PRACA ORYGINALNA

Temperature and concentration dependence of translational diffusion coefficient for human serum albumin in aqueous solutions at different pH

Zależność współczynnika dyfuzji translacyjnej od temperatury i stężenia dla albuminy surowicy ludzkiej w roztworach wodnych przy różnych wartościach pH

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ABSTRACT

BACKGROUND

The aim of the present paper is to investigate the temperature and concentration dependence of the translational diffusion coefficient for human serum albumin in aqueous solutions at neutral pH and at the isoelectric point.

MATERIAL AND METHODS

The viscosity of human serum albumin aqueous solutions at pH 4.7 and 7.0 was measured at temperatures ranging from 5^{o}C to 45^{o}C and in a wide range of concentrations. The measurements were performed with an Ubbelohde-type capillary microviscometer.

RESULTS

The translational diffusion coefficient of a protein in infinitely dilute solutions $D_o(T)$ can be calculated from the generalized Stokes-Einstein equation if the hydrodynamic radius of the studied protein is known. For hydrated human serum albumin, this equation gives $D_o(T)$ in the range of 3.45 x 10^{-11} m^2/s (at 5^{o}C) to 10.0 x 10^{-11} m^2/s (at 45^{o}C). The translational diffusion coefficient for higher concentrations of $D(T,\Phi)$ can be obtained from the relation: $D(T,\Phi) = D_o(T)\eta_o(T)/\eta(T,\Phi)$, where Φ denotes the volume fraction and $\eta_o(T)$ and $\eta(T, \Phi)$ are the viscosities of water and the solution at temperature T, respectively.

CONCLUSIONS

The translational diffusion coefficient of human serum albumin in solutions both at pH 4.7 and 7.0 decreases linearly with increasing volume fraction, when Φ does not exceed the value of about 0.11. The dependence of the translational diffusion coefficient on volume fraction in the whole measured range of Φ is non-linear and can be described by a stretched exponential function. The scaling

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parameters in this function are different at different values of pH. The dependence of the translational diffusion coefficient on temperature, in turn, can be described by the three-parameter Vogel-Tammann-Fulcher's equation.

KEY WORDS
translational diffusion coefficient, viscosity, Stokes-Einstein equation, human serum albumin

STRESZCZENIE

WSTĘP
Celem niniejszej pracy jest zbadanie zależności współczynnika dyfuzji translacyjnej od temperatury i ułamka objętościowego dla albuminy surowicy ludzkiej w roztworach wodnych przy neutralnym pH oraz w punkcie izoelektrycznym.

MATERIAŁ I METODY
Lepkość wodnych roztworów albuminy surowicy ludzkiej przy pH 4,7 i 7,0 zmierzono w zakresie temperatur 5––45^{o}C i w szerokim zakresie stężeń. Pomiary wykonano za pomocą kapilarnego mikrowiskozymetru typu Ubbelohde'a.

WYNIKI
Współczynnik dyfuzji translacyjnej białka dla roztworów rozcieńczonych $D_o(T)$ można obliczyć z uogólnionego równania Stokesa-Einsteina, jeżeli znany jest promień hydrodynamiczny badanego białka. Dla uwodnionej albuminy surowicy ludzkiej równanie to daje $D_o(T)$ w zakresie od $3{,}45 \times 10^{-11}$ m^2/s (w 5^{o}C) do $10{,}0 \times 10^{-11}$ m^2/s (w 45^{o}C). Współczynnik dyfuzji translacyjnej dla wyższych stężeń $D(T,\Phi)$ można otrzymać z relacji: $D(T,\Phi) = D_o(T)\eta_o(T)/\eta(T,\Phi)$, w której Φ oznacza ułamek objętościowy a $\eta_o(T)$ i $\eta(T,\Phi)$ to lepkości, odpowiednio, wody i roztworu w temperaturze T.

WNIOSKI
Współczynnik dyfuzji translacyjnej albuminy surowicy ludzkiej w roztworach wodnych zarówno przy pH 4,7 jak i 7,0 maleje liniowo wraz ze wzrostem ułamka objętościowego, jeżeli Φ nie przekracza wartości około 0,11. Zależność współczynnika dyfuzji translacyjnej od ułamka objętościowego w całym mierzonym zakresie Φ jest nieliniowa i można ją opisać rozciągniętą funkcją wykładniczą. Parametry skalowania w tej funkcji są różne przy różnych wartościach pH. Z kolei zależność współczynnika dyfuzji translacyjnej od temperatury może być opisana przez trójparametryczne równanie Vogela-Tammanna-Fulchera.

SŁOWA KLUCZOWE
współczynnik dyfuzji translacyjnej, lepkość, równanie Stokesa-Einsteina, albumina surowicy ludzkiej

INTRODUCTION

Human serum albumin (HSA) is the most abundant protein in human blood plasma. Analysis of the protein in the crystal state by the X-ray method showed that the protein is a 585 amino acid residue monomer that folds into three structurally homologous α-helical domains [1]. These domains assemble to form a heart-shaped molecule [2]. In a solution, the conformation of HSA depends on the pH of the solution and the protein undergoes reversible conformational transformations with a change in pH [3,4,5]. The normal (or N) form is predominant from pH 4.3 to 8.0 and the HSA molecule is then globular. At pH 4.3, the first conformational transition occurs and below this value of pH, HSA conformation changes to a highly charged fast migrating form (or F). At a pH less than 2.7 in turn, the F-form changes to the fully extended form (or E). At higher values of pH, a conformational transition of HSA takes place at pH 8.0 when the N-form changes to the basic form (or B). A conformation of HSA in the normal form is commonly approximated by an ellipsoid of revolution with one long semi-axis (a) and two shorter semi-axes (b). Analysis of viscosity data showed that from a hydrodynamic point of

view, the hydrated HSA molecule in the N form can be treated as a prolate ellipsoid of revolution with semi-axes a = 8.2 nm and b = 2.1 nm [6]. HSA contributes to colloidal osmotic blood pressure, but mainly serves as a transport protein for fatty acids, drugs, hormones, metal ions, dyes, and amino acids, as well as pharmaceutical compounds [7]. In conjunction with it, knowledge about the quantities characterizing transport phenomena, like the translational diffusion coefficient, is very important. Random translational and rotational Brownian motion of protein molecules is necessary to perform most of the physiological functions of the protein. In particular, the translational diffusion phenomenon of proteins plays an important role in passive intracellular transport which regulates such cellular functions as signal transduction [8], self-assembly of supramolecular structures [9], the kinetics of reactions [10] and embryogenesis [11] and plays a crucial role in the transport of small molecules and ions [12]. The translational diffusion coefficient of proteins can be measured by using different experimental techniques such as fluorescence correlation spectroscopy [13,14,15], light scattering [16], pulsed-gradient NMR [17,18,19], "single particle tracking" [20], "fluorescence recovery after photobleaching" [21] and analysis of the distribution of radioactivity [22]. This quantity can also be calculated from theoretical models [23,24,25]. Some sophisticated models, so-called bead models, need the knowledge about proteins structure on an atomic level and require high resolution X-ray crystallographic or NMR data which are not always available [26,27,28].

In the present study, the translational diffusion coefficient of HSA in infinitely dilute solutions has been obtained from the generalized Stokes-Einstein equation and for higher concentrations, from the viscosity measurements of aqueous solutions of HSA and by using the method proposed by Gros [22]. The translational diffusion coefficient of HSA has been obtained in solutions at pH 7.0 (i.e. at pH in the vicinity of physiological pH (7.4) [29]) and at pH 4.7 (i.e. in the isoelectric point [30]). In both cases, the measurements of viscosity have been conducted from diluted solutions up to concentrated ones and at temperatures ranging from 5 to 45^{o}C. The concentration dependence of the translational diffusion coefficient of HSA in diluted solutions is described by a linear function. However, such a dependence in the whole range of measured concentrations is non-linear and is described by using a three-parameter stretched exponential function. The translational diffusion coefficient also depends non-linearly on temperature. Such a dependence in turn, can be described by the three-parameter Vogel-Tammann Fulcher's relation. Those parameters have been obtained in the whole range of measured concentrations for HSA solutions both at pH 4.7 and 7.0.

MATERIAL

The HSA (lyophilized, purity 99% and essentially fatty acid- and globulin-free) for the solutions at pH 4.7 was purchased from Polish Chemical Reagents factories. The HSA for the solutions at pH 7.0 was obtained from Sigma Chemical Co. The material was used without further purification for all the measurements. From the crystalline state, the HSA was dissolved in distilled water. To remove possible undissolved dust particles, the solutions were filtered by means of filter papers. The solutions were cooled in a refrigerator down to 4°C until just prior to viscometry measurements, when they were warmed from 5°C to 45°C, mainly by increments of 5°C.

VISCOMETRY

The viscosity measurements were performed using an Ubbelohde-type capillary microviscometer placed in a water-bath controlled thermostatically at 5^{o}C to 45^{o}C with a precision of ± 0.1^{o}C. The details of the method are described elsewhere [6]. The upper limit of temperature for which the viscosity measurements were made was established by the denaturation temperature of HSA. For HSA the denaturation temperature is only slightly higher than 45^{o}C. For temperatures above, the denaturation viscosity temperature of HSA solutions increases with increasing temperature. The viscosities of the HSA solutions were measured over a wide range of concentrations, from 8.5 kg/m^3 up to 328 kg/m^3 at pH 4.7 and from 22.1 kg/m^3 up to 369 kg/m^3 at pH 7.0. The solution densities were measured by weighing and the albumin concentrations were determined by a dry weight method in which the samples were dried at high temperatures for several hours.

RESULTS AND DISCUSSION

The translational diffusion process of proteins within cells and tissues can be quantitatively described by using the translational diffusion coefficient. It is defined in the usual Fick's diffusion equation. The translational diffusion coefficient of spherical particles immersed in an ideal, homogeneous, isotropic and continuous solvent was calculated by Einstein [31]. In this theory, the size of the solvent molecules is assumed to be negligible in comparison to the size of the dissolved particles. For solutions at infinite dilution, the interactions between the immersed particles can be neglected and the interactions between the particles and the solvent can be replaced by randomly fluctuating

forces. In this limit, Einstein's theory combined with the results of macroscopic continuum hydrodynamics [32] yields the so-called Stokes-Einstein relation:

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

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

It is well known that many proteins are aspherical. The problem of diffusing particles modeled by a prolate or oblate ellipsoid of revolution was solved by Perrin [33]. According to his theory, the hydrodynamic radius of particles can be expressed as:

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

for particles modeled by a prolate ellipsoid, and as:

$$R_h = \frac{\sqrt{a^2 - b^2}}{\tan^{-1}\left(\sqrt{\frac{a^2 - b^2}{b^2}}\right)} \quad (3)$$

for particles modeled by an oblate ellipsoid. Quantities a and b mean the major and minor semi-axes of the ellipsoid. As has been experimentally proved for some proteins in the native state, the hydrodynamic radius does not depend on the solution pH and temperature [34]. Equation (1) with the hydrodynamic radius given by formula (2) or (3) is called the generalized Stokes-Einstein relation.

As mentioned above, the hydrated HSA molecule in the N form can be treated as a prolate ellipsoid of revolution with semi-axes a = 8.2 nm and b = 2.1 nm. From those values and from equation (2), it is easy to calculate the hydrodynamic radius of a hydrated HSA molecule: R_h = 3.89 nm. Because the values of water viscosity η_o can be found in standard physicochemical tables, the diffusion coefficient of HSA in the limit of infinite dilution can now be calculated from the Stokes-Einstein equation (1). The thus obtained numerical values of the translational diffusion coefficient for HSA are gathered in Table I. Those values do not depend on the pH of a solution. Unfortunately, there are very few experimental values of $D_o(T)$ for HSA published in the literature. They lie in the range (5.9––6.32) x 10^{-11} m^2/s [35,36]. As seen in Table I, the $D_o(T)$ obtained for hydrated HSA at t = 25°C from the Stokes-Einstein relation with the hydrodynamic radius calculated from the Perrin formula lies in this range.

In the interior of biological cells, proteins, nucleic acids and other macromolecules are present in a high total concentration. Up to about 40% of the cytoplasmic volume is occupied by those macromolecules [37,38]. Such systems are often called crowded. Under these conditions, the distance between neighbouriong macromolecules is comparable to their size and the diffusion process of macromolecules is highly restricted by intermolecular steric repulsions as well as nonspecific attractive interactions. To understand the transport processes in a crowded environment, knowledge about the translational diffusion coefficient of proteins in concentrated solutions is necessary. For the first time, the translational diffusion coefficient of proteins in concentrated solutions was measured for ovalbumin [39]. For some other proteins, the experimental results of the translational diffusion coefficient in concentrated solutions are also accessible [17,22, 37,38]. A very convenient and simple method of obtaining the translational diffusion coefficient of proteins at higher concentrations, based on viscosity measurements, has been proposed by Wang et al [39]. The authors proposed replacing solvent viscosity η_o in the Stokes-Einstein relation by solution viscosity η (T, Φ), where Φ denotes the volume fraction of the dissolved proteins and it is connected with concentration as shown in the text below. It allows calculation of the translational diffusion coefficient of a protein, at the same concentration, from the following equation:

Table I. Numerical values of translational diffusion coefficient $D_o(T)$ (in 10^{-11} m^2/s) and linear regression coefficient K_D for HSA in aqueous solutions at pH 4.7 and 7.0 calculated on basis of relation (1) and (5), respectively

Tabela I. Wartości liczbowe współczynnika dyfuzji translacyjnej $D_o(T)$ (w 10^{-11} m^2/s) i współczynnika regresji liniowej K_D dla albuminy surowicy ludzkiej w roztworach wodnych przy pH 4,7 i 7,0 obliczone na podstawie relacji, odpowiednio (1) i (5)

t[°C]	$D_o(T)$	K_D at pH 4.7	K_D at pH 7.0
5	3.45	4.869 ± 0.052	4.411 ± 0.029
10	4.08	4.820 ± 0.068	4.382 ± 0.033
15	4.76	4.750 ± 0.062	4.339 ± 0.033
20	5.50	4.716 ± 0.063	4.324 ± 0.025
25	6.29	4.705 ± 0.066	4.318 ± 0.021
30	7.13	4.677 ± 0.055	4.323 ± 0.030
35	8.04	4.754 ± 0.076	4.309 ± 0.029
40	8.99	4.792 ± 0.074	4.286 ± 0.030
45	10.0	4.859 ± 0.074	4.259 ± 0.030

$$D(T,\Phi) = D_o(T)\frac{\eta_o(T)}{\eta(T,\Phi)} \quad (4)$$

The correctness of the above equation has been successfully verified for different proteins such as *Lumbricus* hemoglobin, tetrameric hemoglobin and ovalbumin by Gros [22], and for bovine serum albumin, α2 – macroglobulin and Eartworm hemoglobin by Lavalette et al [14]. As it appears, relation (4) is correct when the size of the solvent molecules is negligible in comparison to the size of the dissolved particles. Deviations from the above equation appear when the molecular mass of the cosolvent is higher than about 10^3, and in such cases, the relation of the form $D = D_o(\eta_o/\eta)^q$ is fulfilled [14]. Exponent q in this relation is less or equal to 1 and it depends on the cosolvent dimension and mass. Deviations from equation (4) increase with an increasing cosolvent molecular mass. In the present paper, the translational diffusion coefficient of HSA at pH 4.7 and 7.0 and in the whole range of measured concentrations and temperatures has been calculated on the basis of relation (4). At low concentrations, i.e. in the case of dilute solutions, the translational diffusion coefficient of proteins depends linearly on the concentration (Fig. 1). It can be presented in the following way [40,41,42]:

$$D(T,\Phi) = D_o(T)(1 - K_D\Phi) \quad (5)$$

Linear regression coefficient K_D is a measure of interparticle interaction. Volume fraction $\Phi = N_A V c/M_h$ where N_A, V and M_h denote Avogadro's number, the hydrodynamic volume of one dissolved protein and its hydrodynamic mass, and c is the concentration in kg/m^3.

The most striking difference between HSA molecules in the crystalline state and in the solution is that in the latter, the molecules are covered by the hydration shell of water molecules. The detailed analysis conducted in our previous paper [6] showed that HSA molecules in a water solution are surrounded non-uniformly by no more than two layers of water molecules. It causes the dimensions and mass of HSA molecules in solution to be greater than in the crystalline state.

The hydrodynamic volume of one dissolved protein modeled as a prolate ellipsoid of revolution is $V = 4/3\pi ab^2$. As mentioned above for the hydrated HSA molecule in the N form, the values of the semi-axes are: a = 8.2 nm and b = 2.1 nm [6], and it gives $V = 155.3\ nm^3$. The hydrodynamic mass of the protein is the sum of the molecular mass of unhydrated protein M_p and the mass of the hydration shell: $M_h = M_p(1 + \delta)$. The level of protein hydration δ denotes the number of grams of water associated with the protein per gram of protein. The molecular mass of unhydrated HSA M_p = 66.479 kDa [1,43] and the level of HSA hydration determined from the NMR study is δ = 0.379 [44]. It gives a hydrodynamic mass of HSA M_h = 91.675 kDa. Taking this into consideration, the HSA volume fraction can be calculated from the relation: $\Phi = 1.02 \times 10^{-3}\ m^3/kg \times c$.

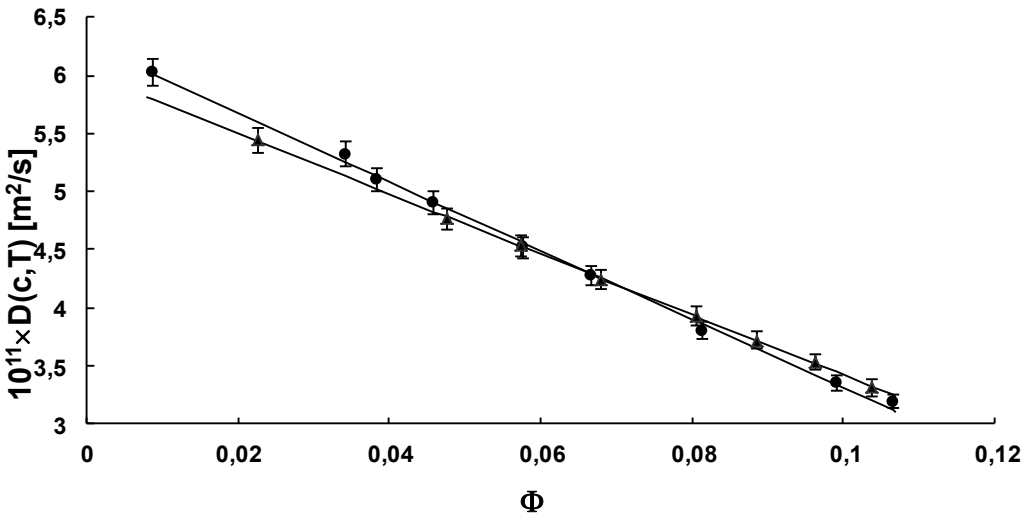

Fig. 1. Plot of translational diffusion coefficient vs. volume fraction of HSA in aqueous solutions at pH 4.7 (•), and at pH 7.0 (▲) and at t = 25°C. Experimental points were obtained on basis of equation (4); straight lines show fit according to equation (5) with parameters: $D_o(T) = 6.26 \times 10^{-11}\ m^2/s$, K_D = 4.71 for HSA at pH 4.7 and $D_o(T) = 6.02 \times 10^{-11}\ m^2/s$, K_D = 4.32 for HSA at pH 7.0.

Ryc. 1. Wykres współczynnika dyfuzji translacyjnej od ułamka objętościowego albuminy surowicy ludzkiej w roztworach wodnych przy pH 4,7 (•) i przy pH 7,0 (▲) oraz w t = 25°C. Punkty doświadczalne otrzymano na podstawie równania (4); linie proste pokazują dopasowanie zgodne z równaniem (5) z parametrami: $D_o(T) = 6{,}26 \times 10^{-11}\ m^2/s$, K_D = 4,71 dla albuminy surowicy ludzkiej przy pH 4,7 i $D_o(T) = 6{,}02 \times 10^{-11}\ m^2/s$, K_D = 4,32 dla albuminy surowicy ludzkiej przy pH 7,0.

Figure 1 shows that the translational diffusion coefficient, calculated from equation (4) for HSA both at neutral pH and at the isoelectric point, depends linearly on the volume fraction up to $\Phi \cong 0.11$. It corresponds to a concentration of HSA of about 100 kg/m^3. As seen in Figure 1, the translational diffusion coefficient of HSA in dilute solutions at neutral pH is very similar to that obtained at the isoelectric point and at the same temperature. The numerical values of linear regression coefficient K_D calculated in the whole range of measured temperatures are gathered in Table I. As can be seen, K_D calculated for HSA in solutions at the isoelectric point is – for a given temperature – – slightly higher than the K_D obtained in solutions at the neutral pH. It means that the rate of decreasing HSA translational diffusion coefficient with an increasing volume fraction is higher in solutions at the isoelectric point. The translational diffusion coefficient of proteins in dilute solutions is often used as a test of the aggregation state of proteins.

The interior of biological cells is a medium with a biological macromolecular volume fraction of up to 40% [38]. Under these conditions, the distance between neighbouring macromolecules is comparable to their size. The diffusion process of macromolecules is then restricted by intermolecular steric repulsions as well as nonspecific attractive interactions. It causes the dependence of the translational diffusion coefficient of macromolecules on the volume fraction to become non-linear and can be described by a stretched exponential function [13,15,45]:

$$D(T,\Phi) = D_o(T)\exp\left(-\beta\Phi^{\nu}\right) \qquad (6)$$

where β and ν are the scaling parameters. Figure 2 shows a plot of the translational diffusion coefficient vs. volume fraction for HSA at neutral pH and at the isoelectric point – for different temperatures – in the whole range of measured concentrations. Different temperatures have been chosen to avoid partial overlapping of the curves. The curves show the fit to the experimental points obtained by using relation (6) in which β and ν were treated as adjustable parameters. The calculations of these parameters were conducted by applying a non-linear regression procedure in a computational statistical program. In the program, approximated values of the parameters are needed. On the basis of those values and by using the iteration procedure, the program calculates the mean value and standard error of the parameters.

The thus obtained numerical values of those parameters for HSA at neutral pH and at the isoelectric point are presented in Table II. As can be seen, scaling parameter β – for HSA both at neutral pH and at the isoelectric point – slowly decreases with increasing temperature up to 30^{o}C, and for higher temperatures it practically does not change. At the same time, parameter β for HSA at the isoelectric point – for a given temperature – is higher than β at neutral pH. A previous study conducted on ovalbumin, HSA and human IgG immunoglobulin showed that parameter β – for a fixed temperature – increases with an increasing protein hydrodynamic mass [46]. The second parameter, ν in turn, is for HSA both at neutral pH and at the isoelectric point – in the whole range of measured temperatures – constant (in the range of estimated errors). At the same time, as for parameter β, parameter ν for HSA at the isoelectric point – for a given temperature – is higher than ν at neutral pH. The above results, and the results obtained earlier for some mammalian serum albumins [47] suggest that both parameters reach the highest values in solutions at the isoelectric point.

The translational diffusion coefficient of proteins strongly depends on temperature. This dependence is commonly described by Vogel-Tammann-Fulcher's equation [48,49]. This equation, with modifications made by Angell [50], has the following form:

$$D(c,T) = A(c)\exp\left[-\frac{F(c)T_o(c)}{T-T_o(c)}\right] \qquad (7)$$

Parameters A(c), F(c) and T_o(c) in the above equation depend on the concentration. The numerical values of these parameters are needed to fit the translational diffusion coefficient from the above formula to the values of D(c,T) obtained at different temperatures from relation (4). For each fixed concentration, they were calculated by using the non-linear least squares method. The results of those calculations for HSA in solutions both at neutral pH and at the isoelectric point are shown in Figures 4–6. It can be seen that each parameter depends on the concentration in a quite different manner. Figure 3, in turn, shows the values of the translational diffusion coefficient in the measured range of temperatures for HSA for an infinitely diluted concentration (in this case D(c,T) does not depend on pH), and for one concentration at neutral pH and at the isoelectric point. The curves show a fit to the experimental points according to equation (7). It can be seen that this equation gives a very good fit over the whole range of temperatures.

Parameter A(c) represents the high-temperature limit of the translational diffusion coefficient, i.e. the translational diffusion coefficient HSA molecules would have at an infinitely high temperature. Figure 4 shows that this parameter (in logarithmic scale) depends linearly on the concentration of HSA for solutions both at neutral pH and at the isoelectric point. The straight lines in Figure 4 correspond to the relation:

$$\ln A(c) = -a_1 + a_2 c \qquad (8)$$

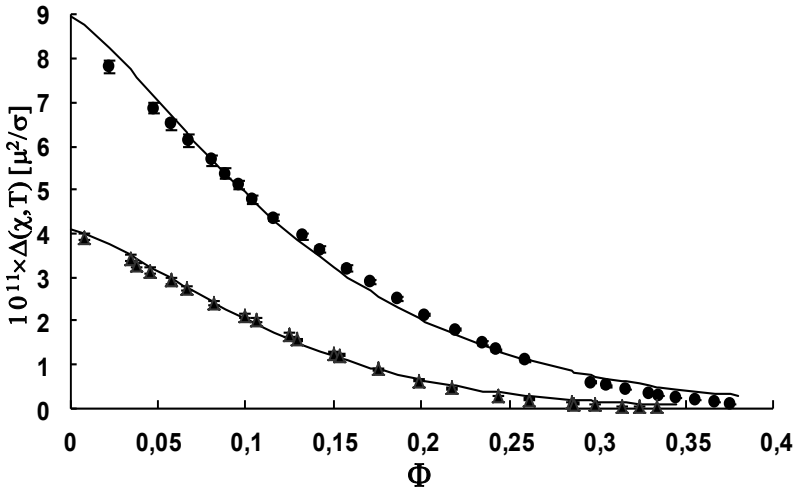

Fig. 2. Plot of translational diffusion coefficient vs. volume fraction of HSA in aqueous solutions at pH 7.0 and t = 40°C (●), and at pH 4.7 and t = 10°C (▲). Experimental points were obtained on basis of equation (4); curves show the fit according to equation (6) with parameters: $D_o = 8.99 \times 10^{-11}$ m²/s, $\beta = 12.06$ and $\nu = 1.299$ at pH 7.0 and $D_o = 4.08 \times 10^{-11}$ m²/s, $\beta = 18.01$ and $\nu = 1.414$ at pH 4.7.
Ryc. 2. Wykres współczynnika dyfuzji translacyjnej od ułamka objętościowego dla albuminy surowicy ludzkiej w wodnych roztworach przy pH 7,0 i t = 40°C (●) oraz przy pH 4,7 i t = 10°C (▲). Punkty doświadczalne zostały otrzymane na podstawie równania (4); krzywe pokazują dopasowanie zgodne z równaniem (6) z parametrami: $D_o = 8{,}99 \times 10^{-11}$ m²/s, $\beta = 12{,}06$ i $\nu = 1{,}299$ przy pH 7,0 oraz $D_o = 4{,}08 \times 10^{-11}$ m²/s, $\beta = 18{,}01$ i $\nu = 1{,}414$ przy pH 4,7.

Table. II. Numerical values of scaling parameter β and ν from equation (6) for HSA in aqueous solutions at pH 4.7 and 7.0
Tabela. II. Wartości liczbowe parametru skalowania β i ν z równania (6) dla albuminy surowicy ludzkiej w roztworach wodnych przy pH 4,7 i 7,0

t[°C]	HSA at pH 4.7		HSA at pH 7.0	
	β	ν	β	ν
5	18.48 ± 1.36	1.414 ± 0.035	12.64 ± 0.81	1.291 ± 0.032
10	18.01 ± 1.30	1.414 ± 0.034	12.42 ± 0.80	1.288 ± 0.032
15	17.65 ± 1.25	1.413 ± 0.034	12.26 ± 0.79	1.287 ± 0.032
20	17.36 ± 1.21	1.412 ± 0.033	12.24 ± 0.78	1.293 ± 0.032
25	17.18 ± 1.16	1.412 ± 0.032	12.24 ± 0.76	1.299 ± 0.031
30	17.08 ± 1.12	1.413 ± 0.032	12.09 ± 0.74	1.294 ± 0.031
35	17.08 ± 1.05	1.412 ± 0.030	12.16 ± 0.72	1.302 ± 0.030
40	17.21 ± 1.01	1.412 ± 0.028	12.06 ± 0.73	1.299 ± 0.031
45	17.27 ± 1.03	1.402 ± 0.028	12.18 ± 0.72	1.306 ± 0.030

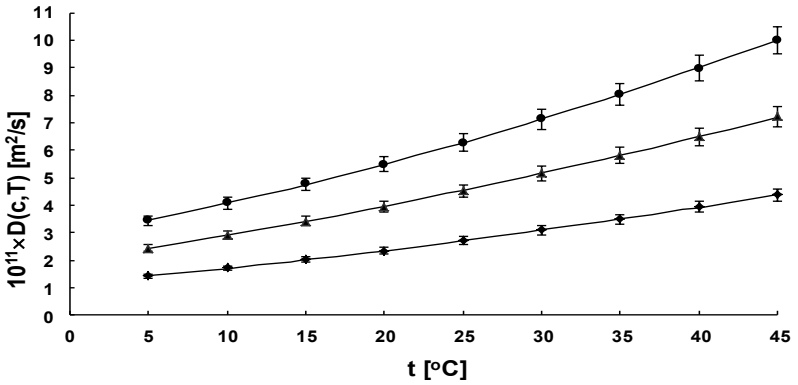

Fig. 3. Temperature dependence of translational diffusion coefficient of HSA in aqueous solutions for concentrations: c → 0 (●), 56.5 kg/m³ and pH 4.7 (▲), 129 kg/m³ and pH 7.0 (♦). Curves show fit obtained by using equation (7) with parameters: $A = 4.022 \times 10^{-9}$ m²/s, F = 4.752 and $T_o = 139.2$ K (c → 0); $A = 1.293 \times 10^{-9}$ m²/s, F = 2.453 and $T_o = 172.1$ K (c = 56.5 kg/m³); $A = 1.208 \times 10^{-9}$ m²/s, F = 3.303 and $T_o = 159.5$ K (c = 129 kg/m³).
Ryc. 3. Temperaturowa zależność współczynnika dyfuzji translacyjnej albuminy surowicy ludzkiej w roztworach wodnych dla stężeń: c → 0 (●), 56,5 kg/m³ i pH 4,7 (▲), 129 kg/m³ i pH 7,0 (♦). Krzywe pokazują dopasowanie otrzymane zgodnie z równaniem (7) z parametrami: $A = 4{,}022 \times$ x 10^{-9} m²/s, F = 4,752 i $T_o = 139{,}2$ K (c → 0); $A = 1{,}293 \times 10^{-9}$ m²/s, F = 2,453 i $T_o = 172{,}1$ K (c = 56,5 kg/m³); $A = 1{,}208 \times 10^{-9}$ m²/s, F = 3,303 i $T_o = 159{,}5$ K (c = = 129 kg/m³).

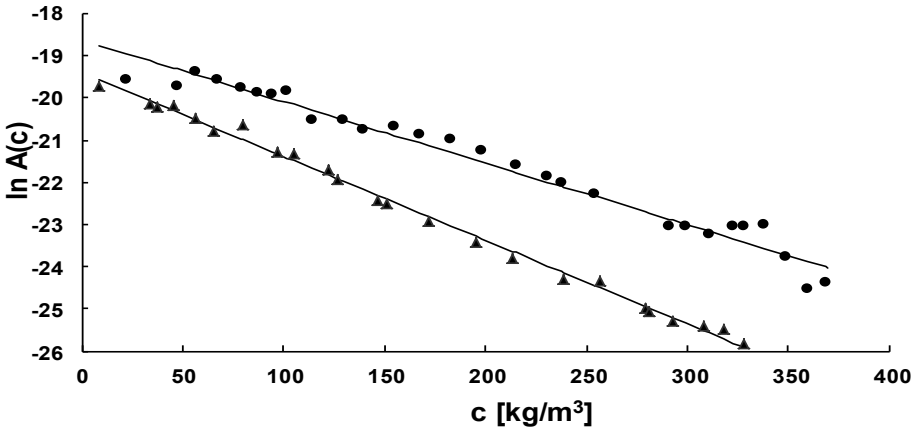

Fig. 4. Plot of parameter A(c) in Vogel-Tammann-Fulcher's equation versus concentration in log-normal plot for HSA at pH 7.0 (•) and pH 4.7 (▲); straight lines show fit according to relation (8) with the linear regression coefficients given in the text.
Ryc. 4. Wykres parametru A(c) z równania Vogela-Tammanna-Fulchera od stężenia na wykresie logarytmiczno-normalnym dla albuminy surowicy ludzkiej przy pH 7,0 (•) i pH 4,7 (▲); linie proste pokazują dopasowanie zgodne z relacją (8) z współczynnikami regresji liniowej podanymi w tekście.

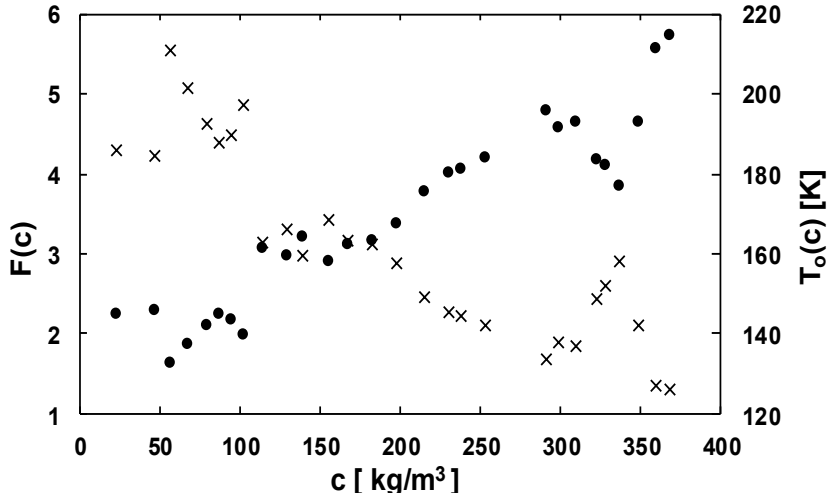

Fig. 5. Plot of ideal glass transition temperature $T_o(c)$ (•) and fragility parameter F(c) (×) versus concentration, for HSA in aqueous solutions at pH 7.0.
Ryc. 5. Wykres temperatury idealnego zeszklenia $T_o(c)$ (•) i parametru kruchliwości F(c) (×) od stężenia dla albuminy surowicy ludzkiej w roztworach wodnych przy pH 7,0.

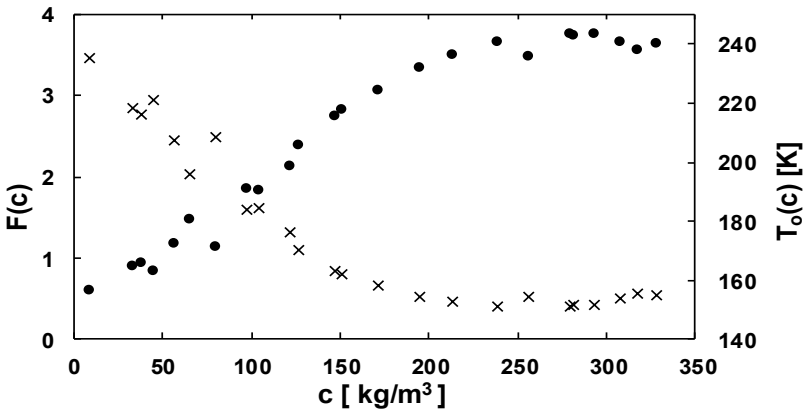

Fig. 6. Plot of ideal glass transition temperature $T_o(c)$ (•) and fragility parameter F(c) (×) versus concentration, for HSA aqueous solutions at pH 4.7.
Ryc. 6. Wykres temperatury idealnego zeszklenia $T_o(c)$ (•) i parametru kruchliwości F(c) (×) od stężenia dla albuminy surowicy ludzkiej w roztworach wodnych przy pH 4,7.

Coefficients a_1 and a_2 were calculated by using the least squares method. The following values were obtained: $a_1 = 18.63 \pm 0.13$, $a_2 = (1.459 \pm 0.057) \times 10^{-2}$ $m^3 kg^{-1}$ for HSA at neutral pH, and $a_1 = 19.41 \pm 0.06$, $a_2 = (1.982 \pm 0.028) \times 10^{-2}$ $m^3 kg^{-1}$ for HSA at the isoelectric point. The correlation coefficients were r = -0.981 and r = -0.998, respectively.

Parameter T_o is called the ideal glass-transition temperature. It means the temperature at which the molecular mobility of a supercooled liquid is stopped [51 and references therein]. As seen in Figure 5, the ideal glass-transition temperature for HSA solutions at neutral pH depends on the concentration in rather an irregular manner. Figure 6 in turn, shows that T_o for HSA solutions at the isoelectric point increases non-linearly with increasing concentration up to about c = 220 kg/m^3 and for higher concentrations it is nearly constant. Earlier studies of some proteins showed that the ideal glass-transition temperature may depend on concentration non-linearly for the solutions of some proteins and linearly for the others [51 and references therein]. It suggests that the concentration dependence of the ideal glass-transition temperature is influenced by specific biochemical and biophysical properties of the dissolved proteins. Parameter F(c) from equation (7) is called the fragility parameter. It was originally introduced into Vogel-Tammann-Fulcher's equation by Angell [50] in order to classify liquids in respect to their rate of changes in viscosity with temperature. According to this conception, liquids whose viscosity does not decrease much with increasing temperature above the glass transition temperature are called "strong". Contrary to this, liquids which show a strong decline in viscosity with increasing temperature above the glass transition temperature are called "fragile". Similar to T_o, the fragility parameter for HSA solutions at neutral pH depends on the concentration in an irregular way (see Fig. 5). For HSA solutions at the isoelectric point, however, the fragility parameter decreases non-linearly with increasing concentration up to about c = 220 kg/m^3, and for higher concentrations it is nearly constant (see Fig. 6). The numerical values of the fragility parameter lie in the range of 1.3 to 5.56 for HSA solutions at neutral pH, and in the range of 0.531 to 3.46 for HSA solutions at the isoelectric point. It proves that the studied HSA solutions belong to the extremely fragile class of liquids. According to Angell's classification, for extremely strong liquids, the fragility parameter attains a value of about 100 [50].

CONCLUSIONS

The translational diffusion coefficient for human serum albumin in an aqueous solution – in the limit of zero concentration – calculated from the Stokes-Einstein equation with a hydrodynamic radius calculated on the basis of the Perrin formula decreases with increasing temperature. This quantity increases with temperature from 3.45×10^{-11} m^2/s (5^{o}C) up to $10.0 \times \times 10^{-11}$ m^2/s (45^{o}C). In the range of dilute solutions, i.e. when the HSA volume fraction does not exceed a value of approximately 0.11, the translational diffusion coefficient decreases linearly with increasing concentration. The linear regression coefficient calculated for HSA in solutions at the isoelectric point is – for a given temperature – slightly higher than its value obtained from solutions at neutral pH.

However, the concentration dependence of the translational diffusion coefficient of HSA obtained from dilute to concentrated solutions appears to be non-linear and can be described by a two- parameter stretched exponential function. The numerical values of those parameters calculated for HSA at the isoelectric point – for a given temperature – are higher than their values obtained for HSA at neutral pH. The temperature dependence of the translational diffusion coefficient for HSA at the isoelectric point and at neutral pH, in turn, can be described by the three-parameter Vogel-Tammann-Fulcher's equation. One of those parameters, which represents the high-temperature limit of the translational diffusion coefficient, depends linearly on concentration. Analytical description of the other two parameters, i.e. for the ideal glass-transition temperature and fragility parameter is not possible. The numerical values of the fragility parameter showed that HSA solutions at the isoelectric point and at neutral pH belong to the extremely fragile class of liquids.

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