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Thermodynamic modelling of the effect of pH upon aggregation transitions in aqueous solutions of the poloxamine, T701

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Abstract

Differential scanning calorimetry has been used to investigate the effect of pH upon the temperature interval over which aggregation occurs in aqueous solutions of the ethylene oxide (EO)/propylene oxide (PO) block co-polymeric compound tetronic T701 (molecular formula $(EO_4PO_{13})_2NCH_2CH_2N(PO_{13}EO_4)_2$). Self association, in this system, occurs at elevated temperatures because the PO blocks become increasingly non-polar and, as a consequence, self associate because of the hydrophobic effect. pH titration shows that the central ethylene diamine moiety is di-basic with pK_a values of 3.8 and 8. Thus, in aqueous solution when the pH value is below 8 a necessary pre-condition for self association is deprotonation. A theoretical model is developed—based upon this hypothesis—which attempts to replicate, with some success, the effect of pH upon the calorimetrically observed T701 self association process.

Keywords: Poloxamine; Co-polymer; Calorimetry; pH titration; pK_a; Self association

1. Introduction

In a previous publication [1] the effect of changes in pH upon the calorimetrically derived temperature interval over which micellar aggregation in aqueous solutions of the poloxamine T701 occurs was reported. The poloxamines are block co-polymers consisting of ethylene oxide (EO) and propylene oxide (PO); their molecular structure is shown in Fig. 1. The physical and chemical properties of these compounds are altered by changes in the molecular size of the EO and PO blocks. The surface activity of these compounds arises from the differing solvent preferences of the hydrophilic EO and hydrophobic PO blocks. Of particular interest in this family of compounds is the presence of the central ethylene diamine moiety which provides such molecules with some pH functionality. For example it has been shown that both micellisation and adsorption at the hexane/water interface is affected by changes in pH [1,2]. It has been conjectured that reductions in pH provide the driving force for de-micellisation [2]. This is, presumably, due to the protonation of the nitrogen atoms in the ethylene diamine moiety and the subsequent electrostatic repulsion that arises between the PO blocks. Since these repulsive forces arise in the middle of the PO block the self assembled PO blocks in micellar aggregates are obliged to separate from each other thereby facilitating de-micellisation. Such simple triggers to alterations in self assembly are of potential interest in drug delivery systems.

Herein differential scanning calorimetry (DSC) has been used to examine the effect of pH changes on the thermodynamic parameters associated with aggregation in poloxamine T701. It is suggested that a necessary pre-requisite for micellar aggregation in these systems is that the molecules must first undergo proton dissociation before they can self

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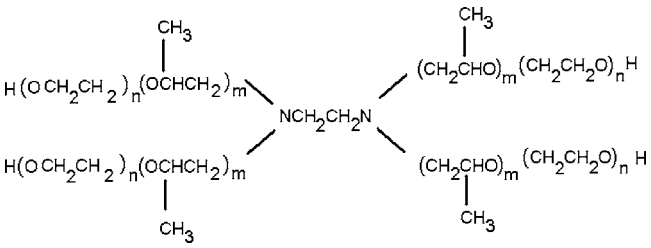

Fig. 1. Schematic of the molecular structure of the poloxamine family of block co-polymers.

assemble. A thermodynamic model is formulated and used to simulate the DSC output obtained for micellar aggregation in T701 solutions.

2. Experimental

2.1. Chemicals

Poloxamine T701 [molecular formula $(EO_4PO_{13})_2NCH_2CH_2N(PO_{13}EO_4)_2$, molecular mass = 3700 g mol^{-1}] was a gift from ICI Chemicals Ltd., (Middlesbrough, Cleveland, UK.) donated under the trade name of Synperonic T^{TM} non-ionic surfactants. As this co-polymer 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 co-polymer in 10 ml of cold de-aerated solvent (277 K), about 1 h prior to loading into the DSC cell. The following buffer systems were used for the pH experiments: glycine/hydrochloric acid (pH 2.52); succinic acid/sodium hydroxide (pH 4.31); citric acid/sodium hydrogen phosphate (pH 4.98); citric acid/phosphate (pH 5.50); phosphate (pH 6.75); phosphate (pH 8.09) and sodium hydroxide and glycine (pH 11.08). Ionic strength was not controlled in these systems. The solvent was first de-aerated by purging with dry nitrogen, as purging the surfactant solution resulted in foaming.

2.2. Titrimetric analysis

Potentiometric titration of T701 was performed using a pH meter (model number PW 9418 *ex* Philips, UK) and a Gelpas (BDH, UK) glass electrode at 298 K. 0.01 M hydrochloric acid (from BDH; Analar grade, Poole, UK) was initially added to approximately 0.01 M poloxamine solutions which were then subsequently titrated with 0.0095 M sodium hydroxide. The sodium hydroxide solution was standardised against sodium hydrogen phthalate.

2.3. Differential scanning calorimetry

Calorimetric measurements were carried out using a Microcal MC-2 instrument (Microcal Inc., Amherst, MA, USA) 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 of 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 experiment, and scans performed at a scan rate of 60 K h^{-1}. For down-scan experiments samples, scanned at a rate of 30 K h^{-1}, were equilibrated for 90 min at a temperature approximately 20 K above the T_m of the observed transition prior to the down-scan. 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}.

3. Results and discussion

3.1. Titration results

The titration data obtained for T701 are shown in Fig. 2. Two inflection points are observed, both of which correspond to proton dissociation from the nitrogen atoms on the central ethylene diamine moiety. The acid dissociation values were readily obtained by fitting the potentiometric data to the following equilibrium model.

Any point on the potentiometric curve is characterised by the following electroneutrality expression [3]:

$$[H^+]+2[PH_2{}^{2+}]+[PH^+]+[Na^+]=[Cl^-]+[OH^-] \quad (1)$$

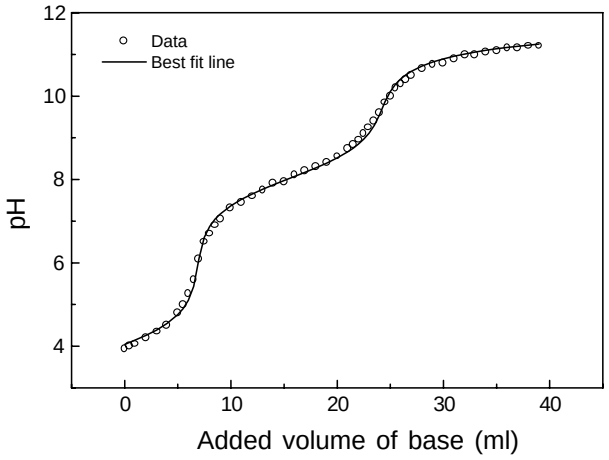

Fig. 2. Experimental titration data obtained for T701 and the outcome of the potentiometric model fitting process.

where $[PH^+]$ and $[PH_2{}^{2+}]$ represent the concentrations of the mono- and di-protonated forms of the poloxamine molecules, respectively.

The acid dissociation constants $K_{a,1}$ and $K_{a,2}$ are given by the following expressions:

$$K_{a,1} = \frac{[PH^+][H^+]}{[PH_2{}^{2+}]} \tag{2}$$

and

$$K_{a,2} = \frac{[P][H^+]}{[PH^+]} \tag{3}$$

The concentration of un-ionised poloxamine molecules, [P], is given by:

$$[P] = \frac{C_{poloxamine}}{1 + ([H^+]/K_{a,2}) + ([H^+]^2/K_{a,2}K_{a,1})} \times \left(\frac{V_{initial}}{V_{initial} + V_{base}}\right) \tag{4}$$

$C_{poloxamine}$ is the concentration of poloxamine chains in solution and the initial solution volume, $V_{initial}$ is given by the combined volume of poloxamine solution $V_{poloxamine}$ and volume of acid solution $V_{acid}V_{base}$ is the volume of added sodium hydroxide solution.

The concentration of $[PH_2{}^{2+}]$ is given by:

$$[PH_2{}^{2+}] = \frac{[P][H^+]^2}{K_{a,2}K_{a,1}} \tag{5}$$

The concentration of $[PH^+]$ is given by:

$$[PH^+] = \frac{[P][H^+]}{K_{a,2}} \tag{6}$$

The concentrations of sodium and chloride ions are given by:

$$[Na^+] = C_{base}\left(\frac{V_{base}}{V_{initial} + V_{base}}\right) \tag{7}$$

and

$$[Cl^-] = C_{acid}\left(\frac{V_{initial}}{V_{initial} + V_{base}}\right) \tag{8}$$

In addition:

$$[OH^-] = \frac{10^{-14}}{[H^+]} \tag{9}$$

For the model fitting procedure Eqs. (4)–(9) are substituted into Eq. (1) which is then solved for $[H^+]$. Values for the parameters $K_{a,1}$, $K_{a,2}$, V_{acid} and $V_{poloxamine}$ were then optimised in the model fitting procedure using the potentiometric data. Model fitting was accomplished using the software package Scientist™ (obtained from MicroMath Scientific Software, Salt Lake City, UT, USA). 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 calculated values from the model and the experimental data. This is combined with the provision of a robust and efficient root finder, which permits Eq. (1) to be solved—at any volume of sodium hydroxide—for $[H^+]$. The pK_a values obtained from the data fitting process for T701 are: $pK_{a1} = 3.8$ and $pK_{a2} = 8$.

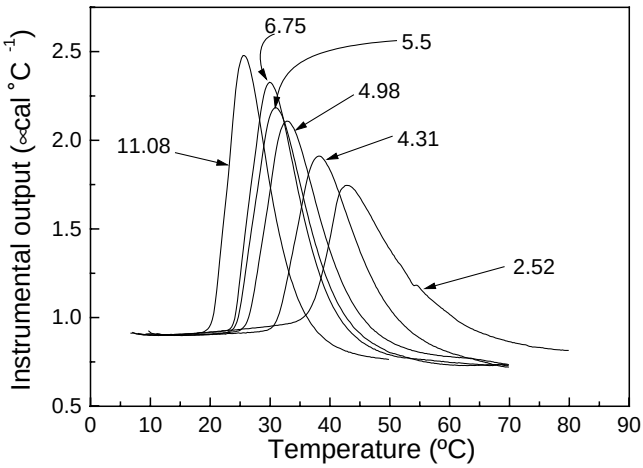

Fig. 3. The impact of pH on the thermally induced, calorimetrically observed, aggregation transitions of T701. Solution concentrations were 5.0 mg ml^{-1} and the scan rate was 60 K h^{-1}.

3.2. DSC data

The DSC scans for T701, as a function of pH, are shown in Fig. 3. It should be noted that changes in ionic strength can have an important impact upon the temperature range over which thermally induced aggregation occurs in EO–PO block copolymers [4]. In our experimental runs the ionic strength was uncontrolled. However the buffer solutions were sufficiently dilute to have a negligible impact upon the transition temperature [4] in terms of ionic strength changes.

The general shape of the curves—an asymmetry characterised by a sharp leading edge followed by a gradually declining tail—is indicative of an association transition [5–7]. It is therefore evident that the thermal event observed by DSC represents micellar aggregation of poloxamine chains in response to the increasing (temperature driven) hydrophobicity of the PO blocks in the poloxamine molecules [8,9]. In addition as the pH of the aqueous poloxamine solutions is altered from acidic to basic conditions, with the consequent loss from the poloxamine chains of their cationic charge, aggregation becomes facilitated. This hypothesis is plausible because aggregation in poloxamine solutions—as with other EO/PO block co-polymers—is driven by the increased hydrophobicity of the PO blocks at elevated temperatures [8,9]. However the coulombic repulsion arising from the presence of positively charged amine group(s) at the centre of the PO blocks will prevent aggregation. Thus, at pH values below pK_{a1} and pK_{a2} a necessary pre-condition for aggregation is proton dissociation. The following equilibrium expression for mono-protonated chains captures the essence of the

deprotonation–micellisation process. The equilibrium constant for micellar aggregation, K, is given by the expression:

$$K = \frac{[P_n]}{[P]^n} \tag{10}$$

where [P] is the concentration of unaggregated chains, n the aggregation number and $[P_n]$ is the concentration of aggregates.

If Eq. (3) is used the following expression for the aggregation of mono-protonated chains is obtained:

$$K_{\text{micellisation}} = KK_{a,2}^n = \frac{[P_n]}{[P]^n} \times \left(\frac{[P][H^+]}{[PH^+]}\right)^n = \frac{[P_n][H^+]^n}{[PH^+]^n} \tag{11}$$

$K_{\text{micellisation}}$ is defined here as the product of the equilibrium constant characterising aggregation and the dissociation constant describing the loss of a proton from the mono-protonated poloxamine molecule. As such it is related to the free energy requirement for removal of a proton plus the free energy change that occurs on aggregation. In other words, it describes the total free energy change on going from the mono-protonated non-aggregated form of the poloxamine molecule to the neutral aggregated form.Rearrangement of Eq. (11) gives:

$$\frac{[P_n]}{[PH^+]^n} = \frac{K_{\text{micellisation}}}{[H^+]^n} \tag{12}$$

Eq. (11) demonstrates that aggregation is favoured (i.e. the ratio of the aggregated to un-aggregated forms of the poloxamine is high) when either $K_{\text{micellisation}}$ is large and/or $[H^+]$ is small. Micellisation is an endothermic event [6,7] thus as the temperature of the aqueous poloxamine system rises $K_{\text{micellisation}}$ becomes progressively larger, reflecting the increasing favourability of the aggregation process. Evidently as the hydrogen ion concentration increases (pH decreases) a larger $K_{\text{micellisation}}$ value is required in order to bring about aggregation. These increased values are obtainable at higher temperatures and phenomenologically this is observed as micellisation being shifted to higher temperatures in response to a reduction in pH.

3.3. Modelling the effect of pH upon the temperature interval over which aggregation occurs

In order to model the signal obtained using DSC it should be appreciated that the observed change in enthalpy with respect to temperature, for a process under strict thermodynamic control, is given by [7,10]:

$$\frac{dq_p}{dT} = \phi C_{p,xs} = \frac{d}{dT}(\alpha(\Delta H_{cal}(T_{1/2}) + \Delta C_p(T - T_{1/2}))) \tag{13}$$

In Eq. (13) q_p is the heat change at constant pressure; T the temperature; $\phi C_{p,xs}$ the apparent excess heat capacity (i.e., the difference in heat capacity between the reference and sample cells); α the extent of change in the system; $\Delta H_{cal}(T_{1/2})$ is the experimentally determined enthalpy change (i.e. the integrated area of the HSDSC curve), $T_{1/2}$ is 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. Given the temperature independence of $T_{1/2}$ and $\Delta H_{cal}(T_{1/2})$ and assuming ΔC_p is independent of temperature for the system of interest, Eq. (13) may be rewritten as:

$$\phi C_{p,xs} = \frac{d\alpha}{dT}\left(\Delta H_{cal}\left(T_{1/2}\right) + \Delta C_p\left(T - T_{1/2}\right)\right) + \alpha \Delta C_p \tag{14}$$

Modelling the effect of pH upon the temperature induced aggregation of T701 requires an elaboration of how the composition of the aqueous system varies with both temperature and pH. In particular it is necessary to evaluate the way in which α varies with both temperature and pH. In order to understand the effect that pH has upon the temperature range over which the aggregation transition occurs the following equilibrium model was formulated. The temperature dependence of the equilibrium constant describing the aggregation transition is described by a combination of the van't Hoff and Kirchoff equations [11]:

$$\left(\frac{\partial \ln K}{\partial T}\right)_p = \frac{\Delta H_{vH} + \Delta C_p(T - T_{1/2})}{RT^2} \tag{15}$$

K is the equilibrium constant, T the temperature, p the pressure, ΔH_{vH} the van't Hoff enthalpy, ΔC_P the isobaric heat capacity, $T_{1/2}$ is the temperature at which half the poloxamine chains have been incorporated into aggregates and R the gas constant.

Eq. (15) can be integrated to provide the following expression:

$$\ln\left(\frac{K(T)}{K(T_{1/2})}\right) = \frac{\Delta H_{vH}}{R}\left(\frac{1}{T_{1/2}} - \frac{1}{T}\right) + \frac{\Delta C_p}{R}\left(\ln\left(\frac{T}{T_{1/2}}\right) + \frac{T_{1/2}}{T} - 1\right) \tag{16}$$

α is the extent of incorporation of poloxamine chains into aggregates and can be defined as:

$$\alpha = \frac{n[P_n]}{C_{\text{poloxamine}}} \tag{17}$$

Rearrangement gives:

$$[P_n] = \frac{\alpha C_{\text{poloxamine}}}{n} \tag{18}$$

The corresponding expression for [P] is given by:

$$[P] = (1 - \alpha)C_{\text{poloxamine}} \tag{19}$$

Recalling that at $T_{1/2}$, $\alpha = 0.5$ Eqs. (17)–(19) can be used to provide the following expression for $K(T_{1/2})$:

$$K(T_{1/2}) = \frac{1}{0.5^{n-1} C_{\text{poloxamine}}^{n-1} n} \tag{20}$$

Combining Eq. (20) with Eq. (17) provides the following expression for the temperature dependence of $K(T)$:

$$\ln\left(\frac{K(T)}{0.5^{n-1}C_{\text{poloxamine}}^{n-1}n}\right) = \frac{\Delta H_{\text{vH}}}{R}\left(\frac{1}{T_{1/2}}-\frac{1}{T}\right) + \frac{\Delta C_p}{R}\left(\ln\left(\frac{T}{T_{1/2}}\right)+\frac{T_{1/2}}{T}-1\right) \quad (21)$$

The variation in the acid dissociation constants with temperature is given by the familiar van't Hoff expression for a process in which the heat capacity change is considered to be zero:

$$K_{\text{a}}(T) = K_{\text{a}}\,(298.15)\,\exp\left(\frac{\Delta H_{\text{a}}}{R}\left(\frac{1}{298.15}-\frac{1}{T}\right)\right) \quad (22)$$

K_{a} (298.15) is the acid dissociation constant which is found by titration (as explained above) and ΔH_{a} is the enthalpy of dissociation. Values for the enthalpies of dissociation for ethylene diamine were obtained from Christensen et al., [12] and used in the modelling calculations.

To obtain a value for α it is necessary to formulate the following mass balance equation for the various chemical forms of the poloxamine chains:

$$C_{\text{poloxamine}} = [\text{PH}_2{}^{2+}] + [\text{PH}^+] + [\text{P}] + n[\text{P}_n] \quad (23)$$

Substituting Eqs. (5), (6) and (10) into Eq. (23) and recalling that the equilibrium constants are temperature dependent, provides the following equation:

$$C_{\text{poloxamine}} = \frac{[\text{P}][\text{H}^+]^2}{K_{\text{a},2}(T)K_{\text{a},1}(T)} + \frac{[\text{P}][\text{H}^+]}{K_{\text{a},2}(T)} + [\text{P}] + nK(T)[\text{P}]^n \quad (24)$$

Model simulation involves specifying the calorimetric data required to evaluate the equilibrium constants at any particular temperature. Using these parameter values permits Eq. (24) to be solved for [P] whose value is then used to compute the concentrations of the other poloxamine species.

Fig. 4 shows how the concentrations of species vary with temperature at several different pH values. Table 1 shows the data used to produce the speciation diagrams. The data used was obtained for T701 at a pH of 11.8 because at this pH T701 is in a non-protonated state. Several features emerge from the speciation diagrams, which are worthy of comment. In particular it is worth noting that at a pH of 3, at low temperatures, the di-protonated form of the poloxamine molecule is predominant. As the temperature is increased a proton is lost and the mono-protonated form of the molecule predominates. At higher temperatures the second proton is lost and the molecules aggregate. At no temperature does the un-protonated form emerge as an important component species of the system. It would therefore seem to confirm our hypothesis that at low pH values the protonated form of the poloxamine molecule prevents aggregation. At a pH of 9 on the other hand the neutral poloxamine molecule is predominant which results in the onset of aggregation occurring at a low temperature because now there are no protons preventing aggregation.

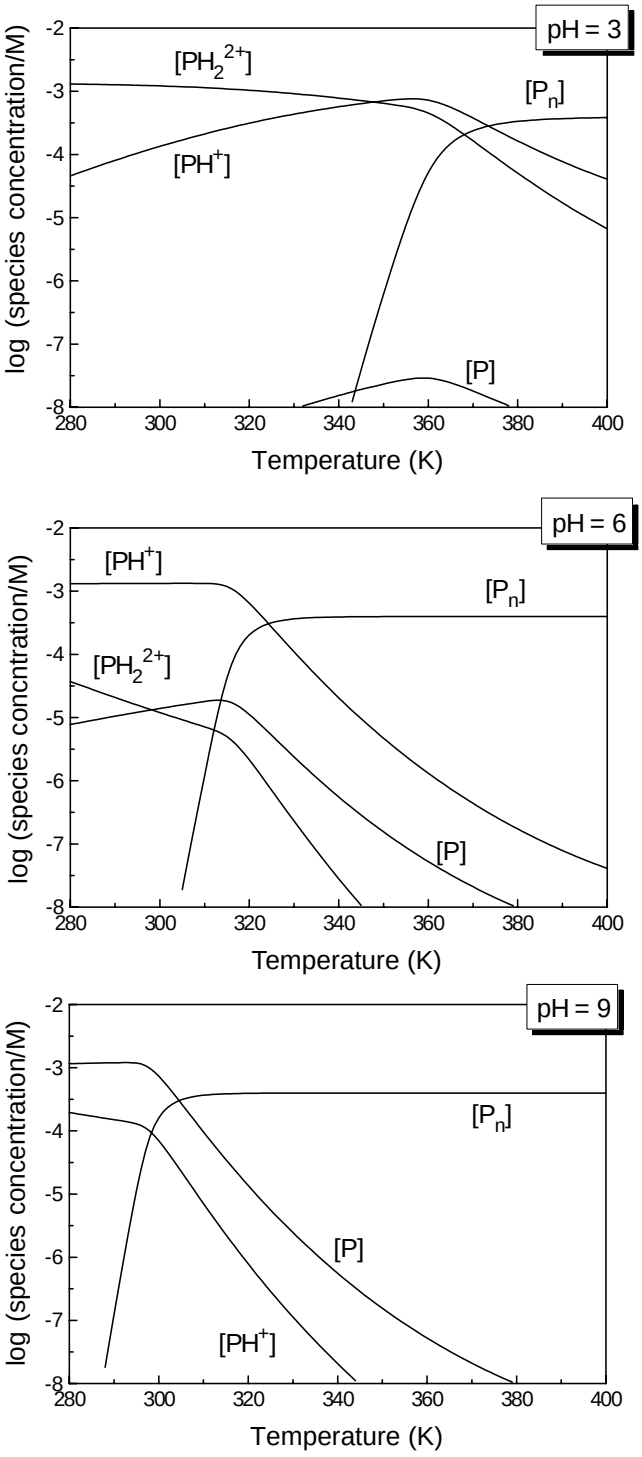

Fig. 4. Thermal speciation diagrams showing how the concentrations of the protonated, de-protonated and aggregated forms of the poloxamine molecule vary with temperature at different pH values.

Table 1
Parametric values used for the simulation of the T701 DSC signals

Parameter	Value
ΔH_{cal}	408 kJ mol^{-1}
ΔH_{vH}	615 kJ mol^{-1}
$T_{1/2}$	300.6 K
n	3.4
ΔC_p	−4.2 kJ mol^{-1} K^{-1}
p$K_{a,1}$	4
p$K_{a,2}$	8
$\Delta H_{a,1}$	40 kJ mol^{-1}
$\Delta H_{a,2}$	20 kJ mol^{-1}

The enthalpy of the system at any particular temperature is given by the following expression [11]:

$$H_{xs} = \frac{[PH]}{C_{total}}\Delta H_{a,1} + \frac{[P]}{C_{total}}(\Delta H_{a,1} + \Delta H_{a,2}) + \cdots \frac{n[P_n]}{C_{total}} \times (\Delta H_{a,1} + \Delta H_{a,2} + \Delta H_{cal} + \Delta C_p \frac{\Delta H_{cal}}{\Delta H_{vH}}(T - T_{1/2})) \quad (25)$$

In order to calculate the apparent excess heat capacity the following centred difference equation was used [13]:

$$\phi C_{p,xs}(T) = \frac{H_{xs}(T + \delta T) - H_{xs}(T - \delta T)}{2\delta T} \quad (26)$$

where $\delta T = 5 \times 10^{-6}$ K.

The simulated DSC signals are shown in Fig. 5. The important feature to note is that the outlined model captures the pH dependence of the DSC signal. It therefore seems reasonable to assert that the pH dependence of the temperature interval over which aggregation occurs is due to the necessary requirement that before the PO blocks in the T701 chains can self associate any protons bound to the central ethylene diamine moiety must be removed. However it is clear that the simulations over-exaggerate the effect of low pH. In the simulations the modelled signal obtained for pH 3 suggests that aggregation occurs at much higher temperature interval than is observed experimentally. This temperature interval can be reduced, by simulation, if the enthalpy values are increased. This is effected either by increasing the values of the enthalpy of proton dissociation or by increasing the van't Hoff enthalpy. The van't Hoff enthalpy value that is effectively used in the simulation could be in error because the heat capacity change which is assumed to be a constant may in fact be temperature dependent [10]. It was also assumed that the heats of proton dissociation were temperature independent. The foregoing parameters may also show a temperature dependence which are accompanied by heat capacity changes.

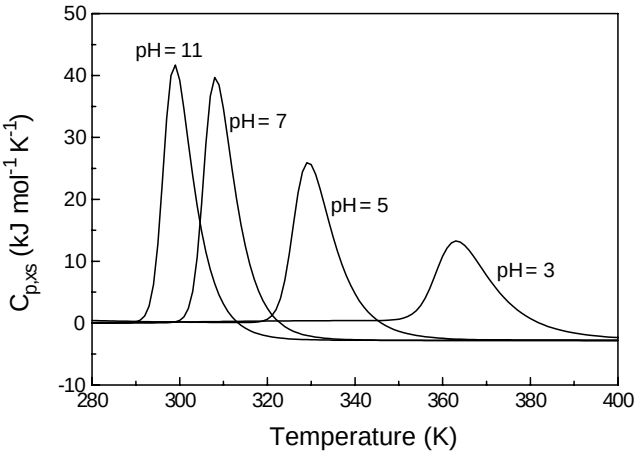

Fig. 5. Simulation of the DSC signals for T701, using the equations outlined in the text and showing the effect of pH upon the temperature range over which thermally induced aggregation occurs.

It is clearly of technological interest to be able to devise systems where micellar aggregation can be induced or reversed simply by changes in pH. Such effects may be of interest in drug delivery systems. It can, however, also be envisaged that micellar solubilisation switching could be used in a variety of new technologies. This paper provides a scheme which can be used to understand how these changes in micellar aggregation can be modelled for a system in which the temperature is slowly changing but the concentration of block copolymer is constant. It should not prove too difficult to create an isothermal model in which the two major forces affecting aggregation are changing poloxamine concentration and alterations in pH.

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