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The effect of pH and concentration upon aggregation transitions in aqueous solutions of poloxamine T701

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Abstract

Thermally induced aggregation transitions have been investigated for aqueous solutions of the poloxamine block copolymer T701—$(OE_4OP_{13})_2NCH_2CH_2N(OP_{13}OE_4)_2$—using differential scanning calorimetry. The calorimetric signals obtained were fitted to a mass action model description of aggregation using a previously reported analytical procedure (Patterson et al., Langmuir 13 (1997) 2219). The presence of a central ethylene diamine moiety in the molecular structure renders the T701 molecule basic; this was confirmed and measured by acid/base titration. Basicity is shown to have an important impact upon aggregation. At low pH (2.5), the poloxamine exists in its protonated form and the bulk solution proton concentration is sufficient to suppress de-protonation, aggregation—as a consequence—is shifted to a higher temperature range. Any increase in pH reduces the temperature range over which aggregation occurs. The derived experimental calorimetric parameters, obtained from model fitting procedures, can be used to compute the fraction of poloxamine existing in an aggregated form, at any particular temperature. The data sets obtained were interpolated to show that at human body temperature (310.6 K) the fraction of poloxamine found in its aggregated form is zero at a pH of 2.5. However at a pH of 6.8, the percentage aggregation increases to about 85%. These aggregation characteristics of T701 have important implications for the design of drug delivery systems, which incorporate poloxamines.

Keywords: Poloxamines; Micellization; Differential scanning calorimetry; Drug delivery

1. Introduction

Poloxamines are tetrafunctional AB block copolymers of polyoxyethylene (POE, the A block)

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and polyoxypropylene (POP, the B block) bonded to an ethylene diamine central group via the POP moiety resulting in four AB blocks per ethylene diamine molecule (Fig. 1) (Schmolka, 1977; ICI, 1991). Poloxamines are also referred too as ethylene diamine alkoxylates, *N,N′,N″,N‴-tetra*[(oxyethylene)-(oxypropylene)] diaminoethylenes, Tetronics™ or Synperonic™ T non-ionic surfactants. The molecular masses of the poloxamines range from 1650 to 30000. Low molecular mass poloxamines are viscous oils or pastes but their higher molecular mass counterparts are amorphous solids. Poloxamines have numerous uses in industry, because of the ease by which the sizes of both the POE and POP blocks can be independently altered giving rise to variations in hydrophilic/hydrophobic balance as well as total molecular mass of the copolymers. Indeed poloxamines can be used as demulsifiers or emulsifiers, anti-foaming agents, and corrosion inhibitors (ICI, 1991). Industrially, poloxamines are generally used at high polymer concentrations and, as a result, most research regarding these polymers has been performed at concentrations of $\gg 10\%$ w/v. Investigations of the poloxamines at low concentrations have generally focussed on their uses in drug delivery systems (Moghimi and Hunter, 2000). There is, however, a paucity of physico-chemical data for the poloxamines. There is some information regarding micellization in aqueous solutions of Tetronic 908 (e.g. Attwood et al., 1990). In particular Attwood et al. (1990) report that micellization is induced by raising the temperature of the aqueous system over a temperature interval of 30–50 °C. Aqueous solutions of POE are probably the most widely investigated of these polyether systems. It has been noted, by a number of researchers, that POE solubility decreases with increasing temperature (Kjellander and Florin, 1981; Karlstrom et al., 1990) and POP has been shown to become insoluble in water above a molecular weight of 750 (Schmolka, 1977). The solubility of POE in water, it has been suggested, is due to hydrogen bond formation between the ether oxygens of POE and water; at elevated temperatures the POE-water system phase separates (Kjellander and Florin, 1981). Similarly the effect of decreased polymer solubility in water, upon heating, is observed for several other non-ionic polymers (Karlstrom et al., 1990). The aforementioned decrease in solubility may be explained by changes in solute–solute, solute–solvent as well as solvent–solvent interactions. In fact phase separation of POE with increasing temperature, from an aqueous system, is also hypothesized to result from a change in conformational equilibria of the polymer chains between polar and less polar conformations (Karlstrom et al., 1990). If we consider the former explanation (hydrogen bonds) it has been proposed Kjellander and Florin, 1981) that POE chains—in water—may be accommodated within an ice like structure. The formation of such a structure produces a favourable (exothermic) enthalpy change but also results in an entropy penalty associated with the enhanced structuring of water. At low temperatures this enthalpy contribution to the free energy of mixing, together with the combinatorial entropy contribution of the chains, outweighs the entropy penalty. However an increase in temperature reverses this balance, giving rise to phase separation.

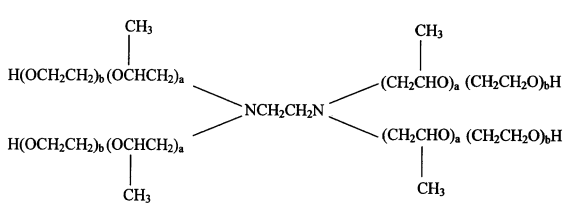

Fig. 1. General molecular formula of a poloxamine.

The same theory may also be used to account for the solubility of POP in water. In this case, however, the pendant methyl group produces a strain in the ice-like structure of water in the hydration sphere, which results in phase separation at lower temperatures Kjellander and Florin, 1981). Such an explanation is not without controversy. Finney and Soper (1994), using neutron scattering, have not found any evidence for water structuring around non-polar methyl groups. Other workers have proposed that the origin of the increasing hydrophobicity of oxyethylene (OE) is the result of changing conformations of OE segments (Karlström, 1985). For the back-

bone segments –O–C–C–O– the preferred orientation about the bonds is trans–gauche–trans (Karlström, 1985; Hergeth et al., 1991). In such polar conformational states, interaction with water is favoured; there being, on average, some two water molecules per OE unit (Hergeth et al., 1991). This state is not only of low energy but also of low statistical weight, there being only two of these conformations (Karlström, 1985). At higher temperatures less polar orientations are favoured. These molecular orientations are not only of higher energy but also of higher statistical weight—there being some 23 non-polar conformations. Self-evidently, the less polar conformations interact less favourably with water. The resulting loss of water at higher temperatures permits POE chains to come together. This model has been used with some success to explain phase separation of POE in aqueous solutions (Karlström, 1985) and non-aqueous solvents (Björling et al., 1991). ^{13}C-NMR has been used to confirm the changes in the conformation of the C–C bonds from gauche to trans, thereby altering the polarity of the POE blocks solvents (Björling et al., 1991; Rassing et al., 1984).

Micellar aggregation in the pluronic or poloxamer family of POE–POP–POE block copolymers is understood to arise for similar reasons (Alexandridis and Hatton, 1995; Alexandridis et al., 1994). As the temperature of the block copolymer solution is raised the POP block progressively loses its hydration sphere resulting in a greater interaction between POP blocks on different chains. The POE blocks on the other hand retain their strong interaction with water. This phenomenon is common to many amphiphilic molecules. As a result the differing phase preferences of the molecular blocks drive the copolymers to form micelles.

Poloxamines are especially interesting because of the presence of the basic ethylene diamine moiety. The ability of the diamine functionality to accept protons suggests that under appropriate pH conditions the presence of a cationic group located in the midst of the POP blocks should prevent aggregation even if the temperature and polymer concentration conditions are such that aggregation is allowed. This aggregation switch, triggered only by pH changes, has to be taken into account when designing drug delivery systems. Proudfoot (1988) has outlined a number of ways in which the bioavailability of drugs may be altered by surfactants. In particular surfactant unimers may alter the membrane permeability in such a way as to increase drug penetration and absorption across the gastrointestinal tract. Whilst drug incorporation in micellar aggregates can impede absorption. Switching the fraction of surfactant present in unimeric or micellar form can therefore have potentially important consequences for drug absorption.

Herein we report a differential scanning calorimetry (DSC) study of the thermally induced aggregation of poloxamine T701 as a function of pH and concentration and, in particular, demonstrate that aggregation behaviour is dependent upon pH conditions.

2. Experimental section

2.1. Chemicals

Poloxamine T701 (molecular formula $(OE_4OP_{13})_2NCH_2CH_2N(OP_{13}OE_4)_2$, molecular mass = 3700 g mol^{-1}) was a gift from ICI Chemicals Ltd., (Middlesbrough, Cleveland, UK) donated under the trade name of Synperonic T™ non-ionic surfactants. As this copolymer is a commercial product, it was used as received, without any further purification. The molecular mass distribution was determined by gel permeation chromatography (GPC) using mixed PL gel columns, a mobile phase of tetrahydrofuran (with antioxidant) at ambient temperature and a flow rate of 1 ml min^{-1} with a refractive index detector. GPC analysis gave a single elution peak. For calorimetric measurements, double distilled water was used for all solutions and samples were prepared by dissolving 50 mg of copolymer in 10 ml of cold deaerated solvent (297 K), about 1 h prior to loading into the DSC cell. The following buffer systems were used for the pH experiments. NaOH and glycine (pH 11.08); Glycine/HCl (pH 2.52); phosphate (pH 6.75); succinic acid/NaOH (pH 4.31); citric/Na_2HPO_4 (pH 4.98); citric/phosphate

(pH 5.50); and phosphate (pH 8.09). Ionic strength was not controlled in these systems. The solvent was first deaerated by purging with dry nitrogen, as purging the surfactant solution resulted in foaming.

2.2. Differential scanning calorimetry

Calorimetric measurements were carried out using a Microcal MC-2 instrument (Microcal Inc., Amherst, MA) and the DA-2 dedicated software package for data acquisition. The reference cell was filled with double distilled water or relevant buffer and all experiments were performed under a pressure of 1 atm nitrogen to prevent bubble formation in the cells and solvent loss by evaporation. Samples were equilibrated in the DSC cells for a minimum of 60 min prior to each run, and scans performed at a scan rate of 60 K h^{-1}. For downscan experiments, samples were scanned at a rate of 30 K h^{-1}, and were equilibrated for 90 min at a temperature ≈ 20 K above the T_m of the observed transition prior to the downscan. The effects of scan rate were studied for poloxamine T701 at a concentration of 5.0 mg ml^{-1} using scan rates of 10, 30 and 60 K h^{-1}. The effects of pH were examined, over a pH range of 2.5–11.1, using dilute buffer systems (0.03 M) and a scan rate of 60 K h^{-1} at a T701 concentration of 5.0 mg ml^{-1}. The effect of copolymer concentration on the observed phase transition behaviour of poloxamine T701 was examined over the range 1.0–20.0 mg ml^{-1} in double distilled water and DSC scans performed at a scan rate of 60 K h^{-1}.

3. Results and discussion

3.1. Titrimetric analysis

Conductimetric and pH curves for the titration of 25 ml of 9.75×10^{-3} M T701 + 25 ml of 1.05×10^{-2} M HCl titrated against 8.43×10^{-3} M NaOH are shown in Fig. 2, measurements were recorded at 0.5 ml addition increments and 0.1 ml increments within 2 ml of each equivalence point. The first equivalence point corresponds to neutralisation of the excess acid added initially and

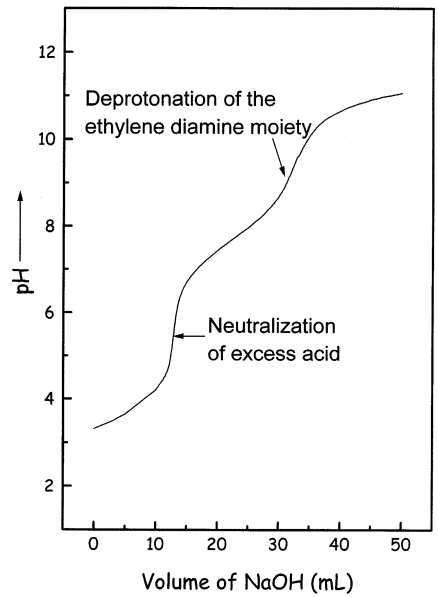

Fig. 2. pH titration plot for 25 ml of 9.75×10^{-3} M T701 and 25 ml of 1.05×10^{-2} M HCl titrated against 8.43×10^{-3} M NaOH.

the second equivalence point corresponds to deprotonation of the ethylene diamine moiety of the poloxamine. It is calculated, by titrimetric analysis, that ≈ 1 proton per poloxamine molecule is removed at the second equivalence point. The pK_a of T701 was determined to be 9.4, which is comparable to pK_a values observed for simple secondary and tertiary amines (Stark and Wallace, 1975). The deprotonation effect observed for the aggregation of T701 in dilute aqueous solution (5.0 mg ml^{-1}) at various pH values by DSC is confirmed by the titrimetric analysis. Below pH 7.4, T701 exists as a protonated ionic species and above pH 11.4 the poloxamine becomes fully deprotonated.

3.2. Differential scanning calorimetry data

Differential scanning calorimetric scans for T701, as a function of concentration and pH are shown in Figs. 3 and 4, respectively. The general shape of the curves – an asymmetric shape characterised by a sharp leading edge followed by a gradually declining tail—is indicative of an association transition (Sturtevant, 1987; Armstrong et

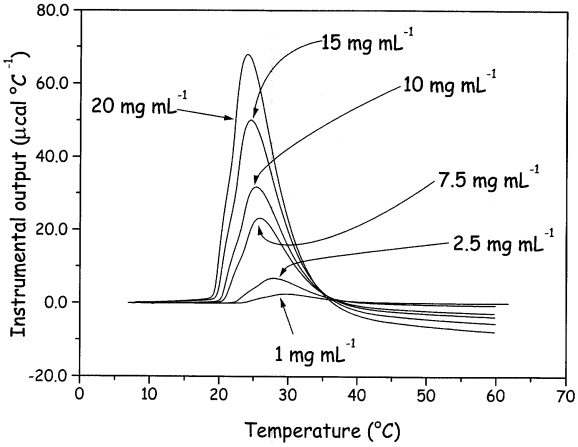

Fig. 3. The impact of copolymer concentration on the thermally induced aggregation transitions of T701 (scan rate: 60 K h^{-1}).

al., 1994; Patterson et al., 1997). It is evident—from Fig. 3—that the transition temperature range also shifts to lower temperatures as the aqueous copolymer concentration is increased. This arises because the process under consideration is an aggregation process, which is readily described by the mass action expression in Eq. (1):

$$K = \frac{[x_n]}{[x]^n}, \quad (1)$$

where K is the equilibrium constant characterising the aggregation process; $[x]$ represents the solution phase concentration of the single unassociated poloxamine molecules; $[x_n]$ represents the concentration of aggregated poloxamine and n is the aggregation number. An increase in the concentration of the non-associated molecules increases the concentration of the aggregated form. Since the association process is endothermic it must, therefore, follow that an increase in concentration results in a decrease in temperature or conversely, for a system at equilibrium, the temperature at which aggregation commences is reduced as the concentration is increased.

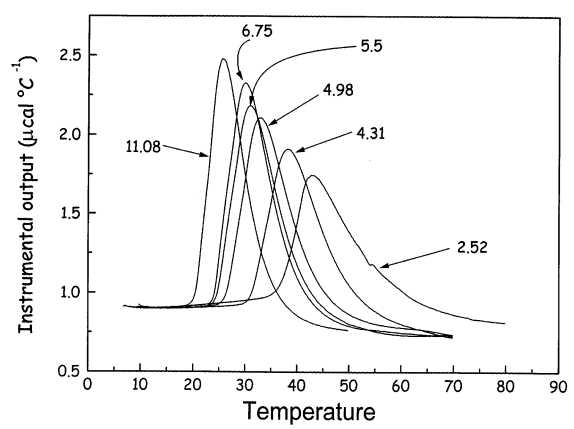

Fig. 4. The impact of pH on the thermally induced aggregation transitions of T701 ([T701] = 5.0 mg ml^{-1}; scan rate = 60 K h^{-1}).

The experimental data for the impact of pH upon the aggregation transition are interesting. It is evident (Fig. 4) that as the pH of the aqueous poloxamine solutions is changed from acid to basic conditions, with the consequent loss from the poloxamine chains of their cationic charge, aggregation becomes facilitated. This seems entirely plausible. Aggregation in poloxamine solutions—in concordance with other POE–POP–POE block copolymers—is driven by the increased hydrophobicity of the POP blocks at elevated temperatures. However the coulombic repulsion arising from the presence of positively charged amine group(s) at the centre of the POP blocks will prevent aggregation. Thus at pH values below pK_a a necessary precondition for aggregation is proton dissociation. The following equilibrium reaction equation captures the essence of the deprotonation–micellization process:

$$nx\mathrm{H}^+ \Leftrightarrow x_n + n\mathrm{H}^+ \quad \text{or} \quad K_{\text{micellisation}} = \frac{[x_n][\mathrm{H}^+]^n}{[x\mathrm{H}^+]^n},$$

where $x\mathrm{H}^+$ is the protonated form of the poloxamine chain. The free energy of the above process (derived from the equilibrium constant, $K_{\text{micellization}}$) is functionally derived from both the free energy of micellization (K in Eq. (1)) and deprotonation (K_a). Clearly as the hydrogen ion concentration increases (pH decreases) the free energy costs of deprotonation–micellization become increasingly expensive and so micellization is shifted to higher temperatures in order to favourably alter $K_{\text{micellization}}$.

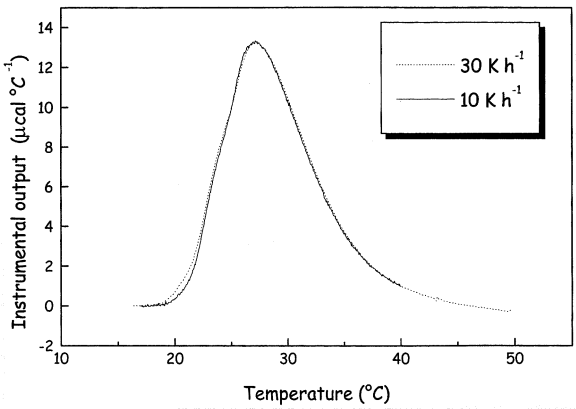

Fig. 5. Superimposability of signals obtained at 10 and 30 K h^{-1} indicating they are independent of scan rate ([T701] = 5.0 mg ml^{-1}).

3.3. Data analysis and model fitting

The techniques used to analyse the calorimetric data has been outlined previously (Armstrong et al., 1994; Patterson et al., 1997). Briefly, with DSC the observed change in enthalpy with respect to temperature for a process under strict thermodynamic control is given by:

$$\frac{dq_p}{dT} = \phi C_{p,xs} = \frac{d}{dT}(\alpha(\Delta H_{cal}(T_{\frac{1}{2}}) + \Delta C_p(T - T_{\frac{1}{2}}))), \quad (2)$$

where q_p is the heat change at constant pressure; T is temperature; $\phi C_{p,xs}$ is the apparent excess heat capacity (i.e., the difference in heat capacity between the reference and sample cells); α is the extent of change in the system; $\Delta H_{cal}(T_{\frac{1}{2}})$ is the experimentally determined enthalpy change at $T_{\frac{1}{2}}$ the temperature at which α equals 0.5 and ΔC_p is the difference in heat capacity between the initial and final states of the system. It is important to note that the absence of any changes in the signal at different scan rates indicates that the aggregation transitions examined are under strict thermodynamic control (Sánchez-Ruiz et al., 1988). As can be seen in Fig. 5, DSC signals obtained at scan rates of 30 and 10 K h^{-1} are clearly superimposable; thereby demonstrating the applicability of using equilibrium thermodynamic analysis model to fit the data.

Given the temperature independence of $T_{\frac{1}{2}}$ and $\Delta H_{cal}(T_{\frac{1}{2}})$ and assuming ΔC_p is independent of temperature for the system of interest, Eq. (2) may be rewritten as:

$$\phi C_{p,xs} = \frac{d\alpha}{dT}(\Delta H_{cal}(T_{\frac{1}{2}}) + \Delta C_p(T - T_{\frac{1}{2}})) + \alpha \Delta C_p. \quad (3)$$

The extent of conversion to aggregates, α, for the aqueous surfactant systems examined in this work is obtained from the temperature dependence of the equilibrium constant describing the incorporation of surfactant unimers into micelles:

$$\left(\frac{\partial \ln K}{\partial T}\right)_p = \frac{\Delta H_{vH} + \Delta C_p \frac{\Delta H_{vH}}{\Delta H_{cal}}(T - T_{\frac{1}{2}})}{RT^2}. \quad (4)$$

Here ΔH_{vH} is the van't Hoff enthalpy. The ratio of the van't Hoff enthalpy and the calorimetric enthalpy provides a measure of the size of the co-operative unit involved in the micellization process. The heat capacity change in Eq. (4) is therefore scaled to reflect this co-operativity in the system since the equilibrium constant reflects those processes involving this co-operative unit. $K(T)$ can be obtained by integrating Eq. (4) and this value can be used in the following form of Eq. (1) to evaluate α-the fraction of surfactant in micellar form:

$$K = \frac{[x_n]}{[x]^n} = \frac{\alpha C/n}{((1-\alpha)C)^n}, \quad (5)$$

where C is the total concentration of copolymer. The evaluation of α at various temperatures permits an evaluation of the temperature dependence of $\phi C_{p,xs}$ in Eq. (3). Using these series of equations provides a mechanism for model fitting the DSC signals and for obtaining numerical values for the various thermodynamic parameters appearing in the above expressions.

It should be stressed that the micellar aggregates continue to increase in size after the initial aggregation event (Attwood et al., 1990). Previously we have argued that in aqueous solutions of ethylene oxide-propylene oxide block copolymers, the initial aggregation (i.e. nucleation) event gives rise to the calorimetric signal and that the subsequent micellar growth phase appears to be calorimetrically silent (Chowdhry et al., 1997).

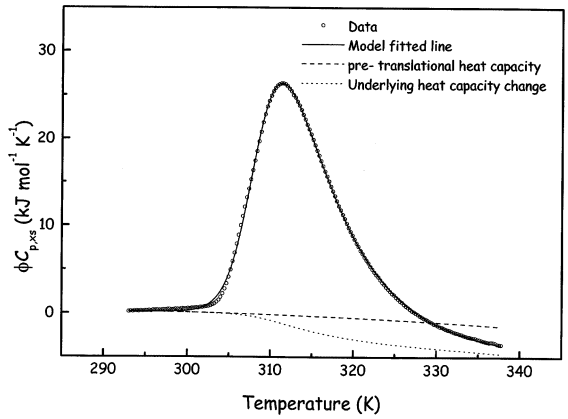

Fig. 6. Results for the calorimetric model fitting process. The data was obtained for a 5.0 mg ml^{-1} solution of T701 scanned at 60 K h^{-1}.

The data were fitted to the model using the software package Scientist™ (obtained from MicroMath Scientific Software, Salt Lake City, UT). The Scientist™ program uses a modified Powell algorithm to find a local minimum and the global minimum of the sum of the squared deviations between the model calculated values and the experimental data. This is combined with the provision of a robust and efficient root finder, which permits Eq. (5) to be solved—at any temperature—for α. An example of the output obtained from the model fitting process is shown in Fig. 6. The model fit is good and is similar to the fits reported for the poloxamer family of oxyethylene–oxypropylene (OE–OP) triblock copolymers (Patterson et al., 1997) and oxypropylene homopolymers (Chowdhry et al., 1997). The fitted calorimetric parameters are shown in Tables 1 and 2. It is clear from these data that the fitted DSC parameters alter with concentration and pH. An explanation for the variation in the characteristic temperature of the aggregation process with concentration has been given in a previous section. The variation in the calorimetric and van't Hoff enthalpies, however, arises because of the negative heat capacity change between the initial and final states of the system. Thus as $T_{\frac{1}{2}}$ increases it would be expected, as a result of the relationship noted by Kirchoff (Kondepudi and Prigogine, 1998), that both ΔH_{cal} and ΔH_{vH} will decrease. A plot of both ΔH_{cal} and ΔH_{vH} as a function of $T_{\frac{1}{2}}$ is shown in Fig. 7. If both sets of data were to be plotted in linear format the data sets would yield good straight lines with R^2 values in the order of 0.97. A modest increase in R^2 values may be obtained if the data sets are fitted by a quadratic expression. However because of the underlying heat capacity, as a function of temperature, the plots in Fig. 7 require to be plotted as curvilinear functions. Such an exercise may be justified by the observation that the model derived heat capacity values appear to be temperature dependent (Fig. 8). The heat capacity values obtained from the experimental data are between 3 and 12 times larger than the model derived values. This is probably accounted for by the large uncertainties in the model derived heat capacity parameter.

The calorimetric information obtained for the effect of pH upon the model derived parameters shows much the same pattern as those for concentration. It is apparent that as the pH increases—thereby decreasing the positive free energy penalty of proton dissociation from the ethylene diamine moiety—the characteristic transition temperature $T_{\frac{1}{2}}$ is reduced (Fig. 9). Again, the negative heat

Table 1
The effect of copolymer concentration upon model derived calorimetric parameters for T701

Concentration (mg ml^{-1})	ΔH_{cal} (kJ mol^{-1})	ΔH_{vH} (kJ mol^{-1})	n	$T_{\frac{1}{2}}$ (K)	ΔC_p (kJ mol^{-1} K^{-1})
1.0	348	541	3.1	304.5	−2.9
2.5	361	580	3.2	302.8	−3.6
7.5	393	636	3.6	301.0	−6.8
10.0	403	641	3.7	300.6	−7.8
15.0	415	686	4.1	299.9	−10.2
20.0	418	698	4.2	299.4	−10.4

Table 2
The effect of pH upon model derived calorimetric parameters for T701 (5.0 mg ml^{-1})

pH (at 298 K)	ΔH_{cal} (kJ mol^{-1})	ΔH_{vH} (kJ mol^{-1})	n	$T_{\frac{1}{2}}$ (K)	ΔC_p (kJ mol^{-1} K^{-1})
2.5	371	592	9.4	324.1	−11.1
4.3	385	481	3.8	314.4	−4.0
5.0	401	499	3.3	308.2	−1.9
5.5	418	498	3.1	306.0	−0.9
6.8	428	525	3.1	305.0	−1.9
8.1	388	594	2.8	298.6	2.6
11.0	408	615	3.4	300.6	−4.2

capacities suggest that as $T_{\frac{1}{2}}$ increases, enthalpy values will decrease. The data in Table 2 demonstrate this to be true. However, in these systems it is inappropriate to plot enthalpies as a function of $T_{\frac{1}{2}}$; This is because the calorimetric signal contains additional information about proton dissociation from the ethylene diamine moiety and its subsequent transfer to the acid anion of the buffer system. The buffer systems employed in this work all have different enthalpies of ionisation. Moreover because of the different buffer systems used, the heat capacities of the initial and final states of each system are not comparable. In a subsequent publication we will outline a data fitting framework which can be used to predict the pH dependence of the calorimetric signal and which can be used to deconvolute the signal into its component contributions.

Using the model derived calorimetric parameters it is a straightforward task to compute the fraction of copolymer in an aggregated state. If Eq. (4) is integrated between the limits of $T_{\frac{1}{2}}$ and T we obtain:

$$\ln\left(\frac{K(T)}{K(T_{\frac{1}{2}})}\right) = \frac{\Delta H_{vH}}{R}\left(\frac{1}{T_{\frac{1}{2}}}-\frac{1}{T}\right)+\frac{\Delta C_p}{R}\left(\ln\left(\frac{T}{T_{\frac{1}{2}}}\right)+\frac{T_{\frac{1}{2}}}{T}-1\right). \quad (6)$$

Noting that at $T_{\frac{1}{2}}$, $\alpha = 0.5$ Eq. (5) can be used to evaluate the left hand side of the expression to yield:

$$\ln\left(\frac{\alpha(0.5)^{n-1}}{(1-\alpha)^n}\right) = \frac{\Delta H_{vH}}{R}\left(\frac{1}{T_{\frac{1}{2}}}-\frac{1}{T}\right)+\frac{\Delta C_p}{R}\left(\ln\left(\frac{T}{T_{\frac{1}{2}}}\right)+\frac{T_{\frac{1}{2}}}{T}-1\right). \quad (7)$$

Using the model derived calorimetric data, Scientist™ was used to solve Eq. (7) to obtain α. In Fig. 10 the fraction of poloxamine in the aggregated form is shown as a function of temperature. It is apparent that altering the pH of the aqueous solutions has an important impact upon the fraction of aggregated poloxamine. If we consider human physiological temperature, essentially, none of the poloxamine is aggregated at a pH of 2.5. On the other hand, some 85% of the copolymer molecules are aggregated at a pH of 6.8. Given the ability of the aggregated forms of POE–POP–POE block copolymers to solubilise hydrophobic solutes (Erukova et al., 2000; Paterson et al., 1999) this pH switch may be of importance in the design of drug delivery systems incorporating poloxamines.

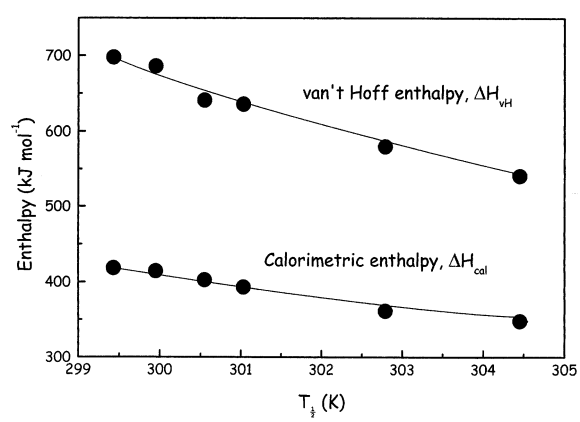

Fig. 7. Enthalpy plotted as a function of $T_{\frac{1}{2}}$. The data was obtained for different aqueous concentrations of T701.

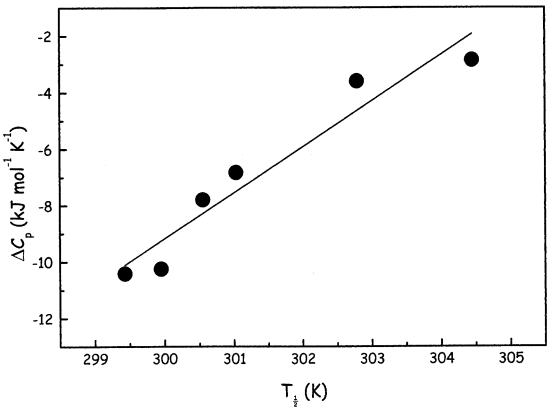

Fig. 8. ΔC_p plotted as a function of $T_{\frac{1}{2}}$. The data was obtained at different aqueous concentrations of T701.

4. Conclusions

Thermally induced aggregation transitions have been examined for aqueous solutions of the poloxamine T701. The signals obtained are similar to those obtained for other OE–OP block copolymeric systems. In particular the temperature range over which the transition occurs is shifted in response to changes in concentration. As the concentration increases, the temperature range decreases. The ethylene diamine moiety is basic with a pK_a value of 9.4. Thus pH is also observed to have an important effect upon the conformational transition temperature range. As the pH is decreased the transition temperature is shifted to higher temperatures. This suggests that a necessary precondition for aggregation is the removal of a proton. The free energy cost is higher at lower pH values, which in turn means that this free energy cost can only be met at higher temperatures. These data can be used to show how the fraction of aggregated poloxamine varies with temperature. At human physiological body temperature, the data also shows that the fraction of aggregated poloxamine at pH 6.8 is some 85%, whereas at a pH of 2.5 the fraction is zero.

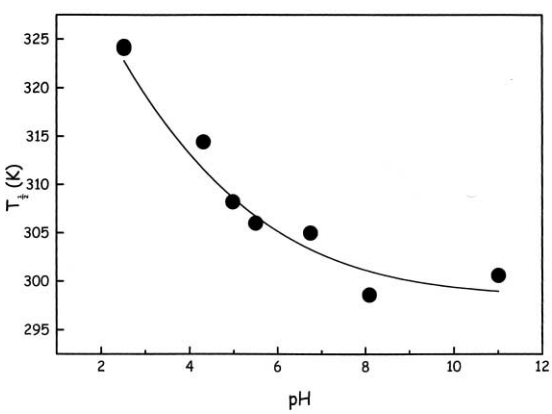

Fig. 9. $T_{\frac{1}{2}}$ plotted as a function of pH for solutions of T701 (5.0 mg ml^{-1}).

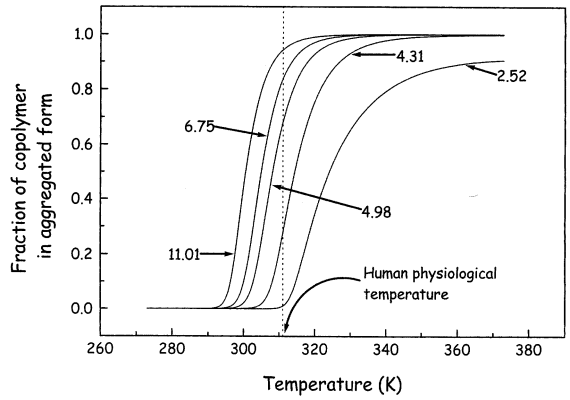

Fig. 10. Fraction of T701 in an aggregated form plotted as a function of temperature. Different outputs are obtained as a function of pH. The broken line indicates physiological human body temperature.

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