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Evaluation of viscosities of aqueous two-phase systems containing protein

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Abstract

The dynamic viscosities of aqueous polyethylene glycol, aqueous bovine serum albumin, and polyethylene glycol–bovine serum albumin–water solutions were measured at temperatures of 15, 20, 25, 30 and 35 °C. To estimate the viscosity values of polyethylene glycol–bovine serum albumin–water solutions, a one parameter Grunberg-like model which was satisfactorily used earlier by the present author for polyethylene glycol-dextran–water solutions was employed. The disposable parameter a for our temperature range was estimated as 3.71. The relative errors varying from 0.29 to 18.98 in absolute value indicates that the Grunberg-like model works perfectly for polymer-protein solutions as well.

Keywords: Grunberg equation; Viscosity; Aqueous two-phase systems; Polyethylene glycol; Bovine serum albumin

1. Introduction

Extraction of biomolecules by making use of aqueous two-phase systems is important simply because it allows the separation and purification of these substances in biocompatible surroundings. Aqueous solutions of two polymers, usually polyethylene glycol (PEG) and dextran, are necessary to form the two aqueous phases. There are certain advantages of using such systems, see [1]. Below, we shall provide a partial list of them so as to make our paper a more self-contained one.

(a) Scale-up can be predicted easily and reliably from small laboratory experiments;
(b) Rapid mass transfer and equilibrium is reached by relatively little input of energy in the form of mechanical mixing;
(c) Continuous processing is readily achievable;
(d) The polymers stabilize the enzymes;
(e) Separation can be made selective and rapid;
(f) Separation can be carried out at room temperature due to the rapid separation;
(g) It has proven to be more economical than other separation processes.

The process of extraction of biomolecules using two aqueous phases can be carried out in an apparatus where contact is continuous or discontinuous. The phase viscosities are important to design a contactor used in industrial scale applications where large volumes of phases must be handled and separated in an efficient manner. Even though the phase viscosities of aqueous PEG–dextran are highly important, there have been only a few studies in the literature [2–5]. However, since PEGs has so many applications in the pharmaceutical, chemical, cosmetic, and food industries, more viscosity data is available for aqueous PEG solutions [6–10].

Aqueous two-phase systems can also be obtained by combining an aqueous solution of a protein with a polymer. Systems with one phase consisting of a concentrated protein solution are of interest for using enzymes in a protein-rich environment since it will imitate their natural environment inside the cell. The opposite phase can be used as a depot for the substrate and/or be a recipient for the product. The phase equilibria of aqueous two-phase systems containing polyethylene glycol–bovine serum albumin was investigated by Johansson in [11]. The partitioning of some mitochondrial enzymes between the phases was also determined. But there seems to be no viscosity data available in the literature for such systems.

In this article, our objective is to use a Grunberg-like model proposed earlier by the present author in [4,5] to evaluate the viscosities of systems containing polyethylene glycol–bovine serum albumin–water. The evaluation of viscosities of such polymer-protein systems is highly important because they can be useful as a base of enzyme reactors [11].

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The simplest form of a binary mixture viscosity equation, see [12], can be written in the form

$$f(\eta_{\text{mixture}}) = x_1 f(\eta_1) + x_2 f(\eta_2). \quad (1)$$

Such additive equations include only constants $f(\eta_1)$ and $f(\eta_2)$ representing the viscosities of the two pure components but have no disposable parameter. In Eq. (1), x_1 and x_2 may denote the weight, mole, or volume fractions of component 1 and component 2, and the viscosity function $f(\eta_{\text{mixture}})$ can be taken as η or $\ln \eta$. Grunberg [13] modified Eq. (1) by adding a term $x_1 x_2 c$, where c is a constant.

Our Grunberg-like equation is a parabolic equation of the form

$$\ln \eta_{\text{mixture}} = c_1 \ln \eta_{\text{PEG}} + c_2 \ln \eta_{\text{BSA}} + c_1 c_2 a \quad (2)$$

where

$$c_1 = \frac{c_{\text{PEG}}}{c_{\text{PEG}} + c_{\text{BSA}}}, \quad c_2 = \frac{c_{\text{BSA}}}{c_{\text{PEG}} + c_{\text{BSA}}},$$

η_{PEG} and η_{BSA} are, respectively, the dynamic viscosities of PEG–water and BSA–water solutions, c_{PEG} and c_{BSA} are, respectively, the weight percentages of PEG and BSA in solution, and a is a disposable parameter.

Table 1
A comparison of experimental and correlated viscosities calculated using Eq. (2), $a = 3.71$ for PEG (M_r 20 000)-bovine serum albumin–water solution. Viscosities are given in units of mPa s

PEG–water		BSA–water		PEG–BSA–water		e_i
c_{PEG} (%, w/w)	η_{PEG}	c_{BSA} (%, w/w)	η_{BSA}	$\eta_{\text{mix}}^{\text{expl}}$	$\eta_{\text{mix}}^{\text{calc}}$	
$T = 15\,^{\circ}\text{C}$						
11.0	16.28	1.0	1.23	18.29	17.44	4.64
10.0	13.20	2.0	1.29	14.78	15.01	−1.59
9.0	11.78	3.0	1.36	13.68	13.78	−0.70
7.0	8.02	6.0	1.59	10.43	9.57	8.22
7.0	8.02	5.0	1.46	10.34	9.72	6.00
7.0	8.02	4.0	1.43	10.14	10.11	0.29
4.0	3.79	8.0	1.87	6.31	5.39	14.50
$T = 20\,^{\circ}\text{C}$						
11.0	13.92	1.0	1.09	14.25	14.95	−4.88
10.0	11.48	2.0	1.15	12.45	13.11	−5.30
9.0	9.94	3.0	1.20	11.45	11.74	−2.52
7.0	6.95	6.0	1.43	9.20	8.43	8.37
7.0	6.95	5.0	1.30	8.18	8.52	−4.12
7.0	6.95	4.0	1.25	8.67	8.80	−1.54
4.0	3.45	8.0	1.71	5.80	4.93	14.95
$T = 25\,^{\circ}\text{C}$						
11.0	11.92	1.0	0.98	12.51	12.85	−2.77
10.0	9.88	2.0	1.04	10.94	11.36	3.86
9.0	8.45	3.0	1.07	9.97	10.12	−1.52
7.0	5.92	6.0	1.28	7.31	7.35	−0.46
7.0	5.92	5.0	1.17	6.80	7.43	−9.17
7.0	5.92	4.0	1.11	6.72	7.62	−13.37
4.0	3.15	8.0	1.55	5.11	4.48	12.33
$T = 30\,^{\circ}\text{C}$						
11.0	9.37	1.0	0.90	9.80	10.23	−4.45
10.0	8.49	2.0	0.95	9.14	9.87	−8.00
9.0	7.53	3.0	0.99	8.99	9.08	−1.02
7.0	5.02	6.0	1.17	7.11	6.46	9.15
7.0	5.02	5.0	1.05	6.08	6.44	−5.92
7.0	5.02	4.0	1.00	5.56	6.61	−18.98
4.0	2.90	8.0	1.43	4.48	4.13	7.75
$T = 35\,^{\circ}\text{C}$						
11.0	7.76	1.0	0.81	7.99	8.54	−6.81
10.0	7.20	2.0	0.84	7.71	8.44	−9.47
9.0	6.41	3.0	0.89	7.66	7.84	−2.41
7.0	4.33	6.0	1.05	6.04	5.67	6.05
7.0	4.33	5.0	0.94	5.47	5.64	−3.10
7.0	4.33	4.0	0.90	4.99	5.78	−15.77
4.0	2.68	8.0	1.26	3.84	3.71	3.44

Derivation of Eq. (2) can be found in [4]. We only remark here that

$$c_1 + c_2 = 1; \quad \eta_{\text{mixture}} = \eta_{\text{BSA}} \quad \text{if } c_{\text{PEG}} = 0;$$
$$\eta_{\text{mixture}} = \eta_{\text{PEG}} \quad \text{if } c_{\text{BSA}} = 0.$$

In [4], Eq. (2) with BSA replaced by Dx was employed to correlate PEG 8000–dextran 580 000–water mixture viscosities, where the experiments were made at temperature 10 °C using equal concentrations of polymers in the range from 1 to 3.5% (w/w). The efficiency of the model was also confirmed by considering the same polymers at different temperatures and varying relative molecular masses and concentrations [5].

Our goal in this work is to check the applicability of Eq. (2) by evaluating the viscosities of PEG–bovine serum albumin–water solutions.

2. Experimental

2.1. Materials

PEG 20 000 (lot #81300) was purchased from Fluka Company (Buchs, Switzerland), and Bovine serum albumin (lot

Table 2
A comparison of experimental and correlated viscosities calculated using Eq. (2) with different a values for PEG (M_r 20 000)-bovine serum albumin–water solution. Viscosities are given in units of mPa s

PEG–water		BSA–water		PEG–BSA–water		e_i
c_{PEG} (%, w/w)	η_{PEG}	c_{BSA} (%, w/w)	η_{BSA}	$\eta_{\text{mix}}^{\text{expl}}$	$\eta_{\text{mix}}^{\text{calc}}$	
$T = 15$ °C and a=3.97						
11.0	16.28	1.0	1.23	18.29	17.78	2.77
10.0	13.20	2.0	1.29	14.78	15.55	−5.23
9.0	11.78	3.0	1.36	13.68	14.44	−5.60
7.0	8.02	6.0	1.59	10.43	10.19	2.26
7.0	8.02	5.0	1.46	10.34	10.34	0.00
7.0	8.02	4.0	1.43	10.14	10.72	−5.73
4.0	3.79	8.0	1.87	6.31	5.71	9.54
$T = 20$ °C and a=3.82						
11.0	13.92	1.0	1.09	14.25	15.06	−5.69
10.0	11.48	2.0	1.15	12.45	13.30	−6.80
9.0	9.94	3.0	1.20	11.45	11.97	−4.50
7.0	6.95	6.0	1.43	9.20	8.65	6.03
7.0	6.95	5.0	1.30	8.18	8.73	−6.72
7.0	6.95	4.0	1.25	8.67	9.01	−3.96
4.0	3.45	8.0	1.71	5.80	5.04	13.01
$T = 25$ °C and a=3.60						
11.0	11.92	1.0	0.98	12.51	12.74	−1.87
10.0	9.88	2.0	1.04	10.94	9.91	−2.23
9.0	8.45	3.0	1.07	9.97	10.12	0.63
7.0	5.92	6.0	1.28	7.31	7.14	2.35
7.0	5.92	5.0	1.17	6.80	7.22	−6.18
7.0	5.92	4.0	1.11	6.72	7.42	−10.42
4.0	3.15	8.0	1.55	5.11	4.37	14.53
$T = 30$ °C and a=3.61						
11.0	9.37	1.0	0.90	9.80	10.16	−3.65
10.0	8.49	2.0	0.95	9.14	9.73	−6.52
9.0	7.53	3.0	0.99	8.99	8.92	0.85
7.0	5.02	6.0	1.17	7.11	6.30	11.37
7.0	5.02	5.0	1.05	6.08	6.29	−3.38
7.0	5.02	4.0	1.00	5.56	6.46	−16.26
4.0	2.90	8.0	1.43	4.48	4.04	9.77
$T = 35$ °C and a=3.57						
11.0	7.76	1.0	0.81	7.99	8.44	−5.66
10.0	7.20	2.0	0.84	7.71	8.27	−7.35
9.0	6.41	3.0	0.89	7.66	7.64	0.26
7.0	4.33	6.0	1.05	6.04	5.48	9.29
7.0	4.33	5.0	0.94	5.47	5.45	0.37
7.0	4.33	4.0	0.90	4.99	5.59	−12.05
4.0	2.68	8.0	1.26	3.84	3.59	6.42

#79H082) was purchased from Sigma Chemical Company (St. Louis, MO, USA). Crystallized and lyophilized BSA was used without purification to prepare a stock solution by weight with an accurately known concentration (10%). Concentrations of PEG stock solution was 25% polymer by weight. The PEG and BSA concentrations were situated between 4.0 and 11.0, and 1.0 and 8.0% by weight for PEG–water and BSA–water solutions, respectively, and for mixtures of PEG–bovine serum albumin–water different concentrations of components in water representing PEG and BSA compositions of homogeneous systems at varying temperatures.

2.2. Apparatus

A Canon–Fenske type viscometer was used to measure the relative viscosities (Cannon Instrument Corporation, PA, USA). Measurements were done at five different temperatures of 15, 20, 25, 30 and 35 °C. The immersion heater (Greiner Scientific Corporation, NY, USA) used in this study is capable of maintaining the temperature of the water bath to an accuracy of ±0.5°C. The dynamic viscosities were then calculated using the dynamic viscosities of water at the specified temperatures [14].

3. Results and discussion

In [5], we reported the viscosity measurements and predictions of PEG 8000–dextran (37 500, 494 000, 2 000 000)–water solutions and observed that Eq. (2) is valid for this polymer–polymer–water system.

We performed experiments at temperatures 15, 20, 25, 30 and 35 °C and considered PEG and bovine serum albumin with varying concentrations. We observed that the proposed model works quite satisfactorily giving results comparable with those in [4,5]. By taking into account data, which are displayed in Table 1, at all temperatures we have calculated a as 3.71 by using regressional analysis via SPSS. The calculated mixture viscosities using Eq. (2) are tabulated in Table 1.

The uncertainty e_i that appears in Tables 1 and 2, is defined by

$$e_i = \left(\frac{\eta_i^{\text{expl}} - \eta^{\text{calc}}}{\eta^{\text{expl}}} \right) \times 100 \tag{3}$$

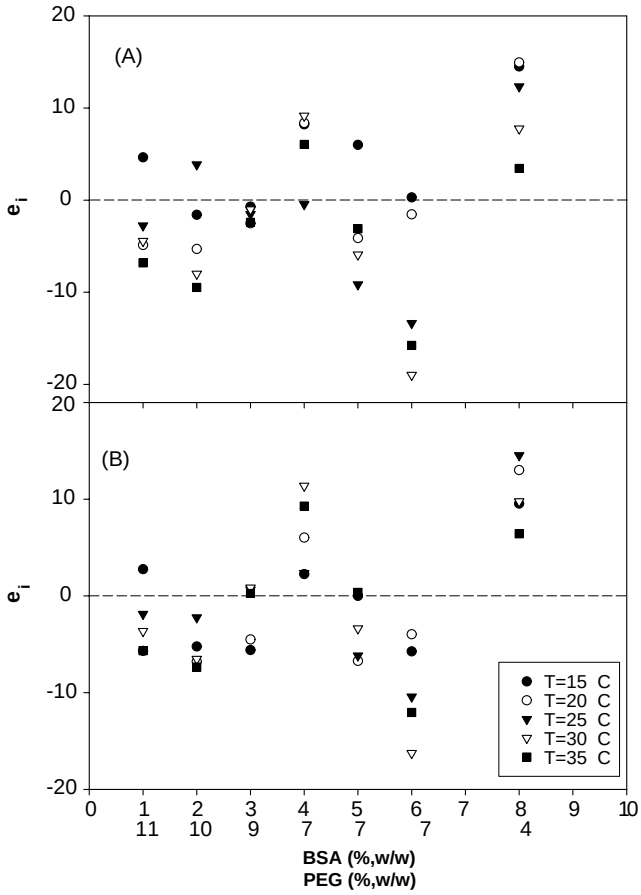

Fig. 1. Uncertainties calculated (A) with $a = 3.71$ and (B) with a values corresponding to different temperatures.

Table 3
Values of c_1c_2 in Eq. (2) for PEG–BSA–water and PEG-dextran–water solutions

PEG–BSA–water			PEG–dextran–water		
c_{PEG} (%, w/w)	c_{BSA} (%, w/w)	c_1c_2	c_{PEG} (%, w/w)	c_{dextran} (%, w/w)	c_1c_2
11.0	1.0	0.076	5.00	0.50	0.083
10.0	2.0	0.139	5.00	1.00	0.139
9.0	3.0	0.188	4.00	1.50	0.198
7.0	6.0	0.249	4.00	2.00	0.222
7.0	5.0	0.243	3.00	2.50	0.248
7.0	4.0	0.231	3.00	3.00	0.250
4.0	8.0	0.222	2.00	3.50	0.231
			2.00	4.00	0.222
			1.00	4.50	0.149
			1.00	5.00	0.138
			0.50	6.00	0.071
			0.50	7.00	0.062

where η^{expl} and η^{calc}, (mPa s), are the measured viscosities and the calculated viscosities, respectively.

Table 1 also displays the experimental and calculated viscosities of PEG 20 000-bovine serum albumin–water solutions, where it is seen that the uncertainties vary between 0.00 and 18.98 in absolute value.

The disposable parameter a has also been calculated by making use of data at each fixed temperature. We have seen that a is respectively equal to 3.97, 3.82, 3.60, 3.61 and 3.57 at temperatures 15, 20, 25, 30 and 35 °C, giving an average value almost the same as 3.71. So, we can say that there is hardly any change in parameter a with respect to the temperature. The constancy of a at all temperatures results in very small variations in uncertainties, indicating another advantage of our model for the viscosity estimation of polymer-protein solutions.

The mixture viscosities calculated using a values at different temperatures together with uncertainties which are changing in absolute value from 0.00 to 16.26 are given in Table 2. The uncertainties can also be seen in Fig. 1. As the uncertainty range is acceptable in engineering applications, our model can be used to estimate viscosity of solutions at different temperatures for large-scale systems. For instance, the model can be employed for viscosity prediction of PEG–BSA–water ternary mixture solutions with varying compositions from the data of PEG–water and BSA–water binary mixture solution viscosities at different temperatures.

Finally, we note that the values c_1c_2 for PEG–BSA–water solutions and PEG–dextran–water solutions are of the same order of magnitude (Table 3). Thinking of $c_1c_2\,a$ as a non-ideality term and comparing the a values [5], we may conclude that the deviation from ideality of PEG–BSA–water solutions is more than that of PEG–dextran–water solutions.

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