expected, that the maxima 4′,4″,3′ and 3″ which form two pairs might not correspond to bromine atoms. This is not astonishing in view of the fact that the value G_4 is the smallest of all G_j values. The six bromine atoms found were taken as a starting point for a three-dimensional Fourier synthesis with cycles of refinement following. These confirmed the correctness of the conclusions arrived at by the SFE method.

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Application of the Hankel Transform Method in Small-Angle X-ray Scattering for the Study of the Internal Structure of Tobacco Mosaic Virus

BY B. A. FEDOROV

Institute of Protein Research, the Academy of Sciences of the USSR, Poustchino, Moscow Region, USSR

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A method developed earlier [Fedorov & Aleshin (1966) *Vysokomol. Soed.* **8**, 5016; translated into English in *Polymer Sci. USSR* (1967) **8**, 1657] which permits the determination of the radial electron density distribution function of cylinders on the basis of the small-angle scattering curve, is applied to the study of the tobacco mosaic virus structure. The results obtained are in good agreement with the X-ray structural analysis of TMV. Questions concerning the extrapolation of the experimental curve, the estimation of the obtained resolution of electron density as well as the possibilities of this method are also considered.

Introduction

Over a period of some years different authors have analysed the angle distribution of intensity of small-angle X-ray diffuse scattering by cylindrical unoriented particles. From some peculiarities of the scattering curve a number of methods of treating small-angle X-ray data were suggested to obtain the main parameters characterizing the cylinder. The most widely known methods are Kratky's method (Kratky & Porod, 1948; Porod, 1948) based on the use of a 'point' collimation and Luzzati's method (Luzzati, 1960; Luzzati, Mathis, Masson & Witz, 1964) which suggests the existence of an 'infinitely' high collimation slit. Both techniques analyse the same section of the scattering curve by a long rigid particle and give similar information on its structure (the electronic radius of gyration of the cylinder with respect to its axis R_q). Kratky's method consists of rearranging the experimental curve into coordinates of $\log [I(\theta)\theta]$ *vs* θ^2 ($I(\theta)$ being the scattering intensity at an angle of 2θ) having, as was shown, a linear dependence in some region of the θ^2 change, and in finding R_q from the slope of this curve. Luzzati's method is based on superposing the experimental curve plotted in a double logarithmic scale on a set of theoretical curves calculated (with some approximations) for long rigid uniform cylinders, the same parameter, R_q, being determined by the coincidence of the curves.

Besides these two well-known methods for study of the structure of unorientated cylindrical particles (macromolecules) it is necessary to mention a simple method suggested by Fedorov & Ptitsyn (1963) for determining the diameter of the cross section of a uniform cylinder by the position of a maximum of the dependence of $I(\theta)\theta^2$ *vs* θ, as well as Kirste's (1964) method which, within the framework of a concrete model of a non-uniform cylinder, allows the evaluation of two of its parameters. In the latter work a form factor of the particle cross section is replaced by the form factor of a uniform sphere surrounded by a vacuum spherical layer. The technique suggested permits the determination of R_q and the relation of the diameter of the sphere with a shell to the diameter of the sphere itself. Of course, this model may have a rather limited application.

If we add to the method mentioned above the usual

'standard curve' method, according to which the diameter of a uniform cylinder is found from the coincidence of the experimental curve with one of the theoretical ones calculated for different diameters, the list of methods employed for treating the curves of small-angle scattering by unoriented cylinders will be exhausted. As can be seen, neither of them give sufficiently detailed information on the electron density distribution within the particle, limiting themselves at best to the determination of the R_q value.

In 1965 the author in collaboration with V. G. Aleshin (Fedorov & Aleshin, 1966) suggested a theory of X-ray diffuse scattering by non-uniform long cylinders with an arbitrary but axially symmetrical electron density distribution function within the particle. On the basis of this theory a method of calculation of the mentioned distribution function using the Hankel transform for the whole experimental scattering curve was developed.

In a recent work by Carlson & Schmidt (1969) this method was subjected to checking for various test functions of the electron density distribution and its effectiveness was demonstrated for the case where the used section of the intensity scattering curve possesses a sufficient length.

A slightly improved method of the Hankel transform was used in the present work for study of the internal structure of tobacco mosaic virus (TMV), and its additional analysis was carried out to check the reliability and accuracy of the data obtained.

The scattering function and the diffuse scattering amplitude

As was shown in Fedorov & Aleshin's (1966) work, for an axially symmetrical cylinder of height b and a radius of a complete fall in electron density a under the limitations imposed on the height of the cylinder $a^2/b^2 \ll 1$ and on scattering angles $\mu b \gg 1$ ($\mu = 4\pi \sin\theta/\lambda$, where λ is the X-ray wavelength and 2θ is the angle of scattering) the following expression for the relative intensity of diffuse scattering $P(\mu)$ is valid:

$$P(\mu) = \frac{4\pi^3}{\mu b m^2}\left[\int_0^\infty \varrho(r) J_0(\mu r) r\mathrm{d}r\right]^2 . \qquad (1)$$

Here $\varrho(r)$ is the radial electron density distribution function of the cylinder, $J_0(\mu r)$ is the Bessel function of the zero order, while $m = 2\pi\int_0^\infty \varrho(r) r\mathrm{d}r$ is the linear electron density of the cylinder.

Expression (1) in the work by Fedorov & Aleshin (1966) is calculated with the aid of Debye's (1927) formula. However, it can be easily derived also from the general theory of X-ray diffraction by a cylinder. Actually, an infinitely long cylinder with the electron density distribution function $\varrho(r)$ can be described, in an inverse space, by only the zero layer line, the scattering amplitude along which (see Vainshtein, 1963) is:

$$F(R) = 2\pi\int_0^\infty \varrho(r) J_0(2\pi R r) r\mathrm{d}r . \qquad (2)$$

$R = 2\sin\theta/\lambda$ is the cylindrical coordinate of the inverse space (corresponding to the coordinate r of the real space), while the intensity of scattering $I(R) = F^2(R)$. Upon arbitrary 'twisting' of the cylinder in relation to the X-ray primary beam the diffraction pattern in the inverse space is smeared so that the intensity $2\pi R I(R)\mathrm{d}R$ included in the layer line, in a ring of width $\mathrm{d}R$ at a distance R from the origin of the inverse space, is uniformly distributed in a circular layer $4\pi R^2\mathrm{d}R$ of the same width. As a result, the intensity of scattering at a distance R will be $I(R)/2R$, or, in terms $\mu = 2\pi R$,

$$I(\mu) = \frac{4\pi^3}{\mu}\left[\int_0^\infty \varrho(r) J_0(\mu r) r\mathrm{d}r\right]^2 \qquad (3)$$

which, with an accuracy of the normalizing factor $1/bm$ leading to relative values of the scattering intensity, agrees with formula (1).

It first follows from expressions (1) and (3) that both the intensity of scattering by a non-uniform cylinder, with restrictions imposed on its height, and the scattering angles can be represented as a square of some inte-

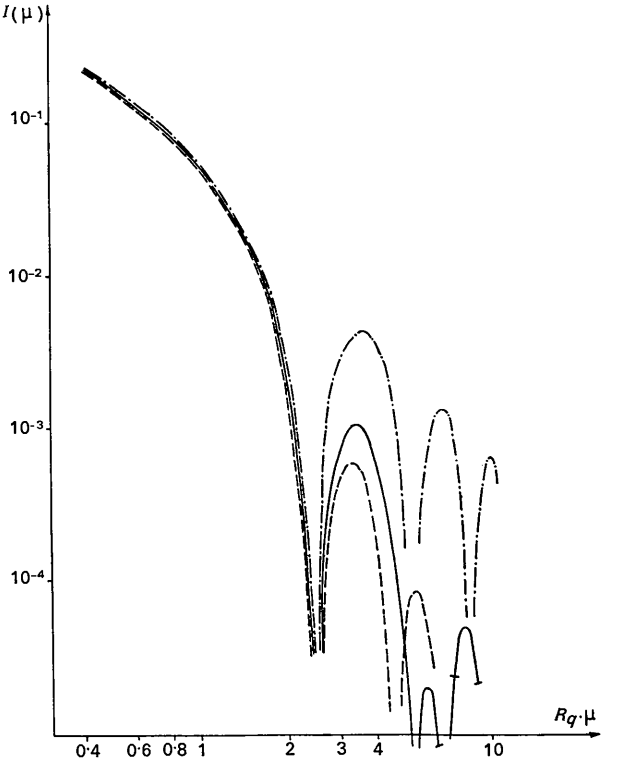

Fig. 1. Theoretical curves of scattering by cylinders with different $\varrho(r)$ function: ----- uniform cylinder; ——— cylinder with a 'hole' (points at which the curve breaks during the study of the error of Hankel transform are marked – see Fig. 6); · · · · hollow cylinder.

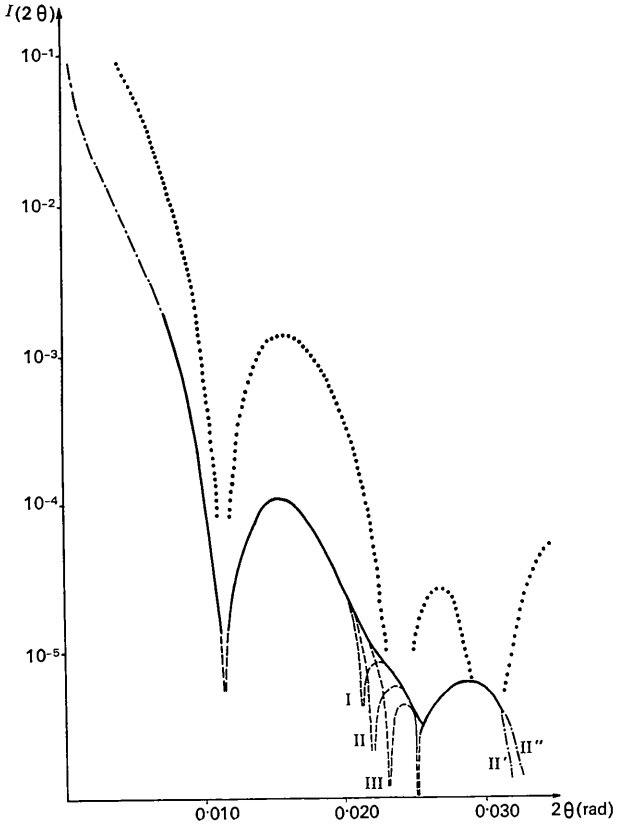

Fig. 2. Scattering curve of TMV according to Malmon's (1957) data. ——— experimental section; -·· · extrapolation section; ----- correction of the experimental curve; ····· theoretical curve of scattering by a cylinder with a 'hole' reconstructed into the corresponding coordinates.

gral depending on $\varrho(r)$. By analogy with X-ray analysis let us term the function

$$A(\mu)= \pm \sqrt{I(\mu)} = 2\pi \sqrt{\pi/\mu} \int_0^\infty \varrho(r) J_0(\mu r) r dr \qquad (4)$$

a diffuse scattering amplitude. We should note in this case that the analogy mentioned is, generally speaking, purely external, since the $A(\mu)$ value determined in such a manner is not a sum of electromagnetic waves scattered by all elements of the object (which would be natural under normal physical determination of the scattering amplitude) but represents a square root of the sum of all the intensities of scattering equidistant from the origin in the inverse space. Nevertheless, such a formal determination appears to be rather convenient inasmuch as the $A(\mu)$ value is fully analogous to the classical scattering amplitude $F(R)$ in terms of mathematical formalism.

A special feature of the diffuse scattering amplitude introduced above is that it represents, like $F(R)$, a real value and reduces the scattering intensity to zero at points of sign alteration. It is true that the ability of the scattering intensity to be transformed into zero at certain μ values is characteristic of only infinitely long and rigid cylinders. Generally speaking equation (1) is inapplicable for cylinders of restricted length, and there are no points of contact $I(\mu)$ with the x axis. However, from general physical considerations, it can be predicted in the first place, that for increasing μ, the minima of the function, showing the intensity of scattering by restricted cylinders, approach the μ axis (the influence of the ends is less felt). Secondly, every given minimum must experience the same tendency with the increase in the length of the cylinder.

Both these properties are distinctly noticeable in the graphs of the curves of scattering by uniform cylinders of finite length calculated numerically at different ratios V of the height to the diameter (Malmon, 1957). For example, at $V=3$ the ordinates of the first and second minima differ by an order ($I(0)\times 10^{-3}$ and $I(0)\times 10^{-4}$, correspondingly). On the other hand, the ordinate of the first maximum of the cylinder with $V=10$ [$I(0)\times 10^{-4}$] also decreases by an order in comparison with the cylinder where $V=3$.

Using the Hankel transform in equation (4)

$$\varrho(r)= \frac{1}{2\pi\sqrt{\pi}} \int_0^\infty A(\mu)\sqrt{\mu} J_0(\mu r)\mu d\mu \qquad (5)$$

we obtain a direct relation between the electron density distribution and the real diffuse scattering amplitude $A(\mu)$ the knowledge of which (*i.e.* the knowledge of the order of sign alteration upon the extraction of the root from the experimental intensity of scattering by long non-uniform cylinders) makes possible the numerical calculation of the desirable function $\varrho(r)$. Evidently, the most essential moment in passing from the experimental scattering curve to $A(\mu)$ is the correct extraction of roots $I(\mu)$ (*i.e.* the points of contact $I(\mu)$ with the μ axis), since it is they that correspond to the region of sign alteration of the diffuse scattering amplitude. The accuracy of the construction of $A(\mu)$ and, consequently, also the calculation accuracy of the $\varrho(r)$ value are mainly responsible for the reliability of identification of the given points and the possibility of their exact separation from other features of the scattering curve.

In the work by Carlson & Schmidt (1969) two kinds of electron density distribution functions [$\varrho_1(r)= \exp(-r/R)$ and $\varrho_2(r)=\exp(-r^2/R^2)$] are given, for which the diffuse scattering amplitude does not change the sign at all and remains positive at all μ values. However, a class of functions with the given property is, probably, small since even a slight variation of the $\varrho_1(r)$ and $\varrho_2(r)$ functions leads to the sign alteration of the $A(\mu)$ value. In fact, even for functions $\varrho^1(r)= r^n \exp(-r/R)$ and $\varrho_2'(r)=r^n \exp(-r^2/R^2)$ the diffuse scattering amplitudes will cause a sign change:

$$A_1(\mu) \simeq \frac{1}{\sqrt{\mu}} \int_0^\infty \exp\{-r/R\} J_0(\mu r) r^{n+1} dr$$

$$= \frac{(n+1)!}{\sqrt{\mu}} \left(\frac{1}{R^2}+\mu^2\right)^{-(n+2)/2} P_{n+1}(1/\sqrt{1+R^2\mu^2})$$

and

$$A_2(\mu) \simeq \frac{1}{\sqrt{\mu}} \int_0^\infty \exp\{-r^2/R^2\} J_0(\mu r) r^{n+1} \mathrm{d}r$$
$$= \frac{1}{2\sqrt{\mu}} R^{n+2} \Gamma\left(\frac{n}{2}+1\right) \exp\{-\mu^2 R^2/4\}$$
$$\times \Phi\left(-\frac{n}{2}; 1; \frac{\mu^2 R^2}{4}\right)$$

where $P_{n+1}(x)$ are Legendre polynomials ('sign-changing' functions at $n>0$), while $\Phi(\alpha;1;x)$ is the confluent hypergeometrical function also possessing zeros at $\alpha<0$. It should be noted that this conclusion is not connected with the appearance of the 'hole' in electron density distribution at $r=0$, since the substitution of $(1+r)^n$ for r^n in functions $\varrho_1'(r)$ and $\varrho_2'(r)$ qualitatively maintains the same result. Thus, the problem of identification of the sign of the $A(\mu)$ value evidently requires, as a rule, a thorough analysis.

Extrapolation of the experimental curve of scattering by a cylinder into the region of smallest angles

For improving the resolution ability of the method let us now consider the extrapolation of the curve of scattering by a cylinder into a non-experimental region of angles. It is clear that a simple break of the experimental curve can be taken as an assumption that scattering intensity of the curve is equal to zero both from its left and from its right. It is also obvious that such an assumption is one of the worst and even a rough extrapolation of the curve would be rather essential. Therefore, let us now attempt to extend the ends of the curve, building up $I(\mu)$ in some physically justified manner.

In this respect, the extrapolation into the region of the smallest angles is more reliable. If the experimental index of scattering has on its left side a section of the curve proportional to μ^{-1}, the extrapolation in this case is extremely simple and consists in extending the experimental curve according to the mentioned law up to $\mu=0$. However, it may appear that even at the smallest experimentally obtainable scattering angles the curve 'feels' the cross-section dimensions of the cylinder and, as a result, the index of scattering is not proportional to μ^{-1} (μ^{-1} is the law of scattering for an infinitely long one-dimensional rod). Let us consider for this case another extrapolation method based on the fact that the section of the scattering curve proportional to μ^{-1} is followed by a sufficiently extended region of the curve depending only on the R_q value. Here, the intensity of scattering is found to be a function of $R_q\mu$. It is clear that indices of scattering by cylinders of different internal structure but of the same R_q value coincide in the given section of the curve. On the other hand, taking the intensities of scattering by cylinders with any $\varrho(r)$ and R_q values in a double logarithmic scale ($\log I(\mu)$ *vs* $\log(R_q\mu)$ we should expect a significantly similar course of the curves at the smallest angles, including also the above mentioned region.

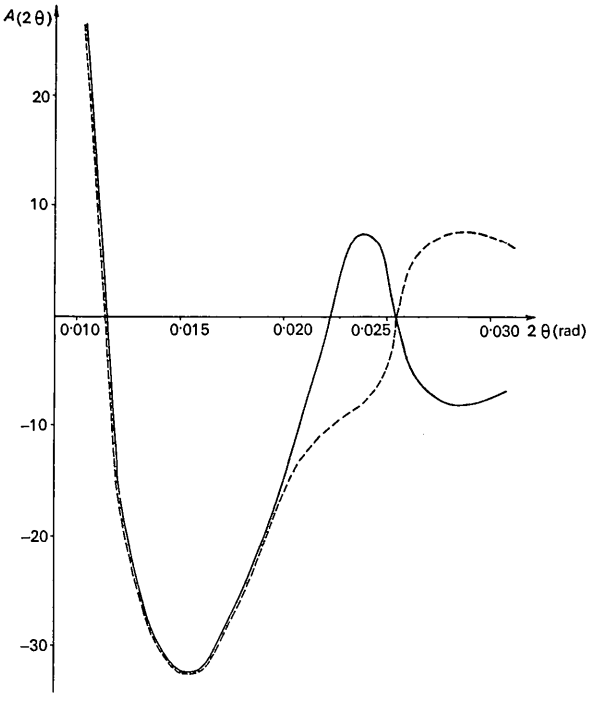

Fig. 3. Diffuse scattering amplitudes calculated on the basis of the TMV experimental scattering curve (Malmon, 1957) with the consideration of two (broken line) and three (solid line) points of contact $2(\theta)$ with the 2θ axis.

The relative values of R_q quantities as well as their absolute values (in the case when one of the R_q values is known) can be easily calculated by a shift of the curves along the $\log\mu$ axis. On the basis of this, the following procedure of extrapolation of the experimental curve, which contains a section depending only on R_q, into the region of the smallest angles of scattering can be suggested. Having plotted the theoretical curve of scattering by any infinitely long cylinder in a double logarithmic scale in a wide range of angles, it should be superposed in the best possible manner on the left part of the considered experimental curve also plotted in a double logarithmic scale. In this case the section of the theoretical curve positioned on the left from the superposed part of the curve is extrapolated for the experimental index of scattering. Here, from the shift of the curves along the $\log I(\mu)$ axis a 'cross-link' constant can be easily determined. It should be noted that although the distribution of $\varrho(r)$ can generally be chosen arbitrarily in calculating the theoretical curve, the closer it is to the electron density distribution in the cylinder studied, the wider the region of superposing of the curves and the more accurate the extrapolation.

As an example of the cited extrapolation procedure let us consider three curves of scattering by cylinders calculated by formula (1) with the following $\varrho(r)$ values:

$$\varrho_1(r)=\begin{cases}1 & r<1\\ 0 & r>0\end{cases}\qquad\text{(uniform cylinder)}$$

$$\varrho_2(r)=\begin{cases}0 & r<0{\cdot}25\\ 1 & 0{\cdot}25<r<1\\ 0 & r>1\end{cases}\qquad\text{(cylinder with a hole)}$$

$$\varrho_3(r)=\delta(1-r)\qquad\text{(hollow cylinder)}.$$

These cylinders possess a sharply different character of radial electron density distribution, which must lead to a noticeable difference in the scattering curves. However, as is seen from Fig. 1, in which the indicated curves are presented, variations start only from the first side maximum. Thus, the extrapolation into the region of small angles by the method cited is possible if the experimental curve contains, at least, a small section on the left from the first minimum.

Calculation of TMV electron density

Let us make use of the developed method of $\varrho(r)$ calculation for study of the internal structure of tobacco mosaic virus. This virus meets necessary requirements of the theory (rigidity and a sufficient length of cylinders) and possesses a sharply non-uniform electron density distribution in the cross section, which makes it quite convenient for the analysis. Besides, TMV has been thoroughly studied by other methods, including X-ray analysis and its structure is common knowledge.

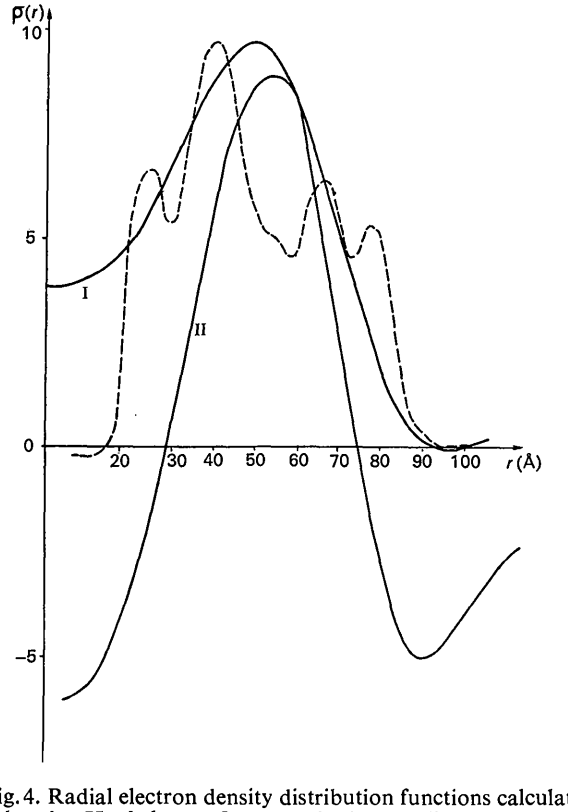

Fig. 4. Radial electron density distribution functions calculated by the Hankel transform method according to the data shown in Fig. 2 with the consideration of only two points of contact $I(\theta)$ with the 2θ axis. Curve I – calculation on the the basis of experimental section and section extrapolated into the region of smallest angles of the curve in Fig. 2; Curve II – calculation on the basis of experimental section of the curve in Fig. 2. ----- $\varrho(r)$ function for TMV according to Caspar's (1956) data.

In a number of works the study of TMV was also carried out by small-angle X-ray diffuse scattering. In the work by Kratky, Paletta, Porod & Strohmaier (1957), the radius of gyration of the TMV cross section (59·6 Å) was obtained on the basis of the mentioned above 'Kratky's method'. This evidence was in good agreement with Caspar's (1956) data (59 Å) obtained by a direct method of X-ray analysis. At the same time, an attempt to find by the scattering curve whether the virus is a solid or hollow cylinder was not then successful. Malmon (1957) employed an incomparably more perfect experimental scattering curve of TMV on which two distinct maxima were observed (see Fig. 2, solid line) and treated it from the viewpoint of the theory of diffuse scattering by uniform non-oriented cylinders. The position of the first (left) maximum corresponds to a TMV radius of ~82 Å agreeing well with Caspar's (1956) data (84 Å), however, the abscissa of the second maximum gives a radius of ~70 Å. The author reasonably explains this difference which obviously exceeds the experimental error by a non-uniform character of electron density distribution within the virus. It was clear that the experimental curve has a greater stock of information on the internal structure of the virus, but owing to the lack of an appropriate theory it was not then possible to obtain such information.

In the calculations given in the present communication we shall make use of the same scattering curve of TMV, the working graph of which was kindly sent to us by Dr Malmon. This curve (solid line) together with the extrapolation section (dash-and-dot line on the left from the solid one) which was plotted according to the procedure cited in the preceding section is demonstrated in Fig. 2 (a uniform cylinder was taken as a 'standard' cylinder for obtaining the theoretical scattering curve).

It is necessary now to solve the main task, *i.e.* to determine the points of contact $I(\mu)$ with μ axis. At first glance the solution seems to be obvious: two sharp minima, being most likely the desired points, are observed on the scattering curve. They must only be slightly corrected (broken line in the region of 0·011 and 0·025 rad, Fig. 2). After this, the plotting of the diffuse scattering amplitude (broken line, Fig. 3) as well as the calculation of the $\varrho(r)$ function by formula (2) with the aid of a computer (curve I, Fig. 4) present no special problems. For convenience in comparison, Fig. 4 gives the TMV radial electron density distribution curve calculated by Caspar (1956) by means of direct methods of X-ray analysis (broken line). We see that the obtained $\varrho(r)$ function describes quite satisfactorily the TMV internal structure, namely: there is a noticeable decrease in electron density at low r values, the maximum of electron density is relatively close to the true one and, finally,

there is a good coincidence of external dimensions of the cylinders. Opportunely, it should be noted that such a result is determined, in the main, by the successful extrapolation of the scattering curve into the region of the smallest angles. In fact, if we restrict ourselves in the calculation only to the experimental section of the considered scattering curve of TMV, the coincidence will be substantially worse (curve II, Fig. 4).

In spite of the quite fair agreement of the calculated function with the true one, a more detailed analysis shows that the diffuse scattering amplitude is most likely plotted incorrectly. Even the shape of experimental scattering intensity (Fig. 2) may raise some doubts as to the correct consideration of only two points of contact $I(\mu)$ with the axis: in the region of angles from 0·020 to 0·025 rad we distinctly notice a 'shoulder' which can be interpreted as an unresolved maximum on the left from which there is still one more zero of the $I(2\theta)$ function. However, we should come more definitely to such a conclusion on the basis of the intensity of scattering by a cylinder, calculated by formula (1) and represented in Fig. 2 by the dotted line. The known size of 'hole' in TMV is used in the calculation as the 'hole' size of the cylinder. We see that between the first and third side maxima on this curve there exists a relatively small second maximum which is slightly shifted with respect to the position of the 'shoulder' on the TMV scattering curve. If we take into account that the index of scattering by such a cylinder with 'hole' is qualitatively close to the index of TMV scattering, the existence of one more maximum in the region of the 'shoulder' on the TMV scattering curve seems quite probable.

For the calculation of the new diffuse scattering amplitude it is necessary to 'correct' preliminarily in an appropriate manner the experimental scattering intensity. Since this procedure cannot be conducted unequivocally, we considered several means for modification of the curve $I(2\theta)$ (curves I to III, Fig. 2) and for every curve we plotted the $A(2\theta)$ value and calculated the $\varrho(r)$ function. Fig. 3 (solid line) represents one of the diffuse scattering curves obtained in such a manner (corresponding to curve II, Fig. 2), while Fig. 5 (curves I to III) gives the desired radial electron density distribution functions.

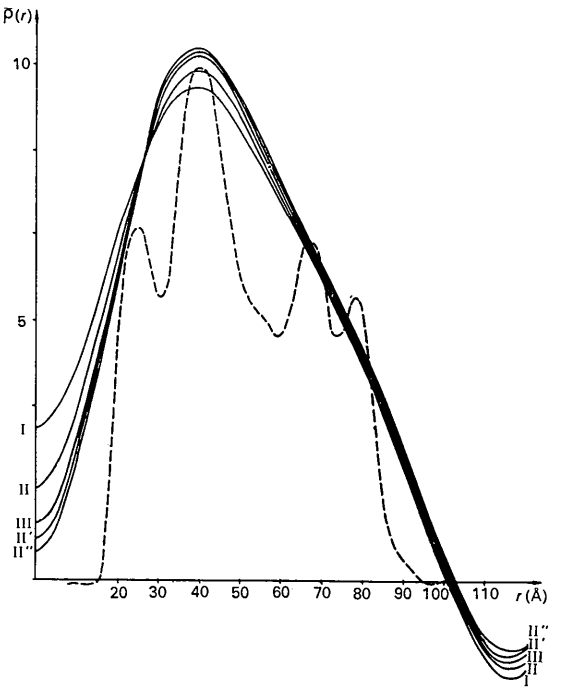

Fig. 5. Radial electron density distribution function calculated by the Hankel transform method according to data given in Fig. 2 with the consideration of three points of contact $I(2\theta)$ with the 2θ axis and extrapolated (into the region of the smallest angles) section of the scattering curve. Curves I to III (corresponding to curves I to III in Fig. 2) – different means of correction of the experimental curve in the region from 0·020 to 0·025 rad; curves II′ and II″ correspond to curve II in Fig. 2 and take into account its extrapolation into the region of larger angles as shown in Fig. 2 (lines II′ and II″). ----- $\varrho(r)$ function for TMV according to Caspar's (1956) data.

Discussion

Undoubtedly, curves I to III shown in Fig. 5 give a better description of electron density distribution in TMV than curve I in Fig. 4. Maxima of the true and calculated functions of $\varrho(r)$ fully coincide and a sharp fall in electron density at low r values is a clear indication of the existence of the 'hole' inside the cylinder. It can be said that the curves thus obtained are a kind of envelope line of the complex true electron density function. It can be also predicted that with the experimental TMV scattering curve being more extended towards large angles, such elements of the TMV structure as a minimum of electron density in the region of 60 Å and, probably, of 30 Å, would resolve. And though recently developed modern experimental technique would permit us at present to obtain more perfect and elongated indices of TMV scattering, nevertheless, if the repolymerized RNA-free TMV was studied, even the given resolution would be enough for its reliable distinction from RNA-containing TMV.

It is seen from Fig. 5 that the curves differing in the manner of 'correction' $I(2\theta)$ are very close to each other, but at the same time differ essentially from the earlier obtained $\varrho(r)$ function (*cf.* Fig. 4). A conclusion can be drawn that the $\varrho(r)$ function is rather sensitive to the number of maxima and minima on the diffuse scattering amplitude and has a relatively slight dependence on the localization of at least one zero of the $A(2\theta)$ value.

As is known, the methods based on the transformation of the whole curve of scattering, though using practically only a part of it, may in principle, lead to errors and even artifacts. The work of Carlson & Schmidt (1969) is devoted to the study of this question as applied to the Hankel transform (for the case of three kinds of the $\varrho(r)$ function). We shall dwell on this

problem only for the estimation of our results. For this purpose let us consider the theoretical curve of scattering by a cylinder with a 'hole' (solid line, Fig. 1) and, breaking the curve at different scattering angles, transform it by formula (2). As is seen from Fig. 6, in which the results of such a 'reverse' calculation are presented, curve III obtained from the theoretical index of scattering with three side maxima satisfactorily describes the initial function. If it is taken into consideration that we made use of just these three side maxima in calculating the TMV electron density shown in Fig. 5 and that the electron density profile of the considered cylinder with a 'hole' must be relatively close to $\varrho(r)$ of TMV, then the conclusion can be drawn that resolution of the curves in Fig. 5 approximately corresponds to curve III shown in Fig. 6. Thus, the occurrence of the artifact is completely excluded in this case. Moreover, a considerable decrease in the $\varrho(r)$ value for curves I and II (Fig. 6) also at low r is not an artifact and qualitatively reflects the electron density distribution though corresponds to a still lesser resolution.

If the extrapolation of the scattering intensity in a small-angle region is a comparatively easy problem, the extension of the experimental curve by 'larger small' angles runs into a principal difficulty - the complete absence of information on the character of behaviour of $I(\mu)$ in this region. Nevertheless, extrapolation in one form or another is needed, since, as was mentioned, simply a break of the curve is the worst way to get out of a difficulty. Therefore, we restricted ourselves to rather arbitrary extrapolation of the curve into the region of $\mu > \mu_{max}$ only up to the intersection of $I(\mu)$ with the abscissa axis. Fig. 2 represents two different examples of extrapolating the curve into the region of larger angles (dash-and-dot lines II′ and II″ on the right from the solid line). These curves (also taking into account the extrapolation into the region of the smallest angles) correspond to curves II′ and II″ in Fig. 5 transformed on $\varrho(r)$. For the remaining curves the large angle extrapolation has not been carried out. As is seen, the mere fact of extrapolation as well as its manner has a negligible influence in our case on the result obtained. However, it can be shown that the mentioned extrapolation plays a more essential role for the first of the considered cases of plotting $\varrho(r)$ where only two points of contact $I(\mu)$ of the experimental curve with the 2θ axis were taken into consideration.

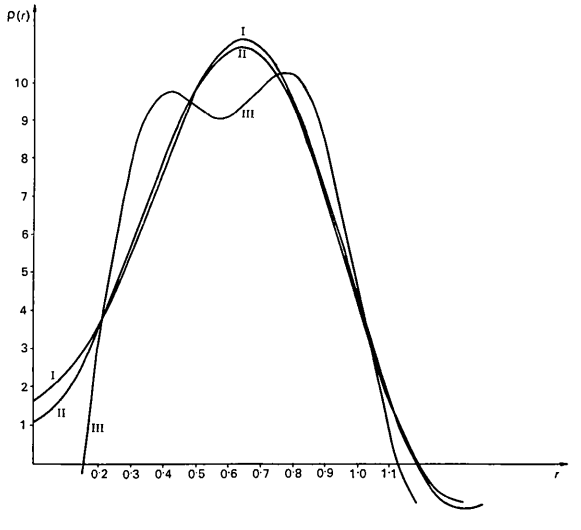

Fig. 6. Calculation of $\varrho(r)$ function for the cylinder with a 'hole' on the basis of the solid curve in Fig. 1 at different angles μ_{max} of its break: $\mu_{max}=0{\cdot}11$ Å^{-1} (curve I); $\mu_{max}=0{\cdot}125$ Å^{-1} (curve II); $\mu_{max}=0{\cdot}150$ Å^{-1} (curve III).

The basic formula (1) was derived on the condition that the electron density of the cylinder was independent of the shift along its axis. Practically however, the particles approximated by cylinders are, as a rule, rather complex helices and the usage of the present method for such helices is determined by the ratio of the step of a helix and its diameter. If the helix is relatively compressed, the layer lines corresponding to it in the inverse space are found to be sufficiently spaced from each other so that the intensity of the first layer line would not make a contribution to the experimental intensity of small-angle scattering. This is so in the case of TMV. The first 'strong' layer line responsible for a TMV helix coil with a step of $l=23$ Å is located at an angle distance of $2\theta=\lambda/l=0{\cdot}067$ rad. This makes it possible to extend (in comparison with the curve in Fig. 2) the experimental region of TMV scattering angles without fear of introducing the intensities of the non-zero layer lines into the index of scattering.

Another limitation of formula (1) is that the axial symmetry of electron density is needed. If there is no such symmetry, the scattering amplitude along the zero layer line will be determined by Bessel functions of all orders, but if the cylinder has a circular symmetry of the N order, the amplitude will be determined by the Bessel function of the nN order. However, the contribution of the Bessel functions of high orders to the scattering intensity is small, and for the cylinder with a sufficiently high axial symmetry formula (1) may be considered to be valid.

Until the present time a technique comparable to that described in this communication was used in small-angle X-ray diffuse scattering as applied only to spheres. In recent years a number of works have been carried out (also on the basis of the Fourier transform of the scattering amplitude) in which a spherically symmetrical electron density distribution function for a series of spherical viruses (Anderegg, 1967) and apoferritin (Fischbach & Anderegg, 1965; Bielig, Kratky, Rohns & Wawra, 1966) was obtained. In the mentioned cases the application of this method proved very successful, the study of apoferritin being conducted independently by the two groups of authors, and led to similar results.

The development of a similar method for long cylinders was made possible, in the sense that in this case as well as in the case of spheres it was possible to express the scattering intensity as a square of some function which we denoted as a diffuse scattering amplitude. As

is known, functions of scattering by other simple geometrical bodies which are widely used for modelling different structures (oblate and prolate ellipsoids of revolution, discs and other short cylinders) have not been given a proper representation and, therefore, the development of an analogous technique for them is apparently impossible.

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Bloch Wave Notation in Many-Beam Electron Diffraction Theory

BY C. J. HUMPHREYS* AND R. M. FISHER

Edgar C. Bain Laboratory for Fundamental Research, United States Steel Corporation Research Center, Monroeville, Pennsylvania, U.S.A.

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There are a number of different Bloch wave labelling systems currently in use in the theory of the diffraction of electrons by crystals. It is suggested that the Bloch wave labelling scheme for electron diffraction which is the simplest and the most logical is an ordered labelling scheme in which the top branch of the dispersion surface corresponds to wave 1, the second branch to wave 2, the third branch to wave 3, and so on. Such a scheme would be consistent with accepted notations in other forms of Bloch wave propagation. The essential mathematical unity of all forms of wave propagation in crystals is discussed, and the use of the proposed notation in describing the critical voltage effect is briefly considered.

1. Introduction

In recent years it has become increasingly clear that the use of a many-beam theory of electron diffraction is essential for the quantitative interpretation of electron micrographs of crystals taken using conventional 100 kV microscopes, and for both qualitative and quantitative interpretation of micrographs taken using higher voltage instruments. However, no satisfactory labelling scheme has been established for identifying the various Bloch waves which represent the fast electron within the crystal. A variety of such schemes exist in the literature, and the purpose of this paper is to examine the situation and to propose a simple but unambiguous method of referring to the individual Bloch waves which it is suggested might be generally adopted. It is also unfortunately the case that the theory of electron diffraction has developed largely independently from that of, for example, lattice vibrations and band theory. In this paper the essential unity of all forms of Bloch wave propagation in crystals will be emphasized, and the proposed notation will be chosen to be consistent with standard notations in related fields.

2. Definition of the problem

Consider an electron incident upon a perfect crystal. The wave function of the electron within the crystal

* On leave from the Department of Metallurgy, University of Oxford, Oxford, England.