Brief Communications

Molecular characteristics of aqueous solutions of biologically active disubstituted derivatives of fullerene C_{60}

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The study of the degree of association of biologically active disubstituted fullerene derivatives at different substance concentrations in an aqueous solution using the diffusion method showed that the nature of substituents exerts the strongest effect on the degree of association of the fullerene derivatives.

Key words: fullerene, amino acid, solubility in water, diffusion, associates, C_{60}-*L*-proline, 2-(*L*-prolinofullerenyl(60))ethanol, eosins Y and B, fluoresceine, alanylhistidine, di-*O*-nitroglycerol.

Research on the biological activity of amino acid derivatives of fullerene C_{60} of different structure[1—3] evoked the problem of establishing the routes of excretion of fullerene derivatives from the organism and determining the time necessary for this process. For these purposes, we intended to use fullerene derivatives with a dye residue, because in this case their localization in one or another organ and the time of their excretion from the organism can be controlled with ease. The fullerene derivatives of eosin and fluoresceine turned out to be very good labels for these purposes. However, before using these compounds for biological studies it was necessary to study their behavior in aqueous solutions. The following compounds were chosen: eosin Y sodium salt ester with methyl *N*-[(2-hydroxyethyl)fullerenyl]-*L*-prolinate (**1**), 3′-(*L*-prolinofullerenyl(60))eosin B disodium salt (**2**), and 3′-(*L*-prolinofullerenyl(60))fluoresceine disodium salt (**3**).

In addition, we studied the behavior in aqueous solution of the following fullerene derivatives manifesting antitumor activity: β-alanylhistidine ester with methyl *N*-[(2-hydroxyethyl)fullerenyl]-*L*-prolinate (**4**) and methyl *N*-(2,3-dinitroxypropylfullerenyl)-*L*-prolinate (**5**).

To study the molecular characteristics, we used the data[4] of measurements of the diffusion coefficient *D*. If its value is known, the stability and possible changes of the molecular structure can be controlled. Having measured the diffusion coefficient in solutions, one can calculate the coefficients of translational friction and the hydrodynamic radius (so-called Stokes radius of molecules). If molecules of the compounds under study have spherical shape in solution and no solvation occurs, one can easily

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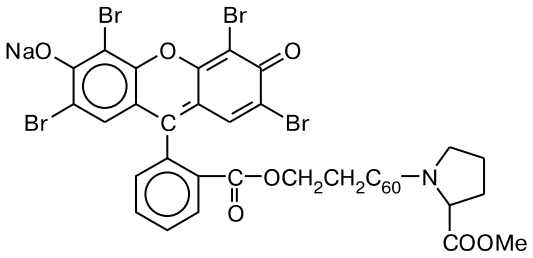

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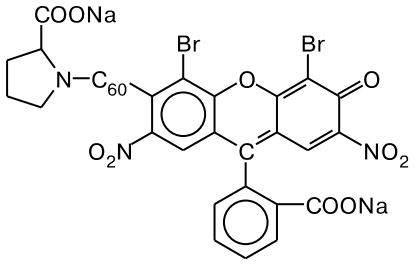

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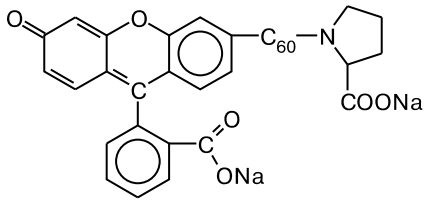

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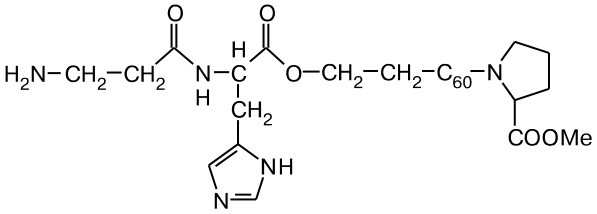

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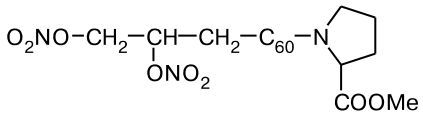

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go from the Stokes radius to the particle volumes and their molecular weight.

Experimental

Experiments were carried out on a MOM-3180 analytical ultracentrifuge (Hungary) using the Philpot—Svensson optics at the rotor temperature 25±0.1 °C. Water was used as the solvent. The diffusion coefficient (D) was measured in a two-sector boundary-forming cell by overlaying the solvent on a solution with the final concentration in a wide concentration range.

The changes in the boundary between the pure solvent and solution at certain time intervals was photographed, thus detecting the extension of the boundary. The rate of rotor rotation (4000—6000 rpm) was chosen in such a way that particles formed no sediments during the experimental time and the boundary extended only due to diffusion. The partial specific volume of particles in solution $\bar{v}$ was determined picnometrically. The density ($\rho_0 = 0.997$ g cm^{-3}) and viscosity of water at 25 °C ($h_0 = 0.8937$ cP) are tabulated values.

The shape of the gradient curves was rather close to Gaussian shape; therefore, the apparent diffusion coefficient D_c (diffusion coefficient at the final concentration) was calculated from the ratio of the surface area Q under the curve to the maximum ordinate H at the moment t (see Ref. 5).

$$D_c = (Q/H)^2/4\pi t. \quad (1)$$

The D coefficient is defined by the coefficient of translational friction f

$$f = kT/D_c, \quad (2)$$

where k is the Boltzmann constant, and T is the absolute temperature (K).

At the same time, for spherical molecules in solution the coefficient of translational friction f is related to the diameter d according to the Stokes equation

$$f = 3\pi h_0 d, \quad (3)$$

where h_0 is the solvent viscosity.

If the diameter of a sphere is expressed through the volume $d = (6V/\pi)^{1/3}$, Eq. (3) can be written as follows:

$$f = 3\pi h_0 (6V/\pi)^{1/3}. \quad (3')$$

In the absence of particle solvation, *i.e.*, $V = M\bar{v}/N_A$ (M is the molecular weight, N_A is Avogardo´s number, and $\bar{v}$ is the partial specific volume),[4] for a spherical particle one has

$$f = 3\pi h_0 (6M\bar{v}/\pi N_A)^{1/3},$$

whence we obtain the formula for the calculation of the molecular weight

$$M = (f/3\pi h_0)^3/(6\bar{v}/\pi N_A). \quad (4)$$

Results and Discussion

Using the experimentally measured values of the diffusion coefficient D_c and the partial specific volume $\bar{v}$, as well as Eqs (2)—(4), for aqueous solutions of the compounds under study we calculated the f values, molecular weights M of the particles (named associates), the number of individual molecules with the molecular weight M_o in the associate $n = M/M_o$, the volumes of associates and individual molecules $V_{ass} = V_{sph}$ and $V_o = V_{ass}/n$, and their diameters $d_{ass} = d_{sph}$ and $d_o = (6V_0/\pi)^{1/3}$.

As can be seen from the data in Tables 1—3, fullerene derivatives of dyes in aqueous solutions behave in different

Table 1. Effect of concentration of compound **1** on the degree of association of its molecules

C /mg mL^{-1}	$D_c \cdot 10^7$ /cm^2 s^{-1}	$f_{sph} \cdot 10^{-7}$ /cm	$R_{sph} \cdot 10^{-6}$ /cm	$V_{sph} \cdot 10^{-18}$ /cm^3	$M_{Dsph} \cdot 10^7$	n	$V_{full} \cdot 10^{-22}$ /cm^3	$R_{full} \cdot 10^{-8}$ /cm	$d_{full} \cdot 10^{-8}$ /cm
1.50	0.84	4.81	2.85	97.4	8.31	53787	18.1	7.56	15.1
1.25	1.58	2.56	2.36	54.8	4.68	30272	18.1	7.56	15.1
1.00	2.05	1.97	1.17	6.7	0.572	3700	18.1	7.56	15.1
0.75	3.45	1.17	0.695	1.41	0.12	778.6	18.1	7.56	15.1
0.50	7.5	0.54	0.321	0.137	0.0117	75.6	18.1	7.56	15.1

Note. The rate of rotation is 8000 rpm, $\bar{v} = 0.706$ cm^3 g^{-1}.

Table 2. Effect of concentration of compound **2** on the degree of association of its molecules

C /mg mL^{-1}	$D_c \cdot 10^7$ /cm^2 s^{-1}	$f_{sph} \cdot 10^{-8}$ /cm	$R_{sph} \cdot 10^{-7}$ /cm	$V_{sph} \cdot 10^{-20}$ /cm^3	M_{sph}	n	$V_{full} \cdot 10^{-21}$ /cm^3	$R_{full} \cdot 10^{-8}$ /cm	$d_{full} \cdot 10^{-8}$ /cm
2.30	9.4	4.37	2.60	7.36	58800	40.7	1.81	7.56	15.0
2.00	10.4	3.95	2.34	5.36	42816	29.6	1.81	7.56	15.0
1.75	12.3	3.34	1.98	3.25	25960	18.0	1.81	7.56	15.0
1.50	13.9	2.95	1.75	2.25	17800	12.4	1.81	7.56	15.0
1.25	14.3	2.87	1.70	2.06	16500	11.4	1.81	7.56	15.2
1.00	15.7	2.62	1.55	1.56	12500	8.6	1.81	7.56	15.1

Note. The rate is 8000 rpm, $\bar{v} = 0.754$ cm^3 g^{-1}.

manner. In spite of structural similarity and a small difference in the partial specific volumes, the magnitude and dependence of the degree of dissociation on the substance concentration in the solution change dramatically, depending on the compound. Compound **1** (monosodium salt with the bridge between the fullerene fragment and eosin residue) has the smallest partial volume of the molecule ($\bar{v} = 0.706$ cm^3 g^{-1}), which indicates a closer packing and higher hydrophobicity compared with those of the derivatives **2** and **3**. These molecules contain directly linked fullerene and dye fragments. In addition, both compounds are disodium salts and have almost equal partial specific volumes ($\bar{v} = 0.754$ and 0.748 cm^3 g^{-1}, respectively). The degree of association of compound **1** decreases dramatically (by several orders of magnitude) upon threefold dilution only. Compounds **2** and **3** are more hydrophilic, because they are disodium salts. Probably, that is why the degree of association of **2** and **3** is much lower than that of compound **1** and decreases by less than ten times rather than orders of magnitude upon dilution.

Derivatives **4** and **5** are promising antitumor drugs.[6] Molecules of compound **4** with free amino groups in the carnosine fragment are very compact (the density of the coil is $\rho_0 = 1/\bar{v} = 1.52$ cm^3 g^{-1}), most likely, due to strong intramolecular interactions. Intermolecular interactions are not very strong, because the degree of association decreases rather sharply upon the dilution of solutions (Table 4). In the case of compound **5**, two nitro groups in the molecule favor the appearance of such a strong intermolecular interaction that the degree of association was measured only at one, very low concentration (Table 5). At higher concentrations, a precipitate was formed.

Thus, our study of the behavior of the fullerene derivatives in aqueous solutions showed that the chemical struc-

Table 3. Influence of the concentration of compound **3** on the degree of association of its molecules

C /mg mL^{-1}	$D_c \cdot 10^7$ /cm^2 s^{-1}	$f_{sph} \cdot 10^{-8}$ /cm	$R_{sph} \cdot 10^{-7}$ /cm	$V_{sph} \cdot 10^{-19}$ /cm^3	$M_{sph} \cdot 10^4$	n	$V_{full} \cdot 10^{-21}$ /cm^3	$R_{full} \cdot 10^{-8}$ /cm	$d_{full} \cdot 10^{-8}$ /cm
0.6	2.31	17.8	10.56	49.10	398	3334	1.47	7.06	14.1
0.5	2.84	14.5	8.59	26.50	214	1791	1.48	7.07	14.1
0.4	3.78	10.9	6.45	11.20	90.6	759	1.47	7.06	14.1
0.3	4.92	8.35	4.96	5.11	41	344	1.48	7.07	14.1
0.2	6.56	6.26	3.72	2.15	17.3	145	1.48	7.07	14.1
0.1	8.41	4.88	2.90	1.02	8.23	69	1.48	7.07	14.1

Note. The rate of rotation is 8000 rpm, $\bar{v} = 0.748$ cm^3 g^{-1}.

Table 4. Effect of concentration of compound **4** on the degree of association of its molecules

C /mg mL^{-1}	$D_c \cdot 10^7$ /cm^2 s^{-1}	$f_{sph} \cdot 10^{-8}$ /cm	$R_{sph} \cdot 10^{-8}$ /cm	$V_{sph} \cdot 10^{-22}$ /cm^3	$M_{D_{sph}} \cdot 10^4$	n	$V_{full} \cdot 10^{-22}$ /cm^3	$R_{full} \cdot 10^{-8}$ /cm	$d_{full} \cdot 10^{-8}$ /cm
1.54	2.3	17.5	104	47500	436	3963	12	6.58	13.16
1.25	3.5	11.5	68.5	13400	123	1119	12	6.58	13.16
1.0	4.0	10.1	59.9	9020	83	753	12	6.58	13.16
0.75	4.6	8.78	52.0	5930	54.5	495	12	6.59	13.18

Note. The rate of rotation is 8000 rpm, $\bar{v} = 0.655$ cm^3 g^{-1}.

Table 5. Parameters characterizing the degree of association of molecules at a low concentration of compound **5**

Parameter	Value	Parameter	Value
C/mg mL^{-1}	0.1	$M_{D_{sph}} \cdot 10^7$	8.53
$D_c \cdot 10^7$/cm^2 s^{-1}	0.796	n	842000
$f_{sph} \cdot 10^{-8}$/cm^5	0.75	$V_{full} \cdot 10^{-22}$/cm^3	13.54
$R_{sph} \cdot 10^{-6}$/cm	3.01	$R_{full} \cdot 10^{-8}$/cm	6.87
$V_{sph} \cdot {}^{10-16}$/cm^3	1.14	$d_{full} \cdot 10^{-8}$/cm	13.7

Note. The rate of rotation is 2000 rpm, $\bar{v} = 0.805$ cm^3 g^{-1}.

ture exerts the greatest effect on the degree of association and on the molecular conformations. The concentration of the solution affects the degree of association to a lesser extent. The data on the change in the degree of association at different concentrations show how to prepare and store aqueous solutions of the compounds discussed for biological studies.

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