

Refinement with SHELXL: free variables, constraints and restraints

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Least-squares refinement

Small molecules are usually refined by *least-squares*. Either:

$$M_1 = \sum_{F > 4\sigma(F)} w(|F_o| - |F_c|)^2 \quad \text{or} \quad M_2 = \sum w(F_o^2 - F_c^2)^2$$

is minimized.

The advantages of M_1 are that $R_1 \ll R_2$, it is slightly faster (less data), and that F is more suitable than F^2 for punched cards.

The advantages of M_2 are that one can use all measured F_o^2 -values, including those that are zero or (because of measurement errors) slightly negative. The weak reflections are particularly important in pseudosymmetry cases.

Calculating structure factors

Each atom j in the unit-cell contributes to the structure factor F that is expressed as a complex number:

$$F_{hke} = \sum_j f_j q \exp[2\pi i(hx_j + ky_j + \ell z_j)]$$

where the term q is $\exp\{-8\pi^2 U [\sin(\theta)/\lambda]^2\}$ for isotropic atoms and $\exp\{-2\pi^2[U_{11}h^2a^{*2} + U_{22}k^2b^{*2} + U_{33}\ell^2c^{*2} + 2U_{23}k\ell b^*c^* + 2U_{13}h\ell a^*c^* + 2U_{12}hk a^*b^*]\}$ for anisotropic. The atomic scattering factor f is complex and takes the form $(f_o + f', f'')$.

Taking anomalous scattering into account

For complex scattering factors $f = (f_o + f', f'')$ we can express the real and imaginary parts A and B of the structure factor as:

$$A_{hke} = \sum_j q \{ (f_o + f')_j \cos[2\pi(hx_j + ky_j + \ell z_j)] + f''_j \sin[2\pi(hx_j + ky_j + \ell z_j)] \}$$

$$B_{hke} = \sum_j q \{ (f_o + f')_j \sin[2\pi(hx_j + ky_j + \ell z_j)] + f''_j \cos[2\pi(hx_j + ky_j + \ell z_j)] \}$$

so we can write:

$$F_{hke} = A + iB; \quad \tan(\phi_{hke}) = B/A; \quad F_{hke}^2 = A^2 + B^2$$

It follows that F_{hke}^2 and $F_{-h-k-\ell}^2$ are equal (Friedel's law) only if:

- f'' is negligible for all atoms, or
- the structure is centrosymmetric (sin terms cancel), or
- all atoms have the same (complex) scattering factors.

Least-squares refinement algorithms

Least-squares refinement minimizes $\sum w(F_o - F_c)^2$ or $\sum w(F_o^2 - F_c^2)^2$ by refining the parameters (x, y, z, B etc.) that describe the model. The standard mathematical procedure used in small-molecule refinements involves the accumulation and inversion of the full least-squares normal matrix (L.S. instruction in SHELXL). This requires storage of about $N_p^2/2$ elements (4 or 8 bytes each) and the time taken is of order $N_p^2 N_R$ for matrix buildup and N_p^3 for matrix inversion.

Conjugate gradient solution of the sparse normal equations (CGLS in SHELXL) is a robust alternative for all but the final cycle (for calculating esds) of large structures. The matrix can be generated 'on the fly' so storage requirements are small. Conditioning is required and SHELXL remembers what happened in previous cycles to accelerate convergence.

What can we do about disorder?

- **Avoid it:** low temperature data collection, avoid loss of solvent when mounting crystals (and **never pump off all the mother liquor**), use only one solvent, avoid H_2CCl_2 and ClO_4^- .
- **Fourier transform affected regions and subtract F_c from the observed data (Ton Spek's SQUEEZE program).** This assumes that the calculated phases don't change so works best for centrosymmetric structures. A serious objection is that modifying the observed data invalidates the statistics and leads to wrong esds.
- **Include continuous disordered solvent directly in the model to be refined.** This is often done for proteins, assuming that the density in the solvent region is flat.
- **Model it as disorder with the help of constraints and restraints!**

Constraints and restraints

Constraints are exact mathematical conditions that lead to a reduction in the number of parameters. Examples are rigid groups and riding hydrogen atoms.

Restraints are additional observational equations involving target values T and their standard deviations σ that are added to the quantity to be minimised:

$$M = \sum w_x (F_o^2 - F_c^2)^2 + \sum w_r (T_{\text{target}} - T_c)^2$$

To bring the X-ray weights w_x onto an absolute scale, they are normalised so that the mean $w_x (F_o^2 - F_c^2)^2$ is unity. $w_r = 1/\sigma^2$ should then be structure and resolution independent.

w_x increases as the agreement between F_o^2 and F_c^2 improves during the course of refinement.

Types of constraint in SHELXL-97

Constraints for special positions: the necessary constraints on coordinates, occupancies and U_{ij} are derived automatically.

Rigid groups (AFIX 6 ... AFIX 0): the 3 positional parameters per atom are replaced by 3 rotations and 3 translations for the whole rigid group. Atoms may not be in more than one rigid group.

Riding hydrogen atoms (AFIX mn): $x_H = x_C + \Delta x$
– no extra positional parameters.

Fixed parameters: add 10 to x, y, z, occ, U etc. Typically occupancies are fixed at 1.0 by adding 10, i.e. given as 11.0

Free variables: can be used to add extra linear constraints to the usual refinement parameters and also be used instead of restraint target values. This provides a convenient way of getting target values with esds for use as restraints in other structures.

Special position constraints

Example: Atom on twofold axis in space group C2. The two positions related by the twofold axis (x, y, z : $-x, y, -z$) coincide when $x = 0$ and $z = 0$. Since we still wish to sum over all symmetry operators in the structure factor calculation, the **occupancy is fixed at 0.5**. The probability ellipsoid used to describe the anisotropic motion should not be changed by the 180° rotation:



$[U_{11}, U_{22}, U_{33}, U_{23}, U_{13}, U_{12}] \equiv [U_{11}, U_{22}, U_{33}, -U_{23}, U_{13}, -U_{12}]$ which is only true if $U_{23} = 0$ and $U_{12} = 0$. All these **constraints** are generated automatically by SHELXL for all special positions in all space groups.

Note the use of SPEC -1 (before the atom) and SPEC 0.2 (after) to prevent SHELXL from moving an atom exactly onto a special position (rarely needed and PART -N also does this).

Free variables

Free variables are an extremely concise but effective way of applying linear constraints to atom parameters (especially occupancies), restraint targets etc. The parameter x is given as $(10m+p)$, which is interpreted as follows:

$m = 0$: refine normally, starting at value p

$m = 1$: fix at value p

$m > 1$: $x = p * fv(m)$

$m < -1$: $x = p * [fv(-m) - 1]$

e.g. 30.25 ($m = 3, p = 0.25$) means $0.25 * [fv(3)]$ and -30.25 ($m = -3, p = -0.25$) means $0.25 * [1 - fv(3)]$, which could be used to constrain two occupancies to add up to 0.25 (only one parameter, free variable #3, is refined). The starting values for the free variables are given on the FVAR instruction (but free variable #1 is the overall scale factor).

Rigid group constraints

In SHELXL, rigid groups are defined by three rotations about the first atom in the group and by three translations of the group as a whole. Special position constraints may be applied to the first atom and restraints and riding hydrogens are allowed on all atoms in the group. Note that the esds of bond lengths and angles but not of co-ordinates within a rigid group come out as zero from the L.S. matrix algebra.

AFIX 6 rigid group – all
... bond lengths and
atoms angles fixed
...
AFIX 0

AFIX 9 variable metric
... rigid group - angles
atoms fixed, bond lengths
... multiplied by the
AFIX 0 same factor

Two cations sharing the same site

The best strategy is to **constrain** the positions and displacement parameters to be the same, and refine the occupancies so that their sum is **constrained** to be unity:

```
EXYZ MG CA
EADP MG CA
FVAR 1.0 0.6
..
PART 1
MG 6 0.37041 0.34874 0.03824 21.0 0.20936
PART 2
CA 7 0.37041 0.34874 0.03824 -21.0 0.20936
PART 0
```

If the cations were sharing a special position on a twofold axis, their occupancies would be specified as 20.5 and -20.5. For three atoms (or molecules) sharing a site, it is better to tether each occupancy to a free variable (e.g. by 31, 41 and 51) and to **restrain** the sum of these free variables to unity:

```
SUMP 1.0 0.001 1.0 1 1.0 2 1.0 3
```

Types of restraint in SHELXL-97

DFIX, **DANG** and **SADI** - distances and 'angle distances'

FLAT and **CHIV** - planarity and chiral volumes

BUMP - antibumping

NCSY - non-crystallographic symmetry (NCS)

DELU, **SIMU** and **ISOR** - (an)isotropic displacements

SUMP - general 'free variable' restraint (e.g. for the sum of occupancies of side-chains with three disorder components)

DEFS sets default restraint esds and **SAME** can generate **SADI** restraints. **CHIV**, **BUMP**, **SAME**, **NCSY** and **DELU** make use of the connectivity array.

The connectivity list

The **connectivity list** is used for the automatic generation of hydrogen atoms and some restraints. Non-hydrogen atoms *i* and *j* are considered to be 'bonded' if:

$$d_{ij} < r_i + r_j + 0.5 \text{ \AA}$$

The **CONN** instruction may be used to modify *r* and to set a maximum connectivity for an atom (e.g. 0 for water). A shell of symmetry equivalents is generated automatically around the unique atoms. Bonds may be added with **BIND** or deleted with **FREE**.

PART N controls the generation of bonds for disordered groups. Most atoms have *N* = 0; multiple conformations have *N* = 1, 2 etc. Bonds are generated only when the *N* are equal or one *N* is zero. If *N* is negative, bonds are not made to symmetry equivalents.

DFIX or SADI?

The **DFIX** restraint is able to restrain bond lengths to target values but sometimes the target is uncertain. For example the P—O distance in a phosphate may vary with the pH and the extent of libration. **SADI** can be very useful in such cases, e.g.

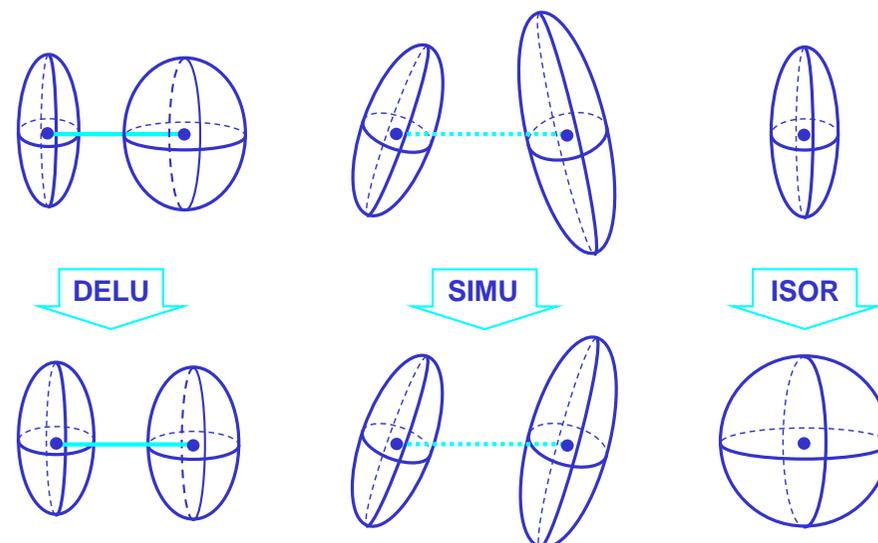
```
SADI P O1 P O2 P O3 P O4
SADI O1 O2 O1 O3 O1 O4 O2 O3 O2 O4 O3 O4
```

ensures that the phosphate will be a regular tetrahedron, but allow the bond length to refine.

The same can however be achieved by an **AFIX 9** constraint or by using **DFIX** with a free variable, e.g.

```
FVAR ..... 1.55
DFIX 31 P O1 P O2 P O3 P O4
DFIX 31.6330 O1 O2 O1 O3 O1 O4 O2 O3 O2 O4 O3 O4
```

Restraints on ADP's



The rigid-bond restraint DELU

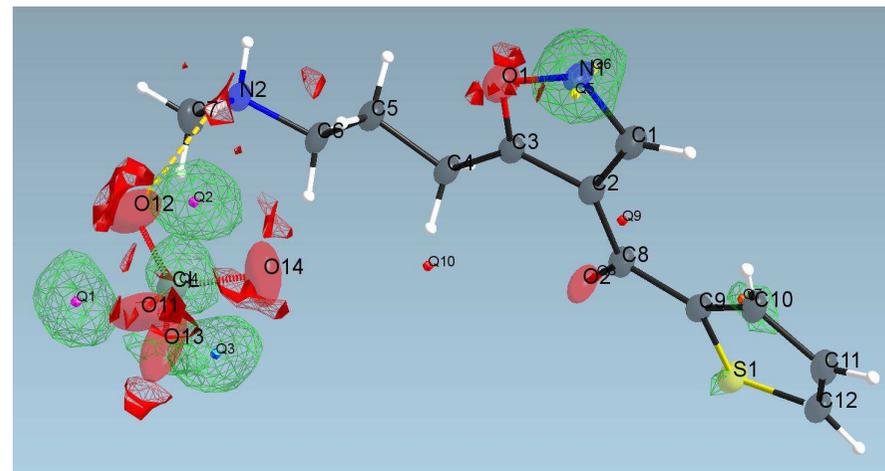
In SHELXL-97, the DELU restraint is a strict rigid-bond restraint, i.e. the components of the anisotropic motion of two atoms along the line joining them are restrained to be equal. Rollet (1970) was probably the first to use this restraint.

Analysis of accurately determined structures by Hirshfeld, Trueblood, Dunitz and Bürgi has shown that these rigid bond restraints should hold accurately and thus may be given a low esd (high weight), but also that they are not applicable for bonds between heavy and light atoms.

Note that this also applies to the Hirshfeld rigid bond test in CheckCIF!

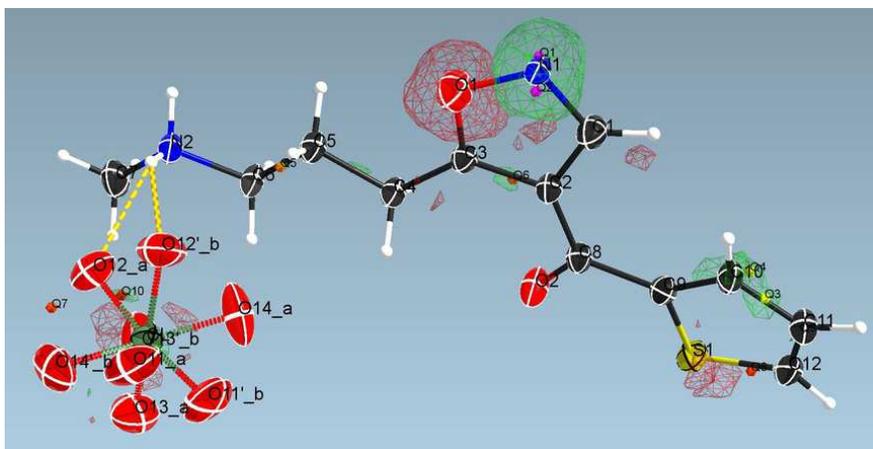
Although DELU is a reliable restraint and so can be given a small e.s.d., there are not as many DELU restraints as U_{ij} , so it may be necessary to supplement them with the less accurate but more numerous SIMU and ISOR restraints with larger e.s.d's.

Difference MAPS can be useful!



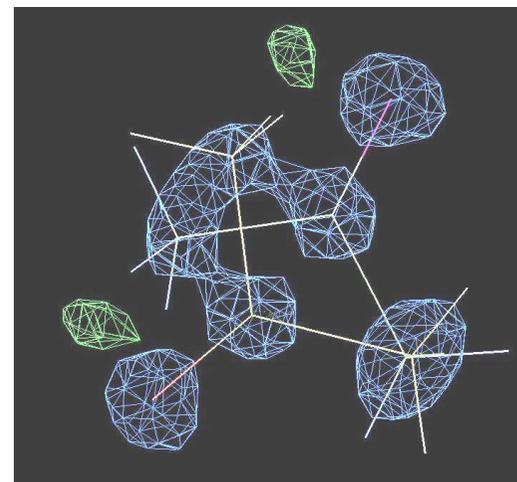
This shelXle screenshot shows that the ClO_4^- anion has a second component (green peaks). In this case all that is needed is to rename Q1..Q4 as oxygen and give them occupancies of 0.5 and the original 4 oxygens 0.5. DELU and SADI restraints (cf. phosphate) can be used (but are not essential here). Something is wrong with O1 and N1!

Element assignment



Modeling the disordered perchlorate has cleaned up the difference density except for N1 and O1. Switching these two atoms eliminates their difference density too.

Two half acetone molecules on a twofold

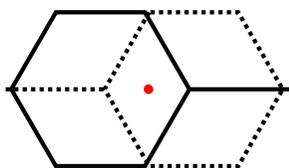


```
SIMU O1 > C3
DELU O1 > C3
SADI C1 C2 C1 C3
SADI O1 C2 O1 C3
CHIV C1
HFIX 127 C2 C3
. . . .
PART -1
O1 . . . . . 10.5 . . .
C1 . . . . . 10.5 . . .
C2 . . . . . 10.5 . . .
C3 . . . . . 10.5 . . .
PART 0
```

The second acetone molecule is generated by the crystallographic twofold axis, so all occupancies are fixed at 0.5. The PART -1 is required for the correct functioning of DELU, CHIV and HFIX.

Toluene on an inversion center

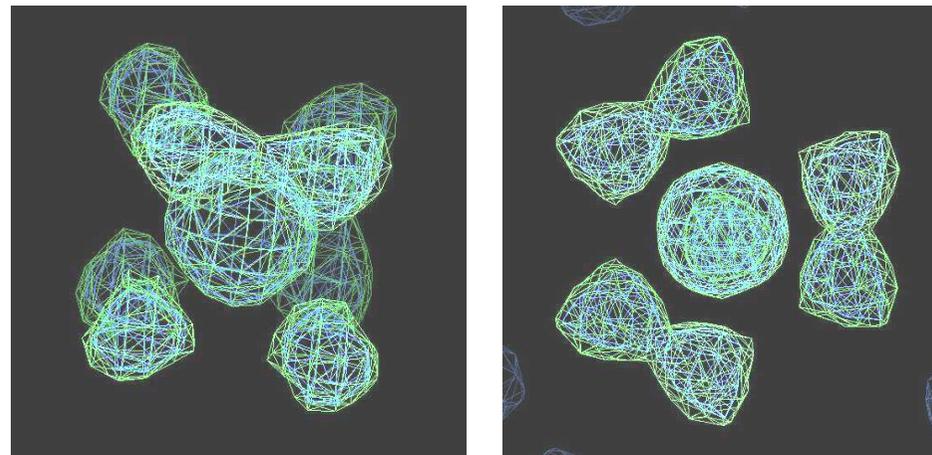
Toluene is a good solvent for growing crystals because of its long liquid range, but it simply cannot resist inversion centers:



This can be handled with one complete toluene molecule with occupancies of 10.5 (fixed at 0.5) and PART -1. Equivalent 1,2- and 1,3-distances can be restrained to be equal with SADI and a FLAT restraint applied to all 7 carbons, or a rigid hexagon can be used for the 6-membered ring (plus two SADI and one CHIV for the CH₃). SIMU and DELU are recommended. The hydrogens should be set with HFIX in a later job:

```
HFIX 43 C1 > C5 (generates 5H with occupancies of 0.5)
HFIX 123 C7      (generates 6H with occupancies of 0.25)
```

Yet another disordered perchlorate



This perchlorate anion is disordered so that the oxygen atoms occupy alternate corners of a cube. The chlorine lies on a position of 32 (D₃) symmetry. Viewed down the 3-fold axis (right view) we can see the each component has one oxygen on the 3-fold, which relates the other 3; the 2-fold axis transforms one disorder component into the other.

The SHELXL difference peaks

```
REM R1=0.2222 for 1415 Fo>4sig(Fo), 0.2225 for all 1426
REM 89 parameters refined using 0 restraints
REM Highest difference peak 21.541, deepest hole -2.151
```

Q1	1	0.1250	0.3750	0.6250	10.16667	0.05	21.54	Cl
Q2	1	0.1498	0.8502	0.3502	10.33333	0.05	4.80	
Q3	1	0.0819	0.4324	0.6771	11.00000	0.05	3.57	O4
Q4	1	0.1790	0.3210	0.6790	10.33333	0.05	3.28	O3
Q5	1	0.1219	0.3739	0.7381	11.00000	0.05	3.18	
Q6	1	0.1221	0.3784	0.7194	11.00000	0.05	2.17	
Q7	1	0.3787	0.3713	0.8750	10.50000	0.05	1.62	
Q8	1	0.1217	0.3591	0.7747	11.00000	0.05	1.58	
Q9	1	0.1298	0.8750	0.6202	10.50000	0.05	1.45	
Q10	1	0.1498	0.4071	0.8643	11.00000	0.05	1.02	

The strongest difference peak (Q1) is the chlorine atom, and lies on a position of 32 (D₃) symmetry and so has an occupancy of 1/6. Q4 is the oxygen on the 3-fold and should also have an occupancy of 1/6. Q3 represents the remaining 3 oxygens related by the 3-fold; these require occupancies of 1/2. The remaining peaks are spurious!

ENVIRONMENT OF Q1

```
Q3 1555 1.364
Q3 6456 1.364 110.6
Q3 12565 1.364 110.6 110.6
Q3 13456 1.364 166.9 58.4 81.3
Q3 18545 1.364 58.4 81.3 166.9 110.6
Q3 24555 1.364 81.3 166.9 58.4 110.6 110.6
Q4 1555 1.439 108.4 108.4 108.4 71.6 71.6 71.6
Q4 13456 1.439 71.6 71.6 71.6 108.4 108.4 108.4 180.0
```

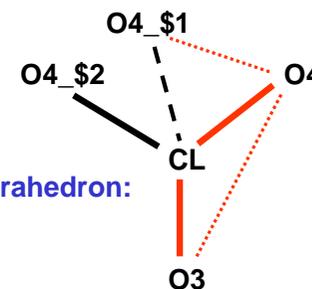
SYMM

```
...
6555. = 0.5000+Z, 0.5000-X, -Y
...
```

So the following restraints ensure a regular tetrahedron:

```
SADI CL O3 CL O4
EQIV $1 Z-0.5, 0.5-X, 1.0-Y
SADI O3 O4 O4 O4_$1
```

Modeling the disorder



Refinement of the disorder model

ANIS

CL	5	0.1250	0.3750	0.6250	10.16667	0.05	21.54
O3	4	0.1790	0.3210	0.6790	10.16667	0.05	3.28
O4	4	0.0819	0.4324	0.6771	10.50000	0.05	3.57

Refinement of the above three unique atoms with the 2 SADI restraints results in a much better R-factor and the spurious difference peaks have mysteriously disappeared!

REM R1=0.0422 for 1415 Fo > 4sig(Fo), 0.0426 for 1426
REM 103 parameters refined using 2 restraints
REM Highest difference peak 0.259, deepest hole -0.368,
1-sigma level 0.063

Q1	1	0.1984	0.3481	0.8878	11.00000	0.05	0.26
Q2	1	0.1856	0.3208	0.6278	11.00000	0.05	0.22
Q3	1	0.0990	0.8901	0.4847	11.00000	0.05	0.21
Q4	1	0.2519	0.4981	0.8750	10.50000	0.05	0.21
Q5	1	-0.0296	0.3750	0.7796	10.50000	0.05	0.20

The goodness of fit (*GooF*) and weights

SHELXL fits the 'goodness of fit' (*GooF*) to unity to put the esds of the reflection data onto an absolute scale, so the *GooF* is not useful as a quality indicator:

$$GooF = \{ \sum w (F_o^2 - F_c^2)^2 / (N_R - N_P) \}^{1/2}$$

The restraint esds are assumed to be already on an absolute scale. This procedure has the advantage that when the model is a long way from convergence and the agreement is poor, the reflection data are weighted down relative to the restraints.

Experience indicates that the major remaining systematic errors in the data (e.g. inadequate absorption corrections) are proportional to the diffracted intensity, so SHELXL uses:

$$w = 1 / [\sigma^2(F_o^2) + (gP)^2] \quad \text{where } P = (F_o^2 + 2F_c^2)/3$$

Least-squares algebra and standard uncertainties

In non-linear least-squares refinement, the parameter shifts each cycle are calculated by $\delta x = A \cdot B^{-1}$ where:

$$A_j = \sum w (F_o^2 - F_c^2) (\partial F_c^2 / \partial x_j) \quad \text{and} \quad B_{ij} = \sum w (\partial F_c^2 / \partial x_i) (\partial F_c^2 / \partial x_j)$$

where the summations are over all reflections. The esds (now called *standard uncertainties* by the IUCr) are then given by:

$$\text{esd}(x_j) = [(B^{-1})_{jj} \sum w (F_o^2 - F_c^2)^2 / (N_R - N_P)]^{1/2}$$

provided that $\sum w (F_o^2 - F_c^2)^2$ is normally distributed, i.e. shows no systematic trends with intensity, resolution and other factors. N_R is the number of reflections and N_P the number of parameters.

These estimates only take random errors into account. Since systematic errors can never be completely eliminated, such esds are always underestimated. Comparison of independent determinations of the same small molecule structures suggest that coordinate esds are underestimated by a factor of about 1.5 and esds in U or U_{ij} by a factor of about 2. Nevertheless, for a single structure determination they are by far the best estimates that we have.

Acknowledgements

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Standard SHELX reference:

Sheldrick, G.M. (2008). *Acta Cryst.* A64, 112-122.

SHELXL book:

Müller, P., Herbst-Irmer, R., Spek, A., Schneider, T.R. & Sawaya, M.R. (2006). *Crystal Structure Refinement: A crystallographer's guide to SHELXL*. IUCr/Oxford University Press.