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DETERMINATION OF MICELLE/WATER PARTITION COEFFICIENTS AND ASSOCIATED THERMODYNAMIC DATA FOR DIALKYL PHTHALATE ESTERS

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Micelle/water partition coefficients for three dialkyl phthalate esters – dimethyl phthalate ester (DMP), diethyl phthalate ester (DEP) and dipropyl phthalate ester (DPP) were obtained by micellar liquid chromatography (MLC). Experiments were conducted over a temperature range which led to calculation of a Gibbs free energy, enthalpy and entropy of transfer for the phthalate esters. In addition, small angle neutron scattering (SANS) experiments were conducted with no substantial change observed in micelle size before and after phthalate ester incorporation.

Overall, a novel method for obtaining thermodynamic information, based on partitioning data, has been developed for dialkyl phthalate esters using micellar liquid chromatography.

Keywords: *micellar liquid chromatography (MLC), micelle size, partitioning, phthalate esters, thermodynamics*

Introduction

Dialkyl phthalate esters are a group of contaminants commonly detected in sediment, soil and water [1]. Although beneficial to society as plasticisers, they have been linked with a variety of toxic effects including their ability to mimic oestrogen [2]. Phthalate esters are still used extensively in the processing of polyvinyl chloride (PVC) resins to produce a range of flexible products from the rigid polymeric material [3]. These compounds impart flexibility and are particularly useful because of their stability, fluidity and low volatility. PVC resins have been used for more than forty years and are currently used in building materials, clothing, transportation, medical products [4], perfumes, cosmetics [5], food packaging and pharmaceuticals.

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 enter the environment during use and disposal causing disruption of lipid bilayers and non-specific toxicity in organisms [6]. Phthalate esters consist of a benzene ring with two adjoining ester groups, permitting alkyl chain extension thus creating a series of compounds – dialkyl phthalate esters. The first three in the series are dimethyl phthalate ester (DMP), diethyl phthalate ester (DEP) and dipropyl phthalate ester (DPP).

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 [7]. It should be noted that hydrophobic compounds, such as dialkyl phthalate esters, can move across biological membranes in two distinct ways – either through passive or active movement with the former being the focus of this work.

An important physicochemical parameter for defining the hydrophobicity of a chemical, which in turn influences toxicity, is the partition coefficient (P). The simplest method for in vitro determination of a partition coefficient is the 'slow stir' method, allowing equilibrium for a compound to be reached between octanol and an aqueous phase whilst minimising the formation of emulsions that can confound experimental results. However, there is limited reliability concerning the octanol-water partition coefficient data available for dialkyl phthalate esters [8]. This is because of the hydrophobic nature of the compounds that introduces practical difficulties with measurement. In addition to the physical constraints upon measurement, octanol may not truly represent biological systems as there are significant structural differences. For example, it has been previously found that

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for many solutes octanol/water partition (P_{OW}) values were about five times the corresponding values for the erythrocyte membrane/water system [9]. Thus, there is a clear necessity for developing an alternative model system which may reflect biological partitioning more closely than the octanol/water method, for example, through the application of micellar liquid chromatography (MLC).

MLC is a form of high performance liquid chromatography involving the use of micelles in the mobile phase. The presence of micelles creates a situation in which different solutes can experience various micro-environment polarities in a given mobile phase. Solutes can remain outside the micelle, associated with the polar head of the surfactant, penetrate into the micelle core or can even form part of the outside palisade layer [10]. Limited previous MLC research has applied the technique to related concerns, for example, the determination of the adsorption coefficient on soil for environmentally significant compounds [11, 12]. Chromatographic methods are suited for the determination of partition coefficients as they often require no sample purification, only a small sample size and achieve a high level of precision [13, 14]. In addition, using micellar liquid chromatography provides an opportunity to calculate a micelle/water partition coefficient [15], (P_{MW}), through Eq. (1).

$$1/K' = \{[(P_{MW} - 1)VC_M]/P_{SW}\Phi\} + (1/P_{SW}\Phi) \quad (1)$$

where K' is the capacity factor, C_M is the micelle concentration (i.e. total surfactant concentration – CMC), P_{SW} is the solute partition coefficient between water and the stationary phase and Φ is the chromatographic phase ratio (ratio of the volume of the stationary phase to the mobile phase in the column). Normalised values are frequently calculated instead of using retention times as the retention time is dependent upon factors such as the flow rate through the system. The normalised value of the capacity factor K', is the time taken for the solvent front to reach the detector (t_0) minus the peak retention time (t_R), divided by t_0, i.e. Eq. (2).

$$K' = (t_R - t_0)/t_0 \quad (2)$$

Experimental K' results over a range of surfactant concentrations provide data to facilitate a plot of $1/K'$ *vs.* C_M. The plot obtained is linear with a measurable slope and intercept hence permitting calculation of P_{MW} using Eq. (3).

$$P_{MW} = s\text{lope}/\text{intercept} \quad (3)$$

Thus, in addition to the retention factor, K', the micelle/water partition coefficient, P_{MW}, can be obtained chromatographically.

To fully understand any chemical system the thermodynamics behind processes that occur within the system must be clearly identified and characterised. As previously discussed, it is possible to determine micelle/water partition coefficients for phthalate esters with MLC. Using the data obtained also permits calculation of the Gibbs free energy from the partition coefficient for each phthalate ester. This is because the change in the Gibbs free energy (ΔG) upon partitioning from one phase into another can be expressed through Eq. (4). Where R is the gas constant and equal to 8.314 J K^{-1} mol^{-1} and T is the temperature at which partitioning is measured in Kelvin.

$$\Delta G = -RT\ln P_{MW} \quad (4)$$

A thorough thermodynamic investigation also requires enthalpic, H, and entropic, S, data. To obtain such data, partition coefficients can be measured over a temperature range and then analysed through the application of the van't Hoff isochore, thus analysis of the partitioning process can be made from a thermodynamic perspective.

It is known that micelle size can be influenced by many factors including the presence of certain compounds [16]; concern was expressed that this size change may occur during these experiments which could affect partition measurements. To eliminate the possibility that results may be affected by the presence of a dialkyl phthalate ester, micellar size measurements were conducted both with and without DMP present; this was chosen as an example system. Small angle neutron scattering (SANS) measurements provide scattering as a function of modulus momentum transfer thus permitting calculation of a mean micelle radius [17–19]. Therefore, it is possible to determine if inclusion of a dialkyl phthalate ester does significantly affect average micelle size.

In this paper the partitioning of DMP, DEP and DPP was investigated using MLC over a range of temperatures to acquire thermodynamic data providing an insight into the nature and extent of partitioning for each compound between two immiscible phases.

Experimental

For all MLC experiments, the mobile phase consisted of sodium dodecyl sulphate (SDS), used as purchased from Aldrich, UK (98%) and diluted with distilled water as necessary to achieve each desired concentration. DMP, DEP and DPP were all purchased from Sigma, UK with a minimum purity of 99.0%.

Degassed mobile phase solvent was pumped through the system using a peristaltic pump (Milton Roy) with a flow rate of 1.35 μL min^{-1}. The reverse phase cyanopropyl column (Spherisorb 5 μL, 15 cm·4.6 mm i.d.) was immersed in a temperature controlled water bath, initially maintained at 298 K. 50 μL samples of solute were injected via a Rheodyne

injector with the solute immersed in a water bath maintained at the same temperature as the column. UV detection (Varian 2550), set at a wavelength of 230 nm, produced a peak indicating the retention of the solute within the column as a function of time. Data was recorded and then analysed to obtain capacity factors using Eq. (2). Each run was repeated three times to ensure reasonable accuracy and precision were achieved.

The mobile phase consisted of an aqueous solution of SDS, over a concentration range 10–30 mM. The mobile phase was diluted after every third run to the next specified concentration using distilled water, left to equilibrate for thirty minutes with retention times then measured at the new mobile phase concentration. From these results capacity factors were determined.

The solute injected was of a standard dialkyl phthalate ester concentration in a surfactant concentration equal to that of the mobile phase. The concentrations of DMP, DEP and DPP were all maintained at a constant value of 1.5 mM.

The column, mobile phase and sample were all maintained at constant temperature using a temperature controlled water bath, having been left overnight at each temperature to allow equilibration to be achieved. From these results it was possible to calculate the micelle/water partition coefficient for DMP, DEP and DPP at each temperature through plotting the SDS concentration (after subtraction of the CMC) with the inverse of the capacity factor and using Eq. (3). Experiments were conducted for all three dialkyl phthalate esters over the temperature range 293–318 K.

A study of partitioning as a function of temperature involves knowledge of the CMC of the surfactant at each experimental temperature. This means that P_{MW} calculated at each temperature involved subtraction of a different CMC. Previous research [20] has established a CMC for each temperature from 293–318 K therefore these values were used in this analysis. Once P_{MW} had been determined, Eq. (4) was used to calculate the change in Gibbs free energy. Application of the van't Hoff isochore to the partitioning data permitted determination of the change in enthalpy, along with calculation of the associated entropy change.

SANS experiments were carried out on the LOQ instrument at ISIS at the Rutherford Appleton Laboratory, UK. Measurements gave the absolute scattering cross section $I(Q)(\mathrm{cm}^{-1})$ as a function of the modulus of momentum transfer (Q), $Q(\acute{\mathrm{L}}^{-1})(4\pi/\lambda)\sin(\check{\mathrm{R}}/2)$, where λ is the incident neutron wavelength (2.2–10 Ĺ) and Ř is the scattering angle (<7°), resulting in an effective Q range of 0.010→0.249 $\mathrm{Å}^{-1}$. All measurements were taken at 298 K and samples contained either 15 mM SDS or 15 mM SDS and 0.2 mM DMP. The samples were in flat quartz cells (1 mm sample thickness) sealed with Teflon stoppers. The $I(Q)$ data obtained was analysed using simple spherical models available in the FISH fitting program [21] available at the Rutherford Appleton Laboratory. For small particles (or micelles) of volume V_p present at number density n_p, the normalized SANS intensity $I(Q)(\mathrm{cm}^{-1})$ can be expressed as in Eq. (5).

$$I(Q)=n_p(\rho_p-\rho_m)^2V_p^2S(Q)P(Q) \quad (5)$$

where ρ_p and ρ_m are the mean coherent scattering length densities of the dispersed phase (*e.g.* particles) and solvent medium, respectively. $P(Q)$ is the single particle form factor describing the angular distribution of the scattering owing to the size and shape of the particle. $S(Q)$ is the structure factor which arises from spatial correlations between particles. Under these conditions $I(Q)$ is a direct measure of $P(Q)$.

Results and discussion

MLC was used to determine micelle/water partition coefficients (P_{MW}) for DMP at six temperatures over the range 293–318 K using the method described in the previous section. Table 1 displays an example set of the chromatographic data collected, K' was calculated from the recorded retention time (Eq. (2)), no significant peak broadening was observed over the concentration and temperature ranges under consideration.

Table 1 Chromatographic data for DMP at 293 K

$[SDS]_{tot}$	CMC[21]	C_M	K'	1/K'
0.030 M	0.008 M	0.022 M	2.56	0.39
0.025 M	0.008 M	0.017 M	3.03	0.33
0.020 M	0.008 M	0.012 M	3.57	0.28
0.018 M	0.008 M	0.010 M	4.16	0.24
0.014 M	0.008 M	0.006 M	5.55	0.18
0.010 M	0.008 M	0.002 M	8.33	0.12

For each P_{MW} calculated it was also possible to use Eq. (4) to determine the change in Gibbs free energy (ΔG) for DMP. The results of these experiments can be seen in Table 2 along with those for both DEP and DPP.

As expected, for all three dialkyl phthalate esters, P_{MW} decreased with increasing temperature as the compounds decreasingly favoured the micellar phase. In addition, DMP is the least hydrophobic of the three with the comparatively lowest P_{MW} values and DPP is the most hydrophobic of the three with the comparatively highest P_{MW} values. Applying the

Table 2 Calculated P_{MW} and ΔG values for DMP, DEP and DPP using MLC from 293 to 318 K

	293 K	298 K	303 K	308 K	313 K	318 K
DMP P_{MW}	135.1	106.2	110.2	90.6	90.0	80.0
DEP P_{MW}	357.8	337.0	330.3	298.9	296.8	287.1
DPP P_{MW}	5064.4	4856.9	4402.8	4272.7	3866.1	3715.8

van't Hoff isochore to the partitioning data in Table 2 facilitated determination of the molar enthalpy of partitioning for DMP (Fig. 1), DEP (Fig. 2) and DPP (Fig. 3). It should be noted that the error bars present on Figs 1–3 arise from the triplicate of experimental results included in the analysis with mean P_{MW} values quoted in Table 2.

For all three dialkyl phthalate esters, the change in enthalpy associated with the partitioning process (ΔH) is negative, i.e. it is an exothermic process. Values do not appear to follow an overall trend with increasing alkyl chain length yet all fall between –7.0 and –16.0±1.4 kJ mol^{-1}. With Figs 1–3 all displaying a linear fit within each data set, it is assumed ΔH is temperature independent within the range of temperatures under consideration in this work.

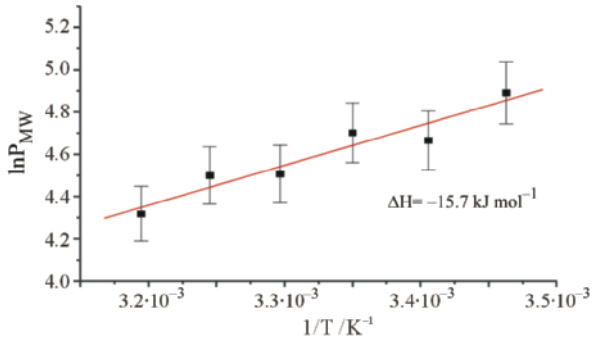

Fig. 1 Application of the van't Hoff isochore for the partitioning of DMP to determine the associated change in enthalpy

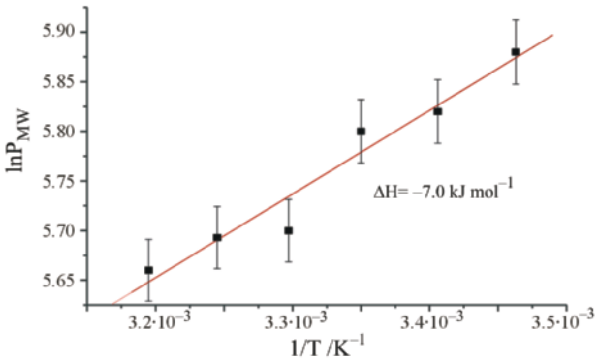

Fig. 2 Application of the van't Hoff isochore for the partitioning of DEP to determine the associated change in enthalpy

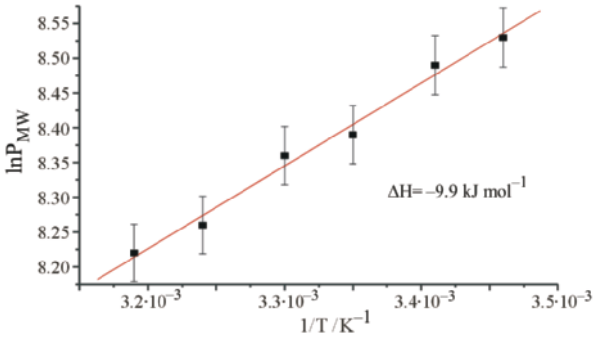

Fig. 3 Application of the van't Hoff isochore for the partitioning of DPP to determine the associated change in enthalpy

Table 3 Thermodynamic data for the micelle/water partitioning of three dialkyl phthalate esters

	ΔG at 298 K/ kJ mol^{-1}	ΔH/ kJ mol^{-1}	ΔS at 298 K/ kJ K^{-1} mol^{-1}
DMP	–11.6±0.2	–15.7±1.4	–0.01±0.01
DEP	–14.4±0.2	–7.0±1.4	0.03±0.01
DPP	–21.0±0.2	–9.9±1.4	0.04±0.01

As previously discussed, if both the change in Gibbs free energy (ΔG) and change in enthalpy (ΔH) are known for a process then entropic data (ΔS) can also be obtained – Table 3.

Based on these thermodynamic values overall it can be seen ΔG values increased in negativity with increasing alkyl chain length, i.e. partitioning of the phthalate became more favourable as the hydrophobicity of the solute increased. However, ΔH values varied with increasing alkyl chain length although all three were negative. This implies the dialkyl phthalate ester partitioned favourably enthalpically for all three. Finally, ΔS values were all small compared with the values calculated for ΔG and ΔH. For DMP the value was marginally negative thus there was a small increase in order in the system yet for DEP and DPP there was a slight decrease overall, i.e. the net process of breaking and reforming bonds had little contribution to the overall thermodynamics implying the hydration shell around the solute played only a minor role in controlling partitioning. Therefore, it can be said that the length of the alkyl chain had no significant effect on the entropy within the system and the partitioning of the solute was enthalpically driven.

Finally, to confirm the presence of phthalate ester had not affected average micelle size, SANS experiments were conducted (Figs 4 and 5).

From the results shown in Figs 4 and 5, there was only a small increase in scattering intensity on inclusion of the DMP due to an increase in micelle size. The fitted lines correspond to a mean micelle radius of 19.0±0.1 and 21.3±0.1 Ĺ for the SDS micelle and

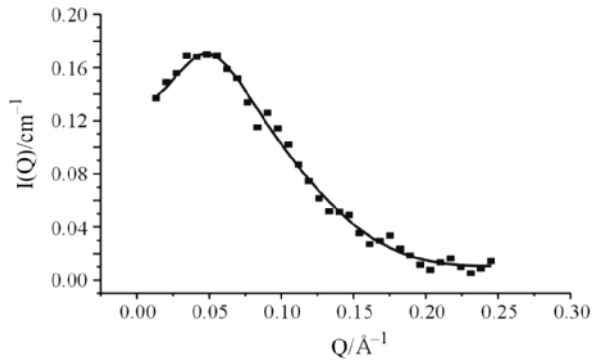

Fig. 4 SANS scattering data for 15 mM SDS micelles in D_2O (squares, raw data; line, FISH fit)

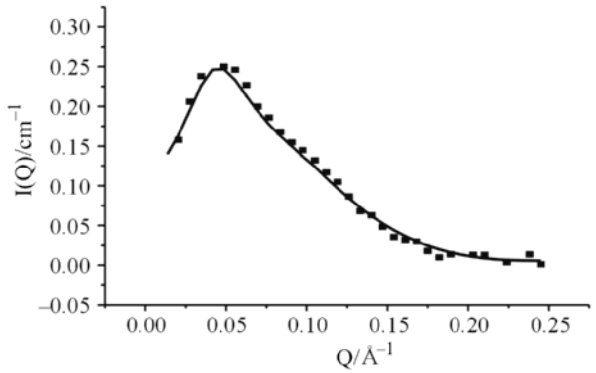

Fig. 5 SANS scattering data for 15 mM SDS micelles in D_2O in the presence of 0.2 mM DMP (squares, raw data; line, FISH fit)

the SDS/DMP system, respectively. This data implies there was no significant change in micelle size before and after dimethyl phthalate ester incorporation which may have affected partitioning measurements and subsequent calculations.

During the course of this work certain assumptions were applied in order to permit calculations to be made. Firstly, the shear rate and stress within an HPLC column can be incredibly high, and may lead to difficulty when comparing with a static technique such as SANS. The agreement in general trend, although actual values differed in magnitude, between partitioning data from these experiments and those for octanol/water partitioning would suggest that this is not a concern in this case. Secondly, the addition of DMP to micelles may have also affected the packing density, aggregation number or any other physical property of the micelles yet it is assumed these events did not occur for the calculation to operate successfully. Thirdly, thermodynamic calculations were based on data from a comparatively small temperature range thus caution should be expressed with respect to their numerical value. Entropic and enthalpic results are primarily presented to display the potential for using MLC to acquire thermodynamic data.

Conclusions

In conclusion, partition coefficients for three dialkyl phthalate esters (DMP, DEP and DPP) have been measured to determine the micelle/water partition coefficient (P_{MW}). A trend can be seen within the three as P_{MW} increases with increasing alkyl chain length.

Changes in Gibbs free energy and enthalpic data were calculated with all three compounds displaying an exothermic partitioning process. Finally, for all three, the change in entropy associated with the process was found to be negligible. However, caution should be expressed when comparing changes in entropy with changes in Gibbs free energy or enthalpy because of the relative size of statistical error in entropy values. It was not possible to compare calculated thermodynamic values with published literature as it is believed no previous work has calculated thermodynamic data for dialkyl phthalate esters partitioning between water and micelles. However, it can be said that P_{MW} values for DMP, DEP and DPP follow a similar trend to known octanol/water values [22] thus indicating the MLC technique is suitable for partitioning measurements. It cannot easily be shown whether or not the values calculated from MLC, compared with octanol/water partitioning, are closer than those that would be truly observed in a biological system. Nevertheless, MLC does present some clear advantages over alternative methods such as the formation of the hydrophobic micelle interior as a bilayer mimic.

SANS data confirmed there was no significant increase in micelle size upon incorporation of a dialkyl phthalate ester, in this case DMP. Again, it was not possible to compare the radius of the micelle with DMP incorporated with literature as it is believed no previous work has considered this. However, the average radius of the ‘empty’ micelle fits well with known literature values [17].

Overall, a novel method for obtaining thermodynamic information, following determination of partitioning data, has been developed for three dialkyl phthalate esters using micellar liquid chromatography. This technique could be applied to a far wider range of compounds than merely dialkyl phthalate esters although these were the focus of this study because of their potential environmental impact as oestrogen mimics.

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