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ANALYSIS OF THE VISCOSITY-TEMPERATURE-CONCENTRATION DEPENDENCE FOR DIMERIC BOVINE β-LACTOGLOBULIN AQUEOUS SOLUTIONS ON THE BASIS OF THE VOGEL-TAMMANN-FULCHER'S EQUATION.

KAROL MONKOS

Department of Biophysics, Medical University of Silesia, H. Jordana 19, 41-808 Zabrze 8, Poland

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The paper presents the results of viscosity determinations on aqueous solutions of bovine β-lactoglobulin at a wide range of concentrations and at temperatures ranging from 278 K to 328 K. The solutions viscosity has been measured by using an Ubbelohde-type capillary microviscometer. The viscosity-temperature dependence, at each fixed concentration, has been quantitatively described by the three parameters Vogel-Tammann-Fulcher's equation. A way of calculations of the parameters, based on the least square method, has been presented. The obtained expressions allow calculation of the parameters, if the experimental values of viscosity and temperature are given. The physical meaning and the concentration dependence of those parameters is discussed too. Temperature dependence of the activation energy of viscous flow and the effective specific volume of the studied protein has been demonstrated.

INTRODUCTION

Bovine β-lactoglobulin (BLG) is a major component of cow's milk with a chemical composition $C_{820}H_{1308}N_{206}O_{252}S_9$, which corresponds to a molecular mass M_p = 18 155 Da (Oreccini *et al*., 2001). BLG is a well-characterized globular protein composed of 162 amino acid residues and is predominantly β-sheet protein consisting of a β-barrel of eight continuous anti-parallel β-strands, an additional β-strand and one major and four short α-helices (Kuwata *et al*., 2001). It is able to bind several non-polar components and is thought to be a member of the lipocalin family of hydrophobic carrier molecules (Kuwajima *et al*., 1996). However, its function is not precisely clear. BLG forms dimmers around neutral pH, but dissociates into a monomers below pH 3. It has been the object of physicochemical studies for many years and serves as a model protein for different studies of folding, stability and self-association. The studies have been based on the experimental techniques such as inelastic neutron scattering (Oreccini *et al*., 2001), NMR spectroscopy (Kuwata *et al*., 2001; Gottschalk *et al*., 2003), circular dichroism and absorption spectroscopy (Kuwajima *et al*., 1996), particle beam liquid chromatography, FT infrared spectrometry and electrospray liquid chromatography (Turula *et al*., 1997), differential scanning calorimetry (Relkin, 1998), fluorescence (Fessas *et al*., 2001), gel electrophoresis (Morgan *et al*., 1999), light scattering (Verheul *et al*., 1998), transient electric birefringence (Rogers *et al*, 2006), densitometry (Taulier & Chalikian, 2001) and viscometry (Monkos, 2006).

Viscometry still plays an important role in investigations of both synthetic polymers and biomacromolecules in solution because of its extreme sensitivity and technical simplicity (Harding, 1997 and references therein). This work presents the results of viscosity measurements for dimeric BLG aqueous solutions at temperatures ranging from 278 K to 328 K and at a wide range of concentrations. The temperature-induced variations of the globular proteins solution viscosity – in a broad range of temperatures – can be described either by application of the absolute rate theory to the process of flow, which leads to the modified Arrhenius equation (Monkos, 1996, 2005, 2006, 2007 and references therein) or by using the free-volume concept, which leads to the Vogel-Tammann-Fulcher's (VTF) equation (Monkos, 1996, 2003, 2004; Vinogradov & Malkin, 1980). In the present paper only the VTF equation is discussed. Based on the non-linear least square method, the three parameters of the VTF equation have been calculated. The physical meaning and the concentration dependence of those parameters has been presented too. Besides, the activation energy of viscous flow and the effective specific volume of

dimeric BLG – in the whole measured range of temperatures – have been calculated.

MATERIALS

The native BLG was purchased from Sigma Chemical (L-6879) and was used without further purification. From the crystalline form the protein was dissolved in distilled water and then filtered by means of filter papers in order to remove possible undissolved fragments. The solutions were stored at 277 K until just prior to viscometry measurements, when they were warmed from 278 to 328 K by steps of 5 K. The pH values of such prepared solutions changed only insignificantly with the mean value 7.2.

VISCOMETRY

Viscometric measurements with BLG solutions were carried out using an Ubbelohde-type capillary microviscometer. The temperature was controlled thermostatically by a circulating water-bath at the range from 278 to 328 K with a precision of 0.1 K. The same viscometer was used for all measurements and it was mounted so that it occupied the same position in the bath. The viscosities of the BLG solutions were analyzed for concentrations from 119 kg/m^3 up to 444 kg/m^3. In this range of concentrations and for temperatures from 278 to 328 K, BLG molecules exist in the dimeric form (Aymard *et al*., 1996). For most concentrations the viscosity measurements were made at 5 K intervals. Solutions densities were measured by weighing and protein concentrations were determined by a dry weight method in which samples were dried at high temperature for several hours.

RESULTS AND DISCUSSION

Liquids - being cooled sufficiently slowly - crystallize below their melting point. However, if the cooling process realizes fast enough it is possible to overcool the fluid substantially below its melting point without crystallizing. It then solidifies, forming a non-crystalline state that is usually called glass. The process occurs at the characteristic temperature – the so called glass-transition temperature T_g (Martinez & Angell, 2001). At this temperature properties of the material change from liquid-like to solid-like. Recently, the glass-transition behaviour of many molecular liquids, synthetic polymers and biopolymers, including proteins and polysaccharides, has been studied (Sartor *et al*., 1995; Noel *et al*., 1995; Biliaderis *et al*., 1999; Baysal & Atilgan, 2002; Lazaridou & Biliarderis, 2002; Borde *et al*., 2002; Lubchenko *et al*., 2005; Kawai *et al*., 2006; Lubchenko, 2006).

Viscosity-temperature dependence for glass forming liquids is usually discussed on the basis of free volume concept (Vinogradov & Malkin, 1980). According to this idea a liquid flows thank to the jumps of molecules into the holes created by surrounding molecules owing to density fluctuations. Development of the free volume idea by Williams, Landel and Ferry leads to the viscosity-temperature relationship which can be applied for temperatures from T_g to (T_g + 100 K). For temperatures close to T_g the viscosity reaches the value of 10^{13} poise. The temperature at which infinite viscosity is reached is called the ideal glass transition temperature T_o. In reality, T_g is always higher than T_o and $T_g - T_o = \kappa^{-1}$, where κ is the coefficient of thermal expansion of free volume (Vinogradov & Malkin, 1980). Formally, free volume is defined as the difference between the specific volume of the liquid and the specific volume occupied by the molecules of the substance. At temperature T_o the free volume is equal to zero. The assumption that for temperatures higher than T_o the free volume increases linearly with temperature leads to the VTF equation. It was originally proposed for oils (Vogel, 1921), supercooled organic liquids (Tammann & Hesse, 1926) and for molten inorganic glasses (Fulcher, 1925). Angell proposed a classification of the glass-forming systems onto "strong" and "fragile" liquids and introduced the so-called fragility parameter (Angell, 1988). Taking it into consideration, the VTF equation for solutions – for which viscosity depends both on temperature and concentration of the solution – can be presented in the following form:

$$\eta(c,T) = W_s(c)\exp\left[\frac{F_s(c)T_{o,s}(c)}{T - T_{o,s}(c)}\right], \qquad (1)$$

where $W_s(c)$, $F_s(c)$ and $T_{o,s}(c)$ are parameters which depends on concentration. To fit the viscosity from the above formula to the experimental values of viscosity obtained at different temperatures the values of these parameters are necessary. In the present paper, numerical values of these parameters have been calculated, for all concentrations of BLG, by applying the non-linear least square method described in the appendix. Figure 1 shows the results of viscosity measurements at various temperatures for BLG aqueous solutions, for three concentrations. The curves presents the fit to the experimental points according to the above equation with the parameters $W_s(c)$, $F_s(c)$ and $T_{o,s}(c)$ calculated from the expressions (A2-A4). As seen a very good fit over the whole range of temperatures has been

obtained. The parameters $W_s(c)$, $F_s(c)$ and $T_{o,s}(c)$ appear to be dependent on concentration in a quite different way and the results of their calculations are shown in Figures 2 and 3.

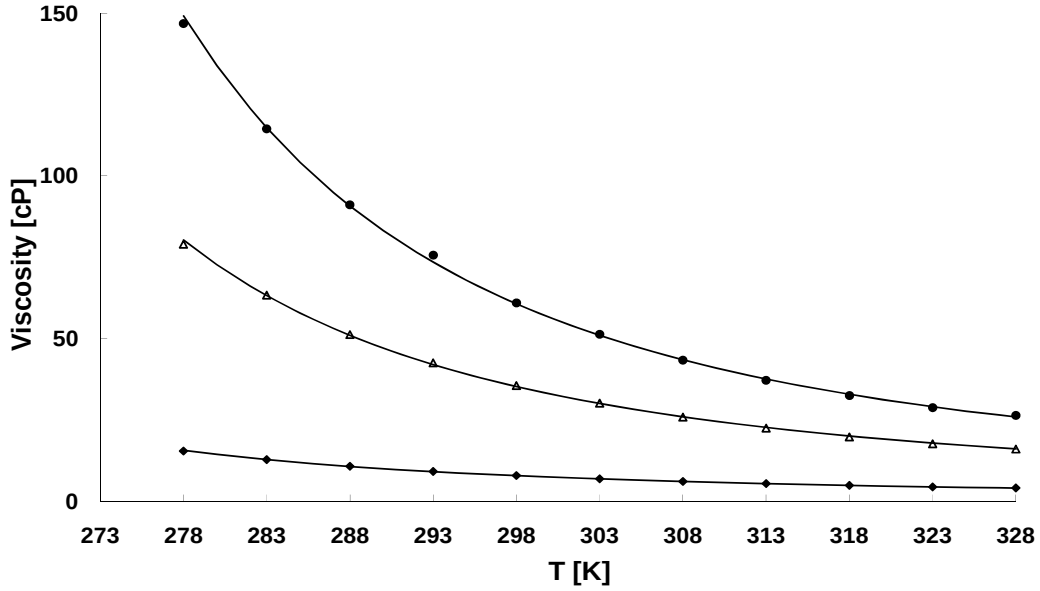

Fig. 1. Temperature dependence of the viscosity of BLG aqueous solutions for concentrations: c = 433 kg/m^3 (•), c = 407 kg/m^3 (Δ) and c = 294 kg/m^3 (♦). The curves show the fit obtained by using equation (1) with the parameters: $W_s(c)$ = 1.299 cP, $F_s(c)$ = 2.1 and $T_{o,s}(c)$ = 192.8 K for c = 433 kg/m^3; $W_s(c)$ = 0.956 cP, $F_s(c)$ = 2.08 and $T_{o,s}(c)$ = 190.8 K for c = 407 kg/m^3; $W_s(c)$ = 0.353 cP, $F_c(c)$ = 2.37 and $T_{o,s}(c)$ = 189.3 K for c = 294 kg/m^3.

Apart from the free-volume model, the theoretical basis of VTF equation is the theory of entropy introduced by Adam and Gibbs (Adam & Gibbs, 1965). This theory is based on the notion of the configurational entropy: $S_c = k\ln\Omega$, where k is the Boltzmann constant and Ω denotes the number of configurations available to the system of N molecules. The authors have assumed that a liquid's flow requires collective rearrangements of some number of molecules. By assuming that the energy required to the rearrangement increases in proportion to this number, Adam and Gibbs obtained the formula for viscosity, which – at the equilibrium state – leads to the VTF equation. The ideal glass transition temperature is then identified with the Kauzmann temperature, i.e. the temperature where configurational entropy is equal to zero (Adam & Gibbs, 1965).

As seen in Figure 2, $T_{o,s}(c)$ obtained for the solutions of dimeric BLG increases nonlinearly with increasing concentration of dissolved proteins. Because of the lack of any theoretical explanation of such changes, only the phenomenological description is possible. It is natural to assume that, if solution concentration c tends to 0, the ideal glass transition temperature of the solution $T_{o,s}(c)$ should tend to the ideal glass transition temperature of water ($T_{o,w}$). On the other hand, when c tends to the infinity the $T_{o,s}(c)$ should tend to the ideal glass transition temperature for dissolved proteins ($T_{o,p}$). The relationship fulfilling the above conditions has the form (Monkos, 2004):

$$T_{o,s}(c) = \frac{c}{\gamma + c}\left(T_{o,p} - T_{o,w}\right) + T_{o,w}, \quad (2)$$

where γ is a parameter. The ideal glass transition (as well as parameters W and F) for water one can obtain from equation (1) taking viscosity values of water from the standard physicochemical tables. Taking the viscosity values in the range of temperatures from 278 K to 328 K, and applying once more the least square method to the equation (1), one can obtain $T_{o,w}$ = 147 K. Figure 2 shows the fit of $T_{o,s}(c)$ obtained from the above equation to the experimental values of $T_{o,s}(c)$, in the case when $T_{o,p}$ and γ are taken as adjustable parameters. The best fitting was obtained for, $T_{o,p}$ = (291 ± 8.5) K and γ = (970 ± 76.5) kg/m^3. It is worth noting that the highest value of $T_{o,s}(c)$ - obtained for the solution of

BLG at c = 444 kg/m^3 - is equal to 194 K and it means that the viscosity measurements for BLG solutions were

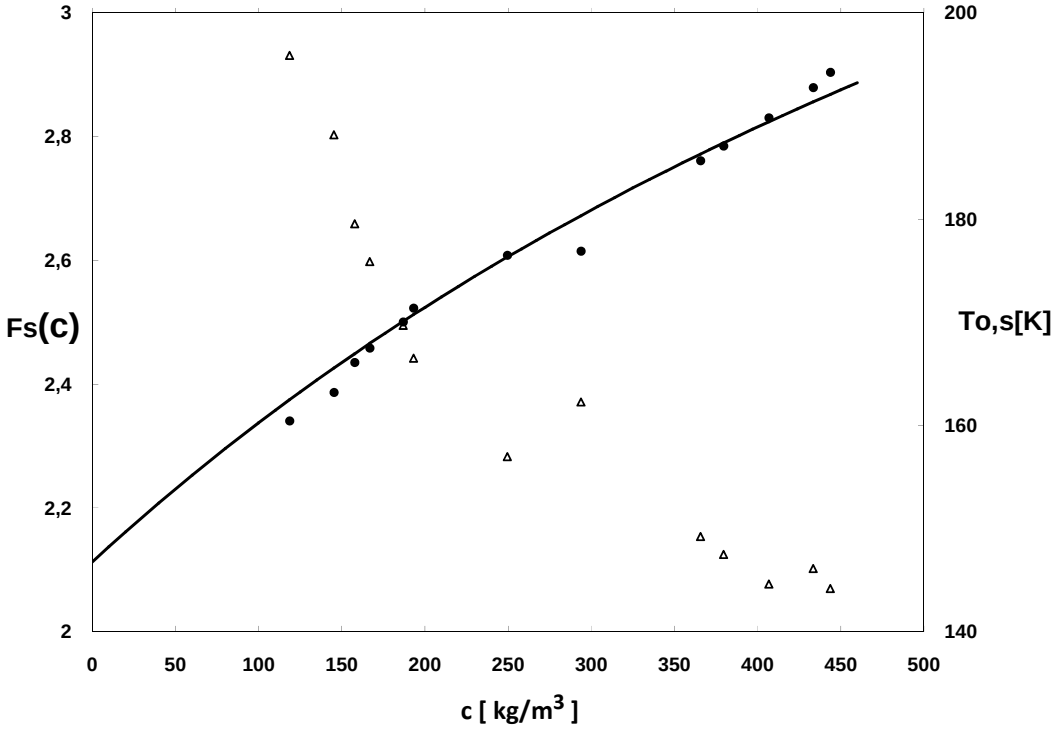

Fig. 2. Plots of the ideal glass transition temperature $T_{o,s}(c)$ (•) and fragility parameter $F_s(c)$ (Δ) versus concentration of BLG in aqueous solution; the curve shows the fit according to expression (2).

made at least 84 K above the $T_{o,s}(c)$. It shows that the above presented fit is not the best one, because the precise values of the parameters can be obtained only from viscosity measurements conducted in the close vicinity of the ideal glass transition. However, in practice it is impossible to conduct such measurements in the deeply supercooled range. It is worth to add that a nonlinear dependence of $T_{o,s}(c)$ versus concentration for bovine serum albumin (Monkos, 1996), human serum albumin (Monkos, 2004) and some carbohydrate aqueous solutions (Rampp *et al*., 2000) has also been obtained.

The temperature variations of the viscosity differs significantly for different liquids. As mentioned above Angell (Angell, 1988) suggested a classification of the glass-forming systems onto "strong" and "fragile" liquids: those with milder variations of viscosity were named as "strong" and those with sharp decreases of viscosity were designated as "fragile". The fragility parameter F is useful in this classification. According to the Angell's conception "strong" liquids are those for which viscosity does not decrease much with increasing temperature above T_g. On the other hand, according to the Adam-Gibbs model, liquids – in this case – have highly constrained structures which have a low density of configurational states. Contrary to this, the "fragile" liquids 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 above T_g.

Figure 2 shows numerical values of the fragility parameter obtained on the basis of VTF relation and from equation (A4). As seen, $F_s(c)$ decreases with increasing concentration from the value of about 2.9 (c = 119 kg/m^3) up to the value of 2.1 (c = 444 kg/m^3). However, it is difficult to find functional description of these changes. The fragile parameter for water – obtained on the basis of equation (1) and relation (A4) - is: F_w = 3.57. The obtained values of this parameter shows that water and all solutions studied here belong to

the extremely fragile class of liquids. For the extremely strong liquids, the fragility parameter tends to the value of about 100.

It is clear from equation (1), that the parameter $W_s(c)$ represents the viscosity liquid would have at an infinitely high temperature. As seen in Figure 3, this parameter (in the logarithmic scale) depends linearly on concentration. The straight line shown in Figure 3 was obtained by the least squares method and corresponds to the relation:

$$\ln W_s(c) = -d_1 + d_2 c, \tag{3}$$

where $d_1 = 4.16 \pm 0.06$, $d_2 = (1 \pm 0.02)\times 10^{-2}$ m^3kg^{-1} and $W_s(c)$ is given in centipoises then.

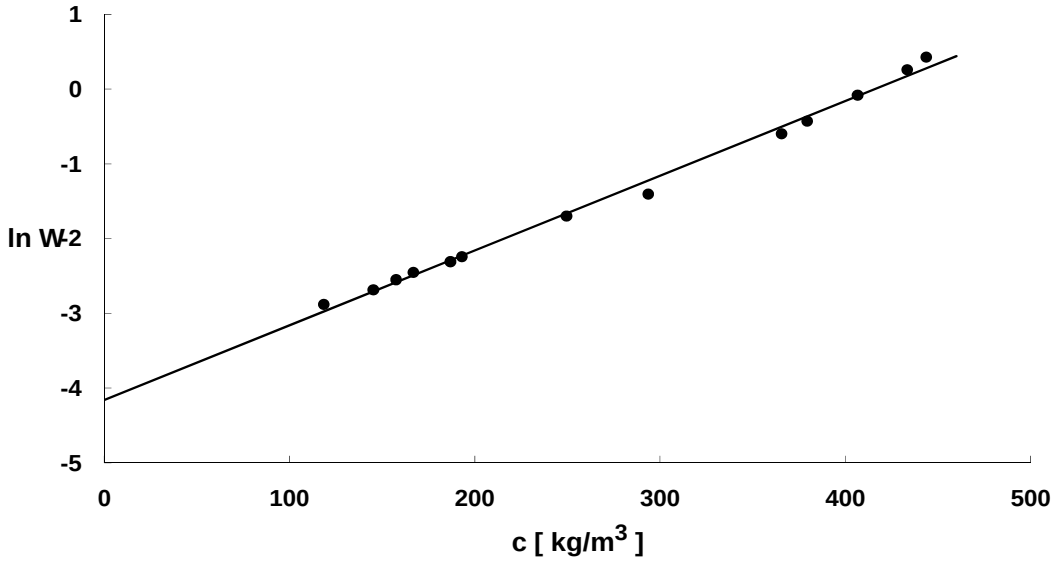

Fig. 3. Plot of the parameter $W_s(c)$ from the Vogel-Tammann-Fulcher's equation versus concentration of BLG in a log-normal plot; a straight line shows the fit according to relation (3).

One of the most important hydrodynamic parameters characterizing the flow of viscous liquid is the activation energy of viscous flow. In the case of Arrhenius behaviour of viscosity this quantity is usually obtained from the slope of the straight line in the Arrhenius plot, i.e. the plot of $ln\eta$ versus T^{-1}. In the case of non-Arrhenius behaviour of viscosity, the activation energy depends on temperature and should be calculated for each temperature individually. For solutions, where viscosity depends both on temperature and concentration, the following definition of the activation energy can be used:

$$\Delta E(c,T) = R\frac{d\ln\eta(c,T)}{dT^{-1}}. \tag{4}$$

The VTF equation gives the functional dependence of the viscosity on temperature. So, one can insert the function from equation (1) into the above definition. After differentiation and simple transformations the following formula for the activation energy of viscous flow of a solution can be obtained:

$$\Delta E(c,T) = \frac{RF_s(c)T_{o,s}(c)T^2}{\left[T - T_{o,s}(c)\right]^2}. \tag{5}$$

The values of $\Delta E(c,T)$ calculated from the above equation – for three temperatures – are shown in Figure 4.

Moreover, as has been shown earlier (Monkos, 1996), the activation energy of a solution – at each individual temperature – can be treated as a superposition of the activation energy of dissolved protein molecules at this temperature $\Delta E_p(T)$ and the activation energy of water molecules at the same temperature $\Delta E_w(T)$. This reasoning yields the following relation for the activation energy of a solution:

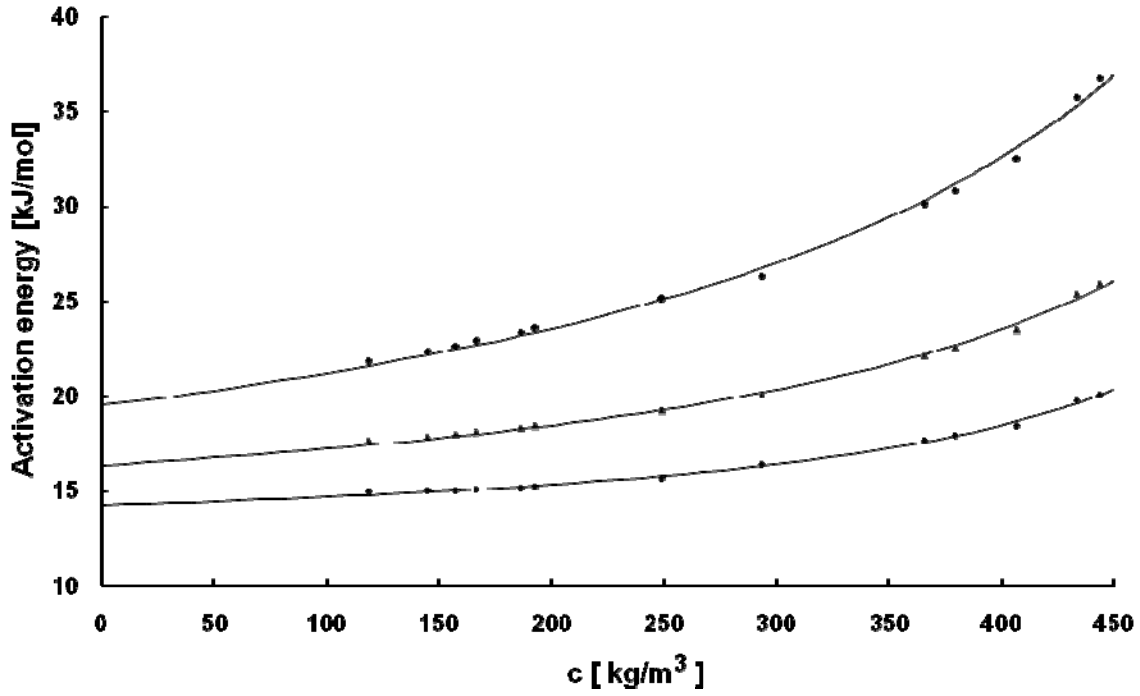

Fig. 4. Plot of the solution activation energy $\Delta E(c,T)$ versus concentration c at T = 278 K (•), T = 303 K (▲) and T = 328 K (♦) for BLG. Experimental points were obtained on the basis of equation (5); the curves show the fit according to equation (6) with the parameters: $\alpha = 2.743\times10^6$ kg/m^{3}and: $\xi = 1.38\times10^{-3}$ m^3/kg, $\Delta E_p(T) = 4.018\times10^4$ kJ/mol, $\Delta E_w(T)$ = 19,52 kJ/mol at T = 278 K; $\xi = 1.454\times10^{-3}$ m^3/kg, $\Delta E_p(T) = 2.054\times10^4$ kJ/mol, $\Delta E_w(T)$ = 16.36 kJ/mol at T = 303 K; $\xi = 1.614\times10^{-3}$ m^3/kg, $\Delta E_p(T) = 1.025\times10^4$ kJ/mol, $\Delta E_w(T)$ = 14,25 kJ/mol at T = 328 K.

$$\Delta E(c,T) = \\ = \frac{c}{\alpha - \beta c}\left[\Delta E_p(T) - \Delta E_w(T)\right] + \Delta E_w(T), \quad (6)$$

where $\alpha = \rho_w M_h/M_w$ and $\beta = \alpha\xi - 1$. The quantities ρ_w, ξ, M_h and M_w denote the water density, the effective specific volume of a protein and the molecular masses of the dissolved protein and water, respectively. At c = 0 the parameters $F_s(0) = F_w = 3.57$, $T_{o,s}(0) = T_{o,w} = 147$ K and equation (5) allows calculation of the activation energy of water molecules at a given temperature. As can be easily shown $\Delta E_w(T)$ changes from 19.52 kJ/mol (T = 278 K) up to 14.25 kJ/mol (T = 328 K).

Hydrodynamic mass of BLG is a sum of the molecular mass of unhydrated BLG and the mass of hydration shell of water surrounding the BLG molecules in solution: $M_h = M_p(1 + \delta)$, where δ means the amount of grams of water associated with the BLG per gram of BLG. As has been shown by using the inelastic neutron scattering method $\delta = 0.4$ for D_2O-hydrated BLG

Table 1. Numerical values of the activation energy of viscous flow $\Delta E_p(T)$ and the effective specific volume ξ of dimeric BLG obtained from equation (6) in which $\Delta E_p(T)$ and ξ are adjustable parameters.

T [K]	$\Delta E_p(T)$ [MJ/mol]	$\xi\times10^3$ [m^3/kg]
278	40.18 ± 0.55	1.380 ± 0.062
283	35.09 ± 0.50	1.387 ± 0.068
288	30.69 ± 0.45	1.398 ± 0.072
293	26.65 ± 0.41	1.413 ± 0.081
298	23.49 ± 0.39	1.432 ± 0.085
303	20.54 ± 0.36	1.454 ± 0.092
308	17.95 ± 0.34	1.480 ± 0.099
313	15.66 ± 0.33	1.509 ± 0.107
318	13.64 ± 0.31	1.541 ± 0.117
323	11.64 ± 0.30	1.576 ± 0.131
328	10.25 ± 0.29	1.614 ± 0.138

(Oreccini *et al*., 2001). It is easy to calculate that this corresponds to $\delta = 0.36$ for H_2O-hydrated BLG and it gives the molecular mass of hydrated BLG in dimeric form M_h = 49.382 kDa and in consequence α = 2.743 kg/m^3. Now, the only unknown quantities in equation (6) are $\Delta E_p(T)$ and ξ. They can be treated as adjustable parameters. Numerical values of them, obtained by using the least square method, are gathered in Table 1.

As seen, the activation energy of viscous flow for dimeric BLG strongly depends on temperature. At 278 K is about 4 times greater than at 328 K. Contrary to this, the effective specific volume of the protein slowly increases with increasing temperature. This quantity is a coefficient of proportionality between the effective molar volume and the molar mass of a macrosolute. The effective specific volume is needed, among others, in calculations of the thermodynamic activity of cellular components in cells (Zimmermann & Trach, 1991). The method presented in this paper is one of few ones which enables on experimentally obtaining of this parameter. Unfortunately, the theoretical values of the effective specific volume was obtained on the basis of the hard sphere model and from analysis of virial expansion only for serum albumins (Zimmermann & Minton, 1993). The authors obtained the values of ξ in the range from 0.8×10^{-3} m^3/kg (at pH 5.1) up to 1.7×10^{-3} m^3/kg (at pH 7.6). Unexpectedly enough the values obtained for dimeric BLG (Tables 1) lie inside of this range. However, as far as I know there is no paper in which the temperature dependence of the effective specific volume would be discussed. Experimental values of ξ are highly desirable because they should give more accurate values of the activity coefficients and should provide a test for any theoretical treatment of the effective specific volume.

CONCLUSIONS

The viscosity of dimeric BLG aqueous solutions at temperatures up to 328 K and in a wide range of concentrations may be quantitatively described by the three parameters VTF's equation (1). One of these parameters – the ideal glass transition temperature T_o – increases nonlinearly with increasing concentration of dissolved proteins. The proposed phenomenological description of this dependence allows to obtain the ideal glass transition temperature for dimeric BLG: $T_{o,p}$ = (291 ± 8.5) K. The fragility parameter, in turn, decreases with increasing concentration of BLG. The numerical values of this parameter indicate that the solutions studied here belong to an extremely fragile class of liquids. Activation energy of viscous flow of dimeric BLG molecules decreases with increasing temperature from 40.18 MJ/mol (at T = 278 K) to 10.25 MJ/mol (at T = 328 K). The effective specific volume, in turn, slowly increases with increasing temperature from 1.38×10^{-3} m^3/kg (at T = 278 K) to 1.614×10^{-3} m^3/kg (at T = 328 K).

APPENDIX

The best way for obtaining the parameters in the VTF equation is to use the least square method. To do this, it is convenient to transform the equation into the form $FT_o = (ln\eta - lnW)(T-T_o)$ and find the minimum of the square form:

$$\chi = \sum_{i=1}^{n}\left[\left(y_i - lnW\right)\left(T_i - T_o\right) - FT_o\right]^2, \qquad (A1)$$

where $y_i = ln\eta_i$, with respect to F, T_o and lnW. A simple calculations show that:

$$T_o = \frac{\left[\sum_{i=1}^{n} y_i T_i^2 - \frac{1}{n}\sum_{i=1}^{n} y_i T_i \sum_{i=1}^{n} T_i\right]\left[\sum_{i=1}^{n} y_i T_i - \frac{1}{n}\sum_{i=1}^{n} y_i \sum_{i=1}^{n} T_i\right] - \left[\sum_{i=1}^{n} T_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} T_i\right)^2\right]\left[\sum_{i=1}^{n} y_i^2 T_i - \frac{1}{n}\sum_{i=1}^{n} y_i T_i \sum_{i=1}^{n} y_i\right]}{\left[\sum_{i=1}^{n} y_i T_i - \frac{1}{n}\sum_{i=1}^{n} y_i \sum_{i=1}^{n} T_i\right]^2 - \left[\sum_{i=1}^{n} y_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} y_i\right)^2\right]\left[\sum_{i=1}^{n} T_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} T_i\right)^2\right]} \qquad (A2)$$

$$lnW = \frac{\sum_{i=1}^{n} y_i^2 T_i - \frac{1}{n}\sum_{i=1}^{n} y_i T_i \sum_{i=1}^{n} y_i - T_o\left[\sum_{i=1}^{n} y_i^2 - \frac{1}{n}\left(\sum_{i=1}^{n} y_i\right)^2\right]}{\sum_{i=1}^{n} y_i T_i - \frac{1}{n}\sum_{i=1}^{n} y_i \sum_{i=1}^{n} T_i}, \qquad (A3)$$

$$F = \frac{1}{nT_o}\sum_{i=1}^{n}\left(y_i - lnW\right)\left(T_i - T_o\right). \qquad (A4)$$

Putting the experimental values of η_i and T_i, for a given concentration, into relations (A2), (A3) and (A4) we have obtained numerical values of T_o, F and lnW which are presented in Figures 2 and 3.

REFERENCES

Adam G. & Gibbs J. H. (1965). On the temperature dependence of cooperative relaxation properties in glass-forming liquids. *J. Chem. Phys.,* **43,** 139-146.

Angell C. A. (1988). Perspective on the glass transition. *J. Phys. Chem. Solids,* **8**, 863-871.

Aymard P., Durand D. & Nicolai T. (1996). The effect of temperature and ionic strength on the dimerisation of β-lactoglobulin. *Int. J. Biol. Macromol.,* **19,** 213-221.

Baysal C. & Atilgan A. R. (2002). Relaxation kinetics and the glassiness of proteins: the case of bovine pancreatic trypsin inhibitor. *Biophys J*., **83**, 699-705.

Biliaderis C. G., Lazaridou A. & Arvanitoyannis I. (1999). Glass transition and physical properties of polyol-plasticised pullulan-starch blends at low moisture. *Carbohydr. Polym.,* **40**, 29-47.

Borde B., Bizot H., Vigier G. & Buleon A. (2002). Calorimetric analysis of the structural relaxation in partially hydrated amorphous polysaccharides. I. Glass transition and fragility. *Carbohydr Polymer,* **48**, 83-96.

Fessas D., Iametti S., Schiraldi A. & Bonomi F. (2001). Thermal unfolding of monomeric and dimeric β-lactoglobulins. *Eur. J. Biochem.,* **268,** 5439-5448.

Fulcher G. S. (1925). Analysis of recent measurements of the viscosity of glasses. *J. Am. Ceram. Soc.,* **8,** 339-355.

Gottschalk M., Nillson H., Roos H. & Halle B. (2003). Protein self-association in solution: The bovine β-lactoglobulin dimer and octamer. *Protein Science,* **12,** 2404-2411.

Harding S. E. (1997). The intrinsic viscosity of biological macromolecules. Progress in measurements, interpretation and application to structure in dilute solution. *Prog. Biophys. Mol. Biol.,* **68,** 207-262.

Kawai K., Suzuki T. & Oguni M. (2006). Low-temperature glass transitions of quenched and annealed bovine serum albumin aqueous solutions. *Biophys. J.,* **90,** 1-7.

Kuwajima K., Yamaya H. & Sugai S. (1996). The burst-phase intermediate In the refolding of β-lactoglobulin studied by stopped-flow circular dichroism and absorption spectroscopy. *J. Mol. Biol.,* **264,** 806-822.

Kuwata K., Li H., Yamada H., Batt C. A., Goto Y. & Akasaka K. (2001). High pressure NMR reveals a variety of fluctuating conformers in β-lactoglobulin. *J. Mol. Biol.,* **305,** 1073-1083.

Lazaridou A. & Biliaderis C. G. (2002). Thermophysical properties of chitosan, chitosan-starch and chitosan-pullulan films near the glass transition. *Carbohydr. Polymer,* **48**, 179-190.

Lubchenko V. (2006). Quantitative theory of structural relaxation in supercooled liquids and folded proteins. *J. Non-Cryst. Solids,* **352,** 4400-4409.

Lubchenko V. & Wolynes P. G., Frauenfelder H. (2005). Mosaic energy landscapes of liquids and the control of protein conformational dynamics by glass-forming solvents. *J. Phys. Chem. B,* **109,** 7488-7499.

Martinez L. M. & Angell CA. (2001). A thermodynamic connection to the fragility of glass-forming liquids. *Nature*, **410,** 663-667.

Monkos K. (1996). Viscosity of bovine serum albumin aqueous solutions as a function of temperature and concentration. *Int. J. Biol. Macromol*., **18,** 61-68.

Monkos K. (2003). A method of calculations of the parameters in the Vogel-Tammann-Fulcher's equation: An application to the porcine serum albumin aqueous solutions. *Current Topics in Biophysics,* **27,** 17-21.

Monkos K. (2004). The ideal glass transition temperature and fragility parameter for human serum albumin aqueous solutions. *Physica Medica,* **20,** 108-110.

Monkos K. (2005). A comparison of solution conformation and hydrodynamic properties of equine, porcine and rabbit serum albumin using viscometric measurements. *Biochim. Biophys. Acta,* **1748,** 100-109.

Monkos K. (2006). On the hydrodynamics of dimeric bovine β-lactoglobulin solutions from viscometry approach. *Polish J. of Environ. Stud.,* **15,** 88-90.

Monkos K. (2007) Studies of proteins solution conformations using viscometric measurements. [In:] Uversky E.A. & Permyakov E.A. (eds.) *Methods in protein structure and stability analysis: Conformational stability, size, shape and surface of protein molecules*, Nova Science Publishers, NY, pp. 355-387.

Morgan F., Léonil J., Mollé D. & Bouhallab S. (1999). Modification of bovine β-lactoglobulin by glycation in a powdered state or in an aqueous solution: effect on association behavior and protein conformation. *J. Agric. Food Chem.,* **47,** 83-91.

Noel R. N., Parker R., Ring S. G. & Tatham A. S. (1995). The

glass-transition behaviour of wheat gluten proteins. *Int. J. Biol. Macromol.,* **17**, 81-85.

Oreccini A., Paciaroni A., Bizzarri A. R. & Cannistraro S. (2001). Low-frequency vibrational anomalies in β-lactoglobulin: contribution of different hydrogen classes reveiled by inelastic neutron scattering. *J. Phys. Chem. B,* **105**, 12150-12156.

Rampp M., Buttersack C. & Lüdemann H-D. (2000). c,T-Dependence of the viscosity and the self-diffusion coefficients in some aqueous carbohydrate solutions. *Carbohydr. Res.,* **328,** 561-572.

Relkin P. (1998). Reversibility of heat-induced conformational changes and surface exposed hydrophobic clusters of β-lactoglobulin: their role in heat-induced sol-gel state transition. *Int. J. Biol. Macromol.,* **22,** 59-66.

Rogers S. S., Venema P., van der Ploeg J. P. M., van der Linden E., Sagis L. M. C. & Donald A. M. (2006). Investigating the permanent electric dipole moment of β-lactoglobulin fibrils, using transient electric birefringence. *Biopolymers,* **82,** 241-252.

Sartor G, Hallbrucker A. & Mayer E. (1995). Characterizing the secondary hydration shell on hydrated myoglobin, hemoglobin, and lysozyme powders by its vitrification behavior on cooling and its calorimetric glass→liquid transition and crystallization behavior on reheating. *Biophys J*., **69,** 2679-2694.

Tammann G. & Hesse W. (1926). Die abhängigkeit der viskosität von der temperature bei unterkühlten flüssigkeiten. *Z. Anorg. Allg. Chem.,* **156,** 245-257.

Taulier N. & Chalikian T. V. (2001). Characterization of pH-induced transitions of β-lactoglobulin: ultrasonic, densimetric, and spectroscopic studies. *J. Mol. Biol.,* **314,** 873-889.

Turula V. E., Bishop R. T., Ricker R. D. & de Haseth J. A. (1997). Complete structure elucidation of a globular protein by particle beam liquid chromatography-Fourier transform infrared spectrometry and electrospray liquid chromatography–mass spectrometry. Sequence and conformation of β-lactoglobulin. *J. Chromatogr. A,* **763,** 91-103.

Verheul M., Roefs S. P. F. M. & de Kruif K. G. (1998). Kinetics of heat-induced aggregation of β-lactoglobulin. *J. Agric. Food Chem.,* **46,** 896-903.

Vinogradov G. V. & Malkin A. Y. (1980). Rheology of polymers. Moscow, Mir Publishers.

Vogel H. (1921). Das temperature - abhägigketsgesetz der viscosität von flüssigkeiten. *Phys. Zeit.,* **22,** 645-646.

Zimmerman S.B. & Trach S.O. (1991). Estimation of macromolecule concentrations and excluded volume effects for the cytoplasm of *Escherichia coli,* J. Mol. Biol., **222,** 599-620.

Zimmerman S. B. & Minton A. P. (1993). Macromolecular crowding: biochemical, biophysical, and physiological consequences. Annu. Rev. Biophys. Biomol. Struct. **22,** 27-65.