Joint influence of the pH and the ionic strength of a solution on the degree of association of *N*-(monohydroxyfullerenyl)-DL-alanyl-DL-alanine in aqueous solution

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It has been found that the degree of association of the alanylalanine derivative of fullerene in neutral and alkaline media at an ionic strength of 0.1 is virtually independent of the pH of the solution but is a function of only its ionic strength, whereas in acidic media, the pH of the solution has a crucial influence on the degree of association of this fullerene derivative.

Previously, it has been shown that amino acid and peptide derivatives of fullerene 60 in aqueous solutions are able to form associates and that the degree of association depends on the concentration, pH and the ionic strength of the solution.[1,2] However, the variation of the degree of association has been studied as a function of only one parameter, either pH or ionic strength. The existing data on the biological activity of amino acid and peptide derivatives of fullerene[3] have expanded the task to a study of the behaviour of water-soluble fullerene derivatives in more complex systems, the composition of which is close to that of a living cell. For this purpose, using the alanylalanine fullerene[†] derivative as an example, we have studied the variation of the degree of its association as a function of both pH and ionic strength of the solution at various concentrations.

The experiments were carried out using a MOM-3180 analytical ultracentrifuge (Hungary) and Philpot–Svennson optics at a rotor temperature of 25±0.1 °C. Water was used as the solvent, and the concentration varied in the range 0.7–1.5 g dm^{-3} (higher concentrations afford non-transparent solutions which are too dark, whereas lower concentrations cannot be studied due to the too small increment in the refractive index). The diffusion coefficients (D) were measured using a boundary-forming cell by layering the solvent onto a solution of the studied concentration that falls within the concentration range mentioned above. Pictures of the boundary between the pure solvent and the solution were taken at regular intervals, and its broadening was thus monitored. The rotation frequency of the rotor (4000 rpm) was chosen in such a way that sedimentation of the particles did not occur during the experiment, and the boundary broadened only due to the diffusion. The partial specific volume of the substance in the solution ($\bar{v}/\mathrm{cm}^3\,\mathrm{g}^{-1}$) was determined by picnometry (see Table 1). The viscosity of the solvents η_0 was determined by picnometry using a modified Ubellohde viscosimeter with known constants. The η_0 value was found to be identical (0.8917 cps) within the accuracy of determination for all the solvents used. It is known that measurements of diffusion coefficients in solutions make it possible to determine the coefficient of the forward friction of molecules $f_0 = kT/D$, which is directly related to the size and shape of the latter. If the species of the compounds under study are spherical or nearly spherical in solution, one can easily pass from the friction coefficient to the size of the species, *i.e.* to their radius and volume and then to the molecular weight. Previously, it has been found by electron and tunnelling microscopy[4,5] that associates of amino acid and peptide derivatives of fullerene exist in aqueous solutions as ellipsoid particles incorporating a slight amount of the solvent, and the spherical model is quite applicable to them.

Using the diffusion coefficients D_c and the partial specific volumes of particles $\bar{v}$ measured experimentally, we calculated the forward friction coefficients f and molecular weights M of the associates, the number n of individual molecules with molecular weight M_0 incorporated in an associate, the volumes of associates and of individual molecules V_{ass} and V_0 as well as their diameters d_{ass} and d_0 from the equations reported previously.[6] The results of the calculations are presented in Table 1 and in Figure 1.

It can be seen from the data presented in Table 1 and in Figure 1 that at the same concentration (for example, 1.5 g dm^{-3}), the maximum degree of association of the fullerene derivative of alanylalanine is attained in acidic media, whereas in neutral and alkaline media, it sharply decreases and remains virtually constant as the pH increases. This result differs from our previous data,[2] according to which the degree of association increased with increase in the pH of the solution containing no KCl (Table 3 in ref. 2). As we have shown previously,[2] the higher the degree of dissociation of the amino acid or peptide fragment, the higher the solubility of fullerene derivatives of amino acids and peptides. A relative high ionic strength of the solution also decreases the solubility of the compound and results in an increase in the degree of association. In acidic media, the decrease in the degree of dissociation of the carboxyl group diminishes the solubility of the compound and increases the degree of association n of the alanylalanine derivative of fullerene. Alkalification of the solution leads to an increase in the solubility of the peptide fragment, because the salt is formed and its solubility is higher than that of the acid, and the degree of association diminishes. However, when the acid has been completely converted into the salt, the ionic strength of the solution, which determines the possibility of dissociation of the salt, becomes the crucial factor. Therefore, in strongly alkaline media, the degree of association of the alanylalanine derivative of fullerene depends only on the ionic strength of the solution.

Study of the concentration dependence under identical conditions has shown (see Table 1) that only in acidic media is

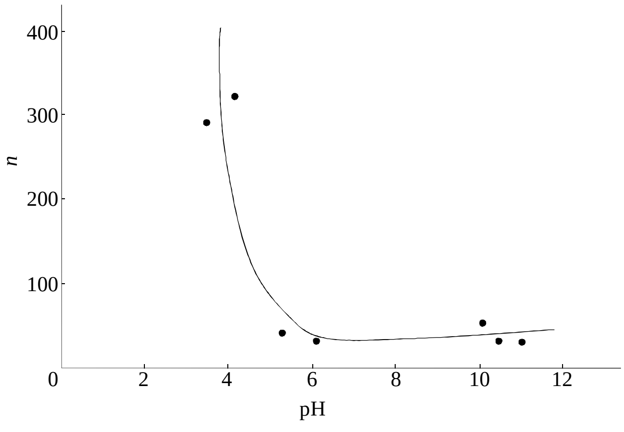

Figure 1 The dependence of the degree of association n of *N*-(monohydrofullerenyl)-DL-alanyl-DL-alanine ($M = 880$) on the pH of the solution in the presence of 0.1 mol dm^{-3} KCl.

[†] $\mathrm{HC_{60}\,NHCH(CH_3)C(O)NHCH(CH_3)COOH}$.

Table 1 Physical constants associated with the picnometric determination.

C/g dm^{-3}	D/10^{-7} cm^2 s	f/10^{-8} g s^{-1}	M/Da	n	V_{ass}/Å^3	V_0/Å^3	d_{ass}/Å	d_0/Å
pH = 3.5; $\bar{v}$ = 0.507 cm^3 g^{-1}								
1.5	6.6	6.227	253070	287.6	213000	740.6	74.1	11.22
1.25	11.9	3.457	43175	49.0	36300	740.8	41.1	11.22
1.0	13.0	3.162	33116	37.6	27880	741.5	37.6	11.22
0.75	14.5	2.834	23853	27.1	20080	741.0	33.7	11.22
pH = 4.1; $\bar{v}$ = 0.567 cm^3 g^{-1}								
1.5	6.15	6.68	281793	320	265000	829	79.5	11.65
1.25	7.15	5.75	178141	202	168000	828	68.4	11.65
1.0	7.78	5.28	138152	157	130000	828	62.2	11.65
0.75	8.15	5.04	120178	137	131000	828	60.0	11.65
pH = 5.25; $\bar{v}$ = 0.728 cm^3 g^{-1}								
1.5	11.65	3.528	32045	36.4	38734	1064	42.0	12.67
1.2	12.0	3.425	29322	33.3	35440	1064	40.8	12.67
0.75	11.0	3.736	38068	43.3	46013	1064	44.5	12.67
pH = 6.1; $\bar{v}$ = 0.722 cm^3 g^{-1}								
1.5	12.7	3.236	24942	28.3	29900	10.55	38.5	12.63
1.25	11.3	3.637	35404	40.2	42440	10.55	43.3	12.63
1.0	12.4	3.314	26796	30.5	32120	10.55	39.4	12.63
0.75	11.2	3.670	36365	41.3	43590	10.55	43.7	12.63
pH = 10.1; $\bar{v}$ = 0.700 cm^3 g^{-1}								
1.5	9.3	4.443	46607	53.0	77380	1461	52.9	14.08
1.25	12.3	3.341	28308	32.2	32900	1027	39.8	12.50
1.0	12.2	3.369	29020	33.0	33730	1023	40.1	12.50
0.75	12.2	3.369	29020	33.0	33730	1023	40.1	12.50
pH = 10.5; $\bar{v}$ = 0.695 cm^3 g^{-1}								
1.5	12.15	3.383	29591	33.6	34140	1015	40.3	12.47
1.25	12.30	3.341	28522	32.4	32910	1015	39.8	12.47
1.0	12.65	3.249	26219	29.8	30250	1015	38.7	12.47
0.75	12.83	3.203	25131	28.6	29000	1015	38.12	12.47
pH = 11.1; $\bar{v}$ = 0.685 cm^3 g^{-1}								
1.5	12.7	3.236	26289	29.9	29900	1001	38.5	12.41
1.25	15.30	2.686	15035	17.1	17100	1001	32.0	12.41
1.0	16.65	2.468	11666	13.3	13270	1001	29.4	12.41
0.75	16.86	2.438	11236	12.8	12780	1001	29.0	12.41

a clear-cut dependence of the degree of association on the concentration of the solution observed. This is accounted for by the extremely low solubility of the substance under these conditions. In neutral and alkaline media where the acid has been completely converted into the salt, the ionic strength of the solution, which determines the possibility of dissociation of the salt and, hence, governs the solubility of the substance and the degree of its association, becomes the main factor. In this case, the dependence of the degree of association on the concentration of the solution is completely levelled by the influence of a substantial ionic strength.

Experimental results made it possible to calculate the diameter of an individual molecule of the substance. As can be seen from the data listed in Table 1, this diameter varies as a function of the pH of the solution. The smallest molecular diameters were found for acidic media; this indicates that the conformation of the molecule changes: in an acidic medium the molecule becomes more compact. This is also confirmed by the marked decrease in the partial specific volume of the substance $\bar{v}$ (the reciprocal of the effective density of the substance ρ), which was constant for all fullerene derivatives[1] or changed only slightly following the variation of only pH.[2] In this case, this parameter was found to depend substantially on the pH of the solution when the ionic strength is relatively high. In acidic media, this value is the smallest, *i.e.* the density of the associates is relatively low. Then, as the solution is alkalified and the medium becomes neutral, this value increases by a factor of approximately 1.5 and thus reaches its maximum. In strongly alkaline media, $\bar{v}$ again slightly decreases. This dependence of the partial specific volume and, correspondingly, the density on the pH indicates that in acidic media at a high ionic strength of the solution, the substance is packed very closely. Apparently, in this case, the solvent is removed almost completely from the associates. Meanwhile, the amount of solvent incorporated in the associates in aqueous solutions reaches 10%.[5] When the solution is alkalified, packing of the substance becomes less close and the solvent penetrates into the associate, which is confirmed by the increase in $\bar{v}$.

Based on the foregoing, the following conclusion can be drawn: the degree of association of *N*-(monohydrofullerenyl)-DL-alanyl-DL-alanine in solution under joint influence of the pH and the ionic strength of the solution depends mainly on the pH in an acidic medium and is a function of the ionic strength of the solution in a neutral or alkaline medium.

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