Journal of Thermal Analysis and Calorimetry, Vol. 80 (2005) 43–47

SATURATION DETERMINATION OF MICELLAR SYSTEMS USING ISOTHERMAL TITRATION CALORIMETRY

Laura J. Waters[1*], *S. A. Leharne*[2] *and J. C. Mitchell*[2]

[1]Department of Chemical and Biological Sciences, University of Huddersfield Queensgate, Huddersfield, HD1 3DH, UK
[2]Medway Sciences, University of Greenwich, Chatham Maritime, Kent, ME4 4TB, UK

A novel method for the determination of the point of micellar saturation has been developed. To exemplify the theory a model system was considered, this being the saturation of two aqueous micellar solvents with dimethyl phthalate ester (DMP).

Upon addition of a hydrophobic compound to an aqueous micellar system partitioning will occur. On further addition, the inner hydrophobic regions will eventually be unable to accommodate any more DMP and, at this specific concentration, the micelle is saturated. With a comparatively large enthalpy change upon partitioning the point of saturation can be determined by a corresponding significant reduction in enthalpy change.

Keywords: *isothermal titration calorimetry (ITC), micelles, partitioning, phthalate ester, sodium dodecyl sulphate*

Introduction

Dialkyl phthalate esters are a group of contaminants commonly detected in sediment, soil and water. They have been linked with a variety of toxic effects including their ability to mimic oestrogen. They are used extensively in the processing of PVC to produce a range of flexible products from the rigid polymeric material. Phthalate esters enter the environment during use and disposal causing disruption of lipid bilayers and non-specific toxicity in organisms [1].

PVC resins have been used for more than forty years and are currently used in building materials, clothing, transportation, medical products [2], perfumes, cosmetics [3], food packaging and pharmaceuticals. Through production, manufacturing, use and disposal, phthalate esters are released into the environment [4].

The widespread production and use of phthalate esters, combined with the fact that phthalates are not chemically bound to the polymeric matrix and are able to migrate from the plastic, makes their environmental fate and subsequent effects relevant. Phthalate esters consist of a benzene ring with two adjoining ester groups, permitting alkyl chain extension thus creating a series of compounds. For example, the basic structure of a dialkyl phthalate ester can be seen in Fig. 1 below.

A number of physicochemical processes affect the distribution of chemicals in the environment along with spatial patterns of use and discharge. Influential factors include biodegradation, hydrolysis, photolysis and photo-oxidation. Knowing the physicochemical properties of phthalates assists in understanding the problems associated with these pollutants. This can be achieved by researching their interaction with micelles. There are two main reasons for such an interest which shall be discussed later, namely surfactant-enhanced subsurface remediation and cell membrane modelling.

A unique characteristic of surfactant molecules is their ability to self-assemble into dynamic aggregates, known as micelles. This phenomenon will only occur at, and above, a certain surfactant concentration, this is known as the critical micellar concentration (CMC) [5]. The CMC is a function of surfactant type and system conditions. There are four main types of surfactant head groups – cationic, anionic, zwitterionic and non-ionic. System conditions include the ne-

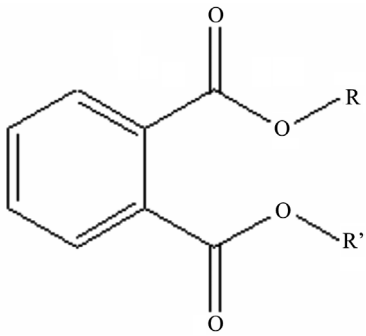

Fig. 1 The structure of a dialkyl phthalate ester (*R* and *R*' are alkyl groups)

* Author for correspondence: l.waters@hud.ac.uk

1388–6150/$20.00

Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

cessity to be above the Kraft temperature [6]. The Kraft temperature is the temperature at which the solubility of the surfactant is equal to it's CMC.

All of these factors combined provide a unique CMC for each surfactant at each temperature. Micelle formation [7–9] distinguishes surfactants from other amphiphilic molecules (for example, octanol) that exhibit a much lower degree of surface activity and do not form micelles. Regardless of size or shape, micelles spontaneously aggregate and disaggregate rapidly within microseconds. The aggregation number is the average number of monomer surfactant molecules per micelle. The surfactant monomers are freely exchanged with monomers in the bulk solution, other micelles as well as surfactants adsorbed on any solid surfaces.

There are two main reasons for an interest in the interaction between dialkyl phthalate esters and micelles. The first of these is the use of micelles as a model biological cell membrane [10]. Controlled transport of molecules and ions across biological membranes is the key to a number of cellular processes. A lipid bilayer provides a barrier that controls the movement of molecules or ions into or out of the cell. The thermodynamic tendency to transport a species through the bilayer is partially determined by an activity gradient across the membrane [11]. An important physicochemical parameter for defining the hydrophobicity of a chemical, which in turn influences toxicity, is the partition coefficient (P). The partition coefficient is a measure of the relative affinity of the solute for the organic and aqueous phases. Traditionally, octanol is used as the non-aqueous phase to mimic the biological membrane. There is a clear need for a more suitable model as there are large discrepancies observed between octanol-water partition coefficients ($P_{o/w}$) and biological membranes [12]. For example, it has been previously found that for many solutes $P_{o/w}$ values were about five times the corresponding values for the erythrocyte membrane/water system [13].

Toxicity can be described in terms of the bioaccumulation factor. The bioaccumulation factor, K_{bio}, can be related to physicochemical equilibrium properties such as the *n*-octanol-water partition coefficient, $P_{o/w}$ [14]. This is based upon the assumption that the entry of the pollutant into the organism is a partitioning process between two phases, the organism and water. All organisms consist of multiple phases but for pollutants, such as phthalate esters, the lipid phase is usually the dominant concentrating phase because of the hydrophobicity of the pollutant.

The second reason for an interest in the interaction between dialkyl phthalate esters and micelles is surfactant enhanced subsurface remediation. Chemical remediation of xenobiotics is an economically and environmentally significant process. Subsurface remediation involves the extraction of subsurface contaminants with above ground treatment for waste processing and management [15]. The hydrophobic sink provided by micelles can significantly increase the mass of contaminant extracted per volume of water pumped thereby overcoming the mass transfer limitations historically experienced in conventional pump-and-treat efforts.

Surfactant selection is paramount to the technical and economic viability of the process. Surfactants that already possess regulatory approval avoid an obstacle as these are already common in food products and other consumer goods, thus deemed acceptable for use.

Many factors must be taken into consideration when choosing a suitable surfactant for remediation as the surfactant chosen must accommodate as much contaminant as possible to maximise subsurface removal. Economically, the surfactant chosen must itself be cheap yet ensure as high a level as possible of remediation is achieved. Therefore, for the process to achieve maximum efficiency, micellar saturation will only occur at a comparatively high xenobiotic concentration.

Isothermal titration calorimetry (ITC) is often used to acquire thermodynamic information on many different systems including binding site interactions [16]. ITC experimental data contains sufficient information to determine the enthalpy change of reaction (ΔH), the association constant (K_a) and the stoichiometry (n) of the interaction. Each interaction has a specific thermodynamic 'signature' and ITC provides a direct route to the complete thermodynamic characterisation of all interactions.

ITC is a differential technique with both reference and sample cell maintained at constant temperature. The reference cell is usually filled with water or buffer and is not involved in the titration. ITC offers many potential advantages over other techniques for characterising chemical and physical interactions. These include high sensitivity, rapid calorimetric response, fast thermal equilibration and high sample through-put [17]. ITC is a convenient tool to determine the critical micellar concentration (CMC) for a surfactant [18, 19] and the enthalpy of micelle formation (ΔH_{mic}) [20, 21].

If a hydrophobic compound is added to an aqueous solution that is sufficiently above the CMC then it is possible to propose a novel application of ITC to determine micellar saturation. Upon addition of a hydrophobic compound to an aqueous micellar system partitioning will occur, similar to that observed in cell membranes. On further addition, the inner hydrophobic regions will eventually be unable to accommodate any more of the hydrophobic compound and, at this specific concentration, the micelle is saturated. As partitioning of a compound between two phases is often accompanied by a change in enthalpy it is possible to investigate the partitioning process using calorimetry.

If the addition of a compound to a purely aqueous solvent involves an insignificant enthalpy of dilution then the point of saturation can be observed by a corresponding apparent reduction in enthalpy change. With the concentration of both amphiphilic molecule and solute known then it is, in theory, possible to determine the saturation limit of a hydrophobic compound.

Experimental

Materials

All ITC experiments were conducted using a Microcal calorimetric unit linked to a Microcal MCS observer. The sample and reference cells were enclosed within an adiabatic jacket and during all experiments were completely filled. The jacket was surrounded by circulating water from a water bath adjacent to the calorimeter maintained at a temperature 10°C lower than that of the experiment.

Experiments initially involved an equilibration period for the sample in the cell. Once equilibration had been achieved the syringe was inserted into the sample cell and stirring commenced, followed by a second equilibration period. Once this equilibration had been achieved injections were made.

For all ITC experiments the reference cell was filled with degassed, distilled water. The mass of the sample was recorded before and after degassing, any discrepancy in mass was replaced with distilled water. Once the sample cell was completely filled a small volume was removed to accommodate the syringe and solution injected. The rotation speed of the syringe was set at 400 rpm, enhancing the mixing of interacting components and reducing the time required for thermal equilibrium to be reached. The enthalpy per injection for the dilution experiments was determined and subtracted from the experimental data. A chemical calibration was conducted prior to every set of experiments utilising the complex formation between barium and 1,4,7,10,13,26-hexaoxacyclooctadecane (18-crown-6) [22]. It should be noted that ITC data provided results in units of cal mol^{-1} therefore all data was multiplied by 4.2 for conversion to J mol^{-1} to simplify analysis of results.

Sodium dodecyl sulphate (SDS), dodecyl trimethylammonium bromide (DTAB) and dimethyl phthalate ester (DMP) were purchased from Sigma-Aldrich Company Ltd., Dorset, United Kingdom, with a minimum purity of 99%.

Experiments were conducted by injecting DMP into an aqueous solution containing SDS micelles. The sample cell was filled with 25 mM SDS in pH 6 phosphate buffer and the syringe was filled with 250 μL of 20 mM DMP in pH 6 phosphate buffer. After 22 injections had been made the solution was left in the sample cell with 0.2 mL s of combined sample removed. The syringe was reloaded with 250 μL of 20 mM DMP in pH 6 phosphate buffer and a second experiment conducted. Again, there was still a significant enthalpic signal at the end of the experiment therefore a further 0.2 mL s of sample was removed from the sample cell and the syringe refilled. The process was repeated until the change in cell feedback signal was insignificant, i.e. less than 2% of the total integrated signal. This was after a total of five experiments.

The second surfactant to be considered was DTAB, a cationic surfactant. The sample cell was filled with 20 mM DTAB in pH 6 phosphate buffer and the syringe was filled with 250 μL of 20 mM DMP in pH 6 phosphate buffer. After 24 injections had been made, the cell feedback signal was still significant therefore the solution was left in the sample cell with 0.2 mL s of combined sample removed. The syringe was reloaded with 250 μL of 20 mM DMP in pH 6 phosphate buffer and a second experiment conducted.

Again, there was still a significant enthalpic signal at the end of the experiment therefore a further 0.2 mL s of sample was removed from the sample cell and the syringe refilled. The three isotherms were then sequentially combined.

Results and discussion

Firstly, ITC experiments focused on SDS as the micellar phase. Experiments followed the protocol outlined in Experimental. After 22 injections had been made, the cell feedback signal was still significant, as can be seen in Fig. 2.

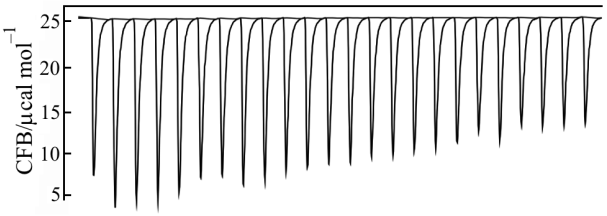

Fig. 2 Raw ITC data for 20 mM DMP injected into 25 mM SDS at 298 K

The process was repeated until the change in cell feedback signal was insignificant, after a total of five experiments, as seen in Fig. 3.

It was assumed that injection of DMP into SDS was athermal when saturation had occurred, thus the saturation limit for DMP in an SDS micelle was calculated. The calculation was based upon calculating the total number of DMP molecules present when the signal became athermal with an aggregation number of 70 and a CMC of 8 mM for SDS. It was calculated that at

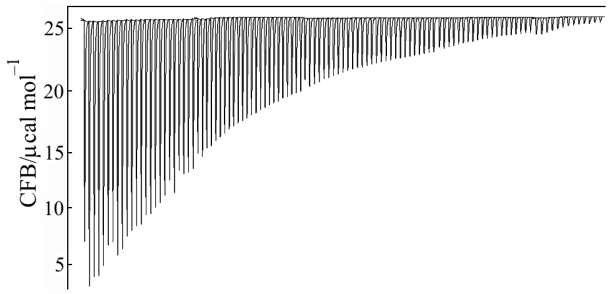

Fig. 3 Raw ITC data for five successive experiments of 20 mM DMP injected into 25 mM SDS at 298 K

saturation, each SDS micelle accommodated 134 molecules of DMP.

Although SDS micelles are clearly capable of accommodating DMP, an alternative surfactant was also considered for comparison – DTAB. After 24 injections had been made, the cell feedback signal was still significant, as can be seen in Fig. 4.

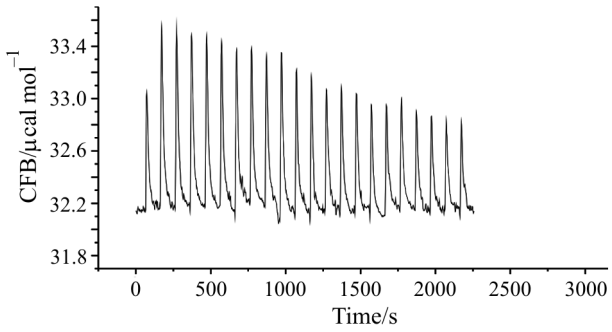

Fig. 4 Raw ITC data for 20 mM DMP injected into 20 mM DTAB at 298 K

The syringe was reloaded and a second experiment conducted. Again, there was still a significant enthalpic signal at the end of the experiment therefore a further set of injections were made. The three isotherms combined can be seen in Fig. 5.

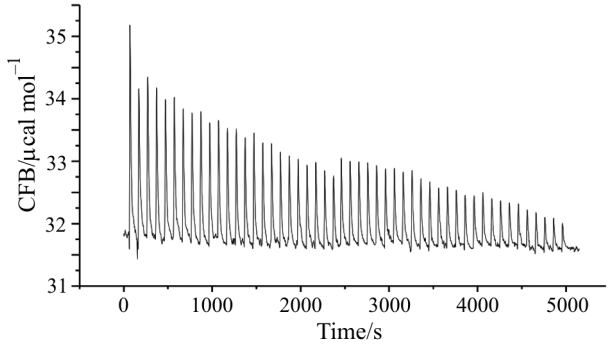

Fig. 5 Raw ITC data for three successive experiments of 20 mM DMP injected into 20 mM DTAB at 298 K

From the results displayed in Fig. 5 it was possible to determine how many molecules of DMP entered each DTAB micelle as the graph displays the cell feedback associated with DMP partitioning into the micelle. It was assumed that injection of DMP into DTAB was athermal when saturation had occurred, thus the saturation limit for DMP in a DTAB micelle was calculated. The calculation was based upon calculating the total number of DMP molecules present when the signal became athermal, an aggregation number of 36 and a CMC of 14 mM for DTAB. It was calculated that at saturation, each DTAB micelle accommodated 58 molecules of DMP.

From these results it can be seen that SDS micelles can accommodate approximately twice as many molecules of DMP per micelle compared with DTAB. It is interesting to note that partitioning of DMP into SDS micelles is an exothermic process whereas for DTAB micelles it is an endothermic process. Knowledge acquired through these experiments aids understanding the partitioning processes involved between DMP and SDS or DTAB micelles. This is subject to further investigation.

It was assumed that injection of DMP into an aqueous micellar solution was athermal when saturation had occurred, thus the saturation limit for DMP in both SDS and DTAB were calculated. This is not the only assumption that must be made for the calculation to be viable; the calculation also assumes that the only thermal event observed is for the solute partitioning into the micelle. This was partially resolved by firstly measuring, then subtracting, the enthalpy of dilution from the final results. The addition of DMP to micelles may have also affected the size, packing density or any other physical properties of the micelle yet it is assumed these events do not occur for the calculation to operate successfully.

Overall, the application of isothermal titration calorimetry to determine micellar saturation with hydrophobic compounds is a novel and useful experimental process.

Acknowledgements

The author wishes to acknowledge Professor A. E. Beezer for useful discussion and the University of Greenwich for financial support.

References

1 D. Brown, R. S. Thompson, K. M. Stewart, C. P. Croudace and E. Gillings, Chemosphere, 32 (1996) 2177.
2 B. Halling-Sørensen, S. Nors Nielsen, P. F. Lanzky, F. Ingerslev, H. C. Holten Lützhøft and S. E. Jørgensen, Chemosphere, 36 (1998) 357.
3 A. M. Api, Food Chem. Toxicol., 39 (2001) 97.
4 P. M. D. Foster, R. C. Cattley and E. Mylchreest, Food Chem. Toxicol., 38 (2000) S97.
5 S. P. Parker, Dictionary of Chemistry, McGraw Hill, New York 1997.
6 A. Blume and T. Zemb, Current Opinion in Colloid Interface Sci., 7 (2002) 66.
7 J. H. Fendler, Membrane Mimetic Chemistry, Wiley, New York 1992.
8 D. W. Armstrong, Sep. Purif. Methods, 14 (1985) 213.
9 D. W. Armstrong, J. Liq. Chromatogr., 3 (1980) 895.
10 R. M. Minardi, P. C. Schulz and B. Vuano, Colloids Surf., A: Physicochem. Eng. Aspects, 197 (2002) 167.
11 P. W. Atkins, Physical Chemistry, 5th Ed., Oxford University Press, Oxford 1994.
12 N. M. Nielson and H. Bundegaard, J. Med. Chem., (1989) 727.
13 P. Seeman, Pharmacol. Rev., 24 (1972) 583.
14 H. O. Esser and P. Moser, Ecotoxicol. Environ. Safety, 6 (1982) 131.
15 D. A. Sabatini, R. C. Knox and J. H. Harwell, Surfactant-enhanced Subsurface Remediation, ACS Symposium Series 594, Washington DC 1995.
16 P. Patnaik, M. Yang and E. Powers, Water Research, 35 (2001) 1587.
17 T. Wiseman, S. Williston, J. F. Brandts and L. Lin, Anal. Biochem., 179 (1989) 131.
18 J. J. Galán, A. González-Pérez and J. R. Rodriguez, J. Therm. Anal. Cal., 76 (2004) 871.
19 A. González-Pérez, J. J. Galán and J. R. Rodriguez, J. Therm. Anal. Cal., 72 (2003) 741.
20 J. J. Galán, A. González-Pérez and J. R. Rodriguez, J. Therm. Anal. Cal., 72 (2003) 465.
21 K. Bijma, M. Blandamer and J. B. F. N. Engberts, Langmuir, 14 (1998) 79.
22 J. R. Horn, D. Russell, E. A. Lewis and K. P. Murphy, Biochem., 40 (2001) 1774.