(*d*) The Fourier maps must be visually inspected. Automated peak search routines can often produce misleading results. Although low-resolution data are very sensitive to the presence of atomic peaks, these can appear slightly shifted from the correct position. This is especially true for structures possessing non-centrosymmetric space groups. Effectively, since general reflections have no restricted phase values, small phase errors associated with the direct-methods application cannot be eliminated, thus producing inaccuracies in the atomic positioning in the *E* map. For these space groups, average atomic displacements of the order of 0.5 Å should be considered normal.

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Simulated Annealing as a Tool for *Ab Initio* Phasing in X-ray Crystallography

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Abstract

Simulated annealing has traditionally been used to refine structural determinations. It is shown in this paper that it can be used for *ab initio* phasing. Several examples are given to illustrate the methodology and capability of this method. The possibility of extending the method to treat macromolecules is discussed.

Introduction

Traditional direct methods are based on the probability distribution of the phases of the structure factors given their magnitudes. The width of the distribution increases with the number of atoms *N* in the unit cell. Therefore, the method is not practical for large *N* (Hauptman, 1986) but there are many interesting large molecules whose structures remain to be resolved. Thus, it is very desirable to explore alternate methods for *ab initio* phasing. Semenovskaya, Khachaturyan & Khachaturyan (1985) have proposed a statistical mechanics approach. They employed stochastic dynamic equations to generate atom positions that optimally fit the measured intensities. In essence, this is a dynamical simulated-annealing approach. They applied this approach to a unit cell containing eight independent atoms. In this paper, we report more extensive calculations of direct structural determination using simulated annealing. Our results suggest that this method is a promising alternate to conventional direct methods. More importantly, it is very straightforward to incorporate symmetry and other partial knowledge about the molecules in this scheme. Thus, it is potentially possible to extend the method to treat macromolecules such as proteins.

This paper is organized as follows: We first discuss the methodology in general. The three molecules on which the method has been successfully tested are then described. Test results on a new system that has not been solved by direct methods are also reported. Based on these results, comparison with conventional direct methods and some alternate methods is made. Finally, we speculate on the possibility of applying the formalism to protein crystallography.

Methodology

The idea of a simulated-annealing approach to the X-ray phase problem is very simple. Basically, the method con-

sists of rearranging the atom positions in the unit cell to minimize the difference between calculated and observed diffraction intensities. In essence, this is a sophisticated least-squares fit of the X-ray data. Simulated-annealing schemes (Kirkpatrick, Gelatt & Vecchi, 1983; Press, Teukolsky, Vetterling & Flannery, 1992) are employed for the minimization procedure. More precisely, the objective function to be minimized is the sum of the square of the difference between the observed and calculated intensities:

$$E(\mathbf{r}_i, \lambda) = \sum_{\mathbf{k}} [\lambda |F(\mathbf{k})| - |F(\mathbf{k})|_{\text{obs}}]^2, \qquad (1)$$

where the structure factor $F(\mathbf{k})$ is calculated from the atomic scattering factor $f_i(\mathbf{k})$ and atom coordinates $\mathbf{r}_i$ in the usual fashion:

$$F(\mathbf{k}) = \sum_i f_i(\mathbf{k}) \exp(i\mathbf{k} \cdot \mathbf{r}_i), \qquad (2)$$

the summation being over all N atoms in the unit cell. Like the particle coordinates $\mathbf{r}_i$, the scale factor λ is determined by the minimization procedure. So far, we have employed the usual Metropolis Monte Carlo scheme (Metropolis, Rosenbluth, Rosenbluth, Teller & Teller, 1953) for updating the particle coordinates and λ. For each simulation, we start from a completely random initial configuration. Then we move each of the N atoms in succession according to the following prescription:

$$\begin{aligned} X &\rightarrow X + \alpha\xi_1 \\ Y &\rightarrow Y + \alpha\xi_2 \\ Z &\rightarrow Z + \alpha\xi_3, \end{aligned} \qquad (3)$$

where X, Y and Z are the fractional coordinates of the atom being displaced, α is the maximum allowed displacement, which is typically a few tenths, and the ξ's are random numbers between -1 and 1. After we move an atom, it is likely to be anywhere within a square of side 2α centered about its original position. Periodic boundary conditions are used, so if the indicated move puts the atom outside the unit cube it re-enters the cube from the opposite side.

We then calculate the change in the energy of the system ΔE due to the move. If $\Delta E < 0$ then the move is accepted. If $\Delta E > 0$, the move is accepted with a probability $\exp(-\Delta E/T)$; *i.e.* we take a random number ξ_4 between 0 and 1 and, if $\xi_4 < \exp(-\Delta E/T)$, the move is accepted. If $\xi_4 > \exp(-\Delta E/T)$, we return the atom to its old position. After all the N atoms have been moved, λ is updated in a similar fashion. This process constitutes a single sweep. Typically, several thousand sweeps are made for a given temperature T to ensure thermal equilibrium. An intial T is chosen so that the acceptance ratio is about 0.5. As T is gradually reduced to zero, the energy E is lowered to a minimum value. If the program succeeds, the atomic configurations at low temperatures should converge to a sensible molecular structure. If not, it may be necessary to repeat the annealing process a few times.

In the presence of any symmetry group, the summation in (2) can be reduced to a summation over the atoms in the asymmetric unit. This significantly reduces the number of atoms needed to be updated. In principle, we can also incorporate the Debye–Waller factors as independent variables to be updated in the annealing procedure. In practice, we have found it adequate to start with an initial guess and then readjust the factors a few times before arriving at the final solution.

The above is a general description of the methodology, further details are discussed in connection with the following specific examples.

Examples

I. $C_{18}H_{22}O_2$

The space group is $P2_1/c$ (monoclinic). The cell constants are $a = 7.97$, $b = 15.29$, $c = 5.84$ Å and $\beta = 92.53°$. The number of formula units per cell $Z = 2$. 1269 reflections are used in the calculation. The H atoms are ignored in the simulation. Since the scattering factors of the O and C atoms are about the same, we treat the O atoms as C atoms initially. Even with this approximation, it is possible to obtain the correct positions. After this, we replace each C atom by an O atom to see if it lowers the objective function. In this way, we can identify the correct positions for the O atoms. The result of the simulation is presented in Fig. 1 in terms of the packing diagram. The structure has been worked out using conventional direct methods. The highly refined molecular structure (Rathore, Bosch & Kochi, 1994) is

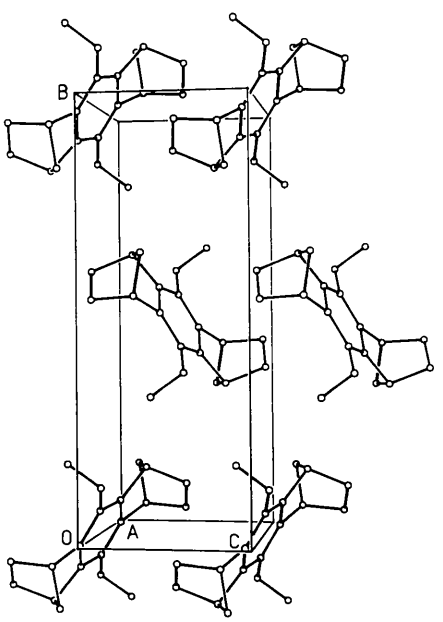

Fig. 1. Packing of $C_{18}H_{22}O_2$ in the unit cell, viewed along the a axis.

reproduced in Fig. 2 for comparison. The result in Fig. 1 was obtained without any prior knowledge of Fig. 2. The atom positions determined by both methods agree with each other within 0.1 Å, even though the R factor of our solution is as large as 0.2.

II. $C_9H_{12}Cl_2N_4Zn$

The space group is *Pbca* (orthorhombic). The cell constants are $a = 12.86$, $b = 14.39$ and $c = 13.18$ Å. The number of formula units per cell $Z = 8$. 2362 reflections are used in the calculation. The H atoms are again ignored and N atoms are treated like C atoms initially. Again, the agreement with the result from direct methods is very good in terms of the atom positions. Fig. 3 shows the packing diagram. The refined molecular structure is displayed in Fig. 4. Compared to example I, example II contains many more atoms in the unit cell. Another significant difference is the presence of heavier atoms, Zn and Cl. Because these heavy atoms contribute more strongly to the objective function than the light atoms, we freeze out the light atoms in the initial stage of simulation. The positions of the heavy atoms are approximately determined at high temperature, which are then used as a starting point for a low-temperature annealing involving all atoms. In this way, we do not have to follow the thermodynamics of simulated annealing all the way from very high temperature down to very low temperature.

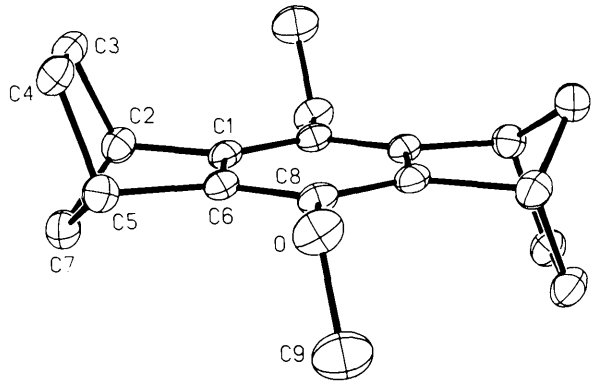

Fig. 2. Refined molecular structure of $C_{18}H_{22}O_2$.

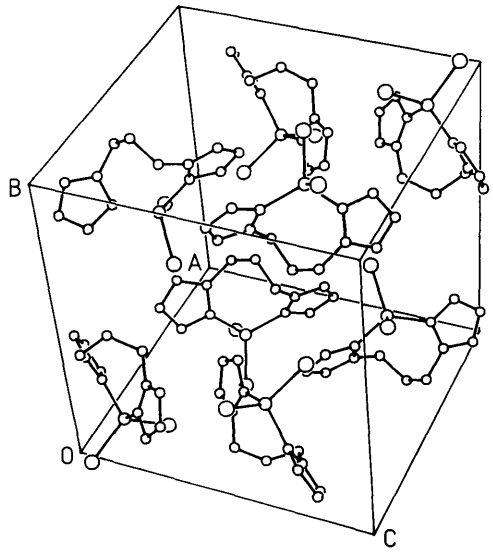

Fig. 3. Packing of $C_9H_{12}Cl_2N_4Zn$ in the unit cell, viewed along the a axis.

III. $C_{32}H_{40}N_4Ta$

The space group is $P\bar{1}$ (triclinic). The cell constants are $a = 8.60$, $b = 10.56$, $c = 16.82$ Å, $\alpha = 88.82$, $\beta = 90.12$ and $\gamma = 105.6°$. The number of formula units per unit cell $Z = 2$. 6778 reflections are used in the calculations. Again, the H atoms are ignored and Ta atoms are first approximately determined at high temperature. N atoms are initially treated as C atoms. In this example, we have a very heavy atom, Ta, which tends to attract a few misplaced atoms. So in the simulation we actually consider more than 37 atoms. For each simulation, we are likely to get some frozen defects or misplaced atoms. But these misplaced atoms are located differently in each simulation. By comparing the results of several simulations and picking only those positions that are robust, we were able to reduce the R factor down to 0.1 and the structure thus determined agrees completely with that obtained by direct methods as displayed in the packing diagram in Fig. 5. The refined molecular structure (Hoffman & Suh, 1994) is shown in Fig. 6. Among the three examples discussed so far, this molecule is the largest. Each simulation takes about half a day with the code running on a DEC 5000 workstation. Without any prior knowledge of Fig. 6, we again succeeded in a complete structural determination.

IV. $Ba_{12}Nb_{26}Si_{12}O_{99}$

The unit cell has hexagonal symmetry. The cell constants are $a = b = 8.995$, $c = 59.12$ Å. The number

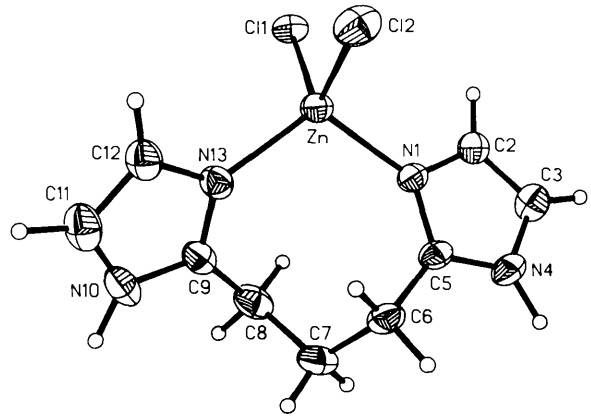

Fig. 4. Refined molecular structure of $C_9H_{12}Cl_2N_4Zn$.

of formula units per cell $Z = 2$. The preliminary space-group assignment for this crystal form is $P6_3cm$, but no refined structure is yet available. For data collected using a copper rotating anode, which is unfavorable because of the adsorption properties of the material, the application of direct methods has not yielded even a partial solution (Krause, 1994). Our simulation, on the other hand, has yielded a partial solution using the same data containing 2138 reflections. We concentrate on Nb atoms first. Starting from several distinct random configurations, we end up with a unique configuration for the Nb atoms. After that, the positions of Ba and Si atoms are determined. Such a configuration of all heavy atoms agrees remarkably well with a structure proposed by Hwu based on homology with two other members of the same barium niobium oxosilicate series $(Ba_3Nb_6Si_4O_{26})_n(Ba_3Nb_8O_{21})$, $n = 1, 2$. These quasi-one-dimensional compounds consist of intergrowth of niobium oxosilicate and niobate layers (Serra & Hwu, 1992). The reflection data obtained so far do not seem good enough to permit a complete determination of all O-atom positions. Details will be published separately (Chen, Su, Krause & Hwu, 1995).

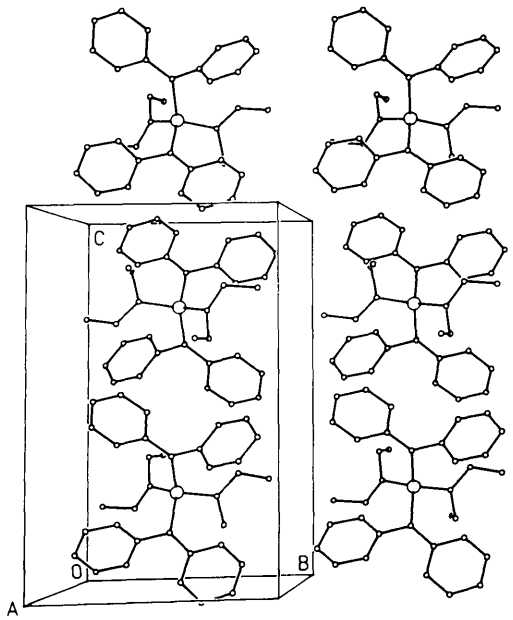

Fig. 5. Packing of $C_{32}H_{40}N_4Ta$ in the unit cell, viewed along the a axis.

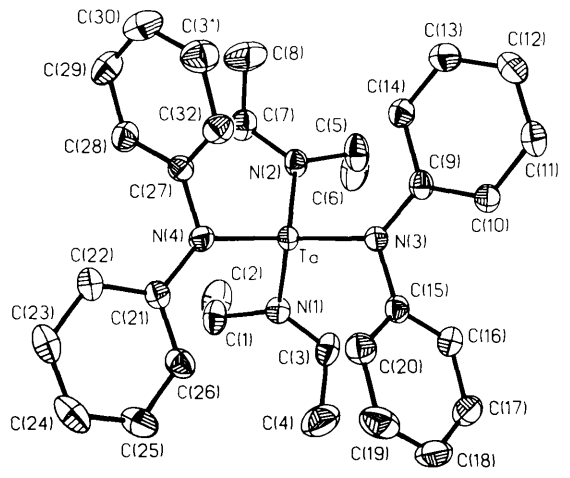

Fig. 6. Refined molecular structure of $C_{32}H_{40}N_4Ta$.

Discussion

The main purpose of this work is to explore simulated annealing as a tool of direct structural determination. Test results reported above clearly demonstrate that it is a respectable method. Some noteworthy features of this method are:

1. The method is very robust and very flexible. In contrast to the shake-and-bake method of Hauptman (Miller *et al.*, 1993), it is based on a genuine variational principle. Any good method of global optimization can be employed to minimize the objective function. In this paper, we have limited ourselves to examples that require minimal computer time but there is no intrinsic limitation on the size of molecules that one can solve. Whether it outdoes the conventional direct methods or not requires more extensive comparison.

2. Partial knowledge of the structure can be incorporated in a straightforward way, either by constraining the configurational space or by introducing penalty functions. In this paper, we have taken only the space symmetry into consideration. In extending the method to treat large molecules such as proteins, one may want to start from the partially known residue structures instead of individual atoms and subsequently updating the positions and orientations of the residues while imposing the correct residue sequence.

We hope this paper will stimulate further applications of the method.

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Structure Determination by Electron Crystallography Using Both Maximum-Entropy and Simulation Approaches

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Abstract

Ab initio structure determination and refinement from electron diffraction data is not a widely used technique in structural science because of the inaccuracies inherent in the process of intensity measurement and because the relative sparseness of the data sets collected makes the structures hard to solve; there are also problems of verifying the correctness of the results. In this paper, the techniques of model building from electron diffraction data were employed to solve the structure. In addition, an *ab initio* solution of the structure of [9,9′-bianthryl]-10-carbonitrile is presented using a routine application of the maximum-entropy method combined with likelihood evaluation employing 150 unique diffraction intensities. The structure thus determined was obtained independently of the model-building studies. The agreement between the two methods is excellent and both agree with a single-crystal X-ray study on the same material. In addition, the high-resolution images agree with the images calculated from the model and with the potential maps after correction for the transfer function and dynamic scattering.

1. Introduction

A major problem for structure analysis of organic crystals, especially polymers, is their small dimensions, making X-ray methods inapplicable. On the other hand, some of the most interesting problems in materials development require detailed information about the direction of molecular dipoles or hyperpolarizabilities in thin films or small crystals. Therefore, knowledge of the molecular conformation and mutual orientation in the unit cell is essential, especially with a view to changing the molecular architecture in order to improve physical properties in a specific manner.

One solution to the problem lies in the improvement of the methods used in electron crystallography combined with high-resolution imaging. In this paper, we demonstrate a successful route to molecular modelling by simulating electron diffraction patterns from different projections. Most importantly, we show that the correct structure is obtained, although the R factor between simulated and experimental diffraction patterns is disappointingly bad by X-ray standards. This problem is well known and has been discussed repeatedly in the literature (Dorset, 1985*a*,*b*, 1991*a*,*b*,*c*, 1993; Dorset & McCourt, 1994). In addition, we adopt another approach to the problem of structure solution with the same experimental

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