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# Relationship between preferential interaction of a protein in an aqueous mixed solvent and its solubility

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**Abstract**

The present paper is devoted to the derivation of a relation between the preferential solvation of a protein in a binary aqueous solution and its solubility. The preferential binding parameter, which is a measure of the preferential solvation (or preferential hydration) is expressed in terms of the derivative of the protein activity coefficient with respect to the water mole fraction, the partial molar volume of protein at infinite dilution and some characteristics of the protein-free mixed solvent. This expression is used as the starting point in the derivation of a relationship between the preferential binding parameter and the solubility of a protein in a binary aqueous solution.

The obtained expression is used in two different ways: (1) to produce a simple criterion for the salting-in or salting-out by various cosolvents on the protein solubility in water, (2) to derive equations which predict the solubility of a protein in a binary aqueous solution in terms of the preferential binding parameter. The solubilities of lysozyme in aqueous sodium chloride solutions (pH=4.5 and 7.0), in aqueous sodium acetate (pH=8.3) and in aqueous magnesium chloride (pH=4.1) solutions are predicted in terms of the preferential binding parameter without any adjustable parameter. The results are compared with experiment, and for aqueous sodium chloride mixtures the agreement is excellent, for aqueous sodium acetate and magnesium chloride mixtures the agreement is only satisfactory.


*Keywords:* Protein; Aqueous mixed solvent; Preferential binding parameter; Solubility

## 1. Introduction

The solvation behavior of a macromolecule such as a protein in a binary aqueous solvent is important in the understanding of such solutions [1–5]. A macromolecule can be preferentially hydrated when the concentration of water in the vicinity of the macromolecule (local concentration of water) is higher than the bulk concentration. The macromolecule can be preferentially solvated when the concentration of the cosolvent in the vicinity of the macromolecule is higher than the bulk cosolvent concentration. A measure of the solvation (or hydration) is the preferential binding parameter [2–6], which can be defined using various concentration scales (component 1 is water, component 2 is a protein and component 3 is a cosolvent):

(1) in molal concentrations

$$\Gamma_{23}^{(m)} \equiv \lim_{m_2 \to 0} \left(\partial m_3 / \partial m_2\right)_{T,P,\mu_3} \tag{1}$$

where $m_i$ is the molality of component $i$, $P$ is the pressure, $T$ the temperature (throughout this paper only isothermal–isobaric conditions are considered), and $\mu_i$ is the chemical potential of component $i$.

(2) in molar concentrations

$$\Gamma_{23}^{(c)} \equiv \lim_{c_2 \to 0} \left(\partial c_3 / \partial c_2\right)_{T,P,\mu_3} \tag{2}$$

where $c_i$ is the molar concentration of component $i$. It should be noted that $\Gamma_{23}^{(m)}$ and $\Gamma_{23}^{(c)}$ are defined at infinite dilution of the protein.

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Many characteristics of a protein in aqueous solvents are connected to its preferential solvation (or preferential hydration). The protein stability is a well-known example. Indeed, the addition of certain compounds (such as urea) can cause protein denaturation, whereas the addition of other cosolvents, such as glycerol, sucrose, etc. can stabilize at high concentrations the protein structure and preserve its enzymatic activity [4–7]. The analysis of literature data shows that as a rule $\Gamma_{23}^{(m)}>0$ for the former and $\Gamma_{23}^{(m)}<0$ for the latter compounds. Recently, the authors of the present paper showed how the excess (or deficit) number of water (or cosolvent) molecules in the vicinity of a protein molecule can be calculated in terms of $\Gamma_{23}^{(m)}$, the molar volume of the protein at infinite dilution and the properties of the protein-free mixed solvent [8]. The protein solubility in an aqueous mixed solvent is another important quantity which can be connected to the preferential solvation (or hydration) [9–13] and can help to understand the protein behavior [9–17].

The aim of the present paper is to establish a relation between: (1) the preferential solvation (or hydration) of a protein and (2) the protein solubility in an aqueous mixed solvent. The obtained relation will be used to predict the protein solubility in an aqueous solvent in terms of the preferential binding parameter.

The preferential binding parameter $\Gamma_{23}^{(m)}$ can be measured experimentally using various methods such as sedimentation [4], dialysis equilibrium [7], vapor pressure osmometry [14], etc. and has been determined for numerous systems [2–7,9–13,18–22]. It is of interest to use these experimental results for the evaluation of protein solubility.

The results obtained will be presented as follows: (1) firstly a relation between the protein solubility and the preferential binding parameter in a binary solvent will be established; (2) secondly the established relation will be used to derive criteria for the effect of cosolvents (salting-in or salting-out), (3) thirdly the experimental data for the preferential binding parameter $\Gamma_{23}^{(m)}$ will be used to predict the protein solubility and the obtained results will be compared with available experimental data.

## 2. Theoretical part

In a previous paper [8], the following expression for the preferential binding parameter $\Gamma_{23}^{(c)}$ was derived on the basis of the Kirkwood–Buff theory of ternary solutions:

$$\Gamma_{23}^{(c)}=\frac{c_1c_3\left(J_{21}V_1-J_{11}V_2^{\infty}\right)}{(c_1+c_1J_{11}+c_3)}+\frac{c_3(c_1+c_3)\left(V_1-V_2^{\infty}\right)}{(c_1+c_1J_{11}+c_3)} \tag{3}$$

where $V_i$ is the partial molar volume of component $i$, $V_2^{\infty}$ is the partial molar volume of a protein at infinite dilution in a mixed solvent,

$$J_{11}=\lim_{x_2\to 0}\left(\frac{\partial \ln\gamma_1}{\partial x_1}\right)_{x_2},$$

$$J_{21}=\lim_{x_2\to 0}\left(\frac{\partial \ln\gamma_2}{\partial x_1}\right)_{x_2},$$

$x_i$ is the mole fraction of component $i$, and $\gamma_i$ is the activity coefficient of component $i$ at a mole fraction scale.

It should be noted that the quantities $\Gamma_{23}^{(c)}$, $V_2^{\infty}$ and $J_{21}$ of Eq. (3) depend on the nature of the protein, while all the other ones are related to the properties of the protein-free mixed solvent.

Eq. (3) can be rewritten as

$$J_{21}=\left(\frac{\partial \ln\gamma_2}{\partial x_1}\right)_{x_2=0}=-\frac{c_3(c_1+c_3)V_1-\left(\Gamma_{23}^{(c)}+c_3V_2^{\infty}\right)(c_1+c_1J_{11}+c_3)}{c_1c_3V_1} \tag{4}$$

Because [2,8,23]

$$\Gamma_{23}^{(c)}=(1-c_3V_3)\Gamma_{23}^{(m)}-c_3V_2^{\infty} \tag{5}$$

and experiment provides $\Gamma_{23}^{(m)}$, Eq. (4) can be recast in the form

$$\left(\frac{\partial \ln\gamma_2}{\partial x_1}\right)_{x_2=0}=-\frac{c_3(c_1+c_3)V_1-\Gamma_{23}^{(m)}(1-c_3V_3)(c_1+c_1J_{11}+c_3)}{c_1c_3V_1} \tag{6}$$

For poorly soluble solids, such as the proteins, one can use the infinite dilution approximation and consider that the activity coefficient of the protein in a mixed solvent is equal to that at infinite dilution. Therefore, for the solubility $y_2$ of a protein (solute, component 2) in a mixed solvent 1–3, one can write the following equation [24]:

$$f_2^S/f_2^L(T,P)=y_2\gamma_2^{\infty} \tag{7}$$

where $\gamma_2^{\infty}$ is the activity coefficient of a protein in a mixed solvent at infinite dilution, $f_2^L(T,P)$ is the hypothetical fugacity of a solid as a (subcooled) liquid at a given pressure ($P$) and temperature ($T$), and $f_2^S$ is the fugacity of the pure solid component 2. If the solubility of the mixed solvent in the solid phase is negligible, then the left hand side of Eq. (7) depends only on the properties of the solute.

The combination of Eqs. (6) and (7) yields the following relation for the solubility of a protein in a mixed solvent

$$\left(\frac{\partial \ln y_2}{\partial x_1}\right)=\frac{c_3(c_1+c_3)V_1-\Gamma_{23}^{(m)}(1-c_3V_3)(c_1+c_1J_{11}+c_3)}{c_1c_3V_1} \tag{8}$$

### 2.1. Salting-in or salting-out?

Eq. (8) allows one to derive a criterium for salting-in or salting-out for small cosolvent concentrations. Starting from the Gibbs–Duhem equation for a binary mixture

$$x_1\frac{d\ln\gamma_1}{dx_1}+x_3\frac{d\ln\gamma_3}{dx_1}=0 \tag{9a}$$

one can conclude that

$$\lim_{x_3\to 0} J_{11}=0 \tag{9b}$$

Eq. (8) can be therefore written for $c_3\to 0$ in the form

$$\left(\frac{\partial \ln y_2}{\partial x_3}\right)=-\left(\frac{\partial \ln y_2}{\partial x_1}\right)=\frac{\alpha}{V_1^0}-1 \tag{10}$$

where $\alpha=\lim_{c_3\to 0}\frac{\Gamma_{23}^{(m)}}{c_3}$ and $V_1^0$ is the molar volume of pure water. Salting-in occurs when

$$\left(\frac{\partial \ln y_2}{\partial x_3}\right)>0, \qquad \text{hence when } \alpha>V_1^0 \tag{11}$$

and salting-out occurs when

$$\left(\frac{\partial \ln y_2}{\partial x_3}\right)<0, \qquad \text{hence when } \alpha<V_1^0 \tag{12}$$

It is well-known [8,19,25,26] that the preferential binding parameter $\Gamma_{23}^{(m)}$ is proportional to the concentration of the cosolvent at least at low concentrations. Consequently the salting-in or salting-out depends on the slope of the curve $\Gamma_{23}^{(m)}$ versus concentration for small $c_3$. The application of the established criteria to salting-in or salting-out in real systems is illustrated in Table 1.

The above criteria (Eqs. (11) and (12)) are valid:

(1) for $c_3\to 0$, hence when a small amount of cosolvent is added to the pure water;
(2) for ternary mixtures (water (1)–protein (2)–cosolvent (3)) (the experimental results regarding the preferential binding parameter) $\Gamma_{23}^{(m)}$ and the solubilities were obtained for mixtures which involve in addition a buffer, and the effect of the buffer is taken into account only indirectly via the preferential binding parameter $\Gamma_{23}^{(m)}$);
(3) for infinite dilution (this means that the protein solubility is supposed to be small enough to satisfy the infinite dilution approximation ($\gamma_2\cong\gamma_2^{\infty}$));
(4) for experimental preferential binding parameters ) $\Gamma_{23}^{(m)}$ and solubilities determined at low cosolvent concentrations (however, the preferential binding parameter $\Gamma_{23}^{(m)}$ and the solubilities were usually determined for molalities larger than 0.5 and those values had to be used for the cases listed in Table 1 because no other experimental data are available).

### 2.2. Simple equation for the protein solubility in a mixed solvent

The combination of Eqs. (4) and (7) leads to the following expression for the solubility of a protein in a mixed solvent

$$\left(\frac{\partial \ln y_2}{\partial x_1}\right)=\frac{c_3(c_1+c_3)V_1-\left(\Gamma_{23}^{(c)}+c_3V_2^{\infty}\right)(c_1+c_1J_{11}+c_3)}{c_1c_3V_1} \tag{13}$$

The integration of Eq. (13) yields for the solubility $y_2$ of the protein in a mixed solvent for a water mole fraction $x_1$ the expression

$$\ln\frac{y_2}{y_2^w}=\int_1^{x_1}\frac{\left(V_1-V_2^{\infty}-\Gamma_{23}^{(c)}/c_3\right)dx_1}{x_1V_1}-\int_1^{x_1}\frac{\left(\Gamma_{23}^{(c)}/c_3+V_2^{\infty}\right)J_{11}}{V_1}dx_1 \tag{14}$$

where $y_2^{w}$ is the protein solubility in cosolvent-free water plus buffer.

Eq. (14) allows one to calculate the protein solubility if the composition dependencies of $J_{11}$, $\Gamma_{23}^{(c)}$ (or $\Gamma_{23}^{(m)}$) and partial molar volumes are available.

Table 1
Application of criteria (Eqs. (11),(12)) for salting-in or salting-out to aqueous solutions of proteins

| Protein | Cosolvent[a] | Experimental data used | | Do the criteria (Eqs. (11) (12)) work? |
|---|---|---|---|---|
| | | Solubility (salting-in or salting-out, conditions, references) | Preferential binding parameter $\Gamma_{23}^{(m)}$ (conditions, references) | |
| Lysozyme | NaCl | Salting-out, $T$=0–40 °C, pH=3–10 [27–31] | pH=4.5 [32], pH=3–7 [12] | Yes |
| Lysozyme | $MgCl_2$ | Salting-out, $T$=18 °C, pH=4.5 [27] | pH=3.0, 4.5 [13] | Yes |
| Lysozyme | NaAcO | Salting-out, $T$=18 °C, pH=4.5, 8.3 [27] | pH=4.5–4.71 [32] | Yes |
| Ribonuclease Sa | Urea | Salting-in, $T$=25 °C, pH=3.5, 4.0 [16] | pH=2.0, 4.0, 5.8 [33][b] | Yes |
| Lysozyme | Glycerol | Salting-in, $T$=25 °C, pH=4.6 [34] | pH=2.0, 5.8 [35] | No |
| β-Lactoglobulin | NaCl | Salting-in, $T$=25 °C, pH=5.15–5.3 [36] | pH=1.55–10 [12] | No |

[a] The term "cosolvent" is also used here for electrolytes.
[b] The preferential binding parameters were determined for ribonuclease A in 30 vol.% glycerol solution.

Eq. (14) can be simplified if one takes into account that at least at low cosolvent concentrations $\Gamma_{23}^{(c)}$ is proportional to the concentration $c_3(\Gamma_{23}^{(c)}=\beta c_3)$ [8,19,25,26] and by assuming in addition that the partial molar volumes $V_2^{\infty}$ and $V_1$ are composition independent. With these two approximations, Eq. (14) becomes

$$\ln\frac{y_2}{y_2^{w}} = \frac{\left(V_1 - V_2^{\infty} - \beta\right)}{V_1}\ln x_1 - \frac{\left(\beta + V_2^{\infty}\right)}{V_1}\left(\ln\gamma_1\right)_{x_2=0} \tag{15a}$$

and hence

$$\ln\frac{y_2}{y_2^{w}} = -\frac{\left(V_2^{\infty} + \beta\right)\ln a_w}{V_1} + \ln x_1 \tag{15b}$$

where $a_w$ is the water activity in the protein-free mixed solvent. Taking into account Eq. (5) and the relation $\frac{\Gamma_{23}^{(m)}}{c_3} = \alpha$, Eq. (15b) can be recast as follows

$$\ln\frac{y_2}{y_2^{w}} = -\frac{\left(\alpha - V_3\Gamma_{23}^{(m)}\right)\ln a_w}{V_1} + \ln x_1 \approx -\frac{\left(\alpha - V_3\Gamma_{23}^{(m)}\right)\ln a_w}{V_1} \tag{16}$$

Because as noted a long time ago [4] $V_3\Gamma_{23}^{(m)}$ "is two order of magnitude smaller" than $\alpha$, $\alpha \gg V_3\Gamma_{23}^{(m)}$, and Eq. (16) can be further simplified to

$$\ln\frac{y_2}{y_2^{w}} = -\frac{\alpha\ln a_w}{V_1} \tag{17}$$

Eqs. (14) (15a) (15b) (16) (17) provide interrelations between the preferential binding parameter $\Gamma_{23}^{(c)}$ (or $\Gamma_{23}^{(m)}$) and the protein solubility in a mixed solvent.

## 3. Calculations

In order to illustrate the results obtained regarding the solubility, several systems, for which experimental data regarding both the preferential binding parameter and the protein solubility in a mixed solvent were available, were selected. The solubilities of proteins were calculated with Eq. (17). In order to predict the solubility of a protein as a function of composition one should have information about $y_2^{w}$, $V^1$, $\alpha$ and the composition dependence of the activity of water $a_w$ in the protein-free mixed solvent. The values of $y_2^{w}$ were taken from the original references regarding the solubilities [28,29], $V_1$ was taken equal to the molar volume of pure water at a given temperature, and $\alpha$ was calculated from the original references regarding the preferential binding parameters [12,32]. The concentration dependence of the activity of water $a_w$ in protein-free mixed solvents were calculated from the experimental data for the osmotic coefficient $\varphi$ [37–39] using the expression [24]

$$\ln a_w = -\varphi M_w m_3 v \tag{18}$$

where $M_w$ is the molar weight of water, $m_3$ is the molality of the cosolvent in the protein-free mixed solvent, and $v$ is the number of ions formed through complete dissociation of the electrolyte.

### 3.1. Water (1)–Lysozyme (2)–Sodium Chloride (3)

The lysozyme solubilities in aqueous solutions of sodium chloride are predicted for pH=4.5 and pH=6.5. In these predictions only the values of the preferential binding parameter were used and no additional (or adjustable) parameters were involved. The results are presented in Figs. 1 and 2 and the experimental preferential binding parameters used are listed in Table 2. The solubilities at pH=6.5 were predicted from the preferential binding parameter determined at pH=7.0 because the values for pH=6.5 were not available. The concentration dependence of the water activity in solutions of sodium chloride was obtained from Eq. (18) using an accurate semiempirical equation for the osmotic coefficient [37].

### 3.2. Water (1)–Lysozyme (2)–Sodium Acetate (3)

The lysozyme solubilities in aqueous solutions of sodium acetate were calculated for pH=8.3 and the results are presented in Fig. 3. The experimental preferential binding parameters are listed in Table 2 (the values for pH=4.68–4.7 were, however, used because those for pH=8.3 were not available). The concentration dependence of the water activity in solutions of sodium chloride was obtained from Eq. (18) using the Pitzer equation for the osmotic coefficient [38].

### 3.3. Water (1)–Lysozyme (2)–Magnesium Chloride (3)

The lysozyme solubilities in aqueous solutions of magnesium chloride were calculated for a pH=4.1 and the results are presented in Fig. 4. The experimental preferential binding parameter is listed in Table 2 (the value for pH=4.5

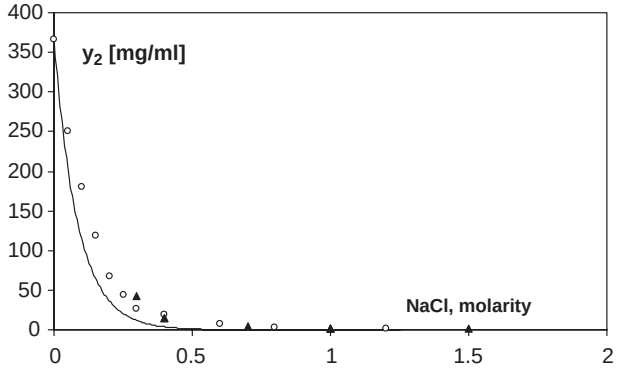


Fig. 1. Lysozyme solubility in aqueous solutions of sodium chloride at pH=4.5. The solid line represents the prediction based on Eq. (17), (o) and (▲) are the experimental data from Refs. [27,28], respectively.

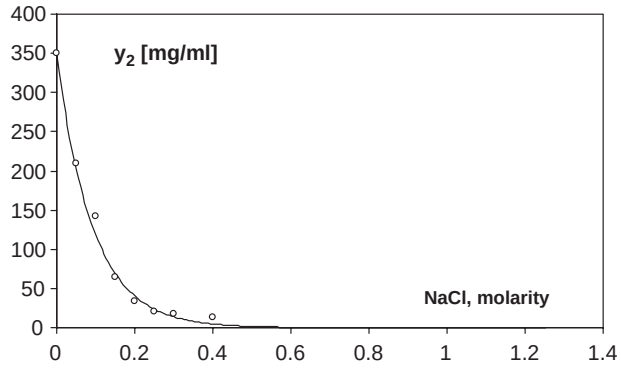


Fig. 2. Lysozyme solubility in aqueous solutions of sodium chloride at pH=6.5. The solid line represents the prediction based on Eq. (17), (o) are the experimental data from Ref. [28].

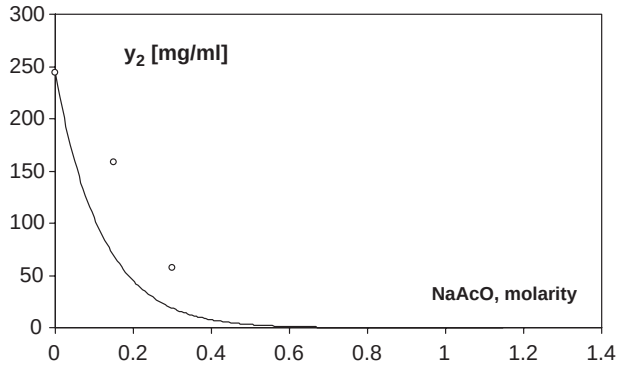


Fig. 3. Lysozyme solubility in aqueous solutions of sodium acetate at pH=8.3. The solid line represents the prediction based on Eq. (17), (o) are the experimental data from Ref. [29].

was used because that for pH=4.1 was not available). The concentration dependence of the water activity in solutions of magnesium chloride was obtained from Eq. (18) using the Pitzer equation for the osmotic coefficient [39]. For this system, the solubility data for $y_2^w$ was not available and therefore the experimental solubility [27] for $m_3=0.4$ (5.8 [mg/ml]) was employed as the lower limit of integration in Eq. (14). Using the approximations involved in the derivation of Eq. (17), one obtains

$$\ln\frac{y_2}{y_2^*} = -\frac{\alpha\ln(a_w/a_w^*)}{V_1} \tag{19}$$

where $y_2^*$ and $a_w^*$ are the molar fraction solubility and the water activity, respectively, both at $m_3=0.4$.

### 3.4. Comments regarding the solubility predictions

The scheme employed to predict the solubility of a protein in a mixed solvent involves a number of simplifications:

(1) The derived equations (Eqs. (14) (15a) (15b) (16) (17)) involve the infinite dilution approximation ($\gamma_2 \cong \gamma_2^\infty$).
2) The equations are established for a ternary mixture (water (1)–protein (2)–cosolvent (3)). However, all the experimental results regarding the preferential binding parameter $\Gamma_{23}^{(m)}$ and the solubility involve in addition to the above three components also a buffer. The effect of the buffer is taken into account only indirectly via the preferential binding parameter $\Gamma_{23}^{(m)}$.
3) The parameter $\alpha$ was determined as the slope of the composition dependence of the preferential binding parameter $\Gamma_{23}^{(m)}$, assuming that the latter is proportional to the concentration.

Table 2
Experimental preferential binding parameters used for solubility predictions

| Protein | Cosolvent | Molality | pH | Preferential binding parameter $\Gamma_{23}^{(m)}$ [mol/mol] | Reference |
|---|---|---|---|---|---|
| Lysozyme | NaCl | 1 | 4.5 | −6.2 | [12] |
| Lysozyme | NaCl | 1 | 7.0 | −5.8 | [12] |
| Lysozyme | NaAcO | 0.5 and 1 | 4.68 and 4.71 | −5.14 and −7.5 | [32] |
| Lysozyme | $MgCl_2$ | 1 | 4.5 | −1.79 | [32] |

## 4. Discussion

In the present paper, a connection between the preferential binding parameter of a protein and its solubility in an aqueous solvent was established. The preferential binding parameter is a measure of the protein / water and protein / cosolvent interaction at molecular level [6,19]. Regarding the preferential binding parameter, Timasheff subdivided the cosolvents into several groups [6]: “When a protein molecule is immersed into a solvent consisting of water and another chemical species (a cosolvent), the interactions between the protein and the

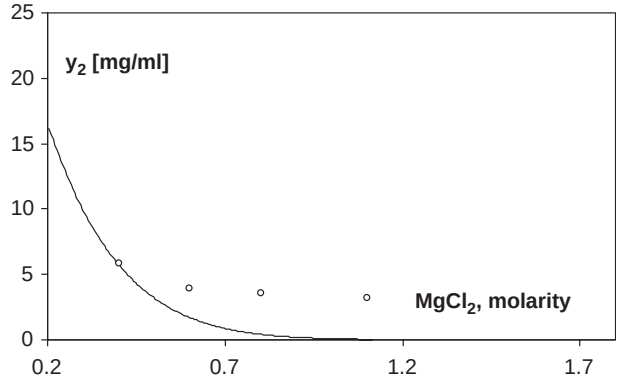


Fig. 4. Lysozyme solubility in aqueous solutions of magnesium chloride at pH=4.1. The solid line represents the prediction based on Eq. (17), (o) are the experimental data from Ref. [27].

solvent components may lead to three possible situations: (1) the cosolvent is present at the protein surface in excess over its concentration in the bulk (this is what constitutes binding); (2) the water is present in excess at the protein surface; this means that the protein has a higher affinity for water than for the cosolvent (this situation is referred to as preferential hydration, or preferential exclusion of the cosolvent); (3) the protein is indifferent to the nature of molecules (water or cosolvent) with which it comes in contact, so that no solvent concentration perturbation occurs at the protein surface".

The present analysis shows that the same classification can be made with respect to the effect of a small amount of a cosolvent on the protein solubility in an aqueous solvent (see Eqs. (11) and (12)). Namely, the cosolvents of the first Timasheff's group (e. g. urea) increase the protein solubility compared to the solubility in water when a small amount is added to water. Compounds of the second group (e. g. salts) decrease the solubility and they are well-known salting-out agents [14]. Substances of the third group (we have no example) do not essentially change the solubility compared to the solubility in pure water.

The present paper emphasizes how the preferential binding parameter is related to the solubility and how the preferential binding parameter can be used to predict the solubility. Eq. (13) (or its equivalent Eq. (8) ) provides the most general equation that connects the preferential binding parameter and the solubility. The integration of this equation leads to Eq. (14) which allows one to predict the protein solubility in a mixed solvent if the composition dependencies of $J_{11}$, $\Gamma_{23}^{(c)}$ (or $\Gamma_{23}^{(m)}$) and partial molar volumes are available. A simplified form of Eqs. (14) and (17), can predict the solubility if information about $y_2^w$, $V_1$, $\alpha$ and the composition dependence of the activity of water $a_w$ in a protein-free mixed solvent is available. Eq. (17) was used in this paper to predict the protein solubility in an aqueous mixed solvent. The results of predictions (Figs. 1–4) demonstrate that the experimental data regarding the preferential binding parameter could be successfully used to predict the solubility of proteins in aqueous mixed solvents. It should be pointed out that no additional parameters (adjustable parameters) were used. However, the present approach involves a number of approximations, among which the infinite dilution approximation deserves an additional comment, because the solubility of some proteins can be relatively large. For example, the solubility of lysozyme in aqueous solutions of sodium chloride at pH=4.5 can be as high as 365 mg/ml. However, in the mole fraction scale, this solubility is smaller than $5\ 10^{-4}$ (2000 molecules of water per molecule of lysozyme), value which seems to be sufficiently low for the system to be considered dilute. The accuracy of the predictions are highly dependent on the quality of experimental data regarding the preferential binding parameter, the solubility and the water activity in protein-free mixed solvents.

## 5. Conclusion

A relationship between the derivative of the activity coefficient of the protein with respect to the mole fraction of water at infinite dilution of protein and the preferential binding parameter was used to connect the solubility of a protein in an aqueous mixed solvent to the preferential binding parameter. This relation was used to examine the salting-in and salting-out effect of various compounds on the protein solubility in water and to predict the protein solubility.

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