

Accuracy of Determination of Position and Width of Molecular Groups in Biological and Lipid Membranes *via* Neutron Diffraction

V. I. GORDELIY^{a,b,†} AND N. I. CHERNOV^c

^aIBI-2, Structural Biology, Forschungszentrum Jülich, D-52425, Jülich, Germany, ^bLarge Scale Structure Group, Institute Laue-Langevin, BP 156, 38042 Grenoble CEDEX 9, France, and ^cDepartment of Mathematics, University of Alabama at Birmingham, AL 35294, USA. E-mail: valentin@ibistr.dnet.kfa-juelich.de

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Abstract

Neutron diffraction combined with the deuterium-labelled molecular groups of biological and model membrane components allows one to detect with high accuracy the structure of these objects. Experiments of this kind are only possible at unique high-flux neutron sources, and the planning of neutron-diffraction experiments must take into account some special requirements primarily related to the duration of the experiment and the accuracy of estimation of membrane structure parameters as a result of finite time of the measurements. This paper deals with the question of statistical accuracy of the position x_0 and width ν of the distribution of deuterium labels in membranes along the normal of their plane, which are determined in a neutron diffraction experiment. It is shown that the accuracy of x_0 and ν estimation does not depend on membrane constitution. It is dependent only on the scattering amplitude of the deuterium label, the label position x_0 and the distribution width ν . Analytic calculations show that the statistical errors Δx_0 and $\Delta \nu$ are inversely proportional to the scattering amplitude of the label and, as usual, to the square root of measurement time. The question of Δx_0 and $\Delta \nu$ dependence on the number of structure factors used in the calculations of x_0 and ν is also studied. It is shown that, the accuracy of x_0 estimation is approximately constant with down to four structure factors used, and, with the number of the factors below four, it deteriorates drastically. Analogous is the behaviour of $\Delta \nu(h_{\max})$ relation with one exception: abrupt deterioration of the accuracy occurs beginning with five structure factors used. One does not have to measure the highest diffraction reflections which takes a much longer time compared with the first ones. It is an important result. All the problems mentioned above have also been considered for the case of two different deuterium labels in membranes.

[†]Permanent address: Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, 141980 Dubna, Moscow District, Russia.

1. Introduction

The efficiency of neutron diffraction in the location of the membrane components and molecular groups along the normal of membrane plane was first demonstrated in 1975 (Zaccai, Blasie & Schoenborn, 1975). This method resulted in the first direct determination of water distribution in membranes (Zaccai *et al.*, 1975). With progress in the technique of labelling membrane components by deuterium it became possible to determine the position in membranes of such molecules as cholesterol, anaesthetics, hexane, *etc.* (Worcester & Franks, 1976; Balgavy, Gordeliy & Syrykh, 1991; King, Chao & White, 1984; King & White, 1986; Wiener & White, 1991*a,b*, 1992).

Electron-density profiles which can be constructed from X-ray diffraction data usually determine membrane structure with canonical resolution $d/h_{\max} \simeq 5\text{--}10 \text{ \AA}$ (d is the repeat distance of multilayer membrane stack and h_{\max} is the number of measured diffraction orders). Only in some special cases (for instance, in determining the position of membrane boundary) one can achieve better accuracy in estimating membrane structure parameters. To be able to do so some assumptions are usually made (McIntosh & Simon, 1987).

Small-angle neutron scattering with single membranes allows one to determine the position of molecular group with the accuracy of $\sim 1 \text{ \AA}$. However, it is only true for very localized labels (Gordeliy, Golubchikova, Kuklin, Syrykh & Watts, 1993). This approach does not allow one to reach conclusions about the width of the label distribution.

Some treatments of electron or neutron scattering density profiles (for instance, *via* a step-function model) can be used for the calculation of membrane structure parameters only within a certain limit (King & White, 1986; Gordeliy & Kiselev, 1995). But even in the best case they give the accuracy no better than $\sim 1 \text{ \AA}$.

Among these approaches neutron diffraction remains the most powerful technique to give a precise membrane structure determination.

One of the most impressive applications of neutron diffraction is the location of almost all the molecular groups in membranes of dipalmitoylphosphatidylcholine (DPPC) with the accuracy higher than 1 Å (Büldt, Gally, Seelig & Zaccai, 1979; Zaccai, Büldt, Seelig & Seelig, 1979). In the papers of Büldt *et al.* (1979), Zaccai *et al.* (1979) the Gaussian distribution was assumed to describe deuterium labels. The whys and wherefores of this approach were considered later (Wiener & White, 1991).

Since the structure of lipid membranes is usually symmetric, one can present its density as

$$\rho_D(x) = t_N \left\{ \exp \left[-\frac{(x - x_0)^2}{\nu^2} \right] + \exp \left[-\frac{(x + x_0)^2}{\nu^2} \right] \right\}, \quad (1)$$

where x_0 is the average position and ν is the width of deuterium label distribution. The amplitude t_N of the distribution (1) deduced from the condition of normalization,

$$\int_{-d/2}^{+d/2} \rho_D(x) dx = 2n(b_D - b_H)/S, \quad (2)$$

is equal to

$$t_N = \frac{n(b_D - b_H)}{\pi^{1/2}\nu S}, \quad (3)$$

where n is the number of D atoms in the label, b_D and b_H are the neutron scattering lengths of deuterium and hydrogen nuclei, respectively, S is the area per lipid molecule in the bilayer surface.

The structure factors of this distribution are then equal to,

$$\begin{aligned} F_D(h) &= \int_{-d/2}^{d/2} \rho_D(x) \cos \frac{2\pi hx}{d} dx \\ &= 2\pi^{1/2}\nu t_N \exp \left(-\frac{\pi^2 \nu^2 h^2}{d^2} \right) \cos \frac{2\pi hx_0}{d}, \quad (4) \end{aligned}$$

where h is the number of the structure factor and d is the repeat distance of multilamellar membranes.

Measuring the structure factors $F_D(h)$ in the experiment one can estimate the label position x_0 and the label width ν via the least-squares method (Büldt *et al.*, 1979; Zaccai *et al.*, 1979).

So far the accuracy of estimating x_0 and ν in a neutron diffraction experiment has not been discussed. However, a number of fundamental problems concerning the structure and properties of membranes can be solved only on the basis of a sufficiently high accuracy of x_0 and ν estimation.

One of these problems is testing of alternative theories of the so-called hydration forces, *i.e.* short-range intermembrane repulsive forces dominating over

the distances of up to 30 Å (Rand & Parsegian, 1989; Israelachvili & Wennerström, 1992; Gordeliy, 1996).

Moreover, the question of accuracy of the x_0 and ν estimations is directly related to the planning of neutron diffraction experiments with membranes. Indeed, these experiments are possible, as a rule, only at unique neutron sources where the measurement time is very expensive.

In this paper we present an investigation of the statistic accuracy of x_0 and ν parameter estimation, its dependence on the position (x_0) and the distribution width of the label (ν), on the degree of deuteration (the number of D atoms in the label), on the type of membranes, on the number of structure factors used in data processing. The same problems are considered for the case of simultaneous presence of two deuterium labels in a membrane. The latter is important, because it reduces the experiment time by half compared with the case where two membranes with different labels are used. Besides, the presence of two labels in one membrane provides a chance to avoid systematic errors related to certain possible differences in the repeat period of two multilayer membranes with different labels (Büldt *et al.*, 1979; Zaccai *et al.*, 1979).

It is necessary to stress that the present paper concerns only statistical accuracy, *i.e.* that part of the errors in membrane structure determination, which is determined by the statistical accuracy of the integral intensities of diffraction reflections. The systematic errors which can occur because of sample or data treatment have not been considered in this paper. However, the investigation of statistical accuracy presented here, which is valuable in itself, gives also a possibility to estimate systematic errors in the neutron diffraction experiment with membranes. It will be discussed in the next paragraph.

It is well known that for the study of membrane structure neutron diffractometers are used both at stationary reactors such as, for instance, ILL, Grenoble (Büldt *et al.*, 1979; Zaccai *et al.*, 1979) and time-of-flight diffractometers at pulsed neutron sources as, for example, the pulsed reactor IBR-2 in JINR, Dubna (Gordeliy *et al.*, 1991). The problems mentioned above are considered for both types of experiments.

2. Model and calculation procedure

In the programme realization of the calculations of the accuracy in estimating the position and label distribution width the step-function model (SFM) was used for the modelling of the scattering amplitude density along the bilayer normal (Fig. 1). Fig. 1 is relevant to membranes of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC).

The deuterium label itself (or two of them) was modelled by equation (1) (or its generalization in the case of two labels).

Assuming that neutron diffraction measurements are carried out with various amounts of deuterated water in membranes, it is important to investigate how its concentration χ affects the accuracy of x_0 and ν estimation.

Let us define the structure factor of membranes by an analytic equation,

$$F(h) = \int_{-d/2}^{+d/2} [\rho_w(x) + \rho_B(x) + \rho_D(x)] \cos \frac{2\pi hx}{d} dx$$

$$= F_w(h) + F_B(h) + F_D(h), \quad (5)$$

where $\rho_w(x)$, $\rho_B(x)$, $\rho_D(x)$ are the neutron scattering densities for water, bilayer and label, respectively, and $F_w(x)$, $F_B(x)$, $F_D(x)$ are the corresponding structure factors.

It follows from (5) that,

$$F_w(h) = -\frac{d}{\pi h} [\rho_{\text{H}_2\text{O}}(1-x) + \rho_{\text{D}_2\text{O}}x] \sin\left(\frac{2\pi h Z_3}{d}\right), \quad (6)$$

where $\rho_{\text{H}_2\text{O}}$ and $\rho_{\text{D}_2\text{O}}$ are the scattering amplitude densities for the water and heavy water, respectively, x is the degree of heavy-water substitution for water ($x \in [0, 1]$), Z_3 is the coordinate of water/layer interface relative to the bilayer center.

In the same manner we get from (5) that,

$$F_B(h) = \frac{d}{\pi h} \sum_{i=1}^3 (\rho_i - \rho_{i+1}) \sin\left(\frac{2\pi h Z_i}{d}\right), \quad (7)$$

where ρ_i is the scattering amplitude density, and Z_i is the coordinate of the corresponding SFM parts for the bilayer (Fig. 1). To ensure (7) holds true one must suppose that ρ_4 equals zero.

To calculate the statistic accuracy of x_0 and ν estimation it is necessary to know the dependence of integral intensity $I(h)$ measured in the experiment of Bragg reflections on membrane structure factors $F(h)$. Besides, two cases should be considered. The first case is when in order to get diffraction pattern one uses monochromatic radiation ($\lambda = \lambda_0 = \text{const.}$, stationary reactor), and diffraction spectrum is obtained, for instance, *via* θ - 2θ scanning with the sample and detector over the scattering angle (Büldt *et al.*, 1979;

Zaccai *et al.*, 1979). And the second case is when polychromatic radiation is used [pulsed neutron sources, time-of-flight diffractometers (Gordeliy, Kiselev, Klose, König & Zweynert, 1991) with the stationary geometry of experiment: $\theta = \theta_0 = \text{const.}$]. Moreover, for each of these sets of experiments one has to consider two different types of $I(h)$ dependence on $F(h)$ for the two types of samples used in experiments: highly oriented and non-oriented membranes (Büldt *et al.*, 1979; Zaccai *et al.*, 1979). The corresponding expressions are given in Table 1.

Here $\varphi(\lambda)$ is the neutron flux on the sample, V is the volume of the sample, N is the number of elementary cells in a volume unit (in our case $N = 1/d$, *i.e.*, N is the number of membranes in a length unit), θ and θ_0 are the Bragg angles, τ is the measurement time.

To make calculations in the case of monochromatic neutrons the parameters of neutron diffractometer D16 in ILL, Grenoble, have been used, $\varphi_0 = 10^7$ neutron $\text{cm}^{-2} \text{s}^{-1}$ and $\lambda = 4.5 \text{ \AA}$, and in the case of polychromatic radiation the parameters [primarily the spectrum of neutrons falling on the sample $\varphi(\lambda)$] of neutron diffractometer DN-2 at the pulsed reactor IBR-2, Dubna (Gordeliy *et al.*, 1991) have been used, and the Bragg angle was $\theta_0 = 8^\circ$. Apart from that, the sample volume was set to 10^{-2} cm^3 , which corresponds to a sample mass of 10 milligrammes. The numerical values of ρ_i , Z_i parameters for SFM corresponded to those of DPPC membranes in the gel-phase (Büldt *et al.*, 1979; Zaccai *et al.*, 1979) with the repeat distance $d = 58 \text{ \AA}$.

The estimation of Δx_0 , $\Delta \nu$ parameter accuracies requires an adequate probabilistic model of the experiment. Our starting point is the assumption that the measured values of the integral intensities are distributed according to the Poisson law, whose averages $I(h)$ are presented in Table 1. The expressions for $I(h)$ show that $F(h)$ is always equal to $\gamma(h)I(h)^{1/2}$, where $\gamma(h)$ is a non-random factor.

Furthermore, we use the following fact: if y is a Poisson random value with parameter α , then $y^{1/2}$ has almost constant variance, approaching 0.25 as $\alpha \rightarrow \infty$. Although this fact is not a canonical one in the probability theory, it can be easily checked numerically or rigorously proved via the approximation of the Poisson law by a Gaussian one with parameters (α, α) .

Therefore, the variance of $F(h)$ is approximated by $\text{Var } F(h) \simeq 1/4\gamma(h)$. Since the value of $F_M(h)$ in (4) is measured by the difference between $F_1(h)$ and $F_2(h)$ in the observations of the samples with and without label respectively, the variance of $F_M(h)$ is,

$$\text{Var } F_M(h) = \text{Var } F_1(h) + \text{Var } F_2(h) = 1/2\gamma(h), \quad (8)$$

[Note that the factor $\gamma(h)$ is the same for both samples].

The estimation of parameters x_0 and ν *via* the least-squares method consists in the minimization of the function,

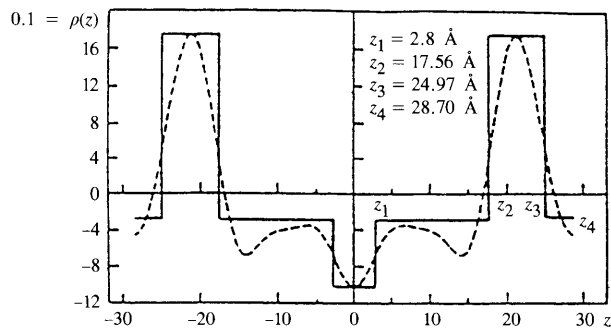


Fig. 1. The step function model of DPPC lipid membranes.

Table 1. *The relation between integral intensities of diffraction reflections $I(h)$ and structure factors $F(h)$ for two types of diffraction measurements, with the use of monochromatic neutrons ($\lambda = \lambda_0$) and polychromatic ones ($\theta = \theta_0$) and two types of samples: highly oriented membranes and those with random orientation (Buras & Gerward, 1975).*

Sample type	Monochromatic neutrons ($\lambda = \lambda_0$)	Polychromatic neutrons ($\theta = \theta_0$)
Random orientation (membrane dispersion)	$\varphi(\lambda_0)VN^2 F(h) ^2\lambda_0^3\frac{\tau}{\sin\theta\sin 2\theta}$	$\varphi(\lambda)VN^2 F(h) ^2\lambda^4\frac{\cos\theta_0}{4\sin^2\theta_0}\tau$
Highly oriented membranes	$\varphi(\lambda_0)VN^2 F(h) ^2\lambda_0^3\frac{\tau}{\sin 2\theta}$	$\varphi(\lambda)VN^2 F(h) ^2\lambda^4\frac{1}{2\sin^2\theta_0}\tau$

$$L_1(x_0, \nu) = \sum_{h=h_{\min}}^{h_{\max}} w_h [F_e(h) - F_M(h)]^2 \rightarrow \infty, \quad (9)$$

where $F_e(h)$ is measured in experiment, $F_M(h)$ is expressed by (4), and $w_h = 2/\gamma(h)$ by virtue of (8). Here we do not impose any condition on the first and last number of measured structure factors h_{\min} and h_{\max} .

We will treat the factor t_N in (1), (4) as an extra unknown parameter to be estimated along with x_0 and ν through (10) [although the value of t_N can be obtained through the normalizing condition (3), but it is not usually done in actual experiments because it is also necessary to measure area S].

Note that in the implementation of the least-squares procedure (9) one need not know the 'weights' w_h exactly. It suffices to define them up to a common factor. That is, in the case of monochromatic neutrons one can take $w_h = 1/\sin\theta\sin 2\theta$ for random orientation and $w_h = 1/\sin 2\theta$ for highly-oriented samples. In the case of polychromatic neutrons one can set $w_h = \varphi(\lambda)\lambda^4$ (see Table 1). However, for the determination of uncertainties of Δx_0 and $\Delta\nu$ one has to know the weights w_h in their full form: $w_h = 2/\gamma(h)$.

Equation (4) 'suggests' the introduction of a new unknown parameter $c = 2(\pi)^{1/2}\nu t_N$ instead of t_N . This parameter is contained in (4) as a linear factor, so it can be easily determined *via* the differentiation $\partial L/\partial c = 0$, that gives,

$$c = \frac{\sum_{h=h_{\min}}^{h_{\max}} w_h F_e(h) f(h)}{\sum_h w_h f^2(h)}, \quad (10)$$

where,

$$f(h) = \exp\left(-\frac{\pi^2\nu^2 h^2}{d^2}\right) \cos\frac{2\pi h x_0}{d}. \quad (11)$$

Substituting the result (10) into (9) we reduce our problem to the maximization of the function,

$$L_2(x_0, \nu) = \left[\sum_h w_h F_e(h) f(h) \right]^2 / \sum_h w_h f^2(h) \rightarrow \sup. \quad (12)$$

Function (12) is a non-linear one in parameters x_0 , ν , c . (11). Therefore, in order to estimate the uncertainties Δx_0 and $\Delta\nu$ we have to use a linear approximation to

(12), *i.e.* to expand $L_2(x_0, \nu)$ into the Taylor series centered at the (unknown) true values x_0^* , ν^* . To this end we must calculate all the first and second partial derivatives of the function L_2 with respect to x_0 , ν and then replace $F_e(h)$ by their 'mean' values $F_m(h) = cf(h)$, where c is already found by (10). As a result the first partial derivatives $\partial L_2/\partial x_0$ and $\partial L_2/\partial\nu$ will be zero and the second ones will form a symmetric 2×2 matrix which will be denoted by $V = \|V_{ij}\|$, $1 \leq i, j \leq 2$. Its entries V_{ij} are non-random values expressed through parameters $\gamma(h)$, d , x_0 and ν . The corresponding expressions are rather complicated and we omit them here.

Then the covariance matrix of our estimates of the parameters x_0 , ν can be easily calculated through the standard properties of the least-squares procedure. The result is

$$\begin{bmatrix} \text{Var } x_0 & \text{cov}(x_0, \nu) \\ \text{cov}(x_0, \nu) & \text{Var } \nu \end{bmatrix} = -2V^{-1}, \quad (13)$$

where we assume that the order of variables in the V matrix is the same as in the left-hand side of (13), *i.e.* $V_{11} = \partial^2 L_2/\partial x_0^2$ etc.

Therefore,

$$\Delta x_0 = (\text{Var } x_0)^{1/2} = \left(\frac{-2V_{22}}{V_{11}V_{22} - V_{12}V_{21}} \right)^{1/2}, \quad (14)$$

and

$$\Delta\nu = (\text{Var } \nu)^{1/2} = \left(\frac{-2V_{11}}{V_{11}V_{22} - V_{12}V_{21}} \right)^{1/2}. \quad (15)$$

Here, one should note that V_{ij} are proportional to the time of measurement τ , then,

$$\Delta x_0, \Delta\nu \simeq 1/\tau^{1/2}. \quad (16)$$

Besides, V_{ij} are proportional to c^2 , so Δx_0 , $\Delta\nu \simeq 1/n$. This means that the use of strongly deuterated labels results in higher precision and statistic accuracy of x_0 and ν varies inversely with the number of D atoms in the molecular group.

One should keep in mind that (14) and (15) are obtained by linear approximation in (12). The smaller the errors Δx_0 , $\Delta\nu$, the more exact is our linear approximation and the more accurate are (15) and (16). But if the uncertainties Δx_0 , $\Delta\nu$ are large,

then (15) and (16) may give very inaccurate results (for instance, Δx_0 and Δv may well exceed d , which is actually impossible due to the natural constraints $|x_0| \leq d/2$, $|v| \leq d/2$). One should treat such 'results' as an indication that the errors Δx_0 and Δv are too large and the linear approximation no longer works.

One should not forget that three unknown parameters are to be estimated: x_0 , v and t_N (or c). Thus, one needs at least three structure factors to measure, *i.e.* $h_{\max} - h_{\min} \geq 2$. Otherwise the values in (15) and (16) may escape to infinity.

An important note is also that the 'weights' w_h must be set exactly as we described above. If one sets the weights in a different manner, then the quality of our linear approximation becomes poorer and the (15) and (16) are no longer adequate. The estimates of x_0 , v also become less accurate because of a wrong setting of the weights.

The case of two labels consisting in determination of their positions x_1 , x_2 and widths v_1 , v_2 is treated in complete analogy to the above problem of single label. We indicate only essential differences between these two cases.

The expressions for the density (1) and the structure factors (4) are now the sums of two terms, corresponding to two labels. Thus, the function $F_M(h)$ in (9) depends on four unknown parameters. The weights w_h are determined in the same way as before.

Special attention must be paid to the choice of an extra unknown parameter (like c in the case of a single label). Now, in (4), there are two independent factors $c_1 = 2n_1(b_D - b_H)/S$ and $c_2 = 2n_2(b_D - b_H)/S$ with two exponents. Here, n_1 and n_2 are the numbers of substituted H atoms in our two labels. The values of c_1 and c_2 are again unknown due to the difficulties in the measurement of S , but their ratio is known to be equal to $n_1 : n_2$. Therefore, it suffices to introduce one extra parameter, *e.g.* $c = 2(b_D - b_H)/S$. As in the case of a single label, this parameter is a linear factor in (4), so it can be easily expressed through others because of the method (10-12).

Finally, we come to the problem (12) of maximization of a function depending on four parameters x_1 , x_2 , v_1 , v_2 . Then we apply linearization as for the case of a single label. The equation, analogous to (13), gives the covariation matrix for the estimates of these parameters. Their diagonal entries are variances $\text{Var } x_1$, $\text{Var } x_2$, $\text{Var } v_1$, $\text{Var } v_2$ of our estimates. Their square roots give the parameter uncertainties Δx_1 , Δx_2 , Δv_1 , Δv_2 . Relation (16) remains valid.

There is a special case of two identical labels, when their widths are known to be equal *a priori*: $v_1 = v_2$. In this case we have one parameter $v = v_1 = v_2$ instead of two, v_1 and v_2 .

After that the investigation is carried on just as described before.

3. Results and discussion

One should note first that the statistic accuracy of position and width estimation of the deuterium-label distribution is inversely proportional [as follows from (16)] to the square root of the measurement time of diffraction spectra, as it is usually the case for random values with a Poisson distribution.

At first sight it seems strange that Δx_0 and Δv_0 depend neither on the type of membrane under investigation nor on the quantity and isotope composition of membrane water.

The accuracy of x_0 and v estimation depends solely on the label (the number of substituted H atoms) and its position and distribution width. This result has been predetermined by the procedure, estimating the label position, which employs only the structure factors of the label, which are equal to the difference between the experimentally established structure factors of membranes with the label and without it. It is this fact that leads to (12), from which we get the values of x_0 and v and the accuracy of their estimation.

It is enough to measure three structure factors to estimate x_0 and v . In the case of two labels four factors are enough if the labels have the same distribution width, and five if they have not.

The results discussed above are valid for the set of experiments at steady state as well as pulsed neutron sources. Moreover, further results which will be discussed in the paper are qualitatively the same for the both cases. For this reason, quantitative estimations of the accuracies will be presented in the following figures only for the case of steady-state reactor.

All numerical calculations have been done for the case of highly oriented samples. In the model calculations we assume the measurement time of an hour for each diffraction reflection. In neutron diffraction experiment the Bragg reflections are usually collected step by step. It means, that the measurement time of h diffraction orders used in the calculation is, in fact, h hours.

Fig. 2 shows the dependence of Δx_0 and Δv on the number of structure factors which are used for two positions of the deuterium label $x_0 = 9$ and 15 \AA . Fig. 3 shows the same kind of dependence. However, data processing in the second case included the experimentally determined values and the accuracy of structure factors for membranes of DPPC with the deuterium label in position C-2 (Zaccai *et al.*, 1979). As it is evident from Figs. 2 and 3, qualitatively the curves are the same in both cases. Approximately, down to four structure factors being used, the accuracy of x_0 estimation is constant and, furthermore, with the number of factors below four it deteriorates drastically. Analogous is the behaviour of Δv (h_{\max} relation with one exception, *i.e.* the abrupt deterioration of the accuracy occurs beginning with five structure factors

used. It is an important observation for the planning of neutron diffraction experiments on determining the position and width of the deuterium labels in membranes. One does not have to measure the diffraction reflections with $h > 6$, which takes considerable measurement time compared to the first ones (Büldt *et al.*, 1979; Zaccai *et al.*, 1979). Moreover, this result is promising for further investigation of membrane structure at time-of-flight instruments with an average resolution, where higher diffraction orders cannot be collected.

The dependence of Δx_0 and Δv on the label position in membranes is demonstrated in Fig. 4. The accuracy deteriorates when the label is in the centre of hydrophilic or hydrophobic areas of membranes.

Fig. 5 shows the accuracy of locating the label position in deteriorating with the growth of its width, and the accuracy of determining the label distribution width itself is, on the contrary, improving.

The estimation accuracy of the label position and its width (Δx_0 and Δv) is inversely proportional to the 'weight' of the label, *i.e.* the number of replaced H atoms.

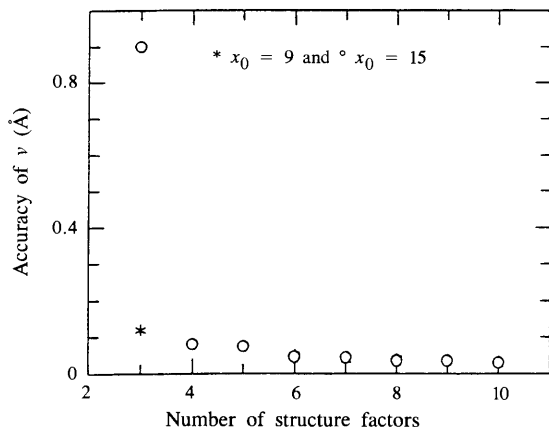
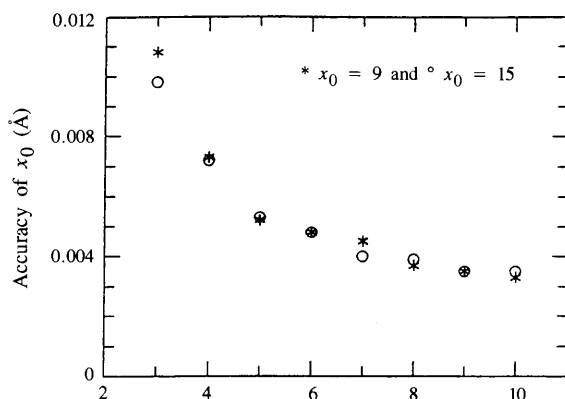


Fig. 2. The dependence of the accuracy parameters Δx_0 and Δv on the number of structure factors, which are used for two positions of deuterium label $x_0 = 9$ and 15 \AA .

The relationships stated for the case of two labels in membrane are in many ways similar to what was just discussed for one label. The dependence of Δv_1 , Δv_2 , Δx_1 , Δx_2 on the interlabel distance is shown in Fig. 6. We used the first five structure factors in the calculations. The parameters similar to those determined for membranes of DPPC (Büldt *et al.*, 1979; Zaccai *et al.*, 1979) were used in these calculations. For the first label with three D atoms in the choline group x_1 was set to 25 \AA . The second label with two D atoms was shifted along the lipid molecule. The values of the label distribution width were set to 3 and 1 \AA respectively (Büldt *et al.*, 1979; Zaccai *et al.*, 1979). With the interlabel distance decreasing, starting from 5 \AA , the x and v estimation accuracy rapidly deteriorates (Fig. 6). The same is true when the second label is put in the centre of hydrophobic area of membranes: $x_2 \approx 0$. The latter result (just as the similar one for the case of one label, in which Δx_0 and Δv deteriorate at a certain label position

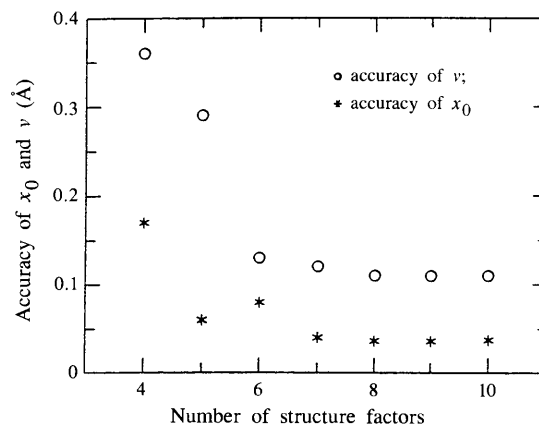


Fig. 3. The dependence of Δx_0 and Δv on the number of structure factors. Here $x = 25.3 \text{ \AA}$ and corresponds to the position of lipid C-2 polar group of DPPC membranes in gel phase with the repeat distance $d = 57.4 \text{ \AA}$ (Zaccai *et al.*, 1979).

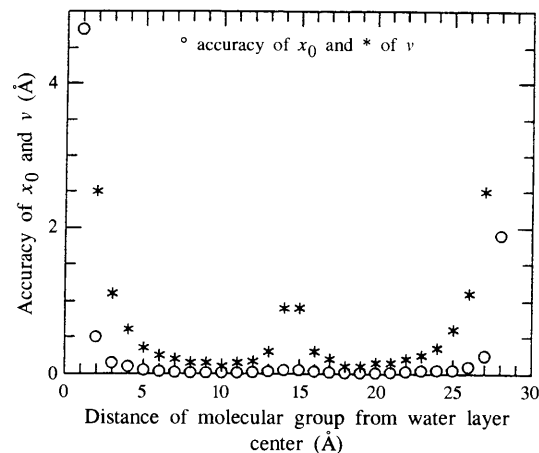


Fig. 4. The dependence of Δx_0 and Δv on the label position in the membrane. Three structure factors were used in the calculations.

x_0^c) is related to a weak dependence of the labels structure factors on their position at certain values of x_0^c . In particular, at $x_0 = 0$ it is true for all structure factors $F_M(h)$. The $\partial F_M(h)/\partial x_0$ derivatives at these points are equal to zero. As is clearly seen from (2), these critical x_0^c positions can be derived from the following condition,

$$2\pi h x_0^c / d = \pi k, \quad (17)$$

where k is a positive integral number. From (18) it follows that,

$$x_0^c = dk/2h. \quad (18)$$

It may seem surprising that the accuracies of x_0 and ν shown in the figures are so high even for short measurements. Indeed, the canonical resolution for neutron (and X-ray) scattering profiles is equal to d/h_{\max} and is usually between 5 and 10 Å. However, this resolution is a measure of the width of the distribution of membrane molecular groups rather than the measure of their spacial separation. The thermal motions of membrane molecular groups determine the label distribution profile and therefore it

can be described by Gaussian function. This is a general property of membranes. It predetermines the choice of the model for describing the label distribution. This point was discussed in detail (Wiener & White, 1991).

It is worthy of notice that the results discussed above allow one to evaluate the role of systematic errors in structure determination. If in neutron diffraction experiment with membranes the total error of x_0 and ν parameters considerably exceeds the statistical accuracy then it means, one has to cope with the sources of systematic errors rather than spend more of the expensive beam time.

To simplify analytical treatment of the problems under discussion the background was not considered. In fact, in any diffraction experiment there is a background component in the scattering intensity. The background is mainly determined by incoherent neutron scattering of H atoms of membranes. The signal/background ratio strongly depends on the sample. It is apparent that this value is very large for the first strong reflections and the statistical accuracy of the integral intensities in this case hardly depends on the background.

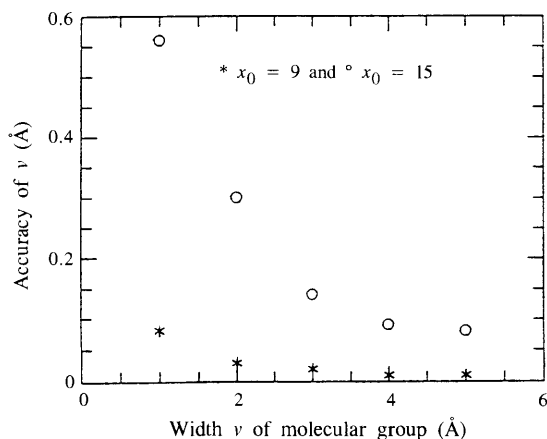
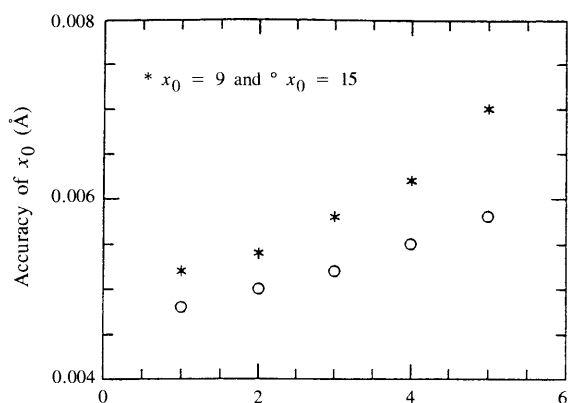


Fig. 5. The dependence of the accuracy parameters Δx_0 and $\Delta \nu$ on the width of the label distribution. Three structure factors were used in the calculations.

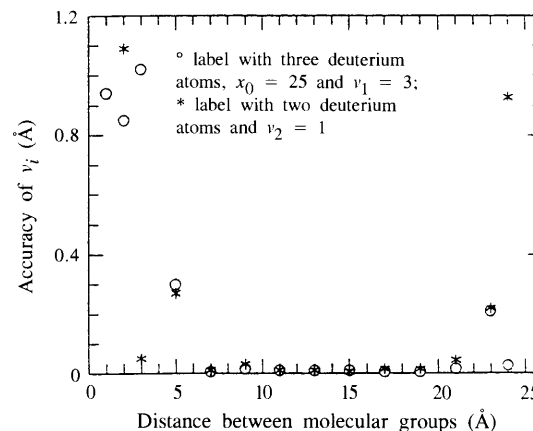
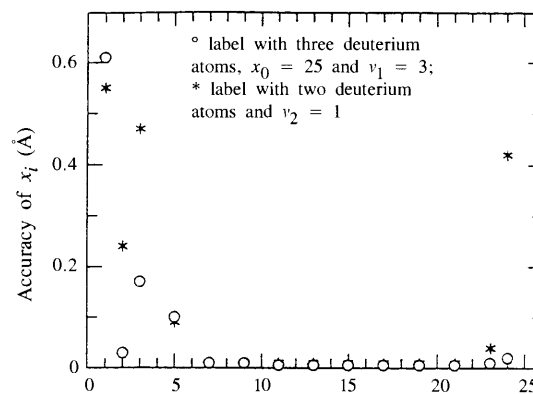


Fig. 6. The accuracy Δx_0 and $\Delta \nu$ in the case of two deuterium labels. One label with three D atoms is fixed at $x_0 = 25$ Å, the other, with two D atoms varies. The dependence of the accuracy on the distance between the labels is shown. Five structure factors were used in the calculations.

In Fig. 7 two dependences of statistical accuracy of experimental integral intensities of diffraction reflections DPPC membranes are shown. The first one corresponds to the real accuracies (calculated with the background taken into account) and the second one corresponds to the calculated accuracy (assuming zero background). As is seen from Fig. 7 the background must be taken into account only for the highest reflections ($h \geq 6$). Thus, consideration of the background does not dismiss the conclusions made above in this paper. Moreover, it gives an additional argument in favour of one of the main conclusions: the measurements of the highest orders do not noticeably improve the statistical accuracy of the estimations of membrane structure parameters.

4. Conclusions

In a general case one does not have to pay too big a price measuring as many diffraction peaks from membranes as possible to get higher accuracy of the determined position x_0 and width ν of molecular groups in membranes.

The accuracy of x_0 and ν improves very quickly with a larger number of structure factors used in calculations. However, it is valid only up to four and five structure factors used for the calculations of x_0 and ν respectively.

These conclusions are valid for any membrane. The accuracy of x_0 and ν does not depend on membrane constitution. However, it strongly depends on the 'power' of deuterium label (*i.e.* the number of D atoms n) and Δx_0 and $\Delta \nu$ vary in inverse proportion to n .

The accuracy of x_0 and ν depends normally on the measurement time τ , it is proportional to $1/\tau^{1/2}$.

It is important to stress here that the accuracy of neutron diffraction experiment (Δx_0 and $\Delta \nu$) depends

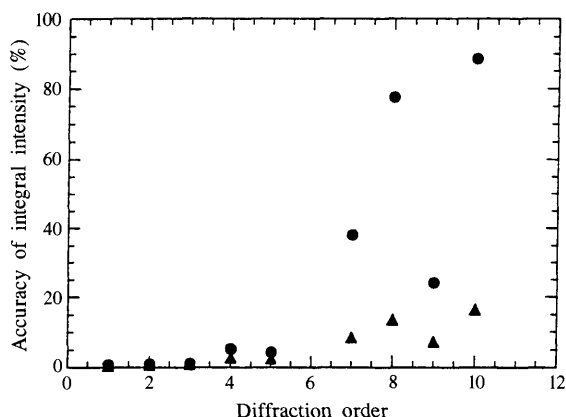


Fig. 7. The dependence of statistical accuracy of integral intensities of the measured diffraction orders of highly oriented DPPC membranes on the number of diffraction order. The calculations were done with the background taken into account (●) and assuming it equal to zero (▲).

also on the position and width of the molecular group in membrane.

The simultaneous use of two deuterium labels in membrane requires the measurement of more structure factors to get a proper accuracy of x_i and ν_i .

There is no qualitative difference between two different sets of experiment: at a steady state reactor and at a pulsed neutron source.

The computer programmes developed here allow one to easily get a quantitative estimation of the accuracy of x_0 and ν and optimize the neutron diffraction experiment with membranes.

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