Neutron Small Angle Scattering of Hemoglobin

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SUMMARY

Neutron small angle scattering experiments were performed on human hemoglobin in various mixtures of H₂O and D₂O. It was possible to observe the interparticle effect without corrections for resolution and to verify the first minimum and the secondary maximum in accordance with the theoretical scattering curve. The accuracy in determining the radius of gyration has been improved over that determined in previous neutron small angle scattering studies. With respect to hydrogen deuterium exchange it was possible to observe the kinetics of the slow exchanging protons with an accuracy of 30 protons. Finally a new and much more precise method for the determination of particle volumes was developed. With this method the density of the scattering lengths of the solvent was altered by varying its D₂O content, and, using a real zero point method, that mixing ratio was determined at which the small angle scattering of the solution vanishes. From the mixing ratio the volume of the nonhydrated hemoglobin was obtained as 84,000 ± 2,000 Å³.

Up to now x-rays have been used almost exclusively for small angle scattering experiments with biological substances. Neutrons are in principle equally suitable, but relatively low intensities attained under previous conditions resulted in bad resolution so that the precision was worse than comparable x-ray experiments (1). Now, however, a much improved apparatus with high neutron intensity is available (2) so that it is possible to explore the full power of the neutron technique. For these investigations we used hemoglobin as a suitable model substance both because experimental results can be compared with earlier x-ray (3, 4) and neutron (5, 6) small angle scattering work, and because the three dimensional structure at atomic resolution is known for this molecule from the work of Perutz (7). Since the experimental small angle scattering curve is not only determined by the structure of the dissolved hemoglobin molecule but also by its complementary pattern, the holes in the solvent water, one must eliminate the solvent scattering in order to get at the intensities, thus information, scattered only by dissolved molecules. For this purpose it is very useful to vary the scattering power of the solvent. In the case of neutron scattering this can easily be done by using mixtures of H₂O and D₂O as solvent. We therefore did a series of measurements of hemoglobin in pure water and in various H₂O/D₂O mixtures. In particular it is possible to choose conditions such that the solvent scattering contribution vanishes. In this way it is possible to measure the pure hemoglobin scattering curve. In x-ray small angle scattering work this curve cannot be measured directly but must be extrapolated, as has been done by Schneider et al. (6). As the same curve can be computed from Perutz’s (7) hemoglobin coordinates, one may compare theoretical and experimental results. As the information on the shape of the molecule, contained in the position and height of the secondary maxima, is found at larger angles the comparison must be extended to such angles. At another mixing ratio of H₂O and D₂O the density of the scattering lengths of the solvent is equal to that of the protein so that the entire small angle scattering curve vanishes. We were able to use this for an improved volume determination of the hemoglobin molecule. The use of H₂O/D₂O mixtures as solvent implies a partial deuteration of the protein which must be taken into account. This deuteration can also be observed by small angle scattering experiments.

EXPERIMENTAL TECHNIQUE

The experiments have been performed at the reactor FRJ-2 (Dido) in Jülich. The small angle scattering apparatus used is shown in Fig. 1. The beam of cold neutrons coming through the neutron guide NG was monochromized by a mechanical velocity selector SE. In this way a wave length distribution, as shown in Fig. 2, was obtained with a mean wave length of λ = 7.3 Å and a root mean square standard deviation of ±0.5 Å. A parallel neutron beam was obtained by the collimator C with a length of 273 cm, an entrance slit of 2.5-5 cm, and an exit slit of 1.5-5 cm. A multidetector system consisting of five position sensitive counters as described by Abend et al. (8) was used. The counters had a diameter of 5 cm and an effective length of 55 cm. Every counter was subdivided into 64 different detector elements. In this way the whole scattering curve could be determined simultaneously. The distance L between the samples mounted in the sample positioning unit M and the detector D could be altered. Measurements were done at two different distances L₁ = 97.3 cm and L₂ = 210 cm, depending on which part of the scattering curve be it larger or smaller angles was desired. Resolution errors were negligible in the essential parts of the scattering curve. This has been controlled by correction procedures using spline functions (9). The scattering vector h is determined by h = 2πξ/λ, with the scattering angle ξ = x/L, where x is the distance of a detector element from the center of the unscattered beam.
Fig. 1. Schematic drawing of the small angle scattering device. NG, neutron guide; SE, mechanical velocity selector; C, collimator; T, scattering tube; SL, slit system of cadmium; MO, monitor counter; SA, sample; M, unit to move the samples in the beam; B, beam stop; D, position sensitive BF$_3$ detector.

Fig. 2. Wave length distribution behind a reference sample of 1 mm H$_2$O measured in a time of flight experiment. The dip caused by the [111] backward BRAGG-reflection corresponds to 7.893 A. $\overline{\lambda}$, mean wave length; $\sigma(\lambda)$, square root of the mean standard deviation.

For the measurements human oxyhemoglobin was used. It was prepared from full blood by repeatedly washing it with 0.9% NaCl solution and subsequent hemolysis with distilled water; ghosts were removed by centrifugation at 16,000 rpm. The hemoglobin solution was dialyzed against distilled water: pH was 7.0, the usual concentration was 45 g per liter. The concentration has been determined from every sample after conversion into cyanmethemoglobin by measuring the optical density at 540 nm. For the scattering experiments the hemoglobin was kept within cuvettes of different thicknesses for H$_2$O-D$_2$O mixtures. For D$_2$O larger path lengths could be used because of its smaller total cross section. In one experiment four samples could be measured, usually two hemoglobin solutions and two appropriate standards with solvent. They were measured repeatedly one after the other and the data accumulated. The total time of such an experiment was 9 (12) hours, the effective measuring time for one sample 126 (168) min depending on the geometry $L = 97.3$ (210) cm. The small angle scattering curve of hemoglobin was obtained as the difference between the data from hemoglobin solution and its solvent standard. The data of the solvent standard have been corrected in order to obtain the effective background scattering. Small deviations of the thicknesses of the cuvettes, which have been calibrated afterwards, and the incoherent scattering of the hemoglobin itself have been taken into account.

We obtain from the measurements the neutron intensity $I(h)$ scattered by hemoglobin into the detector elements. What we want is the scattering curve or the differential cross section $(d\sigma/d\Omega)(h)$. The connection between both is provided by the following equation:

$$I(h) = \Phi_0 \cdot F \cdot D \cdot \exp(-\Sigma_{\text{tot}} \cdot D) \cdot n_{\text{mol}} \cdot (h) \cdot \sigma(\lambda) \cdot \Delta \Omega \cdot P(h) \cdot \Delta \Omega$$

The neutron flux on the sample was $\Phi_0 = 3 \times 10^8$ neutrons per s cm$^2$, the irradiated sample area $F$ was 1.5-5 cm$^2$. $D$ is the thickness of the hemoglobin sample. $\Sigma_{\text{tot}}$ is the total cross section for scattering and absorption. Its magnitude is determined mainly by the incoherent scattering cross section of the solvent. Therefore the term $\exp(-\Sigma_{\text{tot}} \cdot D)$ takes into account this fraction of the neutron beam which is absorbed and scattered by the sample. It has been determined by transmission experiments for all cuvettes and H$_2$O/D$_2$O mixtures used. We obtained for example:

$$\exp(-\Sigma_{\text{tot}} \cdot D) = 0.511 \quad \text{for} \quad D = 1 \text{ mm and H}_2\text{O}$$

$$\exp(-\Sigma_{\text{tot}} \cdot D) = 0.307 \quad \text{for} \quad D = 9 \text{ mm and 89% D}_2\text{O} + 11% \text{ H}_2\text{O}$$

$n_{\text{mol}}$ is the number of hemoglobin molecules per ccm and $\Delta \Omega$ is the solid angle of a single detector element seen from the sample. The effective area of a single element being 4 cm$^2$ we obtain $\Delta \Omega = (4 \text{ cm}^2) / \lambda^2$.

$P(h)$ is the interparticle interference function describing the correlations between the hemoglobin molecules. $P(h)$ is for infinitely diluted solutions equal to 1, but at finite concentrations it diminishes the scattered intensities especially for $h \to 0$. For $P(h)$ there are only crude approximations available, even for the most simple models. Such a model is the hard sphere model, which correlates any two hemoglobin molecules only with respect to a distance of closest approach. This model gives according to Guinier (10) as a first approximation:

$$P(h)_{\text{HS}} = 1 / (1 + 8 \cdot V_{\text{HS}} / n_{\text{HS}})$$

$V_{\text{HS}}$ is the volume of a hemoglobin molecule.

The differential cross section for small angle scattering $(d\sigma/d\Omega)(h)$ is related to molecular quantities by the following relation:

$$d\sigma \over d\Omega (h)_{\text{HS}} = (2\pi a_i - \rho_{\text{HS}} \cdot V_{\text{HS}})^2 \cdot F(h) \cdot |\Gamma(h)|^2$$

Here $\Sigma a_i$ is the sum over all the coherent scattering lengths of the individual atoms of the hemoglobin molecule; it can be computed from the amino acid composition. $\rho_{\text{HS}}$ is the density of the scattering lengths of the solvent. $F(h)$ is called the structure factor.
RESULTS AND DISCUSSION

Interparticle Effect—A first consequence of the point-like geometry of our apparatus was the fact that the interparticle effect could be well resolved, with optimum resolution occurring when sample detector distance \( L_2 = 210 \) cm. At low values of \( h \) the scattering curve deviates more or less from a Gaussian curve, depending on the hemoglobin concentration. This is shown in Fig. 3, and also in Figs. 4 and 5 for hemoglobin concentrations of 98.5, 157, and 44 g per liter. This behavior is described by the interparticle interference function \( P(h) \). We compared in Table I the theoretical values of \( P(0) \) according to Equation 2 and the experimental values as taken from the figure and found good agreement. Considering that at larger angles the scattering curve is not affected by the interparticle effect, it might be useful to characterize the transition between both regions by a characteristic \( h \) value \( h_d \), so that \( P(h) \leq 0.9 \) for \( h < h_d \). These \( h_d \) values depend on concentration as is also shown in Table I.

Large Angles—The behavior of the scattering curve at large angles has been studied with the sample detector distance \( L_1 = 97.3 \) cm. A hemoglobin concentration of 157 g per liter has been used to obtain small statistical errors at large angles. The results are shown in Fig. 4. The theoretical scattering function \( |F(h)|^2 \) of Schneider et al. (11) calculated from the data of Perutz (7) is included. For comparison with the experimental data this scattering curve has been convoluted with the apparative resolution function.

![Fig. 3](image-url)

Fig. 3. The scattered intensity \( I(h) \) from 98.5 g per liter of hemoglobin in pure \( \text{H}_2\text{O} \) is plotted in arbitrary units versus the scattering vector \( h \) in \( \text{A}^{-1} \). (---) Gaussian fit to the experimental data.

![Fig. 4](image-url)

Fig. 4. The scattered intensity \( I(h) \) from 157 g per liter hemoglobin in pure \( \text{H}_2\text{O} \) is plotted on a logarithmic scale in arbitrary units versus the scattering vector \( h \) in \( \text{A}^{-1} \). The theoretical scattering function \( |F(h)|^2 \) (---) as calculated from the data of Perutz (7) by Schneider (5) and the same function after convolution with our apparative resolution function (-----) are included. The experimental data are fitted to the theoretical scattering functions only at Point A.

![Fig. 5](image-url)

Fig. 5. The scattered intensity \( I(h) \) from 44 g per liter of hemoglobin in pure \( \text{H}_2\text{O} \) is plotted in arbitrary units versus the scattering vector \( h \) in \( \text{A}^{-1} \). (---) Gaussian fit to the experimental results between \( A \) and \( B \).

**Table I**

<table>
<thead>
<tr>
<th>Concentration (g/liter)</th>
<th>( P(h = 0) )</th>
<th>( h_d )</th>
</tr>
</thead>
<tbody>
<tr>
<td>44.0</td>
<td>0.72 ± 0.03</td>
<td>0.73</td>
</tr>
<tr>
<td>98.5</td>
<td>0.61 ± 0.03</td>
<td>0.59</td>
</tr>
<tr>
<td>157.0</td>
<td>0.44 ± 0.05</td>
<td>0.47</td>
</tr>
</tbody>
</table>
uation function. At one point (A) the experimental data have been adapted to the scattering functions because in our experiments the cross section has not been determined on an absolute scale. At large scattering angles the hemoglobin scattering is only 1% of the incoherent scattering of the solvent so that tiny corrections of the background become essential. As a consequence of this the experimental errors are large in this region. Nevertheless the position of the first minimum and of the first secondary maximum are clearly visible and are in excellent agreement with the theoretical curve. We do not believe that for molecules like hemoglobin much more accurate results can be obtained in this region by neutron small angle scattering. In the case of larger biological molecules application of the neutron technique might, however, be quite useful.

Radius of Gyration—Up to now we discussed the behavior of the scattering curve at very small angles and at large angles. In the middle region the scattering curve can be approximated by Guinier's law:

\[ \frac{d\sigma}{d\Omega}(h) \sim \exp(-h^2R_g^2/3) \]

\( R_g \) — radius of gyration.

Here the curve is practically independent of concentration and coincides with the curve expected at infinite dilution. We used this part of the curve, indicated in Fig. 5 by the limits A and B corresponding to 0.08 > h > 0.04 A⁻¹, for determining the radius of gyration. The accuracy was best for solutions in pure H₂O and in much D₂O. In these cases the difference between the densities of the scattering lengths of the protein and of the solvent are largest so that one gets high absolute intensities of the scattered neutrons. The values we obtained are shown in Table II. They agree very well with earlier experimental data; however, the accuracy of the present results is much better. We now have both for neutron scattering and for x-ray scattering an excellent agreement of the experimental results with the theoretical radius of gyration calculated from Perutz's coordinates. With the accuracy of the present results we cannot see a difference in the radius of gyration between H₂O and D₂O as solvent. This supports our assumption that as a result of this change in solvent no changes in the quaternary or tertiary structure of hemoglobin occur large enough to be observed by small angle scattering, and therefore that the volume of the molecule remains unaltered. It is known from the work of Tomita and Riggs (12) that hemoglobin alters its function in D₂O. Even remains unaltered. It is known from the work of Tomita and Riggs (12) that hemoglobin alters its function in D₂O. Even if we correct for a difference in the radius of gyration between H₂O and D₂O as solvent, this difference is 0.6 % of the maximum which are just large enough to be observed are the changes which occur upon oxygenation of hemoglobin. They were found to promote a change in the radius of gyration (4, 5).

Deuterium Exchange—Upon changing the solvent from H₂O to D₂O the radius of gyration of the hemoglobin molecule remains constant. The density of scattering lengths, however, changes due to the hydrogen-deuterium exchange. The maximum number of exchangeable hydrogens in human hemoglobin is 950; this number can be computed from the amino acid composition. As the scattering lengths of hydrogen (−0.374·10⁻¹² cm) and deuterium (−0.667·10⁻¹² cm) are quite different, the deuteration of the 950 groups would lead to a marked change of the sum over all scattering lengths \( \Sigma_b \), from 1526·10⁻¹² cm to 2515·10⁻¹² cm. But these protons all exchange with different rate constants, which depend on pH and temperature. The exchange behavior of myoglobin has been studied by Benson and Linderström-Lang (13) and, in much detail, by Englander and Staley (14). The latter were able to identify these different classes of hydrogens and to determine their rate constants. They found that the slow exchanging hydrogens with rate constants greater than 1 hour are the H-bonded peptide hydrogens.

Our experimental conditions were pH 7 and 25°. At pH 7 measurements have been done by Benson and Linderström-Lang (13) at 0° and at 37°, and by Englander and Staley (14) at 4°. From these measurements we can deduce that at a temperature of 25° after 1 hour about 60, after 4 hours about 45, and after 10 hours approximately 35 protons remain unexchanged. This corresponds to 22, 17, and 13% of the maximum number of exchangeable hydrogens in myoglobin (268). If we assume a similar behavior to be true for hemoglobin, which should be a good approximation because of the great similarity of the two proteins, we may then apply these same percentages to hemoglobin. We therefore expect that of the 950 exchangeable protons 90 have exchanged from 1 to 10 hours after mixing with D₂O and that 830 have exchanged after 10 hours.

In one experiment we studied the kinetics of this deuterium exchange. At time zero a concentrated hemoglobin sample was diluted with D₂O, and at certain time intervals thereafter scattering curves were determined. The scattering pattern

### Table II

Radius of gyration \( R_g \): theoretical and experimental values for oxyhemoglobin from x-ray and neutron small angle scattering

<table>
<thead>
<tr>
<th></th>
<th>( R_g ) A</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>X-rays</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical</td>
<td>24.21</td>
<td>11</td>
</tr>
<tr>
<td>Experimental</td>
<td>24.7 ± 1.1</td>
<td>4</td>
</tr>
<tr>
<td>Neutrons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Theoretical</td>
<td>24.14</td>
<td>11</td>
</tr>
<tr>
<td>Experimental</td>
<td>23.8 ± 1.6</td>
<td>5</td>
</tr>
<tr>
<td>( H_2O ) as solvent</td>
<td>23.7 ± 1.0</td>
<td>1</td>
</tr>
<tr>
<td>83% D₂O as solvent</td>
<td>24.0 ± 0.6</td>
<td>This paper</td>
</tr>
<tr>
<td>Mean value of 16</td>
<td>23.7 ± 0.5</td>
<td>This paper</td>
</tr>
<tr>
<td>Measurements</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
consisting of the small angle scattering of hemoglobin and the
incoherent scattering of the solvent was fitted over a constant
intensity contribution by a Gaussian function. This constant
term is actually the solvent scattering. We did not subtract
this solvent scattering as is usually done because it was not
measured simultaneously, but only at certain time intervals.
By the fit one obtains the extrapolated forward scattering
I_{\text{ext}}(0) = I(0)/P(0) and the incoherent scattering of the
solvent I_{\text{sol}}. For the study of the time dependence of the scat-
tering one needs a value which is independent of flux variations
of the reactor. This is usually done by relating I_{\text{ext}} to a monitor
counter. But here it is advantageous to use the relation I_{\text{ext}}/ I_{\text{sol}}
because in this way drifts of the counting system can also be
eliminated. As can be seen from Equation 3 the square root of
this value is proportional to \Sigma a_i and therefore to the number
of exchanged protons. We observe, as shown in Fig. 6, a small
decrease during the time of experiment with an indication of a
faster exchange at the beginning. This time dependent
decrease corresponds qualitatively to the expected exchange.
About 90 exchanging protons are observed between 1 and 10
hours. The accuracy is about 30 protons.

The experiments with HzO-DzO mixtures discussed in the next
paragraph lasted usually 9 or 12 hours, the measurements began
between 1/2 and 1 hour after mixing the hemoglobin solution with
DzO. All the data collected during this time have been summed
up so that the measured intensity corresponds to a medium
degree of exchange. Considering the results on the hydrogen-
deuterium exchange kinetics we may assume that 17% of the
maximum number of exchangeable hydrogens do exchange,
that means 790 protons.

Volume Determination—There are different ways by which
one can obtain the volume of a particle from small angle scat-
ttering experiments. One possibility is provided by Porod's
invariant, an integral over the whole scattering curve (10). In
this manner Conrad et al. (3) obtained 135,000 ± 20,000 Å³
for the volume of hemoglobin from x-ray small angle scattering
experiments. As errors for intensities at larger angles are quite
considerable, this method is not very precise. The volume of a
particle can be determined in another manner from the intensity
scattered in the forward direction. This can be seen from
Equation 3 where in the case of forward scattering the structure
factor F(h = 0) is equal to 1. Therefore, if the density of the
scattering lengths of the solvent psOl is known, and the differen-
tial cross section is determined in its absolute scale, the volume
of hemoglobin V_{\text{Hb}} can be computed. In this way Schelten
et al. (1) obtained V_{\text{Hb}} = 105,000 ± 25,000 Å³ from neutron
experiments. Schneider et al. (6) attempted to improve this
method by varying the density of scattering lengths of the sol-
vent using water-glycine mixtures and obtained V_{\text{Hb}} = 105,000
± 8,000 Å³. But in x-ray experiments psOl can be varied only
in a very limited range between 0.33 and 0.39 el/Å². In neu-
tron small angle scattering work psOl can be varied easily and
by large amounts between \rho_{H_2O} = -0.56 \times 10^6 cm^{-2} and \rho_{D_2O} =
+6.36 \times 10^6 cm^{-2} using mixtures of HzO and DzO. One can,
therefore, use this method as a real zero point method and can
determine with high precision the conditions under which the
small angle scattering vanishes completely. Under such condi-
tions Equation 3 reduces to
\[ \Sigma a_i - \rho_{\text{sol}} V_{\text{Hb}} = 0 \]  
which can now be used for a volume determination.

We then performed a series of measurements with different
HzO-DzO mixtures as solvent. These experiments were done in

![Fig. 7. The square root of the extrapolated and normalized
forward scattering intensity of various hemoglobin samples is
plotted versus the DzO concentration of the solvent.](http://www.jbc.org/)

both geometries, with \( L_1 = 97.3 \) cm and with \( L_2 = 210 \) cm. The
hemoglobin concentration was always about 45 g per liter. A
typical scattering curve of this series is shown in Fig. 5. In the
same way discussed above \( I_{\text{exti}}(0) = I(0)/P(0) \) was extrapolated
from these curves. In order to improve the fit, especially in the
neighborhood of 40% DzO where the small angle scattering
intensity vanishes, a fixed radius of gyration of 24 Å was used.
This \( I_{\text{ext}(0)} \) was normalized and corrected by the following
relation:
\[ \Delta I(h = 0) = I_{\text{ext}(0)}/(\exp(-\Sigma a_i D) \cdot D \cdot n_{\text{Hb}} \cdot \Delta h) \]  
This \( \Delta I(0) \) is identical with \( (d\sigma/d\Omega)(0) \) except for a constant
factor. Equation 3 predicts that the square root of this value
should be a linear function of the quantity \( \rho_{\text{sol}} \) and therefore of
\( \rho_{D_2O} \). We therefore plotted the data in this way as shown
in Fig. 7. All of the data are lying on a straight line going through
zero for \( \rho_{D_2O} = 40.5\% \). It follows then that:
\[ (\Sigma a_i)_{\text{sol}} - \rho_{\text{sol}} V_{\text{Hb}} = 0 \]  
The density of the scattering lengths of the solvent is
\[ \rho_{\text{sol}} = \rho_{H_2O} + (\rho_{D_2O} - \rho_{H_2O}) c_{D_2O} - 2.24 \times 10^{16} \text{ cm}^{-2} \]
If we assume that the deuterium is distributed in a random way
between water and the 790 labile proton sites of the protein, we
obtain:
\[ (\Sigma a_i)_{\text{sol}} = (\Sigma a_i)_{H_2O} + 190 (a_D - a_H) c_{D_2O} = 1859 \times 10^{-16} \text{ cm}^{-2} \]
For the calculation of \( \Sigma a_i \) numbers and kinds of atoms following
from the amino acid composition of human hemoglobin and the
new scattering lengths data of Köster (15, 16) (accuracy +3\%) were
used. With these values the volume of hemoglobin is
\( V_{\text{Hb}} = 83,000 \) Å³. If the assumption of the random distribution
is not true, if the equilibrium constant for the H-D exchange
K is not equal to 1, then the relation between \( \Delta I(0)^{1/2} \) and \( c_{D_2O} \)
is not strictly linear. The linearity is however not very sensi-
tive to changes in K. K values between 0.2 and 5 could prob-
ably be accommodated by our experimental data. It is, however,
known from other deuterium and tritium exchange experiments that \( K \) is not much different from 1. A small equilibrium isotope effect exists though, and it is found that more deuterium is accumulated in the protein than corresponds to a random, unbiased distribution. From the data of Englander and Staley (14) one calculates a value of \( K = 1.15 \). If, instead of \( K = 1 \), this value is used for the calculation of \( \langle \Sigma a_c \rangle_{eq} \), the hemoglobin volume would be larger by 1000 \( \text{Å}^3 \), so that our result would be

\[
V_{Hb} = 84,000 \pm 2,000 \, \text{Å}^3
\]

This new method for the volume determination is very precise. The error is almost exclusively due to the experimental accuracy in determining \( c_{D_{2}O} \). Other errors due to the number of exchanging protons and to the value of \( K \) are smaller and can be neglected.

In contrast to hydrodynamic measurements where the volume of the hydrated molecule is obtained the volume obtained from small angle scattering experiments is the volume of the nonhydrated molecule. The value of 84,000 \( \text{Å}^3 \) obtained from these experiments is in excellent agreement with the value of 83,000 \( \text{Å}^3 \) (17) found from hemoglobin crystals for the nonhydrated molecule. This value is, however, considerably smaller than the values obtained in earlier small angle scattering work, even if one takes into account that in those determinations the error was much larger. The reason for this discrepancy is not yet clear. We hope that this new way for the determination of particle volumes, the reliability and applicability of which we were able to demonstrate in the case of hemoglobin, will be useful for further applications.

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Jakob Schelten, Peter Schlecht, Werner Schmatz and Adalbert Mayer


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