

A METHOD OF CALCULATIONS OF THE PARAMETERS IN THE VOGEL-TAMMANN-FULCHER'S EQUATION: AN APPLICATION TO THE PORCINE SERUM ALBUMIN AQUEOUS SOLUTIONS.

KAROL MONKOS

Department of Biophysics, Silesian Medical Academy, H.Jordana 19, 41-808 Zabrze, Poland.

Viscosity-temperature dependence of the proteins solutions can be quantitatively described by the three parameters Vogel-Tammann-Fulcher's equation. This paper presents a way of calculations of these parameters, based on the non-linear least square method. The obtained formulae allow calculation of the parameters, if the experimental values of viscosity and temperature are given. It has been checked for porcine serum albumin aqueous solutions. The solutions viscosity, over a wide range of concentrations and at temperatures ranging from 5°C to (42–45)°C, has been measured by using an Ubbelohde-type capillary microviscometer. The Vogel-Tammann-Fulcher's equation with such calculated parameters gives a very good fit to the experimental values of viscosity then. As appeared, these parameters depend on concentration in a quite different way. The polynomial's approximations of these dependences have been proposed and the physical meaning of the parameters has been discussed too.

INTRODUCTION

Globular proteins in solutions may exist in the native state only at a relatively narrow range of temperatures. A freezing point of a solution and the temperature where the thermal denaturation occurs are the boundaries from the side of low and high temperatures, respectively. At this range, the temperature-induced variations of the solution viscosity can be described either by application of the absolute rate theory to the process of flow or by using the free-volume concept (Vinogradov & Malkin, 1980). The former one gives a temperature dependence of viscosity in the Arrhenius form. Very recently, the generalized Arrhenius formula was successfully applied to some globular proteins in aqueous solutions (Monkos, 1994, 1996, 1997, 2000; Monkos & Turczynski, 1997, 1999). On the other hand, the free-volume theory leads to the viscosity-temperature relationship in the form of the Vogel-Tammann-Fulcher's (VTF) equation. It was originally proposed for oils, molten inorganic glasses and supercooled organic liquids by Vogel, Fulcher and Tammann and Hesse, respectively. This equation was also widely applied to some polymers (Bondi, 1967), metal and non-metal glasses (Chen, 1986), alcohols (Karger, Vardag & Lüdemann, 1990) and very recently to bovine serum albumin (Monkos, 1996) and some carbohydrates in aqueous solutions

(Rampp, Buttersack & Lüdemann, 2000). For globular proteins in aqueous solutions, at the range of temperatures where they are in the native state, both approaches can be applied. Then, they give a different sort of information about dissolved proteins.

In the present paper, only the VTF equation is discussed. A method of calculations of the parameters in the VTF equation, based on the non-linear least square method, is presented. The obtained results have been used for working out the viscosity data for the porcine serum albumin (PSA) aqueous solutions. The relationships connecting these parameters with the solutions concentration have been proposed too.

Calculation method

The semi-empirical VTF equation describes the dependence between a liquid viscosity η and the absolute temperature T . It has the form:

$$\eta = W \exp\left(\frac{Z}{T - T_0}\right), \quad (1)$$

where W , Z and T_0 are parameters. 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. The iteration methods, used in statistical programs like Statgraphics, Statistica or the others, are the

alternative ones. The best way for obtaining these parameters is to use the non-linear least square method. To do it, it is convenient to transform the VTF equation into the form $Z = (\ln \eta - \ln W)(T - T_o)$ and find the minimum of the square form:

$$\chi = \sum_{i=1}^n [(\ln \eta_i - \ln W)(T_i - T_o) - Z]^2 \quad (2)$$

with respect to Z , T_o and $\ln W$. A differentiation gives the following set of equations:

$$\left. \begin{aligned} \sum_{i=1}^n [(y_i - \ln W)(T_i - T_o) - Z](T_i - T_o) &= 0 \\ \sum_{i=1}^n [(y_i - \ln W)(T_i - T_o) - Z](y_i - \ln W) &= 0 \\ \sum_{i=1}^n [(y_i - \ln W)(T_i - T_o) - Z] &= 0 \end{aligned} \right\} \quad (3)$$

where $y_i = \ln \eta_i$. After some calculations, one can obtain the following expressions:

$$\begin{aligned} \ln W = & \left\{ \left[\sum_{i=1}^n T_i y_i^2 - \frac{1}{n} \sum_{i=1}^n T_i y_i \sum_{i=1}^n y_i \right] \left[\sum_{i=1}^n T_i y_i - \frac{1}{n} \sum_{i=1}^n T_i \sum_{i=1}^n y_i \right] - \right. \\ & - \left. \left[\sum_{i=1}^n T_i^2 y_i - \frac{1}{n} \sum_{i=1}^n T_i y_i \sum_{i=1}^n T_i \right] \left[\sum_{i=1}^n y_i^2 - \frac{1}{n} \left(\sum_{i=1}^n y_i \right)^2 \right] \right\} / \\ & \left\{ \left[\sum_{i=1}^n T_i y_i - \frac{1}{n} \sum_{i=1}^n T_i \sum_{i=1}^n y_i \right]^2 - \right. \\ & - \left. \left[\sum_{i=1}^n T_i^2 - \frac{1}{n} \left(\sum_{i=1}^n T_i \right)^2 \right] \left[\sum_{i=1}^n y_i^2 - \frac{1}{n} \left(\sum_{i=1}^n y_i \right)^2 \right] \right\} \quad (4) \end{aligned}$$

and

$$T_o = \frac{\sum_{i=1}^n T_i y_i^2 - \frac{1}{n} \sum_{i=1}^n T_i y_i \sum_{i=1}^n y_i + \left[\frac{1}{n} \sum_{i=1}^n T_i \sum_{i=1}^n y_i - \sum_{i=1}^n T_i y_i \right] \ln W}{\sum_{i=1}^n y_i^2 - \frac{1}{n} \left(\sum_{i=1}^n y_i \right)^2} \quad (5)$$

$$Z = \frac{1}{n} \sum_{i=1}^n (y_i - \ln W)(T_i - T_o) \quad (6)$$

The application of the above expressions to the fit of the VTF equation to the experimental values of viscosity for PSA aqueous solutions has been presented below.

Material

PSA (Cohn fraction V) was purchased from Sigma Chemical Company (A-2764) and was used without further purification. From the crystalline form the material was dissolved in distilled water and then filtered by means of filter papers in order to remove possible undissolved fragments. The samples were stored at refrigerator until just prior to viscometry measurements, when they were warmed from 5 to (42-45)°C. The pH values of prepared samples were about 6.6 and changed only insignificantly during the dilution of the solutions.

Viscometry

The viscosity measurements were performed using an Ubbelohde (capillary) microviscometer placed in a waterbath controlled thermostatically with a precision of $\pm 0.1^\circ\text{C}$. The same viscometer was used for all measurements. Solutions were temperature-equilibrated and passed once through the viscometer before any measurements were made. The viscosities of the PSA solutions were measured from 5°C to 45°C for concentrations from 34.4 kg/m³ up to 227.48 kg/m³; from 5°C to 43°C for the higher concentrations up to 319.4 kg/m³ and from 5°C to 42°C for the highest concentrations up to 386.16 kg/m³. These ranges of temperatures were chosen because the denaturation temperature of PSA depends on solution concentration and the lower protein concentration the higher denaturation temperature. At temperatures range from 5°C to 40°C the viscosity measurements were done in 5°C intervals. Solutions densities were measured by weighing. Protein

concentrations were determined by a dry weight method in which samples were dried at high temperature for several hours.

RESULTS AND DISCUSSION

In general, the solutions of proteins are polyelectrolytes. At the isoelectric point the surface of the proteins is covered with charges, but the net charge is zero. As the pH is moved from the

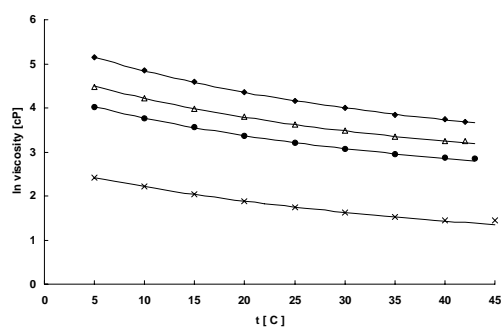


Fig. 1. Temperature dependence of the viscosity of porcine serum albumin aqueous solutions for concentrations $c_1 = 374.86 \text{ kg/m}^3$ (\blacklozenge), $c_2 = 357.91 \text{ kg/m}^3$ (Δ), $c_3 = 319.4 \text{ kg/m}^3$ (\bullet) and $c_4 = 227.48 \text{ kg/m}^3$ (\times). The curves show the fit obtained by using equation (1) with the parameters: $W = 5.697 \text{ cP}$, $Z = 166.98 \text{ K}$ and $T_0 = 229.3 \text{ K}$ for c_1 ; $W = 4.56 \text{ cP}$, $Z = 143.4 \text{ K}$ and $T_0 = 230.03 \text{ K}$ for c_2 ; $W = 3.281 \text{ cP}$, $Z = 141.35 \text{ K}$ and $T_0 = 228.37 \text{ K}$ for c_3 ; $W = 0.785 \text{ cP}$, $Z = 157.7 \text{ K}$ and $T_0 = 218.88 \text{ K}$ for c_4 .

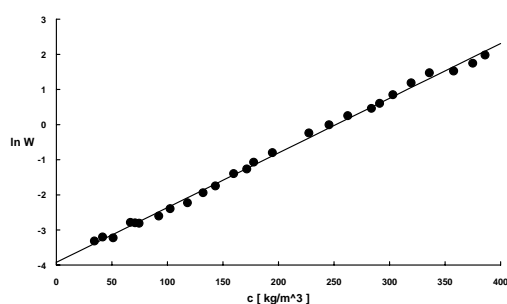


Fig. 2. Plot of the parameter W in the Vogel-Tammann-Fulcher's equation versus concentration in a log-normal plot; a straight line shows the fit according to relation (7).

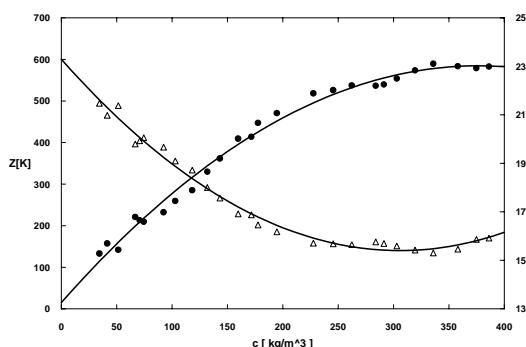


Fig. 3. Plots of the parameters Z (Δ) and T_0 (\bullet) in the Vogel-Tammann-Fulcher's equation versus concentration; the curves show the fit according to expressions (10) and (8), respectively.

isoelectric point, net positive or negative charges on the surface of the proteins increase giving rise to electrostatic repulsion. This repulsion causes the proteins may change conformation and far from the isoelectric point proteins denature giving rise to segmental motion of denatured filaments. Proteins are the most compact and stable near their isoelectric point. For PSA this is the case at pH from 5.1 to 7.0 (Olivieri & Craievich, 1995). The pH values of our samples are within of this range.

An investigation of albumin solutions by small-angle neutron scattering and Monte Carlo simulation showed that even for highly concentrated solutions a molecular mass, shape and dimensions are close to those of monomeric albumin (Sjöberg & Mortensen, 1994). At high temperatures proteins have tendency to aggregate. This heat-induced aggregation causes the thermal denaturation of proteins which reverses upon cooling

(Gorinstein, Zemser, Friedman & Chang, 1995). However, the results of some investigations suggest, that at temperatures below the temperature of denaturation, the size and conformation of proteins do not change with temperature (Ferrer, Duchowicz, Carrasco, de la Torre & Acuña, 2001). So, our measurements were conducted under conditions in which PSA should have stable conformation in the whole range of measured concentrations and temperatures.

Figure 1 presents the results of viscosity measurements at various temperatures for PSA aqueous solutions, for several concentrations. The curves show the fit to the experimental points according to relation (1) with the parameters W , Z and T_0 calculated from the expressions (4-6). As seen a very good fit over the whole range of temperatures was obtained. The parameters appeared to be dependent on concentration and for each concentra-

tion had to be calculated separately. The results of these calculations are shown in Fig. 2 and 3.

The uncertainties of the parameters values depend on the errors of measurements of viscosity and temperature. The errors of the viscosity measurements have changed from about 1.5% at the lower concentrations up to about 1% at the higher ones. By using the standard method of the errors calculation, one can obtain the following uncertainties for Z , W and T_0 : 1.65%, 5.8% and 1.73% for $c = 34.4 \text{ kg/m}^3$, and 1.83%, 5.2% and 0.66% for $c = 386.16 \text{ kg/m}^3$, respectively. The uncertainties of these parameters for the intermediates concentrations lie between the above values.

The parameter W has a simple physical meaning. As seen from the VTF equation, when $T \rightarrow \infty$ the exponent tends to 1. So, the parameter W is a viscosity of a liquid at an infinitely high temperature i.e. the viscosity of a liquid if it would exist in this state. Fig. 2 shows that $\ln W$ depends linearly on concentration. The straight line shown in the figure was determined by the method of least squares and corresponds to the relation:

$$\ln W = -d_1 + d_2 c \quad (7)$$

where $d_1 = 3.922 \pm 0.04$, $d_2 = (1.558 \pm 0.018) \times 10^{-2} \text{ m}^3 \text{ kg}^{-1}$ and W is given in centipoises.

The ideal glass transition temperature T_0 is a temperature in which the free volume i.e. the difference between the specific volume of the liquid and the specific volume occupied by the molecules of the substance is equal to zero (Vinogradov & Malkin, 1980). As seen in Fig. 3, T_0 depends nonlinearly on concentration. The curve shows the fit to the experimental points by the following square function:

$$T_0 = b_1 + b_2 c - b_3 c^2 \quad (8)$$

where $b_1 = (132.6 \pm 2.06) \text{ K}$, $b_2 = (0.518 \pm 0.024) \text{ Km}^3 \text{ kg}^{-1}$ and $b_3 = (6.88 \pm 0.57) \times 10^{-4} \text{ Km}^6 \text{ kg}^{-2}$. It is worth noting that the entire viscosity data end at least 47 K above the T_0 derived from the fits. So, this analysis contains a certain ambiguity, because the precise fit parameters can be obtained only from experimental results in the neighbourhood of T_0 . However, it is impossible to follow the viscosity into the deeply supercooled range. A non-linear dependence of T_0 versus concentration, for some aqueous carbohydrate solutions, has been recently obtained too (Rampp *et al.*, 2000). Unfortunately, the authors did not give the analytical description of this dependence.

The values of T_0 appear to be always below the glass transition temperature T_g . This is one of the characteristic parameters of an amorphous material and means the temperature at which its properties change from liquid-like to solid-like. T_g is not constant; it is dependent on cooling rate, sample history and method of measurements. The difference between T_g and T_0 is equal to the reciprocal of the coefficient of thermal expansion of free volume (Vinogradov & Malkin, 1980). Several authors have studied the glass transition of bulk water, with reported values for T_g in the range 130-145 K and the most often cited value 136 K (Johari, Hallbrucker & Mayer, 1987; Noel, Parker & Ring, 1995; Sartor, Mayer & Johari, 1994; Sartor, Hallbrucker & Mayer, 1995). It is obvious that the coefficient b_1 in equation (8) represents the ideal glass transition for water. As seen, its value is only slightly lower than T_g for water. For glass-forming liquids and polymers the differences between T_g and T_0 are significantly greater.

One can also give some physical meaning to the last parameter Z , in the VTF equation. In the region of very high temperatures, when $T \gg T_0$, the VTF equation transforms into the Arrhenius formula:

$$\eta = W \exp\left(\frac{E_\infty}{RT}\right) \quad (9)$$

where $E_\infty = ZR$ is the limiting value of activation energy of viscous flow and R is a gas constant. In practice, this is the range above the denaturation temperature of PSA i.e. outside the measurable range. As seen in Fig. 3, the dependence of Z on PSA concentration is non-linear. The curve shows the fit to the values of Z , obtained on the basis of the expression (6), by the following square function:

$$Z = a_1 - a_2 c + a_3 c^2 \quad (10)$$

where $a_1 = (600.4 \pm 10.6) \text{ K}$, $a_2 = (3.012 \pm 0.12) \text{ Km}^3 \text{ kg}^{-1}$ and $a_3 = (4.928 \pm 0.295) \times 10^{-3} \text{ Km}^6 \text{ kg}^{-2}$. The limiting value of activation energy for water $E_\infty = a_1 R = 4.992 \text{ kJ/mol}$. For PSA solutions this quantity decreases with increasing concentration to the value of about 1.1 kJ/mol (for $c \approx 330 \text{ kg/m}^3$) and then increases to 1.415 kJ/mol for $c = 386.16 \text{ kg/m}^3$.

The activation energy of viscous flow for water, when measured at temperatures range 5–45°C, is equal to 32 kJ/mol. The activation energy of solution is a superposition of the activation energy of water and protein molecules. As was shown in our

earlier papers (Monkos, 1994, 1996, 1997, 2000; Monkos & Turczynski 1997, 1999) this causes the activation energy of solution increases with increasing concentration of proteins. For PSA solutions this quantity nonlinearly increases up to the value 136.4 kJ/mol. The dependence of E_{∞} on concentration is difficult to explain.

It is worth noting that the parameters W , Z and T_0 were previously obtained for bovine serum albumin (Monkos, 1996). Their numerical values have been calculated by applying a non-linear regression procedure in the computational program Statgraphics. The identical polynomial's approximations as (7), (8) and (10) have been used there. As has appeared the values of the coefficients in the polynomials, for bovine serum albumin and PSA, are slightly different. To check if this polynomial's approximation is universal for different sort of proteins, more theoretical and experimental investigations are needed.

CONCLUSIONS

The non-linear least square method allows to obtain the complicated expressions on the parameters in the VTF equation. These expressions allow calculation of them on the basis of viscosity and temperature data. The VTF equation, with numerical values of parameters obtained in this way, gives very good fit to the experimental values over the wide range of temperatures. It was shown for PSA aqueous solutions. The parameters depend nonlinearly on concentration and the appropriate polynomial's approximations have been given too. However, the physical meaning and interpretation of the relationships is not yet clear.

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