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Micellar chromatographic determination of partition coefficients and associated thermodynamic data for pharmaceutical compounds

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Abstract Micelle/water partition coefficients were obtained for procaine hydrochloride using micellar liquid chromatography (MLC) to illustrate the potential application of this technique to compounds of pharmaceutical interest. Experiments were conducted over a temperature range which led to calculation of a Gibbs free energy, enthalpy and entropy of transfer for procaine hydrochloride. Successful application of this technique was confirmed using a second compound over a range of temperatures, namely caffeine. Overall, this work confirms that MLC can be used to determine precise and accurate partition coefficients that possibly more closely mimic biological membranes than traditional in vitro systems, namely octanol/water.

Keywords Micelle · Partition coefficient · Procaine · Caffeine · MLC

Introduction

An important physicochemical parameter for defining the hydrophobicity of a chemical, which in turn influences pharmacological efficacy, 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. However, there is limited reliability concerning the octanol–water partition coefficient data available for many compounds because of their hydrophobic nature that introduces practical difficulties with measurement [1]. 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 for many solutes octanol/water partition (P_{OW}) values were about five times the corresponding values for the erythrocyte membrane/water system [2]. Thus, there is a clear necessity for developing an alternative model system which may reflect biological partitioning more closely than the octanol/water method [3–6]. To date, this includes some chromatographic methods [7, 8] whereas the work presented in this paper incorporates a novel application of a more recent development, namely 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 [9]. 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 [10, 11] and more recently measurement of partition coefficients for dialkyl phthalate esters [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 MLC provides an opportunity to calculate a micelle/water partition coefficient [15], (P_{MW}), through Eq. 1.

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$$1/K' = (((P_{MW} - -1)V \cdot C_M)/P_{SW}\Phi) + (1/P_{SW}\Phi) \quad (1)$$

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). The normalised value of the capacity factor K', is the peak retention time (t_R) minus the time taken for the solvent front to reach the detector (t_0), 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.

$$P_{MW} = \text{Slope}/\text{Intercept} \quad (3)$$

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

Thermodynamically, calculation of the Gibbs free energy from the partition coefficient is possible 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 methodical 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 should be noted that hydrophobic compounds, such as procaine hydrochloride, can move across biological membranes in two distinct ways—either through passive or active movement, an in vitro simulation of the former being the focus of this work.

Both drugs considered in this work contain ionisable groups, an important consideration when establishing partitioning data. Depending on the degree of ionisation, this will result in an 'apparent' rather than an 'absolute' value for P_{MW}. The ionised drug will have an increased aqueous solubility, compared with the unionised drug, and therefore the 'apparent' P_{MW} will appear lower in value than an 'absolute' value. However, drug absorption in vivo relies upon partitioning across biological membranes and it is therefore the ionised form of each drug that is of relevance to this work.

Initially, procaine hydrochloride was chosen as a model pharmaceutical compound for this research as it is a well recognised and thoroughly characterised material with log P_{OW} values previously established in the range 1.8–2.1 [16–20]. To confirm the successful application of this technique a second, less hydrophobic compound was chosen, namely caffeine with a log P_{OW} value range of −0.07 [21] to −0.08 [22]. This information facilitates a comparative analysis to be made between both literature P_{OW} and measured P_{MW} values.

Overall, this paper investigates the application of MLC to determine partition coefficient values for pharmaceutical compounds over a range of temperatures as a potentially superior alternative to traditionally adopted in vitro techniques.

Materials and methods

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. Procaine hydrochloride and caffeine were used as purchased from Aldrich, UK each with a minimum purity of ≥97%.

Degassed mobile phase solvent was pumped through the system using a peristaltic pump (Milton Roy) with a flow rate of 1.35 mL per minute. The reverse phase cyanopropyl column (Spherisorb 5 μL, 15 cm × 4.6 mm i.d.) was immersed in a temperature controlled water bath. 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 294 nm for procaine hydrochloride and 254 nm for caffeine, 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 30 min 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 concentration (2 mM) in a surfactant concentration equal to that of the mobile phase. The pH of the mobile phase, both with and without solute, was found to be consistently in the range 5–6.

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 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 over the temperature range 295–323 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 [23] has established a CMC for each temperature from 295 to 323 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 for both procaine hydrochloride and caffeine.

Results and discussion

MLC was used to determine micelle/water partition coefficients (P_{MW}) for procaine hydrochloride and caffeine at six temperatures over the range 295–323 K using the method described in the previous section.

Procaine hydrochloride

Table 1 displays an example set of the chromatographic data collected at 310 K, i.e. physiological temperature. K' was calculated from the recorded retention time (Eq. 2), no significant peak broadening was observed over the concentration and temperature ranges under consideration.

A plot of the data displayed in Table 1 provides a linear equation of $A = 79.46X + 0.205$, $R^2 = 0.992$, $n = 5$ and SD = 0.096. Using this data, and Eq. 3, the P_{MW} for procaine hydrochloride at 310 K was found to be 185.8 ± 4.2.

Table 1 Chromatographic data for procaine hydrochloride at 310 K

$[SDS]_{tot}$/M	CMC [16]/M	C_M/M	Av. K'	$1/K'$
0.030	0.008	0.022	7.11	0.14
0.025	0.008	0.017	8.33	0.12
0.020	0.008	0.012	10.00	0.10
0.018	0.008	0.010	14.29	0.07
0.014	0.008	0.006	16.67	0.06

Table 2 Calculated P_{MW} values for procaine hydrochloride using MLC from 295 to 323 K

	295 K	299 K	305 K	310 K	317 K	323 K
P_{MW}	388.6	281.2	201.4	185.8	156.3	121.5

Measurements of $1/K'$ were also determined for procaine hydrochloride over a series of temperatures, namely 299, 305, 310, 317 and 323 K. The resultant P_{MW} values can be seen in Table 2.

It has previously been reported [16–20] that the octanol/water partition coefficient (log P_{OW}) for procaine hydrochloride is in the range 1.8–2.1. MLC provides a similar, yet slightly elevated range for log P_{MW} from 2.59 ± 0.7 (295 K) to 2.08 ± 0.6 (323 K), implying the drug favours the hydrophobic micellar environment slightly more than expected.

For each P_{MW} calculated it was also possible to use Eq. 4 to determine the change in Gibbs free energy (ΔG) for procaine hydrochloride, providing an average value of -13.6 ± 1.0 kJ mol^{-1}. As expected, P_{MW} decreased with increasing temperature as the compound decreasingly favoured the micellar phase. Applying the van't Hoff isochore to the partitioning data in Table 2 facilitated determination of the molar enthalpy of partitioning for procaine hydrochloride (Fig. 1).

The change in enthalpy associated with the partitioning process (ΔH) is -30.0 ± 1.4 kJ mol^{-1}, i.e. it is an exothermic process (assuming ΔH is temperature independent based on the results in Fig. 1).

As previously discussed, if both the change in Gibbs free energy (ΔG) and change in enthalpy (ΔH) are known for a

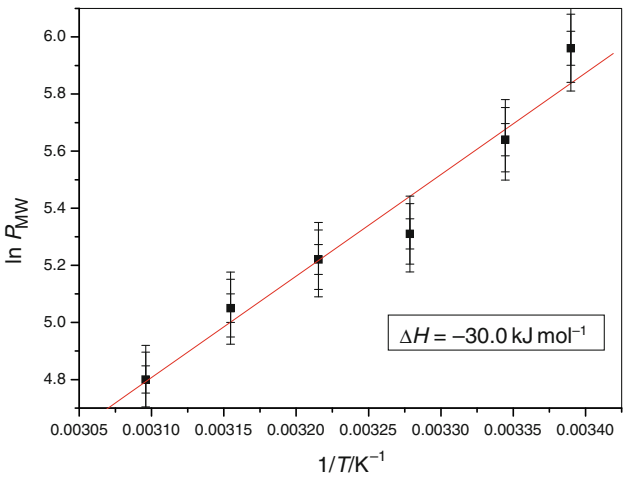

Fig. 1 Application of the van't Hoff isochore for the partitioning of procaine hydrochloride to determine the associated change in enthalpy

process then entropic data (ΔS) can also be obtained; in this case ΔS was found to be equal to -0.05 ± 0.001 kJ K^{-1} mol^{-1} at 298 K. Overall, the negative enthalpy value indicates that partitioning for this compound is enthalpically favourable and that the comparatively small entropy change implies a small increase in order in the system.

It should be noted that the measured pH of the mobile phase is significantly different from the dissociation constants for procaine therefore this work concerns the measurement of the ionised form of the drug. This may result in the partition coefficient appearing slightly lower than expected for the neutral species. However, as MLC is an in vivo mimic it is the ionised form that is of relevance to this work.

Caffeine

To confirm the validity of MLC for measuring partition coefficients for pharmaceuticals a second compound was investigated, namely caffeine. Caffeine was chosen in particular as it is a compound renowned for its hydrophilic nature thus making it an ideal alternative to procaine hydrochloride [24–26]. Again, MLC was utilised to derive P_{MW} values for caffeine over a range of temperatures where Table 3 displays an example set of the chromatographic data collected at 310 K, i.e. physiological temperature.

A plot of the data displayed in Table 3 provides a linear equation of $A = 10.5X + 0.590$, $R^2 = 0.970$, $n = 6$ and SD = 0.025. Using this data, and Eq. 3, the P_{mw} for caffeine at 310 K was found to be 15.9 ± 0.6.

As for procaine hydrochloride, measurements of $1/K'$ were also determined for caffeine over a series of elevated temperatures, namely 305, 310, 317 and 323 K. The resultant P_{MW} values can be seen in Table 4.

Caffeine is renowned for its exceptionally low log P_{OW} value [21, 22] although, based on the results presented in this work, log P_{MW} values appear to be slightly elevated ranging from 1.26 ± 0.01 (299 K) to 1.17 ± 0.01 (323 K). These results imply the drug favours the hydrophobic micellar environment slightly more than in octanol although still significantly less than many other pharmaceutical which tend to have higher log P_{OW} values.

Table 3 Chromatographic data for caffeine at 310 K

$[SDS]_{tot}$/M	CMC [16]/M	C_M/M	Av. K'	$1/K'$
0.030	0.008	0.022	1.15	0.87
0.025	0.008	0.017	1.23	0.81
0.020	0.008	0.012	1.32	0.76
0.015	0.008	0.007	1.39	0.72
0.012	0.008	0.004	1.45	0.69
0.010	0.008	0.002	1.49	0.67

Table 4 Calculated P_{MW} values for caffeine using MLC from 299 to 323 K

	299 K	305 K	310 K	317 K	323 K
P_{MW}	18.3	17.2	15.9	15.0	14.9

The change in Gibbs free energy (ΔG) for caffeine provided an average value of -7.19 ± 0.6 kJ mol^{-1}. As expected, P_{MW} decreased with increasing temperature as the compound decreasingly favoured the micellar phase. As for procaine hydrochloride, applying the van't Hoff isochore to the partitioning data in Table 4 facilitated determination of the molar enthalpy of partitioning for caffeine (Fig. 2).

The change in enthalpy associated with the partitioning process (ΔH) for caffeine is -6.1 ± 0.9 kJ mol^{-1}, i.e. it is an exothermic process (assuming ΔH is temperature independent based on the results in Fig. 2).

In addition, ΔS was calculated to be equal to -0.004 ± 0.001 kJ K^{-1} mol^{-1} at 299 K. Overall, the negative enthalpy value indicates that partitioning for this compound is enthalpically favourable and that the exceptionally small entropy change implies there was no significant increase in order in the system.

As for procaine, it should be noted that the measured pH of the mobile phase is also significantly different from the dissociation constant for caffeine, i.e. partitioning considers the ionised form of the drug. Future work in this area will be concerned with the influence of the mobile phase pH on this experimental system.

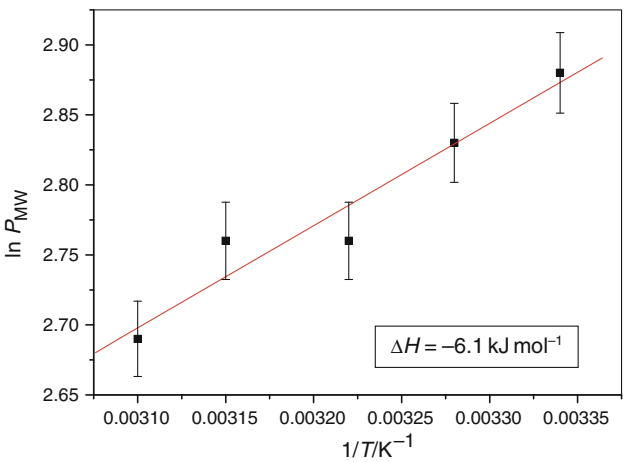

Fig. 2 Application of the van't Hoff isochore for the partitioning of caffeine to determine the associated change in enthalpy

Conclusions

A method for determining the micelle/water partition coefficient for pharmaceutical compounds has been investigated using a model drug to determine the validity of the technique as an alternative to the traditional methods adopted. This has been confirmed using a second compound with both studied over a range of temperatures to derive thermodynamic data. MLC provides several benefits over the standard octanol/water system. Firstly, in that it may more closely mimic a biological membrane, which ultimately leads to more accurate and precise partitioning data. Secondly, it is considerably faster to experimentally determine a partition coefficient value thus speeding up the experimental process. Thirdly, it is comparatively simple to extend the measurements over a temperature range to explore the thermodynamic parameters for each compound thus resulting in enthalpic and entropic data acquisition.

In conclusion, MLC is potentially a suitable and desirable alternative to the traditional methods employed in the determination of partition coefficients, confirmed and exemplified by the results in this work.

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