HEAT CAPACITY OF POLY (ETHYLENE GLYCOL)–WATER MIXTURES: POLY (ETHYLENE GLYCOL)–WATER INTERACTIONS *

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ABSTRACT

The specific heat capacity of the poly(ethylene glycol)–water system has been determined using a drop calorimeter over the full range of composition at 25°C. The system heat capacity responds linearly to change in composition in the dilute solution regime up to 30% (w/w) and is an irregular function at lower water content. The breakpoint in solubility corresponds to a minimal content of 2.5 water molecules per ethylene oxide to complete hydration. We have difficulty in explaining the large excess heat capacity by the presence of a stoichiometrically defined hydrogen-bonded complex between each ethylene oxide group and water molecules. It seems possible that the water of hydration also interacts with bulk water and/or the rest of the polymer chain.

INTRODUCTION

Protein behavior, such as enzyme activity, is a function of the thermodynamic activity and state of hydration of the macromolecule. Most studies of the kinetics and equilibria of biochemical reactions, however, have been done in dilute solution conditions. Many physiological media, in which proteins exist, normally have a very high macromolecular content and the solution state is markedly non-ideal. The descriptions we now have for macromolecular reactions in dilute solutions consequently do not allow us to model the same reactions occurring in the cellular environment.

Theoretical attempts have been made to describe the effects of crowded media on macromolecular structure and reactivity. Deviations from ideality encountered in such media can be expressed in thermodynamic terms as activity coefficients. According to a calculation by Minton [1], such chemical activity or Gibbs energy change can be caused merely by the high volume fraction of the solution occupied by macromolecules even in the absence of any specific interaction.

Hard core macromolecules, as often adopted in calculations, provide only excluded volume effect. In reality, specific interactions such as electrostatic interactions are not unusual. Accumulated evidence now suggests that the polymer poly(ethylene glycol) (PEG) may be useful for experimental studies of crowded macromolecular media. It is a non-ionic polymer which is water soluble over a wide range of chain lengths. Its ability to precipitate globular proteins has been adequately described theoretically [2] and experimentally [3] using the concept of excluded volume. Modelling of the cytosol using PEG has, however, remained limited mainly because of the unclear role of water structures in aqueous PEG solutions.

A considerable amount of work has been done in order to understand the solution characteristics of the water–PEG system, in particular the solubility gap in the phase diagram. Hydrogen bonds between the ether oxygens of PEG and water have been suggested to account for the unusual solubility of the polymer. The questions lie in the number of water molecules per ether bond required for hydration and whether there is more excess structures appearing in the rest of water. Two models have been proposed. The one by Kjellander and Florin [4] emphasized the role of water structuring. These authors suggested that PEG molecules can fit into the water lattice, resulting in an extended water network structure. Any temperature dependence in heat capacity then reflects the melting of the clathrate-like structures in the water network. An alternative model has recently been proposed by Karlström [5] with emphasis on the PEG chain structure. He suggested that the temperature dependence of solubility can be explained by a shift in the polymer chain conformational state distribution.

Our intention in this work is to elucidate further the structural question in the water–PEG system. Heat capacity data as a function of concentration are particularly suited for the present purpose as they tend to reflect structural rearrangements showing large enthalpy–entropy compensation that are not revealed by studies of equilibria.

MATERIALS AND METHODS

Poly(ethylene glycol) 8000 (PEG) from Aldrich Chemicals was used as received and kept in a desiccator over micro sieves. All samples were freshly prepared prior to use by weighing to give the desired composition. Samples

purified according to the procedure described by Ray and Puvathingal [6] showed the same results when compared with untreated commerical samples.

The specific heat capacity of PEG–water mixtures was determined at 25°C using a drop C_p calorimeter designed by Suurkuusk and Wadsö [7]. The drop calorimeter consists of two main parts, a furnace for temperature equilibration of the sample and a receiving calorimeter of the heat conduction type positioned in a water thermostat. The sample, enclosed in a steel ampoule, is thermostatted in the furnace at a well-defined temperature, T_i, being kept constant to better than $\pm 2 \times 10^{-4}$ K. In an experiment, the ampoule is dropped into the receiving calorimeter which is kept constant at a lower temperature, T_f, to about $\pm 5 \times 10^{-4}$ K. The heat transferred, ΔQ, is measured and the heat capacity can then be calculated as $C \Delta Q / (T_i - T_f)$. The calibration constant C accounts for the heat transferred by the ampoule within the same temperature interval. It was determined using water and sapphire, which have well-established heat capacity values. A temperature interval $(T_i - T_f)$ of 4 or 10°C was used.

RESULTS

Figure 1 shows the specific heat capacity of the PEG–water system as determined for PEG contents from 0 to 100% (w/w) at 25.0°C. The value at each PEG content corresponds to an average of 7 or 8 measurements, the typical standard deviation being 0.001 J g^{-1} K^{-1}. Thus, the difference between the linear response from 0 to 30% (w/w) PEG content and the
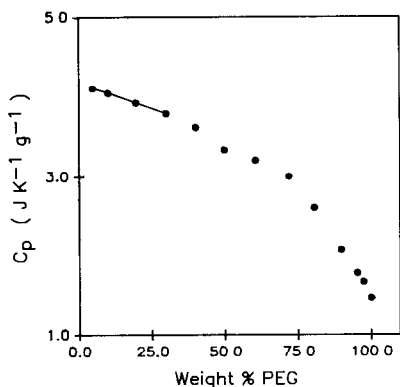

Fig. 1. Plot of specific heat capacity of PEG–water mixtures at 25.0°C as a function of PEG weight content. The measurements were very precise and the typical standard deviation for each point shown is smaller than the size of the symbols used. A linear variation is observed from 0 to 30% (w/w) PEG. The mixture is, however, a homogenous solution up to 50% (w/w).
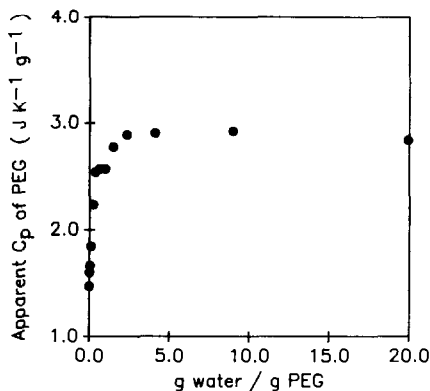
Fig. 2. Plot of the apparent specific heat capacity of PEG as a function of the weight of water bound per gram PEG.

irregular reponse beyond 30% is significant. The linear variation in the C_p values up to 30% (w/w) PEG content as shown in Fig. 1 simply reflects the addition of bulk water to a fully hydrated PEG molecule. The polymer, however, remains soluble up to 50% (w/w). The minimal water content needed to satisfy the basic hydration of the polymer PEG seems to be about 50% (w/w) or 1 g water per g PEG [8–10]. Such water content refers to about 444 water molecules, or about 2.5 for each ethylene oxide group in the present case. This is obviously not enough for complete hydration shells around the polymer chains unless each water molecule participates in the shells of some neighboring chains. This requires a substantial degree of hydrogen bonding to the polymer chain and/or other water molecules and thus introduces large steric constraints on the structure.

It is convenient to express the data at low hydration levels in terms of an apparent specific heat capacity of the polymer component defined for the water–PEG system as

$$\phi C_{p2} = \frac{C_p - w_1 C_{p1}}{w_2} \qquad (1)$$

where w is the weight fraction and the indexes 1 and 2 refer to the water and PEG, respectively. Figure 2 shows the apparent specific heat capacity of PEG, ϕC_{p2}, versus the number of grams of water bound per gram of PEG, defined as $h = w_1/w_2$. The apparent specific heat rises sharply but smoothly and then becomes flat at high h values. As water is added to the dry polymer, the thermal properties change until the polymer–water interaction, as reflected in the apparent specific heat capacity, reaches the dilute solution value after which it remains constant.

For an ideal binary system the specific heat capacity is

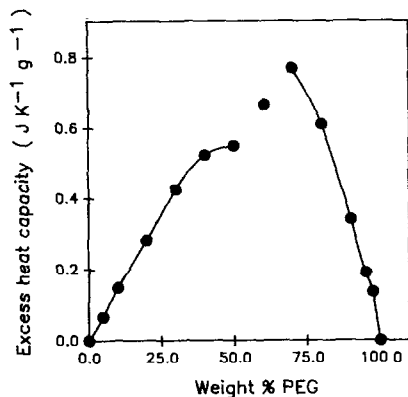$$C_p = w_1 C_{p1} + w_2 C_{p2} \qquad (2)$$

Fig. 3. Plot of the excess specific heat capacity per gram of PEG–water mixture [calculated using eqn. (4)] as a function of the PEG weight content.

where C_{p_i} and w_i correspond to the specific heat capacity and weight fraction of the respective component. For a non-ideal system an excess specific heat capacity can be defined as

$$C_{P_{excess}} = C_p - \left(w_1 C_{p1} + w_2 C_{p2} \right) * \tag{3}$$

where C_p corresponds to the observed specific heat capacity. Combining eqns. (1) and (3),

$$C_{P_{excess}} = w_2 \left(\phi C_{p2} - C_{p2} \right) \tag{4}$$

The quantity on the left-hand side of eqn. (4) thus represents the deviation of the system from ideality. It can also be normalized to unit water or PEG concentration, which then indicates the deviation from ideality for the respective component if only one component is assumed to contribute to the non-ideality.

Figure 3 shows the excess specific heat capacity per gram of water–PEG mixture as a function of the PEG content. The graph can be divided into PEG-rich and water-rich sections. As water is added to dry PEG, the excess C_p, which reflects the thermal properties of the PEG–water interaction, increases. On the other hand, the change in excess C_p per gram water added decreases. One can also start from the water-rich side and obtain the same dependence. The singular point at 60% (w/w) PEG corresponds to the water saturation point. We shall focus in the following discussions only on the dilute solution regime, i.e. the water-rich side, where the hydration process is supposed to be complete. It is also convenient to normalize the excess specific heat to unit PEG or water content as shown in Fig. 4. It is clear

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* C_{p2} corresponds to the specific heat capacity of solid PEG. See the Discussion section for the difference in the heat capacities of solid and liquid PEG.
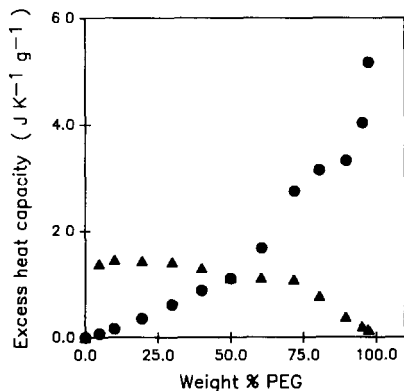
Fig. 4. Plot of the excess specific heat capacity using eqn. (4) but normalized to gram of PEG (▲) or gram of water (●) as a function of the PEG weight content.

from eqn. (4) that the excess C_p would be a linear function of the PEG content (w_2) in the regime where the observed C_p is a linear function of w_2. There is very little variation in excess C_p for PEG up to 40% (w/w) PEG when normalized per gram PEG, which corresponds to the dilute solution limiting case. In assessing the physical significance of such invariance, one should keep in mind that the dilute solution regime refers to completion of the hydration process. This suggests that the non-ideality of the water–PEG system in the dilute solution regime is mostly due to the water and the increase in the excess C_p is probably due to structuring of water molecules. A decrease in conformational freedom of the polymer chain, which is linearly related to the decrease in PEG content, however, cannot be totally excluded.

DISCUSSION

The major aim of the present work is to elucidate the thermodynamic properties of PEG–water interactions in aqueous solution. The heat capacity results presented here, like all the other thermodynamic measurements, do not give direct structural information. On the other hand, any proposed physical or structural model has to be compatible with the measured thermodynamic properties. In aqueous solution of substances such as PEG, which have not only non-polar residues but also polar groups capable of hydrogen bonding with water, the situation is complex. The main question is in what ways are the bulk water structures influenced by the hydration water and polymer. We will attempt in the following discussion to address this question based upon our heat capacity results, at least in a semi-quantitative way.

Two principal observations can be made from the present heat capacity data. First, we have a large variation in excess specific heat capacity over the whole polymer composition range. This is in contrast to a rather small, positive and fairly constant heat of solution of about 6 calories per gram of polymer within the range of 0–40% (w/w) PEG [11]. The other interesting observation is the irregular response of the excess specific heat capacity with respect to the PEG content (see Fig. 3). No such behavior is observed for water activity as a function of PEG contents reported by Herskowitz and Gottlieb [12]. Both of our findings point to the existence of extensive equilibrium structures in an aqueous PEG solution. That these structures are not revealed by Gibbs energy change can readily be reconciled in terms of an enthalpy–entropy compensation phenomenon [13]. The Gibbs energy of transfer of water from bulk water to PEG hydrate can be estimated using the water activity data of Herskowitz and Gottlieb [12]. The PEG hydrate state can be represented by a 52% (w/w) PEG solution, which corresponds to the minimal water content necessary for the hydration of PEG 8000. Although no actual measurements were reported for PEG 8000 at $25\,^\circ$C by Herskowitz and Gottlieb [12], the transfer Gibbs energy per mole of water can be approximated as -180 J mol^{-1} using the 7% decrease in water activity of a 53% (w/w) PEG 6000 solution at $40\,^\circ$C. The enthalpy change which corresponds to transfer of PEG from liquid PEG to PEG–water mixture is the difference between the heat of solution and the heat of fusion. The heat of solution and heat of fusion per mole of ethylene oxide at infinite dilution are 0.29 kJ [14] and 8.65 kJ [15], respectively. The transfer enthalpy per mole of ethylene oxide is thus -8.36 kJ. Using 2.5 mole of water for the hydration of 1 mole of ethylene oxide, the transfer enthalpy per mole of water is -3.34 kJ. As a result, the compensating entropy of water is -10.6 J K^{-1} mol^{-1}.

We see that the hydration equilibrium of the ethylene oxide residue reflects the presence of the ubiquitous compensation phenomenon. Macromolecules dissolved in water are notorious for providing linear Gibbs energy relationships in their interactions with small molecules [16] but the molecular models corresponding to these relationships are many. In the present case, the excess heat capacity can be due to interstate contributions, a shift in the equilibrium between bulk water and stoichiometrically defined hydrates. This, however, cannot explain the deviations of the heat capacity curve from linearity already at 30% (w/w) PEG. The activity of bulk water changes very little if at all in this concentration region and there is no shortage of water for hydration. An alternate source for interstate heat capacity would be the presence of multiple PEG chain conformational substates. The deviations from linearity could be explained in this case by restrictions for conformers due to excluded volume effects. However, in a homopolymer such as PEG, the presence of equilibria between substates instead of statistical random coil has to be due to differential interactions,

stabilization of some substates by water. Thus, the model most consistent with our data and the observed compensation is one with heat capacity contributions due to highly coupled PEG–water states, which is basically the model proposed by Kjellander and Florin [4].

We are now elaborating the present work in two different ways in order to elucidate further the PEG–water network coupling properties. We will determine the excess heat capacity function in solvents that cannot form a hydrogen bond complex with the polymer chain, for example, toluene or chloroform. This will give us some idea of the change in polymer chain mobility once it is incorporated into the water network structure. We will also determine the temperature dependence of the excess heat capacity function at different PEG contents in the aqueous solution regime. If the network or the coupling becomes extended as more PEG is added, such phenomena should give a lower temperature dependence at higher PEG contents.

ACKNOWLEDGEMENTS

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