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c,T - DEPENDENCE OF THE TRANSLATIONAL DIFFUSION COEFFICIENT FOR HEN EGG-WHITE LYSOZYME IN AQUEOUS SOLUTIONS OBTAINED FROM VISCOSITY MEASUREMENTS AND GENERALIZED STOKES-EINSTEIN RELATION.

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Translational diffusion coefficient D of a protein in a solution depends both on temperature (T) and the protein's volume fraction (Φ). At infinitely dilute solutions the translational diffusion coefficient $D_o(T)$ can be calculated from Stokes-Einstein formula, if the hydrodynamic radius of dissolved protein is known. For hydrated hen egg-white lysozyme this quantity is equal to 1.95 nm, and it gives $D_o(T)$ in the range from 6.89×10^{-11} m^2/s (at 5°C) to 24.4×10^{-11} m^2/s (at 55°C). For higher concentrations D can obtain from the relation: $D(T,\Phi) = D_o(T)\eta_o(T)/\eta(T,\Phi)$, where $\eta_o(T)$ and $\eta(T,\Phi)$ are viscosities of water and solution, respectively, at temperature T. To obtain $D(T,\Phi)$ the viscosity of hen egg-white lysozyme aqueous solutions has been measured at temperatures ranging from 5°C to 55°C and for volume fraction from 0.023 to 0.315. The dependence of $D(T,\Phi)$ – obtained indirectly on the basis of viscosity measurements - on Φ (at fixed temperature) has been analyzed on the basis of a stretched exponential function: $D(T,\Phi) = D_o(T)\exp(-\beta\Phi^{\nu})$, where β and ν are scaling parameters. Both parameters decreases with increasing temperature: β from 13.2 (5°C) to 7.83 (55°C) and ν from 1.4 (5°C) to 1.26 (55°C). The dependence of $D(T,\Phi)$ on T (at fixed volume fraction), in turn, has been analyzed on the basis of the Vogel-Tammann-Fulcher's equation.

INTRODUCTION

One of the most important hydrodynamic parameter describing dynamic behavior of proteins in solution is translational diffusion coefficient D. To accomplish most of their physiological functions proteins have to meet and recognize each other. The random translational and rotational Brownian motion is necessary for it. An understanding of the translational diffusion phenomenon of proteins in solutions is needed to correctly modeling of passive intracellular transport. This process regulates such cellular functions as signal transduction (Cluzel *et al*., 2000) or kinetics of reaction (Berry, 2002). Intracellular protein diffusion plays also an important role in the transport of small molecules and ions (Gros & Moll, 1974). Translational diffusion coefficient can be experimentally obtained by using different experimental techniques such as fluorescence correlation spectroscopy (Banks & Fradin, 2005; Lavalette *et al*., 2006; Goins *et al*., 2008), light scattering (Saluja *et al*., 2007) or pulsed-gradient NMR (Nesmelova *et al*., 2002; Lau & Krishnan, 2007; Rampp *et al*., 2000). Some theoretical methods for prediction of translational diffusion coefficient of proteins are also available (Young *et al*., 1980; Tyn & Gusek, 1990; Aragon & Hahn, 2006). In the present study the translational diffusion coefficient of hen egg-white lysozyme (HEWL) in infinitely-dilute solutions has been obtained on the basis of the generalized Stokes-Einstein equation and for higher concentrations indirectly from viscosity measurements of aqueous solutions of HEWL.

HEWL is a small globular protein of the molecular mass M = 14 320 Da (Squire & Himmel, 1979) and well-known structure (Smith *et al*., 1993). It has been the subject of many physicochemical studies by using different experimental techniques for many years (Blanch *et al*., 2000; Gregory *et al*., 1993; Hadden *et al*., 1995; Miura *et al*., 1994; Monkos, 1997; Smith *et al*., 1995; Smyth *et al*., 2001; Turula & de Haseth, 1996) and it serves as a model protein for different biophysical studies. Some advanced theoretical methods have also been applied in those investigations (Halle & Davidovic, 2003; Roth *et al*., 1996; Zhou, 1995). In this paper the translational diffusion coefficient – obtained indirectly from viscosity measurements - for HEWL in aqueous solutions (from diluted up to concentrated ones) at temperatures ranging from 5 to 55^0C is presented. Concentration dependence of such obtained translational diffusion coefficient is discussed by using a two parameters stretched exponential function. Temperature dependence, in turn, is analyzed on the basis of the three parameters Vogel-Tammann-Fulcher's equation. Those

parameters have been obtained in the whole range of measured concentrations, and their physical meaning has been also discussed.

MATERIALS

Crystallized and highly purified HEWL was obtained from Sigma Chemical Co. and was used without further purification for all the measurements. From the crystalline state the material was dissolved in distilled water. Such obtained solutions were treated with filter paper in order to remove possible undissolved dust particles. The samples were cooled in a refrigerator until just prior to viscometry measurements, when they were wormed from 5 to 55^0C, mainly by steps of 5^0C. The pH values of such prepared solutions fluctuated slightly in the vicinity of neutral pH (7.0), i.e. were outside of isoelectric point. The isoelectric point for HEWL is in the range (11 – 11.2) (Young, 1963).

VISCOMETRY

The viscosity measurements were performed using an Ubbelohde-type capillary microviscometer with a flow time for water of 28.5 s at 25^0C. The microviscometer was placed in a water-bath controlled thermostatically at 5 to 55^0C with a precision of ± 0.1 ^{0}C. The upper limit of temperature has been established by the temperature of denaturation, which for HEWL is only slightly higher than 55^0C. The same viscometer was used for all measurements. Measurements started after several minutes delay to ensure the system reached equilibrium. Flow times were recorded to within 0.1 s and – for most concentrations – the viscosity measurements were conducted from 5 to 55^0C in 5^0C intervals. In this range of temperatures, the viscosity has been measured from diluted up to concentrated solutions, i.e. from 24.9 kg/m^3 to 343 kg/m^3. Solution densities were measured by weighing, and protein concentrations were determined by a dry weight method in which samples were dried at high temperatures for several hours.

RESULTS AND DISCUSSION

At the first approximation, proteins – and, in particular, HEWL - in aqueous solutions can be treated as Brownian particles immersed in an ideal, homogeneous and isotropic solvent whose molecular size is so small that it can be practically regarded as continuous. Translational diffusion of such Brownian particles is driven by thermal energy and is hindered by friction experienced by the particles. In the case of solutions at infinite dilution the problem was studied by Einstein in his fluctuation-dissipation theory (Einstein, 1956). In this limit, interactions between immersed particles can be neglected and the interactions between the large particles and the solvent can be replaced by a randomly fluctuating forces. Einstein's theory combined with the results of macroscopic continuum hydrodynamics (Landau & Lifshitz, 1958) gives - for the translational diffusion coefficient of spherical particles at infinite dilution - the so-called Stokes-Einstein relation:

$$D_o(T) = \frac{kT}{6\pi\eta_o(T)R_h} \qquad (1)$$

where k is Bolzmann's constant, T is the absolute temperature, $\eta_o(T)$ is the solvent viscosity and R_h is the hydrodynamic radius of the immersed particles. For a spherical particle, the hydrodynamic radius R_h is equal to its radius.

The diffusion of ellipsoidal particles was studied by Perrin (Perrin, 1936). For particles with a shape of prolate ellipsoid, the hydrodynamic radius is expressed as:

$$R_h = \frac{\sqrt{a^2 - b^2}}{ln\left(\frac{a + \sqrt{a^2 - b^2}}{b}\right)} \qquad (2)$$

where a and b are the major and minor semi-axes of the ellipsoid. For some proteins in the native state has been experimentally showed that the hydrodynamic radius does not depend on solution pH and temperature (Jachimska *et al*., 2008). Equation (1) for non-spherical particles is called generalized Stokes-Einstein relation.

As revealed from X-ray diffraction studies of HEWL in crystals, its molecules can be treated as prolate ellipsoids of revolution with the main semi-axes 2.25 nm and 1.5 nm (Squire & Himmel, 1979). However, the protein molecules in solution are surrounded by a hydration shell of water molecules which have to be taken into account in calculations of some hydrodynamic parameters including hydrodynamic radius. The level of protein hydration – usually marked by δ - denotes the amount of grams of water associated with the protein per a gram of the protein. For HEWL the full hydration is achieved at $\delta = 0.38$ (Gregory *et al*., 1993; Pérez *et al*., 1999). The hydrodynamic volume of one dissolved protein molecule in aqueous solution V is a sum of a volume of the unhydrated protein V_o and a volume of the hydration shell: $V = V_o + M\delta/N_A\rho_w$, where N_A and ρ_w are Avogadro's number and water density, respectively. It gives the hydrodynamic volume of HEWL molecule V = 30.24 nm^3. On the other hand,

analysis of viscosity data of HEWL aqueous solutions shows that the axial ratio of hydrated lysozyme molecule is p = 1.35. Because the volume of an ellipsoid of revolution is $V = 4/3\pi ab^2$, then its semi-axes can be obtained from: $b = (3V/4\pi p)^{1/3}$ and a = pb. It gives for hydrated molecule of HEWL a = 2.36 nm and b = 1.75 nm. One layer of water increases a dimension of the protein semi-axis by approximately 0.3 nm. So, the above results show that the hydration shell of water on the surface of HEWL molecule is not a uniform monolayer but is rather a patchwork of water clusters, covering some atoms in charges groups by water layer while leaving some part of the protein surface uncovered.

From the above calculated values of the semi-axes and from relation (2) it is easy to obtain the hydrodynamic radius of hydrated HEWL molecule: R_h = 1.95 nm. Taking the values of water viscosity η_o from the standard physicochemical tables, one can now calculate - from Stokes-Einstein relation – the translational diffusion coefficient of HEWL in the limit of infinite dilution. The results are gathered in Table I. It is interesting to compare them with the values obtained from different experimental method. In the literature, experimental values of the translational diffusion coefficient are usually given at infinite dilution and at the temperature 20°C. As seen in Table I the Stokes-Einstein relation gives - in this case - for HEWL $D_o(T) = 11.0\times10^{-11}$ m^2/s. The literature values of $D_o(T)$ for HEWL are as follows: 10.6×10^{-11} m^2/s (Dubin *et al.*, 1971), 10.9×10^{-11} m^2/s (Allison & Tran, 1995), 11.1×10^{-11} m^2/s (Banachowicz *et al.*, 2000), 11.2×10^{-11} m^2/s (Saphianopoulos *et al.*, 1962). It proves that the translational diffusion coefficient calculated from the Stokes-Einstein relation with the hydrodynamic radius obtained from Perrin formula for hydrated protein agrees very well with the experimental results obtained in the limit of infinite dilution.

Table 1. The numerical values of the translational diffusion coefficient for hen egg-white lysozyme in aqueous solutions calculated on the basis of relation (1).

t[°C]	$10^{11}\times D_o$ [m^2/s]
5	6.89
10	8.14
15	9.50
20	11.0
25	12.5
30	14.2
35	16.0
40	18.0
45	20.0
50	22.1
55	24.4

To understand the diffusion process of proteins inside cells the knowledge about proteins diffusivity in concentrated solutions is necessary. The first measurements of the translational diffusion coefficient of proteins at high concentrations have been made for ovalbumin (Wang *et al.*, 1954). The results of the investigations of proteins diffusion in concentrated solutions for other proteins are also available in the literature (Nesmelova *et al.*, 2002; Gros, 1978 and references therein). Wang et al also proposed to use the Stokes-Einstein relation to obtain the translational diffusion coefficient at high concentrations. To do it, the

Table 2. The numerical values of the translational diffusion coefficient (in 10^{-11} m^2/s) for hen egg-white lysozyme in aqueous solutions obtained indirectly from viscosity measurements and relation (3) for all measured concentrations.

c[kg/m^3]	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C	50°C	55°C
24.9	6.14	7.29	8.52	9.87	11.4	12.9	14.5	16.4	18.2	20.0	21.9
35.3	5.94	7.07	8.28	9.55	11.0	12.6	14.1	15.8	17.5	19.5	21.2
42.6	5.80	6.87	8.04	9.34	10.7	12.2	13.8	15.5	17.2	19.0	20.8
50.9	5.61	6.66	7.85	9.06	10.4	11.9	13.5	15.0	16.8	18.6	20.3
63.3	5.27	6.27	7.39	8.54	9.77	11.3	12.8	14.5	16.0	17.9	19.5
70.6	5.18	6.13	7.19	8.37	9.64	11.0	12.4	14.0	15.7	17.4	19.0
76.8	4.90	5.85	6.89	7.97	9.18	10.5	11.9	13.4	15.0	16.7	18.3
83.1	4.82	5.73	6.72	7.82	8.99	10.3	11.7	13.2	14.7	16.2	17.8
92.4	4.59	5.48	6.43	7.49	8.62	9.86	11.2	12.7	14.1	15.7	17.3
106	4.24	5.10	6.01	6.98	8.10	9.25	10.5	11.8	13.2	14.8	16.2
109	4.23	5.04	5.94	6.92	7.98	9.15	10.4	11.7	13.1	14.7	16.0
150	3.27	3.97	4.71	5.54	6.43	7.46	8.52	9.63	10.8	12.1	13.3
202	2.19	2.72	3.32	3.98	4.71	5.49	6.34	7.22	8.20	9.22	10.2
209	1.94	2.45	2.99	3.59	4.27	5.02	5.81	6.66	7.53	8.52	9.48
239	1.45	1.86	2.33	2.85	3.44	4.07	4.78	5.51	6.31	7.16	7.94
257	1.10	1.45	1.85	2.31	2.82	3.38	3.98	4.65	5.34	6.10	6.80
296	.657	.922	1.23	1.59	1.99	2.44	2.94	3.49	4.08	4.71	5.28
322	.305	.470	.668	.912	1.20	1.53	1.90	2.31	2.76	3.24	3.67
343	.144	.244	.376	.543	.751	.996	1.27	1.60	1.96	2.34	2.69

viscosity η_o in the Stokes-Einstein relation should be replaced rather by the macroscopic solution viscosity $\eta(c,T)$. So, the following relation should be fulfilled:

$$D(c,T) = D_o(T)\frac{\eta_o(T)}{\eta(c,T)} \qquad (3)$$

Correctness of the above equation has been successfully verified for different proteins by Gros (Gros, 1978). On the basis of relation (3) the translational diffusion coefficient of HEWL in the whole range of measured concentrations and temperatures has been calculated and the results are presented in Table II. It is worth to note that correctness of the above equation has quite recently been also verified by Lavalette (Lavalette *et al*., 2006). The authors showed that equation (3) is correct only in the case when the size of solvent molecules is negligible in comparison to the size of dissolved particles. In the case when the local viscosity is induced rather by the presence of macromolecular co-solutes such as proteins, RNA's etc. with a large molecular mass dispersion, the relation of the form $D = D_o(\eta_o/\eta)^q$ is fulfilled. Experimentally has been proved that the exponent $q \leq 1$ and it depends on the co-solvent's dimension and mass. The deviations from relation (3) appear when the molecular mass of co-solvent is higher than 10^3 Da and become more distinct as the molecular mass of the co-solvent increases.

In the case of dilute solutions the translational diffusion coefficient of dissolved particles depends linearly on concentration, and it is usually presented in the following equation (Brown & Stilbs, 1982; Han & Herzfeld, 1993; Xia *et al*., 1994):

$$D(c,T) = D_o(T)(1 - K_D\Phi) \qquad (4)$$

where the coefficient K_D is a measure of interparticle interaction and Φ denotes the volume fraction of the particles. The volume fraction can be expressed in the following way: $\Phi = N_A Vc/M_h$ where M_h denotes the molecular mass of hydrated particles and c is concentration in kg/m^3.

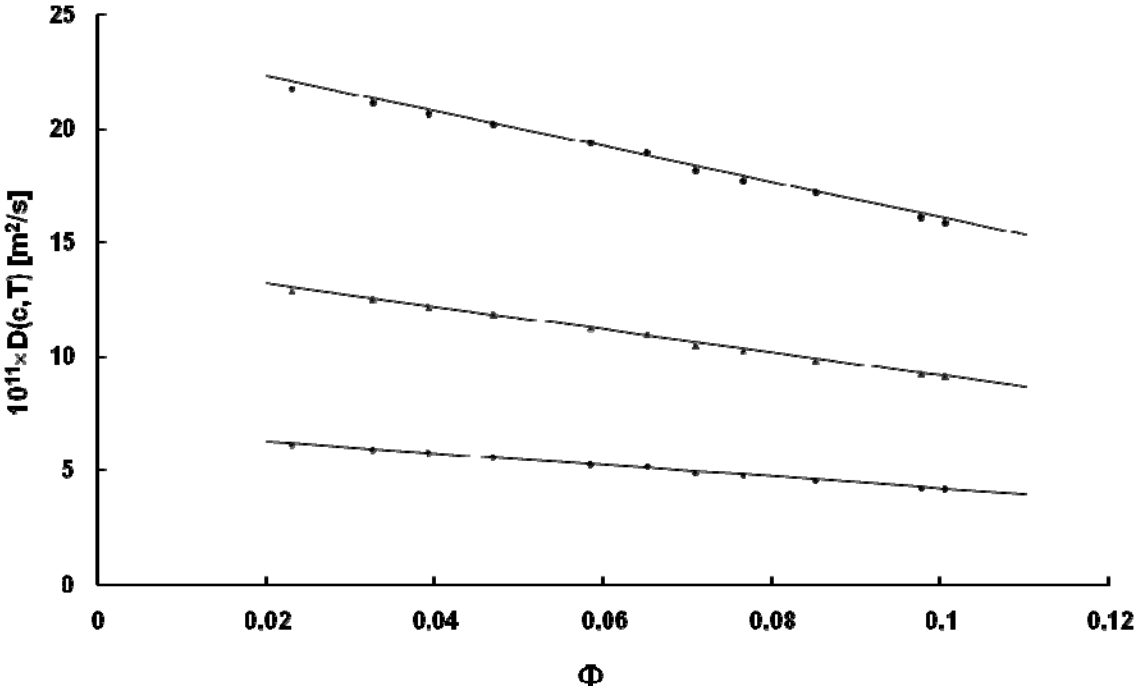

Fig. 1. Plot of the translational diffusion coefficient vs. volume fraction of hen egg-white lysozyme in aqueous solutions at T = 328 K (•), T = 303 K (▲) and T = 278 K (♦). Experimental points were obtained indirectly from viscosity measurements and equation (3); the straight lines show the fit according to equation (4) with the parameters: $D_o(T) = 23.9\times10^{-11}$ m^2/s, $K_D = 3.26$ at T = 328 K; $D_o(T) = 14.2\times10^{-11}$ m^2/s, $K_D = 3.55$ at T = 303 K; $D_o(T) = 6.78\times10^{-11}$ m^2/s, $K_D = 3.79$ at T = 278 K.

As mentioned above, protein molecules in water solution are surrounded by a hydration shell of water molecules. The "bound" water molecules migrate with the protein and therefore contribute to its hydrodynamic

mass. This quantity can be expressed as a sum of the molecular mass of unhydrated protein M and the mass of hydration shell: $M_h = M(1 + \delta)$. For HEWL M = 14 320 Da, $\delta = 0.38$ and it gives $M_h = 19\,762$ Da. So, in this case the volume fraction one can calculate in the following way: $\Phi = 9.22\times10^{-4}$ m^3/kg ×c. Analysis of the obtained values of the translational diffusion coefficient for HEWL shows that – in the whole range of measured temperatures – D(c,T) depends linearly on concentration up to $\Phi \cong 0.1$ (it corresponds to the concentration of about 109 kg/m^3), with the correlation coefficient r = 0.999. For three temperatures it is shown in Figure 1. As seen the slope of the straight lines changes with temperature: the lower temperature the higher rate of decreasing of translational diffusion coefficient with increasing of the volume fraction is. For spherical particles the coefficient K_D should be equal to 1.5 (Brown & Stilbs, 1982). For HEWL, this quantity changes from 3.79 (5°C) to 3.26 (55°C). It indicates that the rate of decreasing of translational diffusion coefficient with increasing of the volume fraction is higher for aspherical particles than for spherical ones.

For higher concentrations the dependence of the translational diffusion coefficient of proteins on concentration becomes non-linear. In the whole range of measured concentrations this dependence can be described be a stretched exponential function (Banks & Fradin, 2005; Dwyer & Bloomfield, 1993; Goins *et al.*, 2008):

$$D(c,T) = D_o(T)\exp\left(-\beta\Phi^{\nu}\right) \qquad (5)$$

where β and ν are scaling parameters.

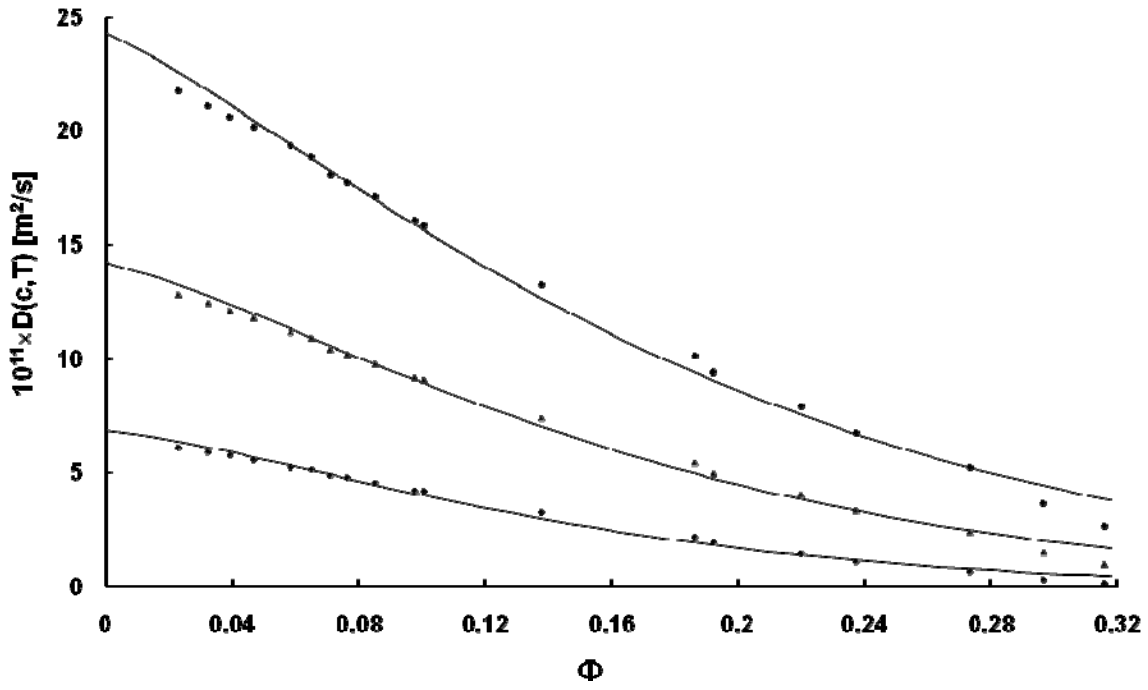

Fig. 2. Plot of the translational diffusion coefficient vs. volume fraction of hen egg-white lysozyme in aqueous solutions at T = 328 K (•), T = 303 K (▲) and T = 278 K (♦). Experimental points were obtained indirectly from viscosity measurements and equation (3); the curves show the fit according to equation (5) with the parameters: $D_o = 24.4\times10^{-11}$ m^2/s, β = 7.83 and ν = 1.26 at T = 328 K; $D_o = 14.2\times10^{-11}$ m^2/s, β = 9.76 and ν = 1.33 at T = 303 K; $D_o = 6.89\times10^{-11}$ m^2/s, β = 13.2 and ν = 1.40 at T = 278 K.

Figure 2 shows a plot of the translational diffusion coefficient vs. volume fraction for HEWL in the whole range of measured concentrations. The curves show the fit to the experimental points obtained using above relation with β and ν treated as adjustable parameters. The numerical values of those parameters obtained in such a way are presented in Table III. As seen both parameters decrease with increasing temperature. Unfortunately, their physical meaning is not clear. Some results suggest only that ν should decrease as the polymer molecular mass increases (Banks & Fradin, 2005). So, experimental values of both parameters for different proteins are highly desirable.

Table 3. The numerical values of the scaling parameters from equation (5) for hen egg-white lysozyme.

t[^{o}C]	5	10	15	20	25	30	35	40	45	50	55
β	13.2 ±1.3	12.2 ±1.1	11.5 ±.97	10.7 ±.87	10.1 ±.78	9.76 ±.70	9.39 ±.66	9.00 ±.60	8.56 ±.56	8.29 ±.54	7.83 ±.53
ν	1.40 ±.05	1.38 ±.05	1.37 ±.04	1.35 ±.04	1.33 ±.04	1.33 ±.04	1.32 ±.04	1.31 ±.03	1.30 ±.03	1.29 ±.03	1.26 ±.03

Temperature dependence of the translational diffusion coefficient is usually described by the Vogel-Tammann-Fulcher equation (Lau & Krishnan, 2007; Rampp *et al*., 2000). With modification made by Angell (Angel, 1988), it has the form:

$$D(c,T) = A(c)\exp\left[-\frac{F(c)T_o(c)}{T-T_o(c)}\right] \qquad (6)$$

where A(c), F(c) and $T_o(c)$ are parameters which depends on concentration. To fit the translational diffusion coefficient from the above equation to the experimental values of D(c,T) obtained at different temperatures the numerical values of these parameters are necessary. They have been calculated – for each fixed concentration - by using the non-linear least square method. Figure 3 shows the values of the translational diffusion coefficient at various temperatures for HEWL, for three concentrations. The curves present the fit to the experimental points according to the above equation. As

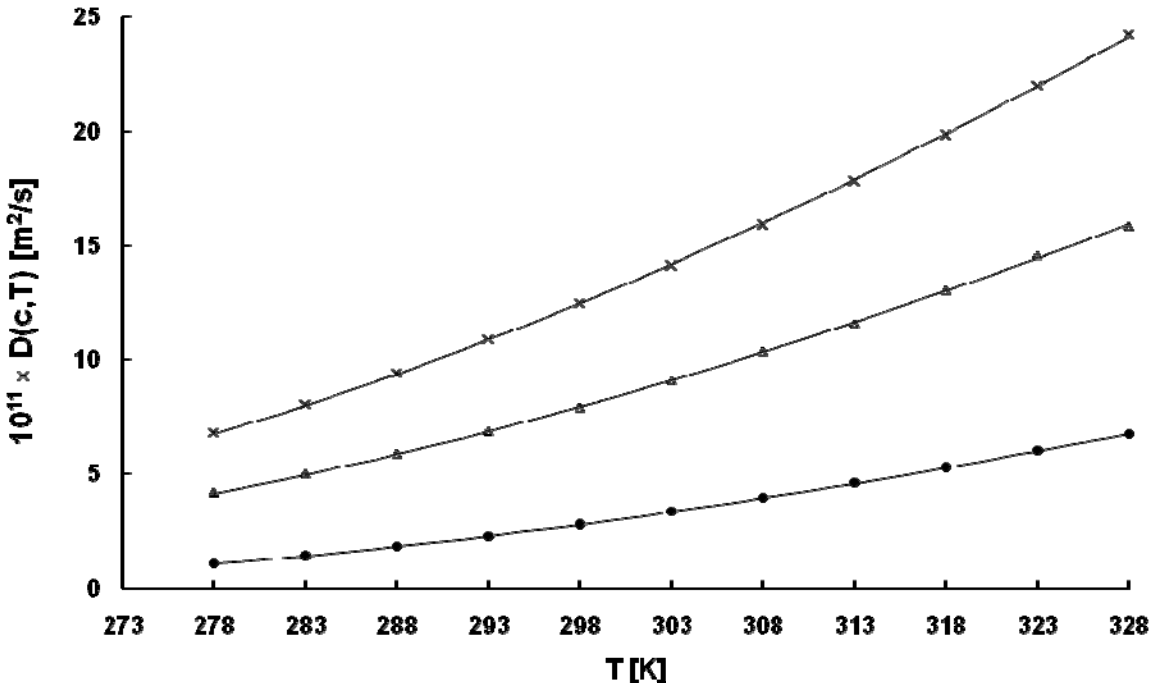

Fig. 3. Temperature dependence of the translational diffusion coefficient of hen egg-white lysozyme in aqueous solutions for concentrations: $c \to 0$ (×), 109 kg/m^3 (Δ) and 257 kg/m^3 (•). The numerical values of D(c,T) for c = 109 and 257 kg/m^3 were obtained indirectly from viscosity measurements and relation (3). The curves show the fit obtained by using equation (6) with the parameters: $A = 6.05\times10^{-9}$ m^2/s, B = 3.77 and T_o = 151 K ($c \to 0$); $A = 4.57\times10^{-9}$ m^2/s, B = 3.83 and T_o = 153 K (c = 109 kg/m^3); $A = 1.36\times10^{-9}$ m^2/s, B = 2.01 and T_o = 196 K (c = 257 kg/m^3).

seen a very good fit over the whole range of temperatures has been obtained. The parameters A(c), F(c) and $T_o(c)$ appear to be dependent on concentration in a quite different way and the results of calculations for F(c) and $T_o(c)$ are shown in Figure 4.

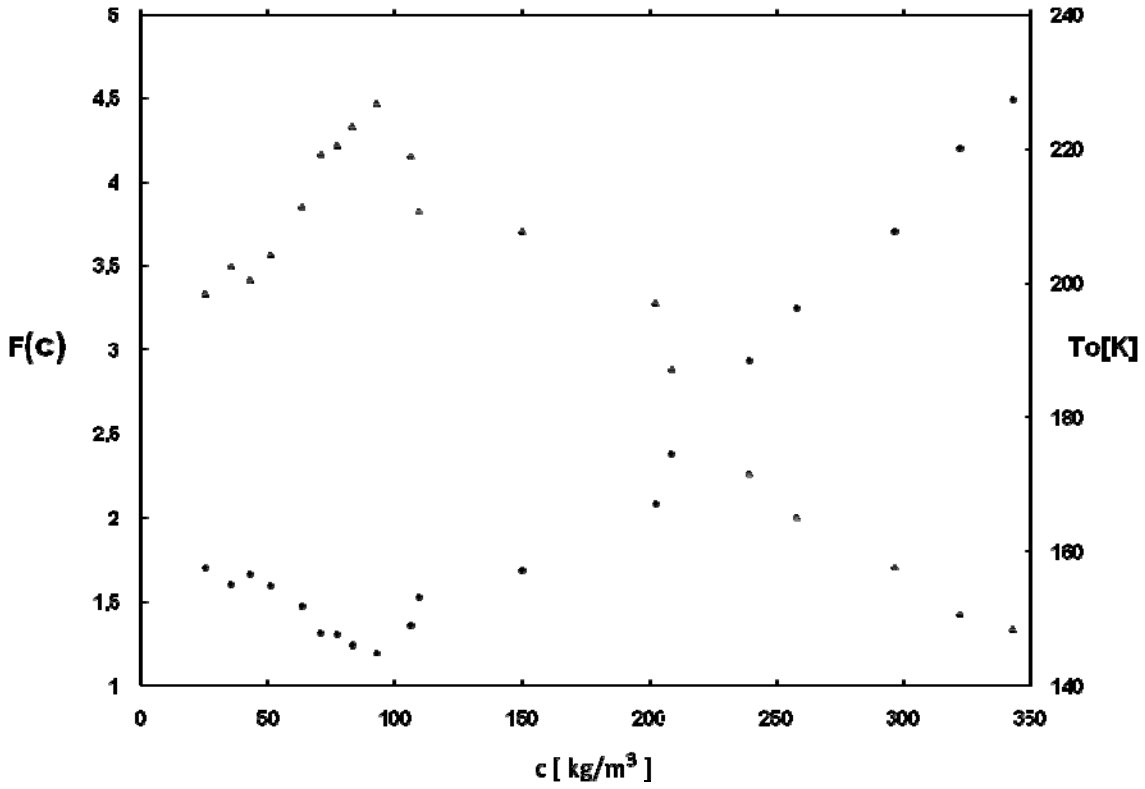

Fig. 4. Plot of the ideal glass transition temperature $T_o(c)$ (•) and fragility parameter F(c) (Δ) versus concentration, for hen egg-white lysozyme aqueous solutions.

The parameter A(c) represents the high-temperature limit of the translational diffusion coefficient. As appears, for dilute solutions A(c) increases with increasing concentration from 4.77×10^{-9} m^2/s to 5.91×10^{-9} m^2/s, and for semi-diluted and concentrated solutions decreases with increasing concentration to the value 5.51×10^{-8} m^2/s (at c = 343 kg/m^3) in rather an unsystematic fashion.

T_o represents the ideal glass-transition temperature, i.e. the temperature in which the molecular mobility of supercooled liquid is completely stopped. The theoretical basis of the Vogel-Tammann-Fulcher equation is the theory of entropy worked out by Adam and Gibbs (Adam & Gibbs, 1965). The theory uses the notion of the configurational entropy: $S_c = k\ln\Omega$ where Ω denotes the number of configurations available to the system of N molecules. By assuming that a liquid's flow requires collective rearrangements of some number of molecules and that the energy required to such rearrangements increases in proportion to this number, the authors obtained – at the equilibrium state – the Vogel-Tammann-Fulcher equation. The ideal glass transition temperature is then identified with the so called Kauzmann temperature, i.e. the temperature where configurational entropy is equal to zero. As seen in Figure 4, the ideal glass transition temperature for the solutions of hen egg-white lysozyme decreases linearly with increasing concentration in the range of dilute solutions, and increases with increasing concentration in the region of semi-diluted and concentrated ones. Contrary to this, the ideal transition temperature obtained for aqueous solutions of some carbohydrate (Rampp *et al*., 2000), porcine serum albumin (Monkos, 2003), human serum albumin (Monkos, 2004) and dimeric bovine β-lactoglobulin (Monkos, 2008) depends – in the whole range of measured concentrations – non-linearly on concentration. Because of lack of any theoretical explanation of such changes, for porcine and human serum albumin and for dimeric bovine β-lactoglobulin some phenomenological description of such dependence has only been proposed.

The parameter F(c) has been originally introduced by Angell (Angell, 1988) for glass-forming liquids in order to differentiate their various temperature dependences of viscosity. According to this conception 'strong' liquids have highly constrained structures which have a low density of configurational states and their viscosity does not decrease much with increasing temperature above

glass transition temperature. The 'fragile' liquids, in turn, have relatively unconstrained structures, so that many configurations become available to them as the temperature raises and they show a strong decline of viscosity with increasing temperature. Figure 4 shows numerical values of the fragility parameter obtained for HEWL on the basis of Vogel-Tammann-Fulcher's equation. As seen, in this case F(c) increases linearly with increasing concentration in the range of dilute solutions, and decreases with increasing concentration in the region of semi-diluted and concentrated ones. All values of fragility parameter lie in the range from 1.34 to 4.47 and it indicates that the studied solutions belong to the extremely fragile class of liquids. For the extremely strong liquids, the fragility parameter reaches the value of about 100.

CONCLUSIONS

Translational diffusion coefficient in the limit of zero concentration obtained for HEWL in aqueous solution from Stokes-Einstein equation with the hydrodynamic radius calculated on the basis of Perrin formula agrees very well with its values obtained from different experimental techniques. This quantity changes with temperature from 6.89×10^{-11} m^2/s (5°C) up to 24.4×10^{-11} m^2/s (55°C). Translational diffusion coefficient obtained here indirectly from viscosity measurements, in the range of dilute solutions - i.e. when the volume fraction of HEWL does not exceed the value of approximately 0.1 - decreases approximately linearly with increasing concentration. Linear regression coefficient changes in this case with temperature from 3.79 (5°C) up to 3.26 (55°C). However, concentration dependence of such obtained translational diffusion coefficient in the whole range of concentrations, i.e. from dilute to concentrated solutions is non-linear and can be described by a stretched exponential function. Two parameters in this function also decrease with increasing temperature. Temperature dependence of such translational diffusion coefficient, in turn, can be described by the three parameters Vogel-Tammann-Fulcher equation. However, the parameters of this equation - for HEWL - depends on concentration in rather an irregular manner.

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