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Water solutions of copper(II) and glycyl-L-histidylglycine contain a large particle of molecular weight of approximately 9000 and also low-molecular-weight particles with an average molecular weight of 430. This is shown in a small-angle X-ray scattering study at 21 ° and neutral pH using a Kratky camera. The concentration of copper(II) was 34-72 mM and the molar ratio of peptide to copper was 1:39 to 1. This study further indicates that the particles contain about ten weight per cent water, and their shapes are an oblate ellipsoid of revolution with an axial ratio of 0.2. The scattering data were analysed with a method similar to that of Mittelbach & Porod [Kolloid Z. Z. Polym. (1965). 202, 40–49]. It was assumed that the particles in solution follow a discontinuous size distribution function dependent on two parameters: one associated with the complex formation constants, and the other with the relative weights of the units building up the complexes. That a very large particle exists is in qualitative agreement with a previous electromotive-force study which indicated that a series of large complexes is formed. The presence of such a large particle in the solution is also in agreement with a single-crystal study on the same system. The crystal structure consists of a three-dimensional network which contains 40% disordered water located in channels limited by rings of six dimers of copper and peptide.

1. Introduction

In an investigation of the copper(II)-glycylhistidylglycine system, using electromotive force (e.m.f.) methods, it was found that complexes of very high nuclearity exist in solution (Österberg, Sjöberg & Söderquist, 1972). At neutral pH, these e.m.f. data indicated that the species CuL2 and Cu3L4, and the series of complexes, Cu3L4(Cu6L6)i, i = 1, 2, 3, ... are formed. (L = ligand, including all the protonated forms that enter the complexes.) Thus, the e.m.f. data yield a thermodynamic indication that very large particles exist in solution. The existence of very large complexes is also in agreement with an X-ray structure investigation of crystals obtained from the same system (Österberg, Sjöberg & Söderquist, 1972). Crystals of copper(II) and glycylhistidylglycine consist of an infinite three-dimensional network which contains 40% disordered water located in channels limited by rings of six dimers, (Cu3L3)6. However, in order to obtain a structural proof regarding the very large complexes found in solutions of copper(II) and glycylhistidylglycine, this small-angle X-ray investigation was performed.

2. Experimental

Solutions used for the small-angle X-ray measurements were made from freshly prepared, and washed, copper(II)hydroxide, glycyl-L-histidylglycine (Miles-Yeda Ltd., Rehovoeth, Israel), and distilled water (Freeman & Taylor, 1965). Through this method of preparation the concentration of counterions was kept to a minimum. The copper concentration was determined by electrolytic deposition, and the ligand concentration by nitrogen analysis using a micro-Dumas method (Kirsten, 1971). Small-angle X-ray measurements were performed for four solutions of the compositions listed in Table 1, all of molar ratio ligand/copper = 1:39. According to the e.m.f. measurements (Österberg, Sjöberg & Söderquist, 1972), the system is most polynuclear at a molar ratio of approximately one; but owing to the low solubility at this ratio, a ratio of 1:39 was used.

During the X-ray measurements the solutions were contained within Mark capillaries. The solutions were found to be sensitive to X-rays, and therefore a special flow device was used by which a fresh solution could be introduced every thirty minutes.

The X-ray measurements were performed with a Kratky small-angle camera (Kratky, 1958; Kratky & Skala, 1958) with an electronic step-scanning device (Leopold, 1968), an X-ray tube with a Cu anode and a line-shaped focus, a proportional counting tube with a pulse-height discriminator, and a Ni filter. The largest angles were measured with the hand-operated goniometer of the Kratky camera. The equipment was installed in a room maintained at 21.0 ± 0.5°C. For each of the, about 100, measured points per curve, 10⁸ pulses were registered. The background scattering, measured with distilled water in the capillary, was subtracted from the scattering of the samples. The intensity of the primary beam, I₀, was determined with the help of a Lupolen platelet (Kratky, Pilz & Schmitz, 1966), calibrated at the Institut für physikalische Chemie, Universität Graz, Austria. The partial specific volume, vₛ, of the solute was calculated by taking the
difference between the densities of the solutions and the distilled water. The densities were determined with a precision digital densitometer (Kratky, Leopold & Stabinger, 1969).

3. Theory and mathematical procedure

3.1. Introduction

In order to treat the small-angle X-ray data obtained from polydispersed systems, certain assumptions must be made of either the size distribution function, or of the form of the particles, or of both; the results depend on these assumptions. For example, the methods given by Shull & Roess (1947), Roess & Shull (1947), and Hosemann (1950) are based on a priori knowledge of the particle shape and type of size distribution function. Another approach to the problem is given by Roess (1946) and Riseman (1952), who showed that, by assuming spherical particles, it is mathematically possible to calculate the distribution function. This method has been further developed by Letcher & Schmidt (1966), see also Brill, Weil & Schmidt (1968).

A third method is based on certain assumptions about both the type of size distribution function and the shape of the particles (Mittelbach & Porod, 1965): the shape is approximated by an ellipsoid of revolution. Thus, it can be described by a single number, the axial ratio, v. The polydispersity distribution function is also assumed to be determined by one parameter. From the experimental curves, characteristic numbers, dependent on both the shape and polydispersity, are calculated; and from these it is possible, under the above assumptions, to obtain information on both the polydispersity and the anisotropy of the particles. The mathematical analysis in this investigation is based on a modification of the method of Mittelbach & Porod (1965).

3.2. Distribution of intersects and characteristic parameters

The intersect distribution function, introduced by Porod (1967), consists of the following: if straight lines pass through a body, in every direction, everywhere, and with both ends lying on the boundary of the body, we obtain a statistical assembly of intersects from zero up to the largest possible diameter of the particle. This assembly may be described by an intersect distribution function, \( A(l) \); such that, \( A(l)dl \) is the probability that an intersect will have a length between \( l \) and \( l+dl \). The function \( A(l) \) will be assumed to satisfy the normalization condition

\[
\int_{0}^{\infty} A(l)dl = 1.
\]

The \( n \)th moment of the intersect distribution function, \( \bar{l}^n \), is defined by

\[
\bar{l}^n = \int_{0}^{\infty} l^n A(l)dl.
\]

From the first six moments, we obtain the following characteristic parameters (Damachsun & Pürschel, 1969):

- the average intersection length, \( d = \bar{l} \);
- the characteristic length, \( l = \bar{l}^2 \);
- the characteristic surface, \( f = (\pi/3)\bar{l}^3 \);
- the characteristic volume, \( V = (\pi/3)\bar{l}^4 \);
- the mean electron distance, \( \bar{a} = \frac{3}{4}\bar{l}^3/\bar{l}^4 \);
- the square of the radius of gyration, \( R^2 = \frac{1}{3}\bar{l}^6/\bar{l}^4 \).

3.3. Characteristic parameters for a polydisperse system

When characteristic parameters are calculated from small-angle X-ray data obtained from a polydisperse system, we will obtain some average values, \( \langle d \rangle \), \( \langle l \rangle \), \( \langle f \rangle \), \( \langle V \rangle \), \( \langle \bar{a} \rangle \), and \( \langle R^2 \rangle \) over the size distribution. The question is now, what type of average values will occur.

If we assume that all the particles have a similar shape, the moments, \( \bar{l}_i^m \), for a particle, \( i \), are defined by the following equation:

\[
\bar{l}_i^m = \bar{l}^m p_i^m,
\]

where \( p_i \) is a linear distance of the particle, \( i \), relative to some particle of reference, and \( \bar{l}_i^m \) is the \( m \)th moment.

Table 1. Experimental results

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper concentration</td>
<td>71.8</td>
<td>55.4</td>
<td>45.3</td>
<td>34.0</td>
</tr>
<tr>
<td>Copper concentration</td>
<td>4.56</td>
<td>3.52</td>
<td>2.88</td>
<td>2.16</td>
</tr>
<tr>
<td>Glycyhypidylglycine concentration</td>
<td>26.83</td>
<td>20.70</td>
<td>16.95</td>
<td>12.69</td>
</tr>
<tr>
<td>Total concentration of the solute, G</td>
<td>31.39</td>
<td>24.22</td>
<td>19.83</td>
<td>14.85</td>
</tr>
<tr>
<td>pH</td>
<td>6.4</td>
<td>6.5</td>
<td>6.6</td>
<td>6.6</td>
</tr>
<tr>
<td>Partial specific volume of the solute, ( \bar{v} )</td>
<td>0.539</td>
<td>0.538</td>
<td>0.538</td>
<td>0.536</td>
</tr>
<tr>
<td>Average intersection length, ( \langle d \rangle )</td>
<td>3.9</td>
<td>3.8</td>
<td>3.8</td>
<td>3.5</td>
</tr>
<tr>
<td>Characteristic length, ( \langle l \rangle )</td>
<td>6.2</td>
<td>6.0</td>
<td>6.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Characteristic surface, ( \langle f \rangle )</td>
<td>58</td>
<td>53</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>Volume, ( \langle V \rangle )</td>
<td>767</td>
<td>629</td>
<td>567</td>
<td>601</td>
</tr>
<tr>
<td>Radius of gyration, ( \langle R^2 \rangle )</td>
<td>10.9</td>
<td>9.9</td>
<td>7.9</td>
<td>7.7</td>
</tr>
<tr>
<td>Weight average molecular weight, ( \langle M \rangle )</td>
<td>734</td>
<td>591</td>
<td>528</td>
<td>526</td>
</tr>
<tr>
<td>Degree of volume swelling, ( q )</td>
<td>1.17</td>
<td>1.19</td>
<td>1.20</td>
<td>1.28</td>
</tr>
<tr>
<td>Water content</td>
<td>8.4</td>
<td>9.3</td>
<td>9.7</td>
<td>13.0</td>
</tr>
</tbody>
</table>

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of the reference particle. Now, taking the average of the characteristic parameters over the size distribution, we use the equation given by Porod (1967):

\[
\langle I^m \rangle = \frac{\langle p^{m+2} \rangle}{\langle p^2 \rangle},
\]

where \( \langle p^2 \rangle \) and \( \langle p^{m+2} \rangle \) are given by the general expression for the average value, \( \langle p^n \rangle \), of \( p_i^j \) over the size distribution:

\[
\langle p^n \rangle = \frac{\sum c_i p_i^n}{\sum c_i}.
\]

In equation (8), \( c_i \) is the molar concentration of the particles \( i \).

From equations (1)–(6) and (7) we obtain the general expressions

\[
\langle d \rangle = d \frac{\langle p^3 \rangle}{\langle p^2 \rangle},
\]

\[
\langle l \rangle = l \frac{\langle p^2 \rangle}{\langle p^2 \rangle},
\]

\[
\langle f \rangle = f \frac{\langle p^3 \rangle}{\langle p^2 \rangle},
\]

\[
\langle V \rangle = V \frac{\langle p^2 \rangle}{\langle p^2 \rangle},
\]

\[
\langle \bar{a} \rangle = \bar{a} \frac{\langle p^2 \rangle}{\langle p^2 \rangle},
\]

\[
\langle R^2 \rangle = R^2 \frac{\langle p^4 \rangle}{\langle p^2 \rangle}.
\]

In equations (9)–(14), \( d, l, f, V, \bar{a}, \) and \( R^2 \) refer to the reference particle. They have been calculated for several types of bodies. Formulae for ellipsoids of revolution are reviewed by Damaschun, Müller, Pürschel & Sommer (1969).

### 3.4. Equilibrium model for the copper(II)-glycyl-L-histidylglycine system. The core-links hypothesis

The equilibria between copper(II) and glycylhistidylglycine, (HA), may be described by the general expression

\[
pCu^{2+} + qH^+ + rA^- \rightleftharpoons Cu_qH_rA_r^{(2p+q-r)}.
\]

In a separate investigation using electromotive-force methods and a copper amalgam electrode, in 3:0 M NaClO\(_4\) medium (Österberg, Sjöberg & Söderquist, 1972), it was found that the data at neutral \( p\text{H} \) could best be described by the following complexes: \( \text{CuL}_2, \text{Cu}_3L_4, \) and an infinite series of complexes \( \text{Cu}_iL_j \) (Cu_{i,j}), \( i = 1, 2, 3, \ldots \) (\( L \) denotes the ligand including all the protonated forms that enter the complexes.) Thus, as a first approximation, it is assumed that the system only contains a series of core-link complexes (Sillén, 1954), of the type \( \text{CORE(LINK)}_i, i = 0, 1, 2, \ldots \)

The formation of the \( \text{CORE unit} \) is described by

\[
pCu^2+ + qH^+ + rA^- \rightleftharpoons \text{CORE}
\]

and a stability constant, \( \beta \). (Charges are not specified.) The step stability constant, \( k \), and also \( s, t \) and \( u \) for the addition of a \( \text{LINK unit} \),

\[
\text{CORE(LINK)}_i + sCu^2+ + tH^+ + uA^- \rightleftharpoons \text{CORE(LINK)}_{i+1}
\]

are assumed to be independent of \( i \), for \( i \geq 0 \). The concentration, \( c_i \), of a complex \( \text{CORE(LINK)}_i \), can now be written as

\[
c_i = \beta b^q h^q a^r (k b^q h^q a^r)^i = K_0 K^i
\]

where \( b, h, \) and \( a \) denote activities of copper(II), protons, and ligand, respectively. \( K_0 \) and \( K \) are constants referring to a certain solution, and \( |K| < 1 \). The molecular weight, \( M_i \), of the same species, is given by

\[
M_i = M_C + iM_L.
\]

\( M_C \) and \( M_L \) are the molecular weights of \( \text{CORE} \) and \( \text{LINK} \), respectively.

The total concentration of the solute, in g \( 1^{-1} \), is given by

\[
G = K_0 \sum_{i=0}^{\infty} K^i (M_C + iM_L) = K_0 \left( \frac{M_C}{1 - K} + \frac{M_L K}{(1 - K)^2} \right).
\]

Equations (8), (15), and (18) give the number average of \( p_i^j \):

\[
\langle p^n \rangle = \sum_{i=0}^{\infty} K_i^j \sum_{i=0}^{\infty} K_i^j (1 + iM_L/M_C)^n.
\]

### 3.5. Determination of \( K, M_L/M_C, \) and the degree of anisotropy, \( v \), of the complexes

Equations (9)–(14) and (19), together with the formulae for the characteristic parameters for ellipsoids of revolution, open the possibility of determining \( K, M_L/M_C, \) and the degree of anisotropy, \( v \), of the complexes. Thus, any combination of equations (9)–(14), combined to give a dimensionless quantity, will be independent of the size of the particles and only dependent on the shape and polydispersity (Porod, 1967).

If the shape is approximated by an ellipsoid of revolution, it can be described by one number, the axial ratio \( v \). There are three unknowns \( (K, M_L/M_C, \) and \( v \); \) the numbers of independent equations are five. In principle it is possible, under the above assumptions, to calculate \( K, M_L/M_C, \) and \( v \).
The choice of the dimensionless numbers is arbitrary, but it is best to use numbers which differ as much as possible in their dependence on the system. In this work, all the different combinations were used:

\[ F_1 = \frac{\langle l \rangle}{\langle d \rangle} = \frac{\langle p^4 \rangle}{\langle p^2 \rangle}, \quad (20) \]

\[ F_2 = \frac{\sqrt{\langle f \rangle}}{\langle d \rangle} = \frac{\langle p^5 \rangle}{\langle p^3 \rangle}, \quad (21) \]

\[ F_3 = \frac{\sqrt{\langle V \rangle}}{\langle d \rangle} = \frac{\langle p^6 \rangle}{\langle p^3 \rangle}, \quad (22) \]

\[ F_4 = \frac{\sqrt{\langle R^2 \rangle}}{\langle d \rangle} = \frac{\langle p^8 \rangle}{\langle p^3 \rangle}, \quad (23) \]

\[ F_5 = \frac{\sqrt{\langle l \rangle}}{\langle l \rangle} = \frac{\langle p^5 \rangle}{\langle p^3 \rangle}, \quad (24) \]

\[ F_6 = \frac{\sqrt{\langle V \rangle}}{\langle l \rangle} = \frac{\langle p^6 \rangle}{\langle p^3 \rangle}, \quad (25) \]

\[ F_7 = \frac{\sqrt{\langle R^2 \rangle}}{\langle l \rangle} = \frac{\langle p^8 \rangle}{\langle p^3 \rangle}, \quad (26) \]

\[ F_8 = \sqrt{\frac{\langle f \rangle}{\langle f \rangle}} = \frac{\langle p^5 \rangle}{\langle p^3 \rangle}, \quad (27) \]

\[ F_9 = \sqrt{\frac{\langle R^2 \rangle}{\langle f \rangle}} = \frac{\langle p^8 \rangle}{\langle p^5 \rangle}, \quad (28) \]

\[ F_{10} = \sqrt{\frac{\langle R^2 \rangle}{\langle V \rangle}} = \frac{\langle p^8 \rangle}{\langle p^6 \rangle}, \quad (29) \]

Combinations containing \( \langle d \rangle \) were excluded because only approximate formulae for the determination of this quantity exist.

Equations (20)-(29) may all be written in the form

\[ F_i = S_i(v) \cdot P_i(K, M_L/M_C), \quad i = 1, 2, 3, \ldots, 10. \]

\( F_i \) are characteristic numbers obtained from the experimental scattering curves. For ellipsoids of revolution, the shape functions, \( S_i \), calculated from the equations described by Damaschun, Müller, Püschel & Sommer (1969) in their Tables 6 and 7, are illustrated in Fig. 1. The polydispersity functions, \( P_i \), for the core-link series of complexes are illustrated in Fig. 2.

The complicated structure of equations (20)-(29) makes a direct solution of the unknowns \( K, M_L/M_C \), and \( v \) very difficult. Therefore, the system of equations were solved with a curve-fitting procedure: ellipsoids of revolution, with different \( v \) values, were tested by comparing \( F_i/S_i \) with computer calculated maps of the polydispersity functions, \( P_i \) (Fig. 2). From the position of the best fit the values of \( \log K, M_L/M_C \), and \( v \) were obtained.

3.6. The mean molecular weight, \( \langle M \rangle \), and the degree of swelling, \( q \)

The molecular weight of the solute is obtained from the formula (Kratky, 1963)

\[ M = \frac{I(0)}{P_0} \cdot \frac{21 \cdot 0a^2}{(z_1 - \bar{v}_1 q_2)^2 G D} \quad (30) \]

where

\[ I(0)/P_0 = \text{the absolute intensity extrapolated to the zero angle;} \]

\[ a = \text{the distance between the sample and the plane of registration, in cm;} \]

\[ z_1 = \text{the number of moles of electrons per g of the solute;} \]

\[ \bar{v}_1 = \text{the partial specific volume of the solute in } \text{cm}^3 \text{ g}^{-1}; \]

\[ q_2 = \text{the number of moles of electrons per } \text{cm}^3 \text{ of the solvent;} \]

\[ G = \text{the concentration of the solute in g cm}^{-3}; \]

\[ D = \text{the thickness of the sample in cm.} \]

For a polydispersed system we obtain the weight average molecular weight:

\[ \langle M \rangle = \frac{\sum_{i=0}^{\infty} c_i M_i^2}{\sum_{i=0}^{\infty} c_i M_i} \quad (31) \]

If \( q \) is the degree of swelling of the volume (Kratky, 1963), and we assume that \( q \) and \( \bar{v}_1 \), the partial specific volume of the solute, are the same for all the particles, we obtain together with equation (12)

\[ q = \frac{0.6023 \langle V \rangle}{\bar{v}_1 \langle M \rangle} \quad (32) \]
Fig. 2. Polydispersity functions for a series of core-link complexes of different ratios \( M_c/M_e \). The curves are marked with numbers referring to the indices used in equations (20)-(29). Thus, curve 1 represents the function \( \langle p^4 \rangle / (\langle p^3 \rangle)^2 \), curve 2 the function \( \sqrt{\langle p^3 \rangle} \langle p^2 \rangle / (\sqrt{\langle p^3 \rangle})^2 \), etc. The quantities \( \langle p^4 \rangle \) were calculated by equation (19).
If the swelling is due to inner solvation, the water content can easily be calculated from $q$.

3.7. Determination of $M_C$, $M_L$, and $K_0$

A combination of equations (15), (16) and (31) gives:

$$\langle M \rangle = 1 + \frac{KM_L/M_C}{M_C} \left( 1 + \frac{M_L/M_C}{1 + K(M_L/M_C - 1)} \right). \quad (33)$$

From the values of $K$ and $M_L/M_C$ obtained above, and the experimental value of $\langle M \rangle$, we can calculate $M_C$ and $M_L$ by using equation (33).

Finally, when $K$, $M_C$, and $M_L$ are known, $K_0$ can be calculated from equation (17).

4. Treatment of the data and results

After smoothing the experimental data with the help of a moving second-degree polynomial and the least-squares criterion, the curves were differentiated by convolution of the equidistant points with the integers $-2, -1, 0, 1, 2$ (Savitzky & Golay, 1964). The smearing effect of the finite length of the primary beam was eliminated as described by Kratky, Porod & Skala (1960), with a computer program written by Heine & Roppert (1962), Heine (1963).

A plot of $I(h)h^4$ resulted in ascending straight lines for large $h$ (Fig. 3). $I$ means the intensity corrected for the collimator effect and $h = 4\pi \sin \theta/2$, where $\theta$ is half the scattering angle and $\lambda$ is the wavelength of the radiation ($1.54 \text{ Å}$). In order to obey Porod's rule (Porod, 1951), the constants defined by the slopes of the curves were subtracted from the scattering data (Luzzati, Witz & Nicolaieff, 1961). Within experimental uncertainty the slopes are proportional to the concentration.

The Guinier plots, Fig. 4, have pronounced curvatures indicating a polydispersed system, or very anisotropic particles, or both.

Table 1 contains the experimental results. They were obtained from the desmeared intensity and from the equations described by Damaschun, Müller, Pürschel & Sommer (1969) in their Table 4, as well as those equations described in the preceding sections of this article. For the calculation of the molecular weights, the following values were used: $a=20.5 \text{ cm}$, $z_1=0.510 \text{ mole g}^{-1}$, $q_2=0.554 \text{ mole cm}^{-3}$, and $D=0.0943 \text{ cm}$. The scattered intensity and the precision of the measurements are reduced when the concentration is decreased. The lowest concentration used in this investigation approaches the limit of the method, and it is included as a supplement to the data obtained at higher concentrations.

In Table 2 are given the results of the curve-fitting procedure (cf. §§ 3.5 and 3.7) for the three most concentrated solutions, with the assumption of a series of core-link complexes. When the values of $F_i/S_i$ were compared with the theoretical curves of $P_i$, Fig. 2, reasonably good fits (within 5%) were obtained in two positions: one for a prolate ellipsoid and one for an oblate ellipsoid. Fig. 5 illustrates an example of this curve-fitting procedure. Equation (33) yields the molecular weight of the core unit, $M_C$, of approximately 50 for the prolate ellipsoid, and of approximately 430 for the oblate ellipsoid. However, in the solutions investigated the lowest molecular weight expected was approximately 270, the weight of the acid-base species of the ligand molecule; therefore, the prolate ellipsoid model was not further considered.

### Table 2. Results of the data treatment with the assumption of a series of core-link complexes

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CORE unit molecular weight, $M_C$</td>
<td>446</td>
<td>408</td>
<td>445</td>
</tr>
<tr>
<td>LINK unit molecular weight, $M_L$</td>
<td>8920</td>
<td>8976</td>
<td>8010</td>
</tr>
<tr>
<td>Degree of anisotropy, $v$</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Constants defining the formation of a CORE unit, $K_0=\beta b^2h^4$</td>
<td>0.0681</td>
<td>0.0582</td>
<td>0.0442</td>
</tr>
<tr>
<td>Constants defining the formation of a LINK unit, $K=kb^h/a^4$</td>
<td>0.00158</td>
<td>0.00090</td>
<td>0.00055</td>
</tr>
</tbody>
</table>
5. Discussion

The smoothed and desmeared experimental points, together with theoretical curves calculated on the basis of the results in Tables 1 and 2, are shown in Fig. 6. For \( h > 0.3 \), there is a small additional scattering which might be due to the existence of other than core-link complexes, or most probably due to acid-base species of the ligand. No attempt has been made to correct for the scattering from acid-base species of the ligand.

The results obtained in this investigation are, in principle, equivalent to those obtained by the e.m.f. investigation (Österberg, Sjöberg & Söderquist, 1972). Highly polynuclear complexes were indicated by both methods. For instance, in order to explain the radii of gyration obtained in this investigation, very large particles must be assumed. The smallest copper complex obtained by the e.m.f. investigation, \( \text{CuL}_2 \), has a molecular weight of approximately 600. In this investigation, the molecular weight of the CORE, \( M_C \), was determined to be approximately 430. Within experimental uncertainty, this value can be explained as a mean of the molecular weights of \( \text{CuL}_2 \) and acid-base species of the ligand (molecular weight approximately 270) and \( \text{CuL} \) complexes (molecular weight approximately 334). However, the molecular weight of the LINK, obtained by the e.m.f. method (approximately 2000) differs from that obtained in this investigation (approximately 8600). This difference might be because the e.m.f. study was performed in a solution containing 3.0M NaClO₄, and this study was performed in pure water. Furthermore, the e.m.f. investigation was performed at a much lower concentration range; and, in order to facilitate the treatment of the small-angle X-ray data, a number of approximations have been made.

Other possible explanations for the differences between the experimental and calculated curves for \( h > 0.3 \), Fig. 6, are as follows: 1. The effect of fluctuations of electron density, in small ranges, was eliminated by subtracting the constant tail end from the whole curve. However, according to Stuhrmann & Kirste (1965), this scattering disappears at the lowest angles. 2. Since extrapolation to zero concentration is not possible in this investigation, no correction was made for the effect of interparticle interference. The above two effects will, however, cause too small scattering at the lowest angles. Therefore the measured molecular weights and radii of gyration can be regarded only as their lower limits.

From the results in Table 2, the concentration of each complex in the series was calculated by using equation (15). Only the two first complexes are of importance. For the most concentrated solution investigated, 97 wt. % of the solute is present in a complex with a molecular weight of approximately 446, and only 3% in a complex with a molecular weight of approximately 9366. Their contributions to the total scattered intensity at zero angle are 59 and 41% respectively. The total contribution of all the other complexes of the series, for the scattered intensity at zero angle, is only 0.2%.

The water content of the complexes obtained in this investigation, about 10%, differs from that in the solid phase, 40% (Österberg, Sjöberg & Söderquist, 1972). This difference can be explained by the fact that the water in the solid phase is contained in channels limited by rings of 12 copper atoms and 12 glycylhistidylglycine molecules. The mononuclear complexes do not contain such channels; therefore the water content must be lower. If, for simplicity, we assume that the stoichiometric composition of the complexes is \( \text{CuL} \), then the 10% water content corresponds to 2-0 H₂O/CuL; the composition \( \text{CuL}_2 \) corresponds to 3-7 H₂O/CuL₂.

![Fig. 5. Comparison of \( F_i/S_i \) with the polydispersity functions, \( P_i \), for the most concentrated solution. The broken curve represents \( F_i/S_i \) and the full-drawn curves represent the polydispersity functions for \( M_L/M_C = 20 \). The position of the straight line of best fit, parallel to the vertical axes, gives \( \log K = -2.8 \) and \( 1/\varepsilon = 5.5 \).](image1)

![Fig. 6. Comparison of experimental data with theoretical curves for the three most concentrated solutions. The full-drawn curves were calculated by assuming a series of core-link complexes defined by the parameters in Tables 1 and 2. The smoothed and desmeared experimental data are indicated by the same symbols as those listed in Fig. 3.](image2)
It should also be noted that in the crystal structure there was only one water molecule coordinated to each copper atom, i.e., one H$_2$O/CuL.

This investigation demonstrates that small-angle X-ray scattering can be used as a complementary method to study the complex formation that leads to relatively large particles. The advantages with this method compared to the e.m.f. methods are that only small amounts of material are needed, and that the measurements can be made at a low ionic strength, or in a medium where e.m.f. methods are not applicable.

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References


Solvent Environment and Mutual Interaction of Simple Solute Molecules in Solution as Revealed by Scattering Experiments. Examples: Hexamethylenetetramine in Water and DNA in Aqueous 1-1-Electrolyte Solution

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Hexamethylenetetramine (HMTA) in water and DNA in 1-1-electrolyte solutions are taken as model systems to obtain quantitative information about the perturbation of water in the vicinity of an uncharged solute molecule and to confirm quantitative information about the distribution of ions around a polyelectrolyte. They are taken as examples for the interaction of uncharged molecules in solution and for the interaction of polyelectrolytes in solution. They are taken because HMTA can be approximated by a spherical and DNA by a cylindrical distribution of scattering density. In these cases transformations from reciprocal to real space and vice versa can be performed by simple one-dimensional Fourier or Hankel transforms unaffected by averaging over all orientations. Fourier transformation of the scattering amplitude of the dissolved HMTA molecule shows the electron distribution within the solute molecule in agreement with its crystal structure and resolves some inhomogeneities of the solvent density around the solute molecule. The 'border area' of solute and solvent is especially well resolved and almost unaffected by termination errors and deviation from spherical symmetry. The obtained