



Understanding the Self-Organisation of Association Colloids

Application Note

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Ultrasensitive Calorimetry for the Life Sciences™

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Abstract

Isothermal titration calorimetry (ITC) is an extremely sensitive technique which measures the heat of reaction of two components after mixing. The release or uptake of heat that can be measured is in the range of a few microcalories. This corresponds to temperature differences of less than 0.000001 K when compared to a reference system.

The systems studied and presented in this paper are physical colloids, also known as association colloids. The particles of these colloids are composed of aggregates of single molecules. Information about the self-organisation (self-aggregation) of such micellar colloids can be easily obtained from ITC, as e.g. the number of molecules associated in the aggregate and the heat of aggregation. A complete thermodynamic description of the micellar colloids can be derived from the ITC data, allowing insight into the formation and stability of these colloidal systems.

1. Introduction

The name *colloid* is derived from the Greek word 'kolla' meaning glue, because it was considered that all colloids are more or less like glue [Jirgensons and Straumanis, 1949, Staudinger 1950].

The first rational classification of colloids was proposed by VON WEIMAR and OSTWALD [Jirgensons and Straumanis, 1949]. The notion of a *dispersed system* was introduced, and particle size was taken as the chief factor in the classification and characterisation of colloids. Thus, colloids are substances consisting of a homogeneous medium and of particles dispersed therein. Any colloid contains particles whose diameter is 10^{-9} to 10^{-7} m [Wedler, 1987]. However, it is to note, that not all 'dispersed systems', a very general term, are colloids.

It is useful to look at colloids from the point of view of the structure of the particles. According to LUMIÈRE and STAUDINGER all colloids can be classified into *molecular* and *association (micellar)* colloids [Jirgensons and Straumanis, 1949, Staudinger 1950]. The particles of molecular colloids are single macromolecules, and their structure is essentially the same as the structure of small molecules: the atoms are joined by true chemical bonds. These molecular colloids are 'true colloids' (also denoted as 'chemical colloids') e.g. starch, polyvinyl chloride, rubber or spherocolloids like glycogen, albumin etc.

The structure of association colloids is quite different. The particles of these micellar colloids are not large molecules but aggregates of many small molecules or groups of atoms which are held together by secondary valencies, i.e. by cohesive or van der Waals forces. This type of colloid is also known under 'physical or association colloids' and will be considered in this paper. One fascinating question is: What are the driving forces of self-organisation of these association colloids?

2. Isothermal Titration Calorimetry

This application note presents examples illustrating how isothermal titration calorimetry (ITC) is used for understanding the self-organisation of amphiphile molecules forming association colloids (micelles). A so-called *demicellisation experiment* is described. The thermodynamic parameters associated with the demicellisation, the change in demicellisation enthalpy (ΔH_{demic}), change in demicellisation entropy (ΔS_{demic}), change in Gibbs free energy (ΔG_{demic}), as well as the change in heat capacity ($\Delta C_{p, \text{demic}}$) during demicellisation are obtained from one and the same ITC experiment.

This is the only technique allowing the user to obtain all these data from one and the same experiment.

In addition to this, the critical micelle concentration (*cmc*) is gained. An example will be presented showing how to derive from the ITC data information concerning the number of molecules forming the aggregate.

ITC is a technique which measures the heat of reaction involved in the interaction of two reactants after mixing (for more detail, please see [Blume and Garidel 1999]).

In the presented examples the colloids 'interact' with water due to dilution of the colloidal systems. As a result, the stability of the colloids is influenced and the micellar colloids disintegrate. This process is called demicellisation. From these experiments information concerning the stability and self-organisation of the micellar colloids are derived.

3. Demicellisation Protocol

Isothermal titration calorimetry (ITC) measurements were performed using the following MicroCal calorimeter units: the MicroCal Omega, the MCS titration calorimeter or the newer VP-ITC. The experimental procedure was described elsewhere [Blume and Garidel, 1999]. Briefly, the reaction cell was filled with degassed water or buffer solution. Typically a removable 250 μl syringe is filled outside the instrument with the micellar solution ($c_{\text{mic}} \gg \text{cmc}$). In steps of 5 or 10 μl aliquots the micellar solution is injected into the reaction cell and the heat flow is measured as a function of time. During the titration experiment the syringe rotates at a speed of approximately 400rpm allowing appropriate mixing. Data evaluation was performed as described recently [Keller et al. 1997, Garidel et al. 2000]. The heat of reaction can be measured as a function of temperature between 10-70 °C.

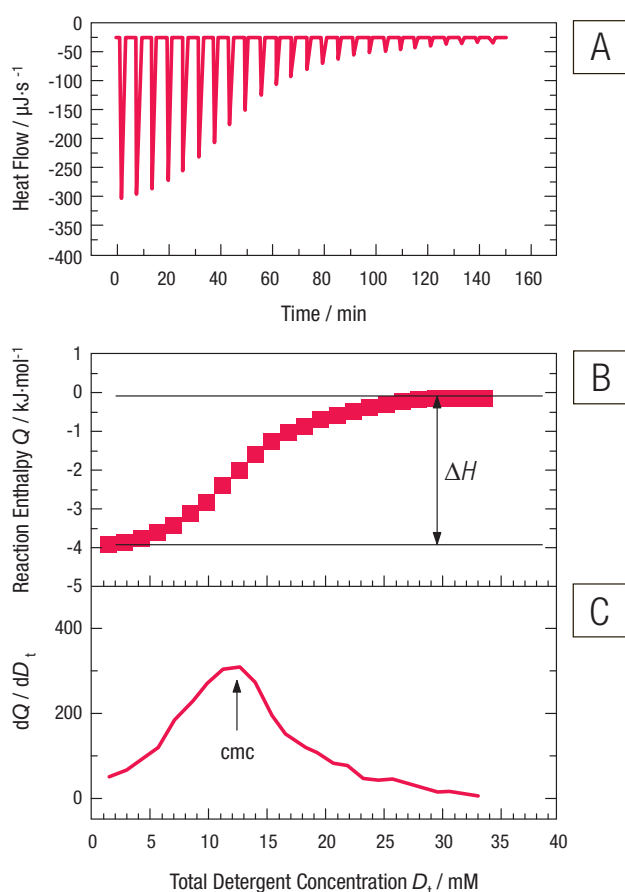


Figure 1. Typical ITC demicellisation experiment for the determination of the critical micelle concentration (*cmc*) of a detergent. (A) calorimetric traces (heat flow vs. time), (B) reaction enthalpy (Q) versus the total detergent concentration (D_t) in the reaction cell, (C) first derivative (dQ/dD_t) of curve B calculated numerically from interpolated values.

In Figure 1, a typical example of an ITC detergent (amphiphile molecule) dilution experiment is shown. The titration consists of 25 injections of 10 μl aliquots of a highly concentrated detergent, i.e. micellar solution. Each injection induces a heat flow as a function of time and, thus a deviation from of the baseline. The next injection is performed after the baseline has reached its initial value, which usually takes a few minutes. The reaction heat of each injection is obtained by integration, as can be seen in Figure 1B. Exothermic heat flows are measured with increasing concentration in the sample cell while the heat flow decreases and becomes constant, i.e. almost zero at the end of the experiment (Figure 1A).

The integrated heat as a function of total detergent in the measuring cell has a sigmoidal shape. The measured heat flows in the ITC experiment correspond to three different processes denoted by B, C and D in Figure 2.

The concentration of the detergent in the syringe (situation A in Figure 3) is much larger than the expected *cmc* of the detergent, that means the solution in the syringe contains micelles in addition to monomers.

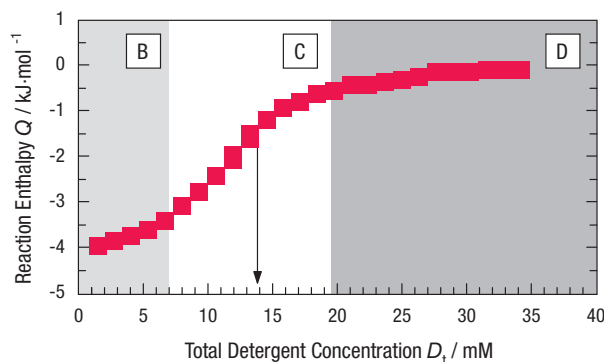


Figure 2. Reaction enthalpy (Q) versus the total detergent concentration (D_t) in the reaction cell.

As a consequence, for the first injection into the measuring cell, which is filled with water, all micelles disintegrate and the corresponding reaction heat for the demicellisation process is measured (situation B in Figure 3). This process is exothermic at the particular temperature as can be seen in Figure 2 and decreases with further injections. Whether this process is exothermic or endothermic in general, depends on the investigated temperature [Garidel et al. 2000]. During the titration experiment, the amount of detergent in the sample cell increases and at a critical concentration, the so called critical micelle concentration *cmc*, the first aggregates are formed (situation C in Figure 3). This critical concentration is easily determined in the ITC experiment by calculating the first derivative of the reaction heat with respect to the total detergent concentration (D_t) in the cell (Figure 1C) [Keller et al. 1997, Garidel et al. 2000, 2003].

The molar reaction heat (ΔH) is obtained as shown in Figure 1B. The relation between the enthalpy of the demicellisation process (ΔH_{demic}) and micellisation process (ΔH_{mic}), is:

$$\Delta H_{\text{mic}} = -\Delta H_{\text{demic}} \quad (1)$$

The large exothermic peak observed for the first injection is the sum of three processes [Garidel et al. 2003]: 1. dilution of monomers ($\Delta H_{\text{mon, dil}}$), 2. dilution of micelles ($\Delta H_{\text{mic, dil}}$) and 3. demicellization, i.e. decomposition of micelles (ΔH_{demic}) (see Figure 3).

$$\Delta H_{\text{demic}} = \Delta H - \Delta H_{\text{mon, dil}} - \Delta H_{\text{mic, dil}} \quad (2)$$

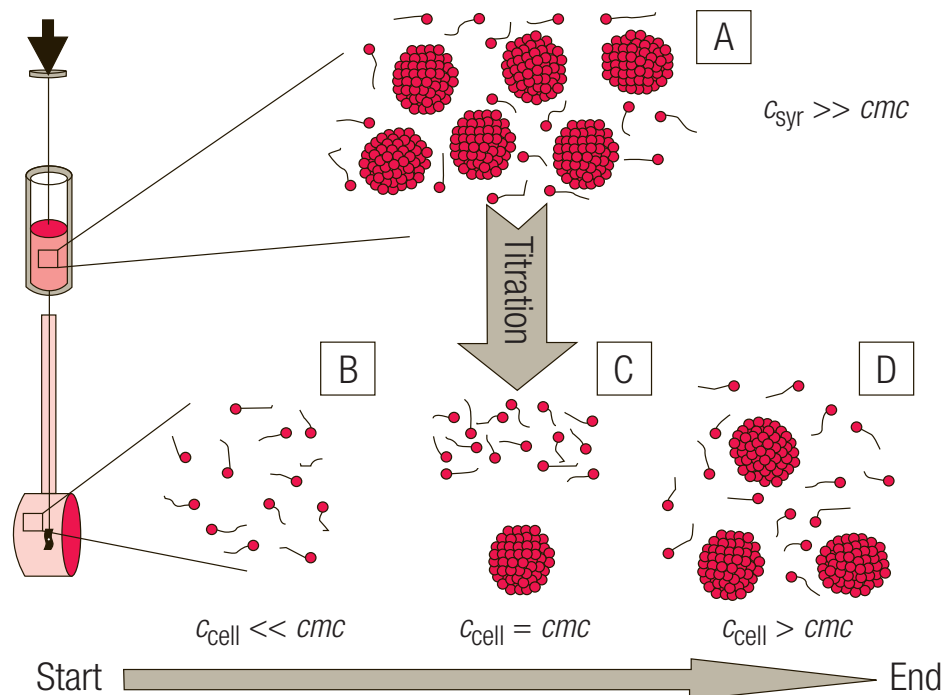


Figure 3. Schematic representation of the demicellisation experiment.

The heat of monomer dilution is obtained by the titration of a highly diluted detergent solution (monomeric solution) to buffer, whereas the second heat contribution is obtained by the injection of a micellar solution into the sample cell containing also a micellar solution (Figure 3D).

From this type of experiment the demicellisation enthalpy as well as the *cmc* is obtained and the thermodynamic parameters can be derived. This will be demonstrated for the following three case studies:

- Soap (sodium oleate)
- Physiological surfactant (bile salt)
- Co-micelle (binary detergent system of bile salt and sodium oleate)

4. Case Study #1: Soap (Sodium Oleate)

In Figure 4, an example of an ITC dilution experiment of sodium oleate (NaO) in water (*pH* 7.5) at 65 °C is shown. At 65 °C the heat flow is endothermic compared to data obtained at lower temperatures, and the heat of reaction is determined at 65 °C with $\Delta H = +18.0 \text{ kJ}\cdot\text{mol}^{-1}$ in contrast to $\Delta H = -18.4 \text{ kJ}\cdot\text{mol}^{-1}$ at 10 °C (see Figure 6).

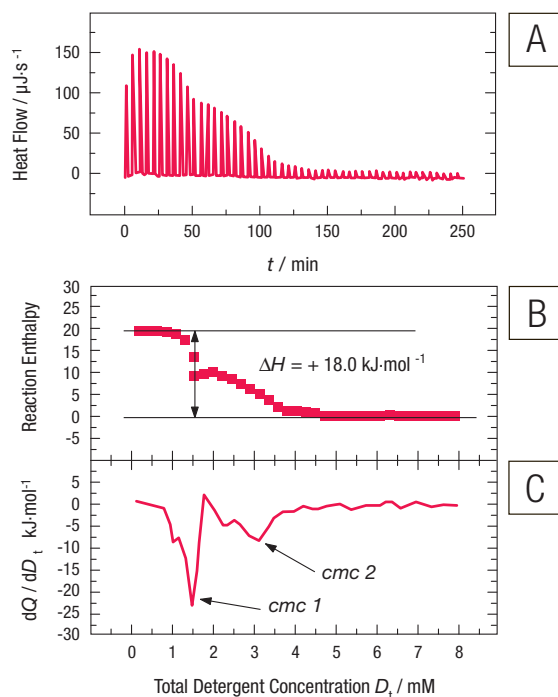


Figure 4. ITC dilution experiment for the determination of the critical micelle concentration (*cmc*) of sodium oleate (NaO) in water at *pH* 7.5 at 65 °C: titration of 40 mM (NaO) in 50 steps of 5 μl . (A) calorimetric traces (heat flow vs. time), (B) reaction enthalpy Q versus the total detergent concentration (D_t) in the reaction cell, (C) first derivative (dQ/dD_t) of curve B calculated numerically from interpolated values (adapted from Hildebrand et al. 2004).

Most detergents, like oligo (ethylene oxide) alkyl ethers [Heerklotz et al. 1996, Heerklotz and Seelig, 2000a], octyl glucoside [Keller et al. 1997], sodium dodecyl sulphate [Paula et al. 1995], bile salts [Garidel et al. 2000, 2003] or the steroid CHAPS [Heerklotz and Seelig, 2000b] show only one extremum in the dQ/dD_t curve of the demicellisation experiment, which corresponds to one cmc value. However, analysing the first derivative (Figure 4C) of NaO clearly shows the appearance of two extrema, indicating two cmc values denoted by $cmc1$ and $cmc2$.

The data for NaO demicellisation were measured over a temperature range between 10 to 70 °C and are shown in Figure 5. The cmc data were fitted with a second order polynomial [Nusselder and Engberts 1992].

The cmc of NaO is much lower compared to bile salt detergents (see case study #2). This is due to the different chemical structure of the amphiphiles.

The temperature dependence of the cmc of amphiphiles usually shows a minimum at room temperature [Blume and Garidel 1999], as is observed for the $cmc1$ of NaO. The second cmc of NaO, ($cmc2$) however, increases continuously with increasing temperature (Figure 5). This means that the second dissociation step of NaO is accompanied by an endothermic process over the whole temperature range, whereas the enthalpy change for the first dissociation is temperature dependent. In the ITC titration curve there is indeed an indication that even at low temperature the second cmc is connected with a slight endothermic effect, because the reaction enthalpy is positive at intermediate concentrations [see Hildebrand et al. 2004].

The two $cmcs$ for the demicellisation of NaO, as observed by ITC, are apparently related to a two step self-aggregation mechanism. Similar results have been observed using proton longitudinal magnetic relaxation times and self diffusion measurements [Mahieu et al. 1991]. It was possible to detect two types of aggregates at a concentration of 1.5 mM and 3.0 mM (room temperature). The first micelles have a spherical shape, whereas at higher detergent concentration a transformation into rod like structures is observed. Detergents with at least two $cmcs$ were denoted by Zimmels and Lin [1974] as “multi- cmc systems”. They are characterised by a stepwise aggregation of monomers with discontinuous transformation of the different aggregate structures. Thus, self-organisation of such systems can be analysed by ITC.

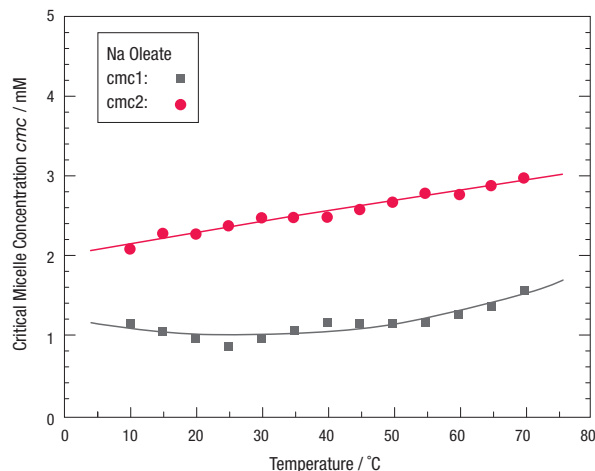


Figure 5. Temperature dependence of the two critical micelle concentrations of sodium oleate, $cmc1$ and $cmc2$ in water at pH 7.5. The lines are obtained using a second order polynomial fit.

According to the pseudo phase separation model, the micelles are considered as a separate phase. For the calculation of the demicellisation enthalpy, the monomer concentration in the syringe has to be taken into account, i.e. $c_{\text{syringe}} = c_{\text{monomer}} + c_{\text{micelle}}$, with $c_{\text{monomer}} = cmc$ [Garidel et al. 2000]. The ΔH_{demic} data are plotted in Figure 6 as a function of temperature. The Gibbs free energy (ΔG_{demic}) for the demicellisation is calculated according to:

$$\Delta G_{\text{demic}} = -RT \cdot \ln cmc' \quad (3)$$

with R = gas constant, T = temperature, $cmc' = cmc$ expressed in mole fraction units.

The $T \cdot \Delta S_{\text{demic}}$ term is obtained using the Gibbs-Helmholtz relation

$$T \cdot \Delta S_{\text{demic}} = \Delta H_{\text{demic}} - \Delta G_{\text{demic}}. \quad (4)$$

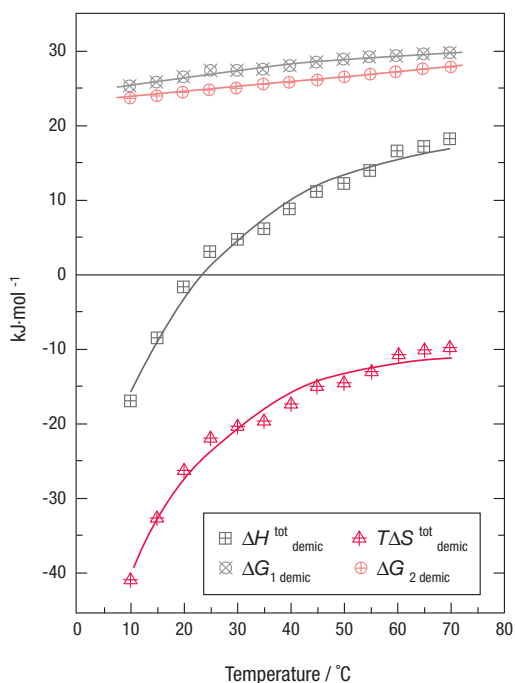


Figure 6. Thermodynamic parameters of demicellisation of sodium oleate (NaO) in water at *pH* 7.5, as a function of temperature.

All thermodynamic data for the demicellisation process are summarised in Figure 6. Two data sets are obtained for ΔG_{demic} because two *cmc* values are found. A strong temperature dependence is observed for the change in entropy and enthalpy, whereas the change in Gibbs free energy is nearly not affected by temperature changes. ΔG_{demic} are positive over the whole investigated temperature range. As can be seen, the total ΔH_{demic} is negative below room temperature and becomes positive above 25°C. At a temperature where $\Delta H_{\text{demic}} = 0$, the formation of micelles is completely entropy driven, which usually happens for ionic detergents at room temperature. At this temperature, the *cmc* shows a minimum in its temperature dependence (Figure 5). At higher temperatures, the driving force for the aggregation becomes more and more enthalpic. The $T\Delta S_{\text{demic}}$ values usually become positive at extremely high temperatures. However, in this case, ΔH_{demic} does not increase linearly with temperature. As a consequence, the $T\Delta S_{\text{demic}}$ plot is also curved and it is not clear whether $T\Delta S_{\text{demic}}$ will ever become zero.

The observed behaviour for the different thermodynamic functions is caused by the hydrophobic effect, which describes an enthalpy-entropy compensation during micellisation [see: Hildebrand et al. 2004].

Information concerning changes in exposed hydrophobic surface area during the demicellisation process is obtained by analysing the change in heat capacity $\Delta C_{p \text{ demic}}$ [Garidel and Blume 1999]. It is defined as:

$$\Delta C_{p \text{ demic}} = (\partial \Delta H_{\text{demic}} / \partial T)_p \quad (5)$$

ΔH_{demic} as a function of temperature (Figure 6) can be fitted by a second order polynomial. Therefore, $\Delta C_{p \text{ demic}}$ shows a linear temperature dependence. $\Delta C_{p \text{ demic}}$ (NaO) in water at *pH* 7.5 is +780 J·mol⁻¹·K⁻¹.

Positive $\Delta C_{p \text{ demic}}$ values indicate a transfer of hydrophobic surfaces into the surrounding water phase [Joliceur and Philip, 1974]. As mentioned previously [Garidel et al. 2000], the contribution of the hydration of the hydrophilic surface areas, and the counter ions dissociation during the demicellisation has to be considered, however the latter is probably very small (~3% of the total contribution of $\Delta C_{p \text{ demic}}$ (NaO)).

Compared to the $\Delta C_{p \text{ demic}}$ values of bile salts, the $\Delta C_{p \text{ demic}}$ (NaO) is much larger [Hildebrand et al. 2004]. This can be explained by the different chemical nature of the amphiphiles.

5. Case Study #2: Physiological Surfactants (Bile Salts)

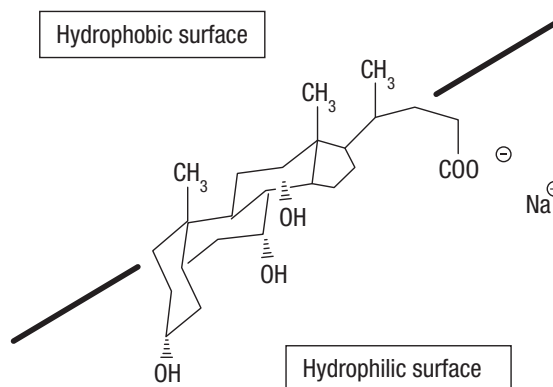


Figure 7. Chemical structure of a bile salt sodium cholate (NaC)

Bile salts are biological surfactants synthesised in the liver by oxidation of cholesterol and are transported via bile into the small intestines. They play a major role in intestinal lipid absorption and are used in pharmaceutical research in formulation development. The behaviour of bile salts is more complex compared to the classical head-tail amphiphiles (see Figure 7).

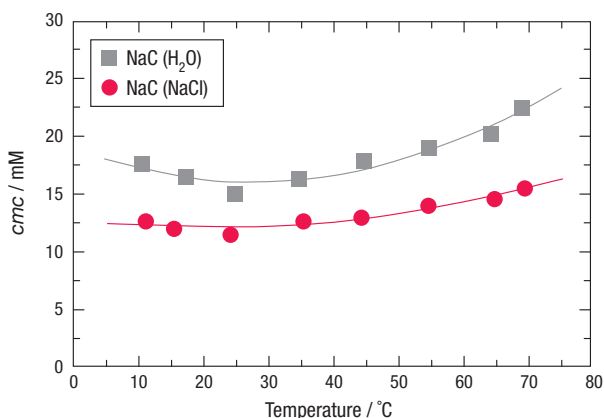
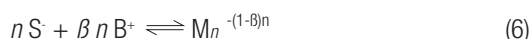


Figure 8. Critical micelle concentration of sodium cholate (NaC) as a function of temperature and ionic strength.

As can be seen from Figure 8, the *cmc* of sodium cholate (NaC) is much higher compared to the *cmc* of NaO (Figure 5). Only one *cmc* is observed for the bile salts. Figure 8 also shows the data for the *cmc* in 0.1 M NaCl. Increasing the ionic strength induces a decrease of the *cmc*. Thus, the micelles are formed at a lower total surfactant concentration, when the ionic strength is increased. This is due to the fact that the higher ionic strength decreases the surface charge of the surfactant micelles by shielding the charges as a result of counter-ion adsorption. As a consequence, it also becomes energetically more favourable for the monomers to self-associate into larger aggregates [see Garidel et al. 2000].

In addition to the analysis presented for the case study #1, it will be shown how the number of molecules associated in the self-organisation of a micelle can be derived from ITC data. The information concerning the aggregation number is obtained from the simulation of the titration curve. The simulation model for the titration curves is based on the mass action model, by additionally taking into account counter-ion binding to the aggregates. The formation of a micelle M_n with n monomers S^- and βn bound counterions B^+ is given by:



The concentrations $[M_n^{-(1-\beta)n}]$, $[S^-]$, $[B^+]$, and the total surfactant concentration c_t^S and the total counterion concentration c_t^B , with $c_t^S = c_t^B = c_t$, are related by:

$$c_t = [S^-] + n[M_n^{-(1-\beta)n}] = [B^+] + \beta n \cdot [M_n^{-(1-\beta)n}] \quad (7)$$

With the equilibrium constant K being defined by:

$$K = [M_n^{-(1-\beta)n}] / ([S^-]^n [B^+]^{\beta n}) \quad (8)$$

one arrives at the following equation:

$$[M_n^{-(1-\beta)n}] = [S^-]^n \{c_t(1-\beta) + \beta [S^-]\}^{\beta n} K \quad (9)$$

The relation between K and ΔG is

$$\Delta G = -R \cdot T \ln K = n \cdot \Delta G_0 \quad (10)$$

with ΔG_0 being the Gibbs energy change of a transfer of a monomer from water into the aggregate.

Equations (7) and (9) are the basis for the simulations of the titration curves.

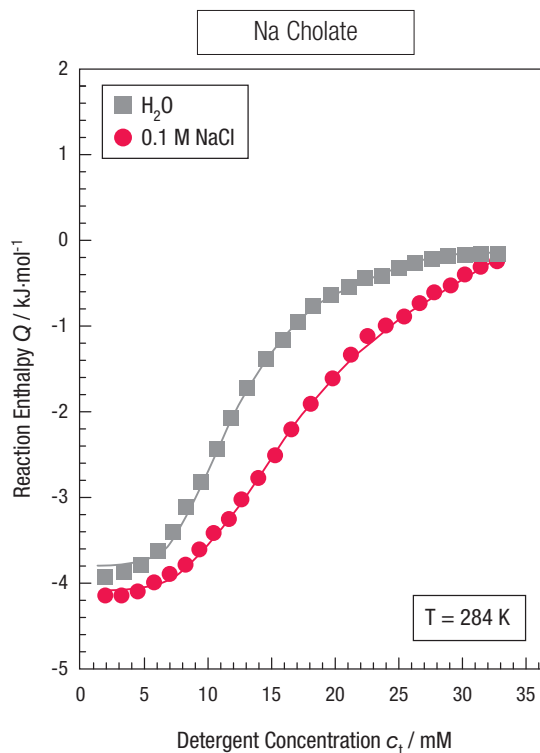


Figure 9. Experimental (data points) and calculated (lines) titration curves for the demicellization process of NaC in H₂O and 0.1 M NaCl (*pH* 7.5) at low temperature. The simulations were performed using the mass action model (adapted from Garidel et al. 2000).

For the simulation of the titration curves' five adjustable parameters, namely the aggregation number n , ΔG_0 , the enthalpy ΔH_{demic} , the dilution enthalpy of monomers $\Delta H_{dil}(mon)$, and the dilution enthalpy of micelles $\Delta H_{dil}(mic)$ were used. The degree of counter-ion binding (β) was introduced as a fixed parameter and values for β were taken from the literature [Coello et al. 1993, 1996].

The experimental data and simulated curves are shown in Figure 9. The parameters obtained from the simulations can be found in Garidel et al. [2000]. For the tri-hydroxy bile salt NaC, the aggregation number at low temperature is $n = 4-5$ and increases only slightly to $n = 6$ with temperature. The influence of the ionic strength is negligible. For NaDC (sodium di-hydroxy cholate) however, the influence of temperature and ionic strength is more pronounced. Aggregation numbers between 5 and 13 are found, depending on the temperature and ionic strength [Garidel et al. 2000]. The calculated values for n are in agreement with previous observations by other groups. Sugioka and Moroi [1998] reported the mean aggregation number for NaC as a function of the total cholate concentration. For $c_{\text{total}} = 16-20$ mM, their values for n are 4-6, in excellent agreement with the data determined by ITC. Figure 10 shows the monomer and micellar concentrations of NaC as a function of the total concentration for measurements at low temperature ($T = 284$ K) as obtained from the simulations. The curves show that the monomer concentration still increases well above the cmc , particularly when no additional salt is present. This shows that the pseudo-phase separation model, in which a constant monomer concentration above the cmc is assumed, is not appropriate.

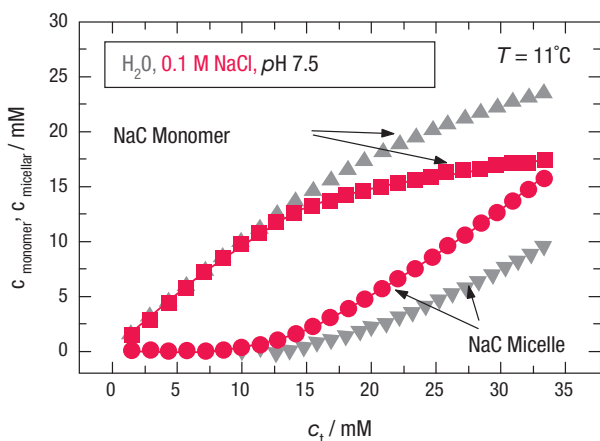


Figure 10. Calculated values for the concentration of detergent in monomeric and micellar form for NaC (pH 7.5) at low temperature. C_{monomer} in H_2O (\blacktriangle) in 0.1 M NaCl (\blacksquare); C_{micellar} in H_2O (\blacktriangledown) in 0.1 M NaCl (\bullet) (adapted from Garidel et al. 2000).

6. Case Study #3: Co-Micelles (Binary Detergent Systems)

Mixed bile salt micelles prepared with phospholipids, or salts of fatty acids like sodium oleate, have been shown to be suitable as drug carriers for parenteral administration because their physiological compatibilities make their application more advantageous as compared to other parenteral formulations. Due to the fact that the colloidal particles are composed of two components, the physical properties of the micelles depend, in addition to environmental conditions (temperature, ionic strength, pH etc), also on the interaction of the two components in the micellar particle. Such co-micelles can be analysed as described in the presented case studies. Here, the properties of preformed co-micelles derived from ITC data concerning the mixing of the components are briefly summarised [for more detail see Hildebrand et al. 2004].

Mixed micelles composed of a bile salt (BS) and NaO at a 5:2 molar ratio, are of great interest because the formation, stability and structure of such co-micelles are formed from two different types of surfactants, the classical “head-tail” detergent NaO and a bile salt with its completely different amphiphilic structure. Furthermore, these mixed micelles are formed during fat digestion.

The ITC demicellisation experiments show a characteristic temperature dependence of the cmc of the mixed micellar systems, with its minimum at room temperature. In general, it is observed that the presence of NaO induces a decrease of the cmc compared to the pure bile salt micelles [Hildebrand et al. 2004]. In contrast to pure NaO, only one cmc is observed.

With the assumption of ideal mixing behaviour, a cmc_{mix} can be calculated using the cmc data of the pure components. These data are then compared to the cmc data as obtained from the experiment to check for the validity of the model and thus gain more insights in the formation of these colloids. Using the model proposed by Lange and Beck [1973] or Clint [1975], respectively, the cmc_{mix} value for ideal mixing is calculated according to:

$$\frac{1}{cmc_{\text{mix}}} = \left(\frac{\alpha}{cmc_{\text{BS}}} \right) + \left(\frac{(1-\alpha)}{cmc_{\text{NaO}}} \right) \quad (11)$$

where α is the total mole fraction of the bile salt.

This relation was adapted for non-ideal mixing behaviour of the components using regular solution theory by Rubingh [1979]. For the non-ideal mixing behaviour an interaction parameter β_I can be calculated from the expression:

$$\beta_I = \left(\frac{\ln \left(\frac{\alpha \cdot cmc_{mix}}{\chi_{BS} \cdot cmc_{BS}} \right)}{(1 - \chi_{BS})^2} \right) \quad (12)$$

where χ_{BS} denotes the mole fraction of the bile salt in the co-micelle, which is obtained by solving the following numerical equation:

$$\chi_{BS}^2 \cdot \ln \left(\frac{\alpha \cdot cmc_{mix}}{\chi_{BS} \cdot cmc_{BS}} \right) = (1 - \chi_{BS})^2 \cdot \ln \left(\frac{(1 - \alpha) \cdot cmc_{mix}}{(1 - \chi_{BS}) \cdot cmc_{NaO}} \right) \quad (13)$$

Thus, a non-ideal mixing behaviour of both detergents is given by $\beta_I \neq 0$. With β_I and χ_{BS} the activity coefficients f_{BS} and f_{NaO} can now be calculated:

$$f_{BS} = \exp(\beta_I (1 - \chi_{BS})^2) \quad (14)$$

$$f_{NaO} = \exp(\beta_I \chi_{BS}^2) \quad (15)$$

According to Rubingh [1979], the cmc values of both detergents have to be corrected by these activity coefficients to calculate cmc_{mix} .

$$\frac{1}{cmc_{mix}} = \left(\frac{\alpha}{f_{BS} \cdot cmc_{BS}} \right) + \left(\frac{(1 - \alpha)}{f_{NaO} \cdot cmc_{NaO}} \right) \quad (16)$$

From the ITC data analysis, it can be seen that the experimental cmc_{mix} lies between the cmc data of the pure components. However, the calculated cmc_{mix} values assuming ideal behaviour are larger than the experimental ones. The difference between the experimental cmc_{mix} and the calculated cmc_{mix} is smaller when the model of non-ideal mixing of Rubingh [1979] is used compared to the simple model assuming ideal [Clint 1975] mixing behaviour.

The difference between calculated and observed cmc_{mix} values increases slightly with increasing temperature. In the temperature range between 20-70 °C, negative β_I data are obtained. Negative values for β_I are characteristic for a deviation from ideal mixing behaviour with attractive interactions of both components. In addition, it was found that with increasing temperature, the mole fraction χ_{NaC} in the mixed micelle increases, i.e. more NaC is

transferred into the mixed micelle. Small deviations from ideal mixing behaviour are observed at low and high temperatures as is derived from the slightly negative β_I . The attractive interaction parameter is attributed to favourable changes in the packing behaviour in the co-micelles and the induced reduction of particle surface charge [Coret et al. 1999].

7. Conclusions

In the presented application note the utility of isothermal titration calorimetry for studying association (physical) colloids, also known as micellar colloids is briefly described. It was not the aim to give a full description of possible ITC experiments, rather to give a feeling how to use ITC.

From the ITC demicellisation experiments, the following data can be obtained or derived from one and the same experiment:

- The critical micelle/aggregation concentration (cmc)
- The demicellisation enthalpy ($\Delta H_{demic} = -\Delta H_{mic}$)
- The demicellisation entropy ($\Delta S_{demic} = -\Delta S_{mic}$)
- The demicellisation Gibbs energy ($\Delta G_{demic} = -\Delta G_{mic}$)
- The aggregation number (n)

In addition, using models describing ideal or non-ideal mixing behaviour, information concerning the interaction and self-association of different amphiphiles in association colloids are obtained.

8. Acknowledgements

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