108	0.340 (4)	−0.025 (4)	0.100 (11)	0.07 (2)	0.15
C2	0.298	0.125	0.163	0.296 (3)	0.129 (4)	0.161 (4)	0.07 (2)	0.05
C3	0.381	0.248	0.103	0.382 (3)	0.256 (3)	0.133 (10)	0.08 (2)	0.24
C4	0.325	0.402	0.120	0.327 (4)	0.408 (4)	0.078 (7)	0.06 (2)	0.32
C5	0.279	0.134	0.358	0.270 (2)	0.137 (4)	0.361 (5)	0.02 (2)	0.12
C6	0.160	0.067	0.413	0.161 (2)	0.058 (4)	0.410 (9)	0.03 (2)	0.08
C7	0.072	0.183	0.353	0.072 (2)	0.176 (4)	0.383 (10)	0.00 (1)	0.23
C8	−0.038	0.105	0.370	−0.038 (3)	0.086 (6)	0.374 (12)	0.11 (3)	0.17

squares model determined using powder X-ray data (Cojazzi *et al.*, 1976).

2.3. Least-squares refinement

After incorporating the electron scattering factor for carbon (Doyle & Turner, 1968), the full-matrix least-squares refinements were carried out using the program *SHELXL97* (Sheldrick, 1997). In this refinement, the quantity

$$R_2 = \sum_{\mathbf{h}} w_{\mathbf{h}} (|F_{\mathbf{h}}^{\text{obs}}|^2 - k|F_{\mathbf{h}}^{\text{calc}}|^2)$$

was minimized, where the weight

$$w_{\mathbf{h}} = [\sigma^2(F_{\text{obs}}^2) + (0.10p)^2]^{-1}$$

and

$$p = \max[(F_{\mathbf{h}}^{\text{obs}})^2, 2(F_{\mathbf{h}}^{\text{calc}})^2/3].$$

Three positional parameters and one isotropic temperature factor were refined for each of the eight unique atoms in addition to an overall scale factor k . All parameter shifts were damped using a factor of 0.5. In addition, a soft restraint was applied in which all the bond distances in the asymmetric unit were initially set at 1.50 Å with an effective standard deviation of 0.05 Å and constrained to remain the same within this permitted standard deviation. No restraints were placed on the bond angles, however.

Also, the refinement only considered the variation of carbon atom parameters, as is the case in the initial refinement

of any typical organic structure against X-ray intensity data (Stout & Jensen, 1968). While hydrogen theoretically has a larger scattering contribution in electron crystallography than in X-ray crystallography (Vainshtein, 1964; Dorset, 1995), the difference still is not great enough to expect a large effect on the heavy-atom refinement.

3. Results

Optimal results were obtained when a set of 120 observed reflections was used as the basis of the structure refinement. The original electron diffraction model (Dorset *et al.*, 1994) found after Fourier refinement against electron diffraction data (Table 1) contained some aberrant bond lengths (Fig. 1*a*). A better agreement (Table 1) was found after four cycles of least-squares refinement under the constraints outlined above, resulting in bond lengths more consistent with the accepted 1.54 Å value for a carbon–carbon single bond (Fig. 1*b*). While there was greater deviation of the bond angles from the ideal tetrahedral value in the final solution than found in the original model, the final values are not so atypical when compared to many X-ray diffraction analyses of polymethylene chain compounds at room temperature. The final crystallographic residual

$$R_1 = \sum_{\mathbf{h}} \frac{||F_{\mathbf{h}}^{\text{obs}}| - |F_{\mathbf{h}}^{\text{calc}}||}{\sum_{\mathbf{h}} |F_{\mathbf{h}}^{\text{obs}}|}$$

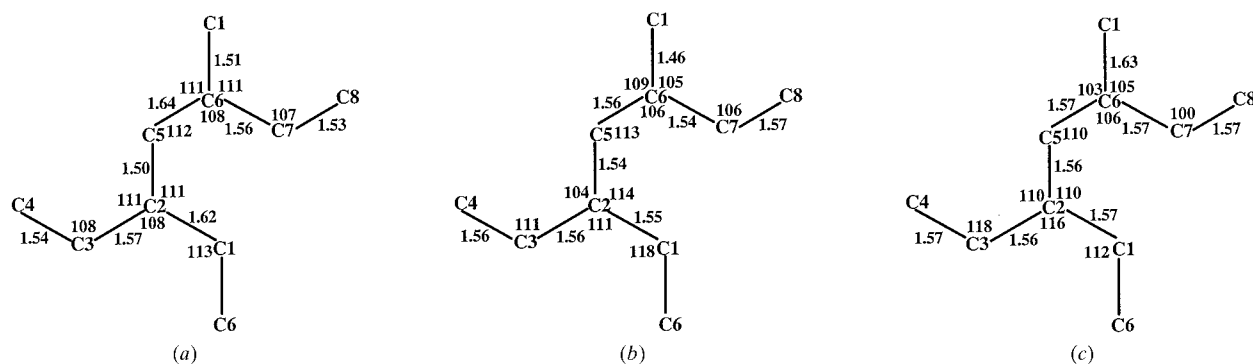


Figure 1

Bond distances and angles for isotactic poly(1-butene). (a) Initial electron diffraction model (Dorset *et al.*, 1994). (b) Refinement against 120 measured electron diffraction intensities. (c) Refinement including estimates of unmeasured reflection intensities.

was 0.185 for the 58 most intense reflections and 0.216 for the complete data set. The weighted residual based on $|F|^2$,

$$wR_2 = \left(\sum_{\mathbf{h}} \{w[(F_{\mathbf{h}}^{\text{obs}})^2 - (F_{\mathbf{h}}^{\text{calc}})^2]^2\} / \sum_{\mathbf{h}} \{w[(F_{\mathbf{h}}^{\text{obs}})^2]^2\} \right)^{1/2}$$

fell from 0.50 to 0.41 for the complete data set. The mean atomic shift was 0.17 Å and the largest shift value was 0.32 Å. The goodness of fit,

$$S = \left(\sum_{\mathbf{h}} \{w[(F_{\mathbf{h}}^{\text{obs}})^2 - (F_{\mathbf{h}}^{\text{calc}})^2]^2\} / (n - p) \right)^{1/2},$$

where p parameters are being refined against n data, was 1.37, which is an indication of correct intensity variances. A final list of observed and calculated structure-factor values is given in Table 2.

When the ideal X-ray model found from an *LALS* fit to powder X-ray data (Cojazzi *et al.*, 1976) was used as the starting model for least-squares refinement, within the standard deviations of the atomic positions, the same model was found again after four cycles of least-squares refinement against the 120 electron diffraction intensities. These result in crystallographic residuals that were virtually identical to the ones reported in the previous paragraph.

If, on the other hand, no restraints were placed on the shift of atomic parameters, the refinement was not stable. Although 'very good' crystallographic residuals were found after four cycles, *e.g.* $R_1 = 0.135$ for the 58 most intense reflections and 0.162 for the complete data set, the bond distances varied between 1.46 and 1.91 Å and bond angles between 89.9 and 114.8°. While it might be imagined that a larger data set, including estimates of 26 unmeasured amplitudes, might improve the refinement, the contrary result was found. (Again, in this case, 'unmeasured' reflections denote the weaker amplitudes within recorded electron diffraction patterns and not reflections in unsampled zones.) With the same damping and restraints as before, a final R_1 value of 0.208 was found for the 58 most intense reflections and the agreement was worse ($R_1 = 0.287$) for the complete data set. One linkage bond distance between two asymmetric units (C1–C6 in Fig. 1c) was quite large whereas one of the bond angles was abnormally small.

4. Discussion

There is very little experience in the use of unconstrained least-squares refinement in electron crystallography. Favorable results have been reported for two inorganic problems (Mo *et al.*, 1992; Weirich *et al.*, 1996) although details of the refinement procedure have not been discussed at length for either application. The result of this study is consistent with an earlier trial with diketopiperazine (Dorset & McCourt, 1994), using three-dimensional oblique-texture electron diffraction data. From the overall low value of the isotropic thermal parameter found from the Wilson plot in the original study (Dorset *et al.*, 1994), it was demonstrated that the data must be somewhat contaminated by dynamical and/or secondary

Table 2

Final observed and calculated structure-factor magnitudes.

<i>hkl</i>	$ F_{\text{obs}} $	$ F_{\text{calc}} $	<i>hkl</i>	$ F_{\text{obs}} $	$ F_{\text{calc}} $
200	14.78	14.80	611	6.14	5.56
400	9.65	6.36	711	3.63	2.13
600	4.26	5.56	021	15.78	18.10
800	5.51	4.76	121	16.91	11.99
10,0,0	4.13	1.19	221	4.38	4.28
110	17.78	19.92	321	4.26	0.51
210	10.27	9.02	421	13.28	12.77
310	8.14	6.09	521	3.88	4.02
410	9.90	9.27	621	5.26	3.33
510	9.52	4.61	821	3.38	4.38
710	2.76	0.24	031	5.51	7.82
810	3.26	2.58	231	4.88	3.14
10,1,0	4.38	3.88	331	10.90	10.73
020	9.77	6.03	431	9.52	6.08
120	17.66	16.31	531	2.88	2.59
220	6.76	6.03	041	4.01	2.41
420	4.51	0.42	141	6.64	5.39
520	6.51	4.88	241	6.51	2.81
620	12.28	10.60	341	4.01	4.22
720	4.64	5.38	541	11.40	9.54
820	3.01	2.12	641	2.25	2.92
920	6.26	7.01	741	2.38	4.56
10,2,0	4.01	2.57	151	4.13	3.67
130	7.77	3.97	251	2.88	2.10
230	4.26	1.88	351	3.38	3.90
330	6.01	2.91	451	3.76	2.27
430	4.13	0.32	751	3.38	0.83
530	13.03	10.89	261	4.64	3.48
630	10.02	9.79	461	3.38	3.39
730	2.63	2.68	271	3.88	2.64
830	2.12	2.33	471	5.14	3.81
930	3.26	3.25	402	3.51	4.67
040	7.26	7.18	502	5.01	7.63
140	7.26	3.98	012	11.52	12.75
240	10.02	5.54	112	13.78	16.17
340	8.02	8.23	412	2.76	1.96
440	11.27	8.96	512	3.76	2.64
540	8.49	6.38	022	5.01	5.50
740	3.51	3.88	122	4.01	4.14
840	3.63	3.19	222	4.88	2.79
940	3.26	1.97	322	5.14	6.31
250	2.88	1.83	422	4.13	2.24
350	6.51	5.11	522	4.64	4.91
450	7.39	9.02	032	7.64	8.37
060	5.64	1.50	432	4.26	1.57
160	5.26	3.83	042	3.13	1.95
360	5.26	5.81	142	2.88	2.49
460	3.51	1.71	242	4.76	4.14
560	4.01	3.12	342	3.38	3.92
170	4.88	3.87	442	3.63	3.48
270	8.14	6.53	642	2.88	2.35
080	2.76	1.33	742	2.76	3.85
201	30.07	26.99	113	10.65	10.09
301	17.79	18.20	223	4.38	4.16
401	12.03	11.86	033	4.38	4.21
501	8.02	6.38	133	4.01	4.01
111	20.29	21.80	233	4.13	3.98
211	6.89	5.03	333	3.51	3.86
311	3.88	3.54	004	20.04	22.01
411	8.27	5.04	114	6.14	7.02

scattering (Dorset, 1995). Nevertheless, constraint of the atomic shifts in each cycle was sufficient to find a local minimum of the crystallographic residual consistent with a more chemically reasonable structure. As in one case explored for this polymer, release of constraints allowed the atoms in diketopiperazine to seek a chemically meaningless global

Table 3
Torsional angles (°).

	X-ray†	Electron diffraction‡	Refined
C1—C2—C3—C4	169.9	168.4	144.4§
C4—C3—C2—C5	-63.7	-70.0	-92.7§
C3—C2—C5—C6	152.8	154.4	161.5
C2—C5—C6—C7	-76.6	-73.1	-86.2
C5—C6—C7—C8	169.9	168.5	162.9
C5—C2—C1—C6'	159.1	165.4	163.6
C3—C2—C1—C6'	-76.6	-73.3	-79.5

† Cojazzi *et al.* (1976). ‡ Dorset *et al.* (1994). § Greatest differences.

minimum, essentially compensating for the multiple-scattering perturbations with a distorted model (Dorset & McCourt, 1994).

It is clear that the best, *i.e.* structurally most reasonable, solution found in this refinement also represents a local minimum, since it is also reached by allowing a strictly ideal structural model to be changed by the recorded data. Aside from the bond geometry values already stated, the structure appears to be chemically reasonable. Many of the torsional parameters are not greatly changed (Table 3), especially in the polymer chain backbone. Most affected is the conformation of one of the ethylene side chains, a change associated with a more consistent van der Waals contact between neighboring molecules than before [starting model: $d(\text{C8—C3}) = 4.31$; $d(\text{C3—C8}) = 3.94 \text{ \AA}$; refined model: $d(\text{C8—C3}) = 4.10$; $d(\text{C3—C8}) = 4.20 \text{ \AA}$].

There are still some difficulties with the best restrained refinement that are due to multiple-scattering perturbations to the observed data. In this case, secondary scattering (Cowley, 1995) may have a larger influence than does dynamical scattering, although the presence of forbidden axial reflections in zonal patterns is not especially noticeable. Polymer crystals are imperfect (Geil, 1963) so that secondary scattering will add to actual dynamical scattering for a composite multiple-scattering perturbation (Dorset *et al.*, 1999). There are, of course, errors in data measurement and from merging individual zones. For example, the use of zonal intensities to merge zones, following the procedure given by Hu & Dorset (1989), reveals some variation of the fit between $h00$ data (average $R = 0.26$) or $0k0$ data (average $R = 0.12$). The range of isotropic thermal parameters for the atoms (see Table 1), for example, is physically unreasonable and demonstrates how the refinement attempts to compensate for such errors, barring large independent shifts of atomic positions. For example, one atom with a nearly zero temperature factor is unrealistic. While the largest thermal parameter occurs for the methyl end of a side branch, it would be expected that the other branch end should have a similar U_{iso} value. In the original study of the polymer, an overall thermal parameter $U_{\text{iso}} = 0.05 \text{ \AA}^2$ was applied to all atoms.

The conclusion from this study is that kinematical least-squares refinements of crystal structures solved from three-dimensional electron diffraction data may be possible under suitable constraints. However, there is always enough error due to nonlinear scattering events in the recorded data to

exacerbate the fact that a very small data set is being considered when compared to normal X-ray data sets. Measurement of electron diffraction data, at best, is tedious. One has to justify the orientation of a particular zonal projection and then establish its self-consistency by showing that R_{merge} of separate diffraction patterns is very low (Dorset *et al.*, 1998). Finding optimal projections for data collection under low-illumination conditions can also be difficult, especially for nonorthogonal reciprocal lattices.

How then can these refinements be improved? There are efforts under way in inorganic electron crystallography to combine multiple-beam dynamical-scattering calculations with the refinement itself (Zandbergen & Jansen, 1998). This is mostly practicable when one principal zone is being used for the structure determination or, at best, a small number of zonal projections. Optimally, a nanoprobe can be used to isolate an essentially flat region of the crystal so that the n -beam dynamical model gives a nearly exact solution. This approach is not envisioned to be of equal utility, however, when selected-area diffraction intensities from a selected microarea must be used instead (in order to minimize radiation damage to organic specimens). In this case, secondary scattering can be just as important as dynamical scattering because of the presence of crystal defects (Cowley, 1995). Nonflatness of the specimen can also be a major concern since strong intensity fluctuations of otherwise symmetric reflections can be observed (Dorset, 1995).

For three-dimensional data, some nonsystematic dynamical interactions can be averaged out by sampling a number of crystal orientations for measurement of true integrated intensities. This was the concept behind oblique-texture electron diffraction experiments (Vainshtein, 1964; Cowley, 1967; Zvyagin, 1993), although the disadvantage of these measurements was similar to powder diffractometry where nearby beams in a reciprocal lattice could overlap. Alternatively, an iterative two-beam dynamical-like correction, resulting in the re-shaping of the electron diffraction scattering envelope, has shown some promise for structures with a dominant heavy atom (Sha *et al.*, 1993; Dorset, 1997) but, unfortunately, the success has not been consistent (Dorset, 1997). The recent development of precession methods (Vincent & Midgeley, 1994) for collecting single-crystal electron diffraction intensities may actually do much to improve the data quality from organic crystals, as it has already in inorganic applications (Gjønnnes *et al.*, 1998), provided that the beam doses can be kept low enough to eliminate the further complication caused by radiation damage. Further experience with this mode of data collection is required before a final judgement can be made on kinematical least-squares refinement in electron crystallography.

A further possibility lies with the least-squares method itself and its relationship to the more general technique of maximum-likelihood (ML) refinement. The least-squares method considered above makes the following assumptions (Bricogne, 1991, 1993; Pannu & Reed, 1996; Pannu *et al.*, 1998):

- (i) The deviation between the observed and scaled calculated structure factors is normal or Gaussian;
 (ii) The mean standard deviation is zero;
 (iii) The standard deviation of the Gaussian is independent of the model, *i.e.* the atomic parameters.

Generally speaking, these assumptions cannot be made. However, in small-molecule X-ray crystallography, the large overdetermination of data to parameters means that any inherent problems with these assumptions are not significant. In situations where data are sparse and the variances of the observations are poorly defined, then ML methods, which do not make these assumptions, should show superior behavior. Indeed, ML refinement is becoming commonplace in macromolecular X-ray crystallography for this reason. What are required are suitable probability distributions to ML refinement that could readily be incorporated into standard least-squares-refinement programs. We will test this possibility at a later date.

Thus, a careful data-collection strategy combined with ML refinement could be the way forward for routine kinematical refinement of structures in electron crystallography. This may be the most realistic strategy for radiation-sensitive molecules where many three-dimensional intensity data must be collected under low-beam-dose conditions and where more stringent tests incorporating many-beam dynamical interactions cannot be considered.

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