COMPARISON OF THE OVERALL MOTION CORRELATION TIMES OF SEVERAL MAMMALIAN SERUM ALBUMINS IN DILUTE SOLUTIONS DETERMINED ON THE BASIS OF MAXWELL EFFECT AND THE DEBYE-STOKES-EINSTEIN EQUATION.

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One of the rarely used ways of determining the overall motion correlation time of proteins is method based on the Maxwell effect. This effect consists in the appearance of a stimulated birefringence in liquids or solutions and induced by the mechanical force like shear stress in a streamline flow. To determine the overall motion correlation time for protein in dilute solution is sufficient to know the molecular mass and the ratio of the principal axes of protein, and an intrinsic viscosity. The intrinsic viscosity has been measured using an Ubbelohde-type capillary microviscometer immersed in a water-bath controlled thermostatically in the range from 5ºC to 45ºC for six mammalian albumins. To check the influence of solution pH on the overall motion correlation time the intrinsic viscosity value of the human serum albumin in solutions at the isoelectric point and beyond of it was measured. The thus obtained correlation times were compared with the times determined on the basis of the Debye-Stokes-Einstein equation.

INTRODUCTION

Rotational correlation times are frequently used source of information on the dynamics of proteins in solution. In particular, they are helpful in understanding many aspects of the processes occurring at the molecular level (Wüthrich, 2003). Knowledge of that times is of significant value in biophysical characterization of proteins in solution. Because rotational correlation times correlate with the volume and molecular mass of folded proteins, they can provide information about the polymerization and can reveal aggregates formed under the chosen conditions (Danielsen *et al*, 1991; Lee *et al*, 2006). In the case of protein solutions rotational correlation times can be set for the water molecules of the solution (bulk water), the water molecules of hydration layer of proteins, protein side-chains or the whole protein (Kakalis & Kumosinski, 1992). In the latter case, the rotational correlation time is called the overall motion correlation time. Depending on the experimental method used correlation times connected with tensors of different rank are obtained. For example, they can be connected with a dipole moment (rank-1 tensor) τ_1 or protein polarizability (rank-2 tensor) τ_2. The exact definition of the correlation time associated with the tensor of arbitrary rank has been given in the earlier work (Monkos, 2007a).

For proteins correlation time τ_1 can be measured by dielectric spectroscopy (Kabir *et al,* 2003; Miura *et al,* 1994; Grant *et al*, 1971; Moser *et al*, 1966) while the time τ_2 in fluorescence anisotropy measurements (Zorrilla *et al*, 2007; Kuttner *et al,* 2005; Zorrilla *et al*, 2004; Feinstein *et al*, 2003; Kamal & Behere, 2002; Ferrer *et al,* 2001; Bialik *et al*, 1998; Takeda *et al,* 1991; Takeda & Yamamoto, 1990; Bucci & Steiner, 1988; Gryczynski *et al,* 1988), EPR spectroscopy (Cavalu *et al*, 2002; Hustedt *et al*, 1993; Steinhoff, 1990; Steinhoff *et al*, 1989; Steinhoff, 1988; Ebert *et al,* 1981), NMR spectroscopy (Yilmaz *et al*, 2014; Yao *et al*, 2008; Luchinat & Parigi, 2007; Budak *et al*, 2006; Lee *et al,* 2006; Korchuganov *et al,* 2004; Denisov *et al*, 2004; Denisov & Halle, 1995a, 1995b; Kakalis & Kumosinski, 1992), perturbed angular correlations of γ-rays method (Danielsen *et al*, 1991) and by using Maxwell effect (Monkos, 2007a; Ferrer *et al*, 2001; Acuña *et al*, 1987). Quite recently a method for determining the correlation times based on modeling and numerical methods has been developed (Rezaei-Ghaleh *et al*, 2015). However, results presented for a given protein in different publications can vary significantly. As far as I know -

for proteins – the correlation times associated with tensors of higher ranks have not yet been measured.

The correlation times of arbitrary rank n are related to the rotational diffusion coefficient D_r by a simple relationship: $\tau_n = [n(n + 1)D_r]^{-1}$. Thus, the correlation times of different ranks are interconnected: $\tau_1 = 3\tau_2 = 6\tau_3$ and so on. In this study, the overall motion correlation time τ_2 for several mammalians albumin was determined based on Maxwell effect. The results were compared with the theoretical values of correlation times obtained on the basis of the Debye-Stokes-Einstein equation.

MATERIALS

The following products of the Sigma Chemical Co. were used in this study: bovine serum albumin BSA (A 4503), equine serum albumin ESA (A 9888), human serum albumin HSA at pH 7.0 (A 1653), ovine serum albumin OSA (A 3264), porcine serum albumin PSA (A 2764) and rabbit serum albumin RSA (A 0639). HSA at pH 4.7 (lyophilized, purity 99% and essentially fatty acid- and globulin-free) was purchased from Polish Chemical Reagents factories. Albumins were used without further purification for all the measurements. From the crystalline form the albumins were dissolved in distilled water and then filtered by means of filter papers in order to remove dust particles and possible undissolved fragments. The samples were stored in a refrigerator until just prior to viscometry measurements, when they were warmed from 5 to 45°C, mainly by steps of 5°C. The pH values of thus prepared solutions were as follows: 5.2 for BSA, 7.4 for ESA, 7.0 or 4.7 for HSA, 7.05 for OSA, 6.6 for PSA and 7.0 for RSA. These values changed only slightly with a change in solution concentration. The above values are the mean values of

pH. The isoelectric point pI of the studied albumins is: (4.7 – 5.4) for BSA, (4.65 – 4.9) for ESA, (4.7 – 4.95) for HSA, (4.6 – 4.9) for OSA, (4.6 – 4.9) for PSA and (4.6 – 5.3) for RSA (Miller & Gemeiner, 1998). Thus, BSA and HSA at pH 4.7 were studied in their isoelectric point, and the other albumins above their isoelectric point.

VISCOMETRY

The viscosity measurements of albumins solutions were conducted using an Ubbelohde-type capillary microviscometer immersed in a water bath controlled thermostatically at (5–45)°C with a precision of ±0.1°C. The microviscometer was calibrated using cooled boiled distilled water and a flow time for water was 28.5 s at 25°C. A solution passed once through the viscometer before any measurements were made. Measurements started after a few minutes delay to make sure that the system has reached thermal equilibrium. They were usually made in intervals of 5°C. Flow times were recorded to within 0.1 s. The upper temperature of viscosity measurement has been selected by the denaturation temperature of albumins. At temperatures slightly higher than 45°C the thermal denaturation of the studied albumins occurs and the lower the albumin concentration the higher the denaturation temperature. For temperatures above the denaturation temperature, the viscosity of albumins solutions increases with increasing temperature.

Solution densities were measured by weighing. Albumin concentrations were determined using a dry method in which the samples were dried at high temperature for several hours.

Table 1. The numerical values of the intrinsic viscosity $[\eta] \times 10^3$ $[m^3/kg]$ for all investigated albumins.

t[°C]	BSA pH = pI	HSA pH = pI	HSA pH 〉 pI	RSA pH 〉 pI	OSA pH 〉 pI	ESA pH 〉 pI	PSA pH 〉 pI
5	6.49±0.16	6.46±0.10	4.90±0.12	5.21±0.13	4.95±0.12	4.38±0.11	4.36±0.11
10	6.26±0.15	6.30±0.10	4.83±0.12	5.12±0.13	4.87±0.12	4.31±0.11	4.28±0.10
15	6.06±0.15	6.18±0.10	4.78±0.12	5.05±0.12	4.80±0.12	4.25±0.10	4.22±0.10
20	5.90±0.14	6.10±0.10	4.73±0.12	4.99±0.12	4.74±0.12	4.19±0.10	4.17±0.10
25	5.78±0.14	6.06±0.10	4.69±0.12	4.94±0.12	4.69±0.12	4.14±0.10	4.13±0.10
30	5.69±0.14	6.07±0.11	4.66±0.11	4.91±0.12	4.65±0.11	4.09±0.10	4.10±0.10
35	5.63±0.14	6.11±0.11	4.63±0.11	4.89±0.12	4.62±0.11	4.05±0.10	4.08±0.10
40	5.60±0.14	6.18±0.12	4.62±0.11	4.87±0.12	4.60±0.11	4.01±0.10	4.08±0.10
45	5.60±0.14	6.29±0.13	4.61±0.11	4.87±0.12	4.59±0.11	3.98±0.10	4.08±0.10

The main purpose of measuring the viscosity of albumins solutions was determination of their intrinsic viscosity. According to the definition, the intrinsic viscosity $[\eta] = \lim_{c \to 0} \eta_{sp} / c$, where the specific viscosity

$\eta_{sp} = \eta_r - 1$ and c is the solute concentration in kg/m^3. The relative viscosity, in turn, $\eta_r = \eta/\eta_o$ and η and η_o denote the viscosity of a solution and the solvent, respectively. The traditional method for determining the

intrinsic viscosity is based on the plotting of η_{sp}/c on the concentration and extrapolating it to the intersection with the axis of coordinates. The value of the intersection is equal to the intrinsic viscosity. In this study we used a different method proposed earlier (Monkos, 1996). This method has been successfully applied to various globular and non-globular proteins (Monkos, 2007b). The numerical values of $[\eta]$ obtained by this method for temperatures from 5°C to 45°C are shown in Table 1 (Monkos, 1996, 2004, 2005a, 2005b, 2013). They will be used for the calculation of the overall motion correlation time of albumins on the basis of Maxwell effect.

RESULTS AND DISCUSSION

τ_2 from the Maxwell effect

A simulated birefringence in liquids or solutions induced by the mechanical force like shear stress in a streamline flow is called Maxwell effect (Raman & Krishnan, 1927). The effect appears when the molecules of a flowing liquid or molecules dissolved in a solution are non-spherical in shape and have anisotropic polarizability (Cwietkow et al, 1968). The flow of a suspension of rigid molecules having the shape of ellipsoids of revolution was examined by Jeffery on the basis of the phenomenological Navier-Stokes equations (Jeffery, 1923). Jeffery showed that the resultant of stresses acting on the surface of such ellipsoid is equivalent to the action of two couples of forces. The first of them is trying to direct the orientation of the principal axis of the molecule according to the direction of liquid flow and the second one is trying to direct its orientation in the direction perpendicular to the flow direction. The simultaneous action of these couples causes that movement of the ellipsoid becomes periodic, ie. its main axis of symmetry precesses around the axis perpendicular to the direction of fluid flow. In a laminar flow rate of precession is dependent on the size of the principal axes of the molecule and the velocity gradient. It appears that the major axes of the molecules form with a flow direction different angles, and the distribution function describing their spatial arrangement becomes anisotropic. At low temperatures, when the Brownian motion is negligible, distribution function takes the maximum value when the direction of the major axis of the molecule is parallel to the direction of flow. In other words, hydrodynamic forces in laminar flow tend to set the major axis of the asymmetric molecules in the flow direction. However, at higher temperatures, this forced orientation of molecules induced by the flow of liquid is weakened by the rotational Brownian motion of molecules. At constant velocity gradient in the solution a stationary distribution of the orientation of the principal axes of the molecules

is established and the privileged direction of the molecules orientation makes some angle with the direction of the liquid flow. When the considered molecules are optically anisotropic their non-uniform distribution leads to a macroscopic anisotropy in the solution.

In the steady-state, the anisotropy of the spatial orientation of the main axis of the molecules can be described by a distribution function of all possible directions which satisfies equation of diffusion type (Cwietkow et al, 1968). If the external mechanical stresses that force the movement of the liquid disappear, anisotropy of the distribution of orientation of the molecules principal axes disappears due to their rotational Brownian motion. The time at which the distribution function is reduced by a factor of e^{-1} is called the overall motion correlation time and it is equivalent to the rotational correlation time τ_2 (Cwietkow et al, 1968).

The overall motion correlation time can be evaluated on the basis of Maxwell effect for prolate ellipsoids of revolution only for the dilute solutions. It uses then the following relationship (Cwietkow et al, 1968; Acuña et al, 1987; Ferrer et al, 2001):

$$\tau_2 = \frac{M[\eta]\eta_o}{RTF(p)} \qquad (1)$$

where M, R and T are the molecular mass of the molecules in solution, gas constant and the absolute temperature, respectively; $F(p)$ is a function of the axial ratio $p = a/b$ of dissolved molecules modeled as a prolate ellipsoids of revolution with one long semi-axis (a) and two shorter semi-axes (b). This function can be represented as $F(p) = \nu/6\Pi$, where ν is a Simha factor (Simha, 1940) and Π is the Perrin function (Perrin, 1934).

In his original work Simha showed how to calculate the value of the factor ν for triaxial ellipsoid. Expressions obtained by him contain elliptic integrals that can be solved numerically. However, for prolate ellipsoids of revolution relationship between ν and p can be determined from the asymptotic relations given by Harding (Harding, 1997):

$$p = -10.71584 + 2.79158\nu + 1.622009\nu^2 +$$
$$+ 0.01556169\nu^3 - 0.192997\nu^4 +$$
$$+ 0.02060718\nu^5 \qquad (2)$$

for $(1.1 \leq p < 2)$,
and

$$p = -3.80413 + 2.8712\,v - 0.3908319\,v^2 +$$
$$+\,0.03612282\,v^3 - 0.001733981\,v^4 +$$
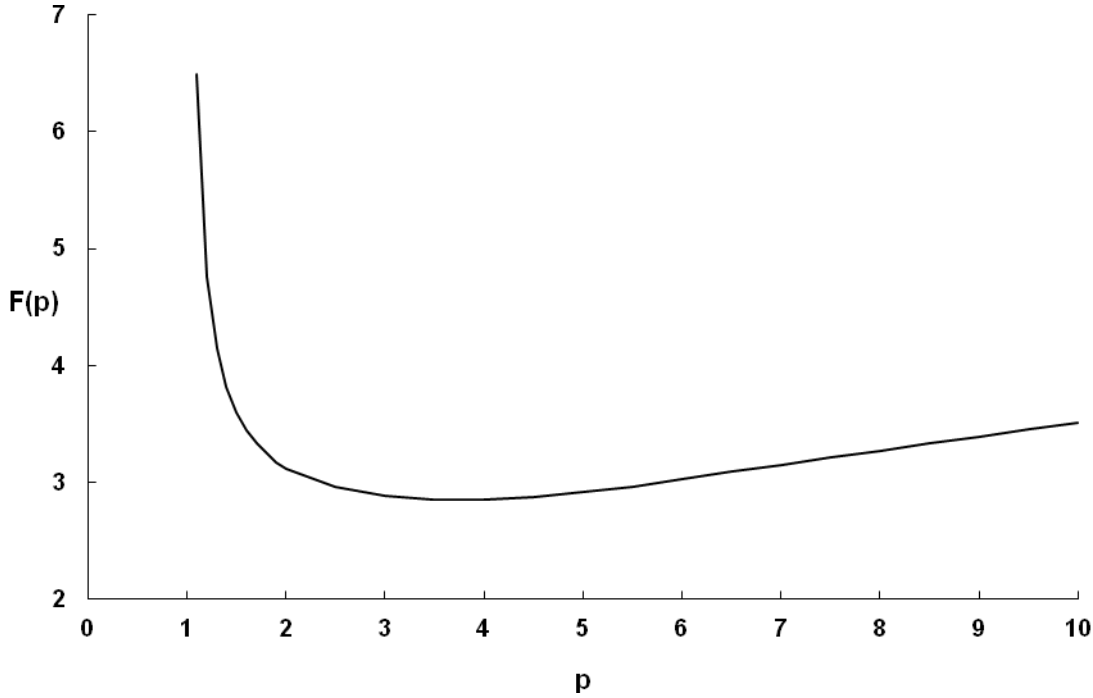$$+\,0.0000332711\,v^5 \qquad (3)$$

for $(2 \leq p < 10)$.

The Perrin function, in turn, has the following form:

$$\Pi \equiv \left[\frac{0.4}{2g_1^{-1} + 4g_2^{-1}} + \frac{0.4}{5g_1^{-1} + g_2^{-1}} + \frac{0.2}{6g_1^{-1}} \right] \qquad (4)$$

where

$$g_1 = \frac{2(p^2 - 1)}{3p(p - S)} \quad , \quad g_2 = \frac{2(p^4 - 1)}{3p(2p^2 - 1)S - p} \qquad (5)$$

and

$$S = \frac{ln\left(p + \sqrt{p^2 - 1} \right)}{\sqrt{p^2 - 1}} \qquad (6)$$

Figure 1 shows the function $F(p)$ for globular proteins, ie. for molecules for which $1 \leq p \leq 10$.

Fig. 1. Plot of the function $F(p) = v/6\Pi$ vs. axial ratio p, according to formulae (2) - (6).

Mammalian serum albumins are moderately large proteins, with primary structure constituted by a single polypeptide chain of about 580 amino-acid residues and molecular mass $M_p = 66.479$ kDa for HSA and $M_p = 66.5$ kDa for the other studied here albumins (Dockal *et al*, 1999). These albumins exhibit high amino-acid sequence identity with each other and this leads to the expectation of the similar (but not identical) overall shape of them. This was confirmed among other by examining the three-dimensional structure of HSA, BSA and ESA by X-ray crystallography (He *et al*, 1992; Ho *et al*, 1993; Carter & Ho, 1994). However, under physiological conditions, albumins are in aqueous environment and their conformation may be different than in the crystalline state. The albumin molecules in aqueous solution are surrounded by a hydration shell of water molecules. These "bound" water molecules migrate with the albumin molecules and contribute to their hydrodynamic mass and volume. A conformation of the experimentally studied hydrated albumins is usually approximated by an ellipsoid of revolution with one long semi-axis (*a*) and two shorter semi-axes (*b*). These values have been previously determined experimentally for hydrated HSA (Monkos, 2004), ESA, PSA, RSA (Monkos, 2005a), OSA (Monkos, 2005b)

and BSA (Nesmelova *et al,* 2002). Obtained results are shown in Table 2.

The measure of protein hydration is the hydration level δ, defined in terms of the time-averaged mass of water associated with a protein per unit anhydrous mass of protein. It does not depend on temperature (Ferrer *et al.* 2001). The molecular mass of hydrated albumin is composed of the molecular mass of unhydrated albumin M_p and the mass of hydration shell of water surrounding the albumin molecule in solution: $M = M_p(1 + \delta)$. For HSA $\delta = 0.379$ (Baranowska & Olszewski, 1996) and for the other studied here albumins $\delta = 0.35$ (Squire & Himmel, 1979). This gives a hydrodynamic mass of HSA $M = 91.675$ kDa and for the other studied here albumins $M = 89.775$ kDa.

Table 2. The numerical values of the major semi-axis (*a*), the minor semi-axes (*b*), the hydrodynamic radius (R_h), the axial ratio *p*, the Simha factor ν (from the relation (3)), the Perrin's function Π (from the formula (4)), and the function $F(p) = \nu/6\Pi$ for discussed albumins.

Albumin	*a*[nm]	*b*[nm]	R_h[nm]	*p*	ν	Π	$F(p) = \nu/6\Pi$
Human	8.2	2.1	3.89	3.95	4.61	0.270	2.85
Bovine	7.7	2.2	3.83	3.50	4.16	0.243	2.85
Equine	7.16	2.27	3.74	3.15	3.84	0.223	2.87
Ovine	7.41	2.42	3.92	3.06	3.76	0.217	2.88
Porcine	7.18	2.31	3.78	3.11	3.80	0.220	2.87
Rabbit	7.75	2.39	4	3.25	3.93	0.229	2.86

The molecular mass and the axial ratio of hydrated albumins, as well as experimentally determined intrinsic viscosity values allow to calculate their overall motion correlation time from equation (1). The numerical values of τ_2 for all investigated here albumins are gathered in Table 3.

Table 3. The numerical values of the overall motion correlation time τ_2[ns] for all investigated albumins obtained on the basis of equation (1).

t[°C]	BSA pH = pI	HSA pH = pI	HSA pH ⟩ pI	RSA pH ⟩ pI	OSA pH ⟩ pI	ESA pH ⟩ pI	PSA pH ⟩ pI
5	134±3.3	137±2.1	104±2.5	107±2.6	101±2.5	90.1±2.2	89.3±2.2
10	110±2.7	113±1.8	86.3±2.1	89.2±2.2	84.3±2.1	74.9±1.8	74.3±1.8
15	90.9±2.2	94.5±1.5	73.1±1.8	75.4±1.8	71.2±1.7	63.2±1.5	62.7±1.5
20	76.6±1.9	80.9±1.3	62.7±1.6	64.5±1.6	60.9±1.5	54.0±1.3	53.7±1.3
25	65.6±1.6	70.3±1.2	54.4±1.4	55.9±1.4	52.7±1.3	46.6±1.1	46.5±1.1
30	56.9±1.4	62.0±1.1	47.6±1.1	48.9±1.2	46.1±1.1	40.6±1.0	40.7±1.0
35	50.0±1.2	55.6±1.0	42.1±1.0	43.2±1.1	40.7±1.0	35.7±0.9	36.0±0.9
40	44.4±1.1	50.0±1.0	37.4±0.9	38.5±0.9	36.2±0.9	31.6±0.8	32.1±0.8
45	39.9±1.0	45.8±1.0	33.6±0.8	34.6±0.8	32.4±0.8	28.2±0.7	28.8±0.7

As can be seen the values of τ_2 obtained - at the same temperature - for the studied albumins in solutions at pH higher than their pI differ little from each other. Comparison of the numerical values of τ_2 for HSA obtained in solutions at pH = pI and pH ⟩ pI shows that the correlation time obtained for the albumin in solution at pH = pI is significantly higher than in the solution at pH ⟩ pI. Furthermore, the correlation times τ_2 obtained for HSA and BSA in solutions at pH = pI have similar values. In solutions at pH ⟩ pI molecules of albumins are charged as a whole. The Coulomb repulsion between two molecules of albumin partially balances the attractive forces caused mainly by dipole-dipole interaction. As a result, the resultant force of interaction between two molecules is low. Contrary to this, in solutions at pH = pI the net charge on the albumins is equal to zero and the attractive forces connected with the dipole-dipole interaction prevail. It is obvious that attractive forces between the molecules slows down their rotational movement. So, the resultant forces between two molecules are greater the rotational correlation times should be longer. Since the resultant forces between the molecules are the greatest in solutions at pH = pI therefore can be hypothesized, that for a given protein and at a fixed temperature the correlation times take then the maximum value.

It should be emphasized that from the relation (1) the overall motion correlation time can be determined only when molecules in solution are approximated as a prolate ellipsoids of revolution. For random coil polymers the overall motion correlation time can be calculated from the following formula (Isihara, 1968):

$$\tau_2 = \frac{2M[\eta]\eta_o}{3RT} \qquad (7)$$

For example, the above equation were used to determine τ_2 for the complex branched polysaccharides: hetero-polysaccharide pullulan (Dais & Vlachou, 2001) and scleroglucan (Vlachou *et al*, 2001). In modeling the dynamics of this type of polysaccharides, two types of motions are considered: (i) the overall rotational motion of the carbohydrate chain as a whole, and (ii), local chain motions. Usually the overall motion is much slower than the chain local motions. Since in ^{13}C relaxation NMR measurements - commonly used in studies of polysaccharides - all carbons in the carbohydrate chain relax via both the overall and local motions this method does not allow separate the overall motion and the local chain motions. However, the overall motion correlation time estimated from equation (7) can help in their separation.

τ_2 *from the Debye-Stokes-Einstein equation*

The problem of the translational and rotational diffusion of spherical molecules immersed in an ideal, homogeneous, isotropic and continuous solvent was considered in fluctuation-dissipation theory by Einstein (Einstein, 1956). In this theory, it is assumed that the size of the solvent molecules is negligibly small compared to the size of the dissolved molecules. It means that the dissolved molecules can be treated as Brownian particles. This condition is satisfied for the aqueous solutions of proteins. This theory assumes then that in dilute solutions the interaction between the solute molecules are negligible and the interactions between these molecules and the molecules of the solvent can be replaced by randomly fluctuating forces. Translational and rotational diffusion of Brownian particles is driven by thermal energy and is hindered by friction experiences by them. Einstein's theory along with the results of macroscopic continuum hydrodynamics (Landau & Lifshits, 1959) allows to determine the coefficients of the translation and rotation diffusion of the macromolecules in dilute solutions. The obtained result for the rotational diffusion coefficient can be used to determine the overall motion correlation time. For the correlation time connected with the l-rank tensor, we get the so-called Debye-Stokes-Einstein (DSE) equation:

$$\tau_l = \frac{8\pi\eta R_h^3}{l(l+1)kT} \qquad (8)$$

where k is Boltzmann constant, η is solution viscosity and R_h denotes the hydrodynamic radius of the macromolecules in solution. For spherical molecules, the hydrodynamic radius R_h is equal to their radius. Generally, the hydrodynamic radius of non-spherical molecule is interpreted as the radius of the spherical molecule with the same diffusion coefficient.

The problem of translational and rotational diffusion of molecules modeled by a prolate or oblate ellipsoid of revolution was also studied by Perrin (Perrin, 1936). According to this theory for molecules modeled by a prolate ellipsoid of revolution, the hydrodynamic radius can be expressed by its major and minor semi-axes in the following way:

$$R_h = \frac{\sqrt{a^2 - b^2}}{ln\left(\frac{a + \sqrt{a^2 - b^2}}{b}\right)} \qquad (9)$$

The size of major and minor semi-axes of molecules otherwise affect the value of their hydrodynamic radius. To see this is sufficient to express the hydrodynamic radius per unit of major and minor semi-axes of molecule by the axial ratio p:

$$\frac{R_h}{a} = \frac{\sqrt{1 - p^{-2}}}{ln[p(1 + \sqrt{1 - p^{-2}})]} \qquad (10)$$

$$\frac{R_h}{b} = \frac{\sqrt{p^2 - 1}}{ln(p + \sqrt{p^2 - 1})} \qquad (11)$$

These relationships are presented graphically in Figure 2.

It has been experimentally proved, for native BSA and HSA, that the hydrodynamic radius does not depend on solution pH and temperature (Jachimska *et al*, 2008). In Table 2 are given the numerical values of the major semi-axis (*a*) and the minor semi-axes (*b*) for hydrated albumins investigated in this work. On this basis, their hydrodynamic radius can be calculated from the equation (9). The results are also shown in Table 2. It is evident that the hydration layer increases the hydrodynamic radius of the albumin molecule compared to the radius of the unhydrated molecule and slows the rotational motion.

DSE relation (Eq. 8) for correlation times connected with 2-rank tensors has the form:

$$\tau_2 = \frac{4\pi\pi\eta_h^3}{3kT} \qquad (12)$$

The correctness of the above equation for globular proteins in dilute solutions has been demonstrated by many authors (Yilmaz *et al*, 2008; Zorrilla *et al*, 2004; Zeeb *et al*, 2003; Van-Quynh *et al*, 2003; Ferrer *et al*, 2001; Lavalette *et al*, 1999; Steinhoff 1990). According

to the above relationship the overall motion correlation time is proportional to solution viscosity and the plot of τ_2 depending on η/T is linear. Deviations from linearity are observed for the relative viscosity of the solution above 2 (Zeeb *et al,* 2003). Deviations from the DSE equation appear also when the assumptions of continuity and homogeneity of solvent are not fulfilled. This situation occurs when the viscosity of the solution of proteins is adjusted by adding viscous cosolvents (like dextrans) of various size and mass. In this case to describe the rotational Brownian motion of proteins in a macromolecular environment the linear dependence of τ_2 on η in the SED equation must be replaced by a power law: $\tau_2 \sim \eta^k$. The exponent "k" of the power law expresses the fact that the protein experiences only a fraction of the hydrodynamic interactions of macromolecular cosolvent and it depends on protein size and cosolvent's mass (Lavalette *et al,* 1999; Steinhoff, 1990).
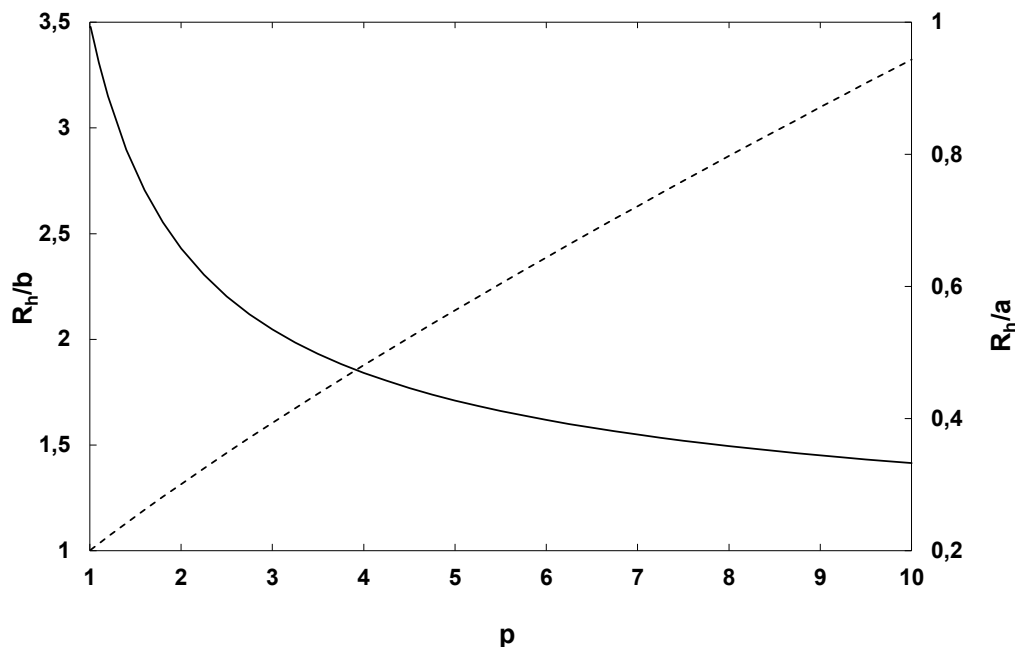

Fig. 2. Plot of the hydrodynamic radius per unit of major axis (——) and minor axis (---) of ellipsoid of revolution vs. axial ratio according to equations (10) and (11), respectively.

Since the size of the water molecules is negligibly small relative to the size of proteins, and in particular to a size of albumin molecules, so the latter can be regarded as Brownian particles. This means that equation (12) can be used to determine the overall motion correlation times for dilute solutions of albumins. Using the values of the hydrodynamic radius of hydrated molecules of albumins from the Table 2, the values of the overall motion correlation time τ_2 for the studied here albumins were calculated. The results are presented in Table 4.

Table 4. The numerical values of the overall motion correlation time τ_2[ns] from DSE equation for all investigated albumins.

t[°C]	BSA	HSA	RSA	OSA	ESA	PSA
5	93.1	97.4	106	99.8	86.7	89.2
10	80.1	82.3	89.7	84.4	73.3	75.4
15	67.5	70.6	76.8	72.3	62.8	64.6
20	58.4	61.1	66.6	62.7	54.4	56.0
25	51.1	53.4	58.2	54.8	47.6	49.0
30	45.0	47.1	51.3	48.3	41.9	43.1
35	40.0	41.8	45.5	42.9	37.2	38.3
40	35.7	37.4	40.7	38.3	33.3	34.2
45	32.1	33.6	36.5	34.4	29.9	30.7

It is interesting to compare these values with the values of τ_2 obtained by using the Maxwell method (see Table 3). As can be seen τ_2 values obtained for a given albumin at a fixed temperature from the DSE equation are very similar (and in some cases nearly identical) to values of the times obtained from the Maxwell method, but only for albumins in solutions at pH > pI. The values of the times τ_2 obtained by the Maxwell effect for BSA and HSA in solutions at pH = pI are substantially greater than the corresponding times obtained from the DSE equation. As mentioned above, in solutions at pH = pI the net charge on the albumins is equal to zero and the attractive forces connected with the dipole-dipole interaction prevail. The obtained results show that in solutions at pH = pI this interaction greatly affects the rotational diffusion of albumins, even in dilute solutions.

The above results clearly show that the value of the overall motion correlation time depends on both the type of albumin, solution pH and temperature. Unfortunately, it is difficult to compare values of τ_2 presented here with the values obtained by other methods, because the authors often do not provide the type of studied albumin as well as the solution pH and temperature. For HSA at 25°C in solution at concentration 0.94 mM and pH 7.4 the value $\tau_2 = 63$ ns has been obtained (Luchinat & Parigi, 2007). For BSA in dilute solution the following values of τ_2 have been reported: 54.8 ns (at t = 18°C) (Kakalis & Kumosinski, 1992); (40±2) ns (Ferrer *et al,* 2001), 48.2 ns and 41.7 ns (at t = 20°C) (Bucci & Steiner, 1988); 41 ns (at t = 27°C) (Van-Quynh *et al,* 2003); and 76 ns (at t = 25°C) (Moser *et al,* 1966). As can be seen, these values are of the same order of magnitude as those obtained by the present study. To my knowledge there is no corresponding data in the literature for the correlation time τ_2 for other types of albumin.

The investigation of rotational motion of proteins through different experimental methods is complicated by the presence of several potentially coupled motions. As appears, proteins exhibit besides Brownian rotation of the entire macromolecular framework, motions ranging from local vibrations of atoms and groups through global changes of relative positions of whole domains to rotations of side chains, over an exceedingly wide time extending from fractions of picoseconds to seconds (Acuña *et al,* 1987). Evidence for internal motions in globular proteins, reflecting the presence of molecular flexibility has come from a number of different experimental approaches. For example, the time-resolved depolarization of the intrinsic fluorescence (tryptophan) of proteins contains a very fast decay time due to the segmental motions of the tryptophan with respect to the rest of the protein as well as a much slower one corresponding to the global Brownian rotation of the protein (Ferrer *et al,* 2001;

Kamal & Behere, 2002). In turn, the EPR spectrum of a bound spin label reflects the rotational motion of the protein itself, the fluctuation of protein domains, or some residual motion of the label (which may be regarded as an artificial amino acid chain) with respect to the whole protein (Steinhoff *et al,* 1989; Steinhoff, 1990). Similarly, in NMR spectroscopy the NMR spin relaxation rates depend on both the global movement of protein and the local movements (Korchuganov *et al,* 2004; Lee *et al,* 2006). The Maxwell method and the DSE equation enable us to determine the correlation time τ_2 which is not susceptible to internal motion of the protein. An accurate knowledge of this time may, in turn, help in the separation of the movements that influence the results obtained by other methods.

CONCLUSIONS

The overall motion correlation time τ_2 of proteins in dilute solutions can be relatively easily and conveniently determine by using the Maxwell effect, if we experimentally determine the intrinsic viscosity. The results show that temperature has a strong influence on the value of τ_2: for each studied here albumin τ_2 at a temperature of 5°C is approximately 3 times longer than at a temperature of 45°C (both in solutions at pH = pI and at pH > pI). The values of the overall motion correlation time obtained for HSA, RSA, OSA, ESA and PSA in solutions at pH > pI - at a fixed temperature - are only slightly different from each other. Similarly, τ_2 determined for the BSA and HSA in solutions at pH = pI are - at the same temperature - very similar, but differ significantly from the value of τ_2 for albumins in solution at pH > pI. This is particularly evident for HSA, for which the values of τ_2 were determined both in solution at the isoelectric point and beyond of it. The results obtained enable to postulate that, for a given protein and at a fixed temperature, τ_2 reaches its highest value in the solution at isoelectric point. The values of τ_2 obtained for the studied here albumins in solutions outside the isoelectric point agree very well with the values obtained using the Debye-Stokes-Einstein equation, wherein the hydrodynamic radius has been calculated for hydrated molecules.

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