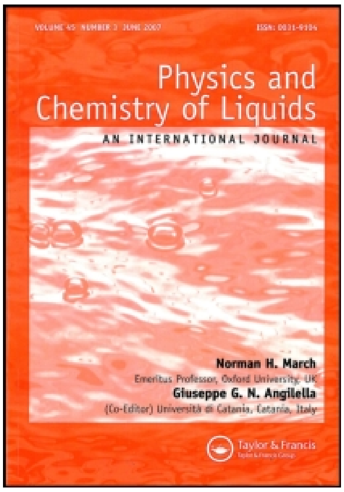

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Steric Exclusion of Salts by Polyethylene Glycol

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The solubility of salt in water decreases in the presence of a water soluble polymer, polyethylene glycol (PEG), and could be expressed by a linear relationship between the logarithm of the solubility of salt (M) and the concentration of PEG (M). The slope of the solubility lines was in agreement with the excluded volume of PEG calculated with the rod-like model of PEG. The solubility limit of each salt in the PEG-free solvent (solvent unoccupied by PEG) remained constant regardless of the concentration of PEG. Therefore, the solubility of salt in PEG solutions may be described by the excluded volume effects of PEG. This suggests that PEG sterically excludes salt, thus salt is concentrated in the accessible solvent up to the solubility limit.

KEY WORDS: Excluded volume, polyethylene glycol, solubility of salt.

1 INTRODUCTION

Polyethylene glycol (PEG) is a biocompatible water-soluble synthetic polymer used widely for fractional precipitation of proteins and other biological preparations.[1–9]

A salt solution becomes turbid when PEG is introduced into the solution in excess of a certain threshold concentration.[10–14] At higher concentrations of PEG, the solubility of salt is lower or equally for lower concentrations of salt, the theshold concentration of PEG is higher. Therefore, the solubility of salt depends on the concentration of PEG. It was the objective of this study to describe and explain this simple and obvious phenomenon.

In this article the solubility of salt was described in terms of the excluded volume effects of PEG. The excluded volume of PEG was calculated using the spherical as well as rod-like models of PEG[15–17] and the molar hydrated volume of PEG was determined by measuring non-freezable bound water of PEG using a differential scanning calorimeter. In addition, the molar surface tension increment of salt was determined to estimate the surface tension of the PEG-free solvent (solvent unoccupied by PEG) in order to explain the solubility limit of salt in the PEG-free solvent.

2 MATERIALS AND METHODS

Preparation of Stock Solutions

PEG with various molecular weights, 300, 600, 1450, 3350, and 8000 (PEG300, PEG600, PEG1450, PEG3350, and PEG8000, Sigma Chemical Co., St. Louis, MO) were used. PEG solutions were prepared by dissolving PEG in deionized water. The concentrations of the stock solutions were 50% (w/v) for PEG1450, PEG3350, and PEG8000 and 80% for PEG300 and PEG600. All PEG solutions were kept at room temperature.

Solutions of ammonium sulfate, sodium sulfate, sodium citrate, potassium citrate, and sodium tartrate (Mallinckrodt Inc., Paris, KY) were prepared in deionized water. Potassium phosphate and sodium phosphate solutions were prepared by dissolving equal moles of their monobasic and dibasic salts in deionized water.

Solubility of Salt in PEG Solutions

The solubility of salt in PEG solutions was determined by the titration.[10] Ten ml of PEG solution (50 or 80%, w/v) was placed into a flask (50 ml). A salt solution was then titrated into the flask under stirring. First a homogeneous solution yielded, but after a certain amount of salt had been added, one further drop of the salt solution caused turbidity. The composition of this mixture was noted and the concentration of salt was considered as the solubility of salt at that particular concentration of PEG. When 1 ml of deionized water was added, then the mixture resumed a clear homogeneous state. The salt solution was then added dropwise until the mixture became turbid again. The composition of this mixture was noted and more deionized water was added to get a clear solution and so forth. The titration was continued until 25 to 50 ml of the salt solution was used. All titrations were conducted at room temperature.

Molar Excluded Volume of PEG

The molar excluded volume of PEG for potassium phosphate (U_{ps}) was calculated for the spherical and rod-like models of PEG using Eqs (1) and (3). When PEG and potassium phosphate are treated as equivalent impenetrable spheres, U_{ps} can be expressed as:[9,15,17]

$$U_{ps} = \frac{1}{2.303}\,\frac{4\pi N}{3}\,(R_p + R_s)^3 \qquad (1)$$

where N is Avogadro's number. R_p and R_s are the equivalent radii of PEG and potassium phosphate (expressed in cm) and estimated from:

$$R^3 = \frac{3vM}{4\pi N} \qquad (2)$$

where v is the partial specific volume (0.84 ml/g for PEG[18] and 0.43 ml/g for potassium phosphate[19]) and M is the molecular weight. Potassium phosphate dissociates in water. A phosphate ion ($R_s =$ 0.25 nm) is larger than a potassium ion ($R_s = 0.13$ nm), this the equivalent radius of the phosphate ion was considered as that of potassium phosphate.

If PEG is considered as a rod with radius R_r and length l, while continuing to regard potassium phosphate as a sphere then[15–16]

$$U_{ps} = \frac{1}{2.303}\,N\pi(R_r + R_s)^2 l \qquad \text{when } l \gg R_r \qquad (3)$$

The length of a PEG rod was estimated using a reported value (9.3 nm per 1,000 dalton) which was calculated from CPK space filling models based on known bond angles and length.[7] The radius of the PEG rod used for calculations was 0.22 nm.[7]

Molar Hydrated Volume of PEG

The molar hydrated volume of PEG, V_h, was determined from:[20]

$$V_h = M(v_2 + \delta_1 v_1) \qquad (4)$$

where v_1 is the specific volume of water and v_2 is the specific volume of PEG (0.84 ml/g). δ_1 is bound water of PEG (g H_2O/g PEG).

Bound water of PEG was determined with a differential scanning calorimeter (DSC) by measuring non-freezable bound water.[21–22] Various concentrations of PEG solutions (20 to 80%, w/v) were weighed in aluminum pans (8 to 15 mg). The aluminum pans were sealed tightly and weighed. Sample pans were cooled to −40°C and heated to 20°C at a rate of 10°C/minute at instrument sensitivity of 20 mcal/sec with a DSC equipped with an Intercooler (Perkin-Elmer DSC-2, Perkin-Elmer, Norwalk, CT). An empty pan was used to balance the heat capacity of the sample. The calibration of the instrument was carried out using indium and deionized water as standards.

Free water was calculated by measuring the enthalpies of melting (peak areas of free water). The content of free water was plotted with the content of total water and non-freezable bound water was determined from the intercept of the plot.

Molar Surface Tension Increment of Salt

The surface tension of salt solutions was measured with a Rosano surface tensiometer (Precision Balance Federal Pacific Electric Co., Newark, NJ) at room temperature. The surface tension of the salt solutions increased linearly with increases in the molar concentration of salt (0.1 to 1.0 M). The molar surface tension increment of salt (σ) was determined from the slope.

The surface tension of the PEG-free solvent was estimated by adding the production of the molar surface tension increment of salt (σ) and the molar concentration of salt in the PEG-free solvent to the surface tension of water (72.3 dyne/cm).[23–25]

3 RESULTS AND DISCUSSION

The logarithm of the solubility of potassium phosphate in PEG solutions decreased linearly with increases in the concentration of PEG as shown in Figure 1. This relationship could be expressed by

$$\log S = \log S_f - \beta C \tag{5}$$

where S is the solubility of salt in PEG solutions, C is the concentration of PEG, and β is a constant. S_f is the solubility of salt in the PEG-free solvent. Equation (5) is identical to that shown also for the effects of PEG on the solubility of proteins and described by the excluded volume effects of PEG.[3,7,9,26]

The slope of the solubility lines, β, increased with increases in the molecular weight of PEG (Table 1). This is due to differences in the size of PEG molecules. The size of PEG molecules increases with increases in the molecular weight, thus the volume occupied by the molecules increases with increases in the molecular weight of PEG.

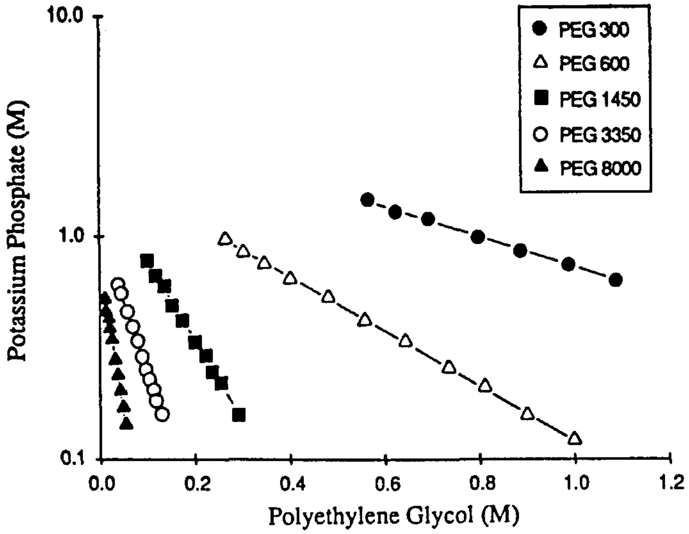

Figure 1 Logarithm of the solubility of potassium phosphate decreases linearly with increases in the concentration of PEG. The slope of the solubility lines increases with increases in the molecular weight of PEG.

The molar excluded volume of PEG for potassium phosphate (U_{ps}) was calculated for the spherical and rod-like models of PEG using Eqs (1) and (3), respectively (Table 2). The slope of the solubility line, β, was larger than the molar excluded volume of PEG calculated for the spherical model of PEG. The difference between them increased with increases in the molecular weight of PEG. This is probably due to

Table 1 Slopes and intercepts of the potassium phosphate solubility lines in PEG solutions

Molecular weight of PEG	β^a (L/mole PEG)	$S_f{}^b$ (M)
300	0.7	3.5
600	1.3	2.3
1450	3.2	1.6
3350	6.7	1.1
8000	13.8	0.9

[a] Slope may represent the molar excluded volume of PEG for potassium phosphate.

[b] Intercept may represent the solubility of potassium phosphate in the PEG-free solvent.

Table 2 Molar excluded volume of PEG for potassium phosphate

Molecular weight	U_{ps}[a] (L/mol PEG)		β[b] (L/mole PEG)
	Spherical	Rod-like	
300	0.4	0.5	0.7
600	0.6	1.0	1.3
1450	1.2	2.5	3.2
3350	2.3	5.7	6.7
8000	4.7	13.5	13.8

[a] Molar excluded volume of PEG calculated using the spherical and rod-like models of PEG with Eqs (1) and (3), respectively.

[b] Slope of the solubility lines in Figure (1).

deviation of the molecular shape of PEG from a sphere with increases in the molecular weight of PEG. Thomas and Charlesby[27] demonstrated that PEG was a rod-like molecule in water and its axial ratio increased with increases in the molecular weight of PEG within the molecular weight range 400 to 4000. Therefore, the spherical model for PEG is not applicable for the calculation of the molar excluded volume of PEG.

The slope of the solubility line, β, was in agreement with the molar excluded volume of PEG calculated for the rod-like model of PEG at high molecular weights of PEG. For PEG8000, β (13.8 L/mole PEG) was the same as the value calculated (13.5 L/mole PEG). Therefore, the slope of the solubility lines of potassium phosphate in PEG solutions, β, may represent the molar excluded volume of PEG.

However, β was larger than the value calculated for low molecular weights of PEG. The difference between them increased with decreases in the molecular weight of PEG. This is probably due to the assumption made for the calculation of the molar excluded volume of PEG for the rod-like model. The length of PEG molecules was assumed to be much larger than the radius of PEG molecules ($l \gg R_r$). If PEG chains are fully stretched, l/R_r (same as twice of the axial ratio of PEG molecules) is 21 per 1,000 dalton.[7] Then, l/R_r at the corresponding molecular weight range of PEG is 6 for PEG300, 13 for PEG600, 30 for PEG1450, 70 for PEG3350, and 170 for PEG8000. Therefore, l/R_r is not sufficiently large to apply Eq. (3) for calculations of the molar excluded volume of PEG at low molecular weights of PEG.

The molar hydrated volume of PEG, V_h, was determined from the nonfreezable bound water of PEG measured with a DSC and the

Table 3 Hydrated volumes and slopes of the solubility lines

Molecular weight of PEG	Bound water[a] (mole H_2O/mole PEG)	V_h[b] (L/mole PEG)	β (L/mole PEG)	V_h/β
300	20	0.6	0.7	0.86
600	40	1.2	1.3	0.92
1450	95	2.9	3.2	0.93
3350	200	6.4	6.7	0.96
8000	400	13.8	13.8	1.00

[a] Non-freezable bound water determined with a DSC.
[b] Molar hydrated volume of PEG.

partial specific volume of PEG reported (0.84 ml/g)[16] (Table 3). The slope of the solubility lines, β, was close to V_h. However, the ratio of V_h to β increased with increases in the molecular weight of PEG. This is probably due to the steric exclusion of potassium phosphate by PEG. The excluded volume of PEG for a solute depends on their relative sizes.[9,17] When the size of PEG is much larger than that of a solute ($R_p \gg R_s$), the excluded volume of PEG is independent of the size of the solute. Therefore, the hydrated volume may be a part of the excluded volume.

Equation (5) can be rearranged as:

$$S_f = S\,10^{\beta c} \tag{5'}$$

where $10^{\beta c}$ is the volume fraction of the solvent available for dissolution of salt (PEG-free solvent).[9,28] If the solubility of salt is proportional to the solvent volume available, S_f represents the solubility of salt in the PEG-free solvent. According to Eq. (5), the solubility of potassium phosphate in the PEG-free solvent can be determined from the intercept of the solubility lines and should remain constant regardless of the concentration of PEG. The solubility of potassium phosphate in the PEG-free solvent determined from the intercept decreased with increases in the molecular weight of PEG (Table 1). This agrees with the phenomenon in phase separation of PEG/potassium phosphate aqueous two-phase systems.[10] The PEG/potassium phosphate aqueous two-phase systems phase-separate at the higher concentrations of potassium phosphate with lower molecular weights of PEG.

The logarithms of the solubilities of all other salts (ammonium sulfate, sodium phosphate, sodium tartrate, and potassium citrate) also decreased linearly with increases in the concentration of PEG (Figure 2). The slopes of the solubility lines were within the range of 10.0 to 13.8

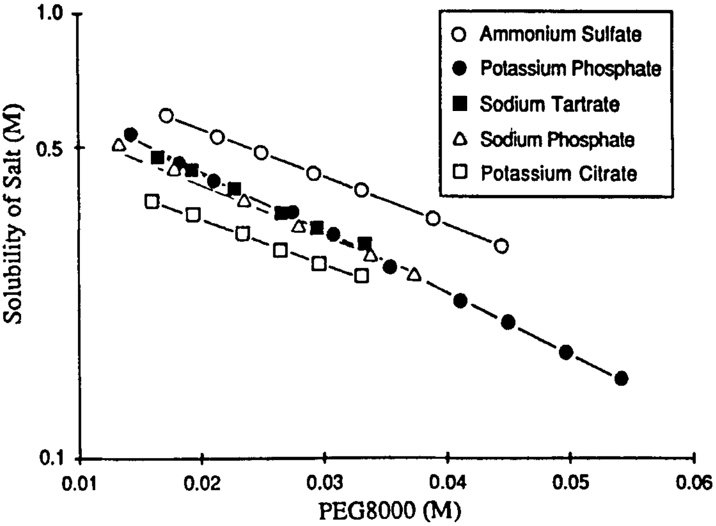

Figure 2 Logarithm of the solubility of salt decreases linearly with increases in the concentration of PEG8000. The intercept (solubility of salt in the PEG-free solvent) varies with the type of salt.

L/mole PEG8000. The excluded volume of PEG for salt, the slope of the solubility lines, was different with different types of salt. This is due to differences in the size of ionized groups for different type of salt and also probably due to changes in the effective size of PEG in different salt solutions.

The solubilities of salts in the PEG-free solvent (S_f) determined from the intercept of the solubility lines decreased with increases in the molar

Table 4 Solubility of salt in the PEG-free solvent of PEG8000 solutions

Salt	S_f[a] (M)	σ[b] (dyne/cm M)	γ[c] (dyne/cm)
Ammonium sulfate	0.91	2.4	74.5
Potassium phosphate	0.85	2.7	74.6
Sodium phosphate	0.77	2.8	74.5
Sodium tartrate	0.75	3.1	74.6
Potassium citrate	0.53	3.5	74.2

[a] Solubility of salt in the PEG-free solvent.
[b] Molar surface tension increment of salt.
[c] Surface tension of the PEG-free solvent at the solubility limit.

surface tension increment of salt (σ) (Table 4). Therefore, the solubility of salt in the PEG-free solvent is determined by the type of salt. On the other hand, the surface tension of the PEG-free solvent at the solubility limit is about the same regardless of the type of salt. This indicates that the solubility of salt in the PEG-free solvent is inter-dependent with the surface tension of the PEG-free solvent.

The solubility of potassium phosphate in the PEG-free solvent decreased with increases in the molecular weight of PEG (Table 1), thus the surface tension of the PEG-free solvent at the solubility limit is lower with higher molecular weights of PEG (Table 5). Therefore, the higher solubilities of salt in the PEG-free solvent with lower molecular weights of PEG is due to the higher tolerance of the lower molecular weights of PEG for the surface tension of the PEG-free solvent.

4 CONCLUSIONS

The solubility of salt in water decreases in the presence of PEG and can be expressed with a linear relationship between the logarithm of the solubility of salt (M) and the concentration of PEG (M). The slope of the solubility lines can be described by the molar excluded volume of PEG calculated with the rod-like model of PEG. Salt is excluded by PEG in the solution thus concentrated in the PEG-free solvent up to the solubility limit.

The solubility of salt in the PEG-free solvent remains constant regardless of the concentration of PEG in PEG solutions but is inter-dependent with the surface tension of the PEG-free solvent. The surface

Table 5 Solubility of potassium phosphate and the surface tension of the PEG-free solvent

Molecular weight of PEG	S_f[a] (M)	γ[b] (dyne/cm)
300	3.55	81.9
600	2.29	78.5
1450	1.58	76.6
3350	1.15	75.4
8000	0.85	74.6

[a] Solubility of potassium phosphate in the PEG-free solvent.

[b] Surface tension of the PEG-free solvent at the solubility limit.

tension of the PEG-free solvent at the solubility limit of salt is independent of the type of salt and higher with lower molecular weights of PEG. However, the concentration of salt providing a given surface tension varies with the type of salt representing the molar surface tension increment of salt. Therefore, the solubility of salt in the PEG-free solvent is determined by both the molecular weight of PEG and the type of salt.

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