# Rapid Estimation of Octanol–Water Partition Coefficients using Deoxycholate Micelles in Capillary Electrophoresis

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Deoxycholic acid micelles have been used successfully in capillary electrophoresis to estimate the octanol–water partition coefficient (log *P*) of a number of drugs; the methodology developed is fast and is suitable for the determination of this physico-chemical parameter for neutral, basic and weakly acidic molecules with a variety of structures.

Octanol is probably the most popular solvent used to model lipid membranes. This choice has been influenced to a large extent by the greater solubility of most organic compounds in the latter solvent rather than in other more hydrocarbonaceous media such as hexane. The hydroxy group in octanol also gives it hydrogen-bond donating and accepting properties in a manner similar to biomembranes. The partition coefficient (*P*) of a drug between water and octanol is usually expressed as the logarithm of the ratio of its equilibrium concentrations in octanol and water.[1,2] Log *P* is determined using the 'shake-flask' technique where a solute is allowed to distribute itself between the two phases of octanol (saturated with water) and water (saturated with octanol) before analysis.

Although the above procedure for the determination of log *P* can give accurate data, the methodology is both laborious and time consuming, and the measurement of this parameter for one single drug can take more than one day. It is often the case in drug design that speed is more important than absolute accuracy. Until recently, reversed-phase high performance liquid chromatography (HPLC) has been the only indirect approach that allows a fast estimation of the lipophilicity of organic molecules.[3–5] This technique has been most successful in determining log *P* values of a set of compounds by comparing capacity factors with those of a structurally related sub-set (usually three or four compounds) whose log *P* has been previously determined by the traditional 'shake-flask' method. In the case of unrelated compounds HPLC leads to very inaccurate estimations of log *P* values mainly due to variation in the behaviour of these molecules in their interaction with free (uncapped) silanol groups of the stationary phase.

The introduction of micellar electrokinetic capillary chromatography (MECC),[6] one of the modes of operation of capillary electrophoresis (CE), has provided other opportunities to characterise the hydrophobicity of organic molecules. Recently we have demonstrated[7] that using sodium dodecyl sulphate (SDS) micelles, the capacity factor of a number of analytes was statistically and significantly related to their corresponding log *P* values. Our work was followed by two other publications on the same subject again using either SDS alone[8] or an emulsion consisting of this surfactant and a mixture of butanol and hexane.[9] In the case of SDS alone the behaviour of a number of compounds containing polar moieties such as nitro- and keto-substituents is anomalous; these molecules migrate slower than the corresponding unsubstituted compounds. The introduction of butanol corrects for these anomalies, and may be due to the formation of smaller micelles containing absorbed alcohol.[9]

Here we show that the capacity factors ($k'$) of a number of solutes obtained in MECC using deoxycholic micelles can be correlated with the corresponding octanol–water partition coefficients. This technique is fast, reproducible and avoids complications arising from the addition of organic solvents to the surfactant in the separation buffer.

Under the conditions (ionic strength and bile salt concentration) of the present study, bile salts are expected to form small primary micelles with an aggregation number of about 3 to 4 monomers.[10–12] The micellar shape has been postulated to consist of monomers packed back-to-back with the hydrophobic hydrocarbon moiety forming the interior of the aggregate and the hydrophilic hydroxy groups in contact with the surrounding

**Table 1** Literature octanol–water log *P* values and capacity factors[a] determined in bile salt micelles

| Standard compounds | Octanol–Water Log *P* | Capacity factor ($k'$) 40 mmol dm$^{-3}$ DC[b] pH 8 | Capacity factor ($k'$) 40 mmol dm$^{-3}$ DC[b] pH 9 | Capacity factor ($k'$) 40 mmol dm$^{-3}$ GDC[a] pH 9 |
|---|---|---|---|---|
| benzylalcohol | 1.10 | 0.14 | 0.14 | 0.13 |
| phenol | 1.46 | 0.21 | 0.28 | 0.25 |
| acetophenone | 1.63 | 0.34 | 0.35 | 0.29 |
| nitrobenzene | 1.85 | 0.33 | 0.36 | 0.87 |
| benzene | 2.13 | 0.39 | 0.42 | 0.36 |
| anisole | 2.11 | 0.50 | 0.55 | 0.46 |
| *p*-nitro-methylbenzoate | 2.06 | 0.57 | 0.59 | 0.58 |
| *o*-toluonitrile | 2.08 | 0.61 | 0.64 | 0.52 |
| *m*-nitro-toluene | 2.37 | 0.95 | 1.01 | 4.92 |
| toluene | 2.73 | 1.36 | 1.46 | 1.20 |
| β-naphthol | 2.84 | 2.03 | 2.68 | 2.02 |
| bromobenzene | 2.99 | 2.61 | 2.74 | 2.34 |
| benzophenone | 3.18 | 4.11 | 4.67 | 3.31 |
| *p*-xylene | 3.15 | 4.46 | 4.47 | 3.71 |
| 1-nitronaphthalene | 3.19 | 5.35 | 4.22 | 13.88 |
| phenylphenol | 3.20 | 5.02 | 5.36 | 4.39 |
| naphthalene | 3.30 | 7.64 | 7.20 | 5.69 |
| butylbenzene | 4.26 | 31.76 | 19.87 | 16.88 |

[a] Electrophoresis conditions as in Fig. 1 legend. [b] DC and GDC represent the potassium salts of deoxycholic acid and 3-β-D-glucopy- ranosyl-5-β-cholan-12-α-hydroxy-24-oic acid, respectively.

aqueous medium.[10,12] The interaction of water with these micelles bears some semblance to that of either octanol–water partitioning or of SDS micelles in the presence of butanol.[9]

Using deoxycholic acid and an β-glycosylated form[13] of this bile acid we have measured the capillary electrophoretic migration of 35 analytes, made up of 18 simple organic molecules and 17 drug substances. The $k'$ values were calculated from migration times by eqn. (1),[6] where $t_r$, $t_o$ and

$$k' = \frac{t_r - t_o}{t_o\,(1 - t_r/t_{mc})} \quad (1)$$

$t_{mc}$ are the migration times of the analyte, the electroosmotic front and the micelle, respectively. The value of $t_o$ was measured from the migration time of methanol which is excluded from the micelle whereas $t_{mc}$ was measured from the migration time of the antimalarial drug, halofantrine which completely associates with micelles.[14] As a matter of interest we calculated (Pomona Software) the log $P$ value for halofantrine. The high value of 8.3 obtained again justified its use as a marker for $t_{mc}$.

Capacity factors determined for the 18 standard compounds under different conditions are presented in Table 1. For deoxycholic acid micelles $k'$ values are similar at pH's 8 and 9, despite the fact that some of the analytes are weak acids with expected p$K$a values around 9. Moreover under both conditions the order of migration of these analytes is the same and overall, $k'$ increases with the corresponding octanol–water partition coefficient. It is noteworthy that, unlike the case of SDS micelles,[7,8] nitro- and keto- derivatives migrate before the unsubstituted solutes, indicating that partitioning across deoxycholate micelles occurs predominantly by hydrophobic

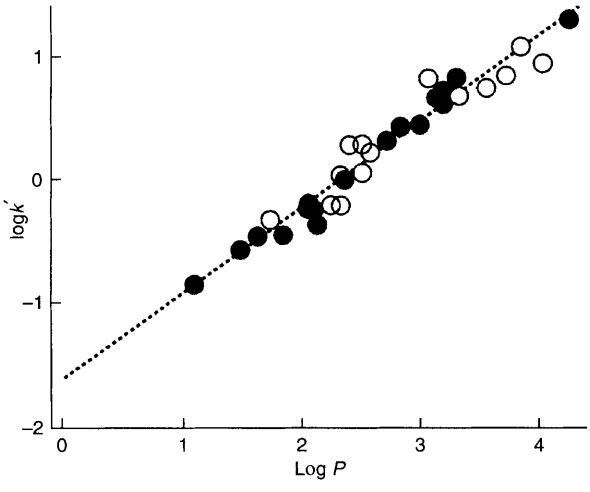


**Fig. 1** Correlation of octanol–water log $P$ with log $k'$ for 32 analytes (●, standard compounds and ○, drug substances). MECC conditions as in Fig. 2, except that separation buffer was carried out at pH 8.

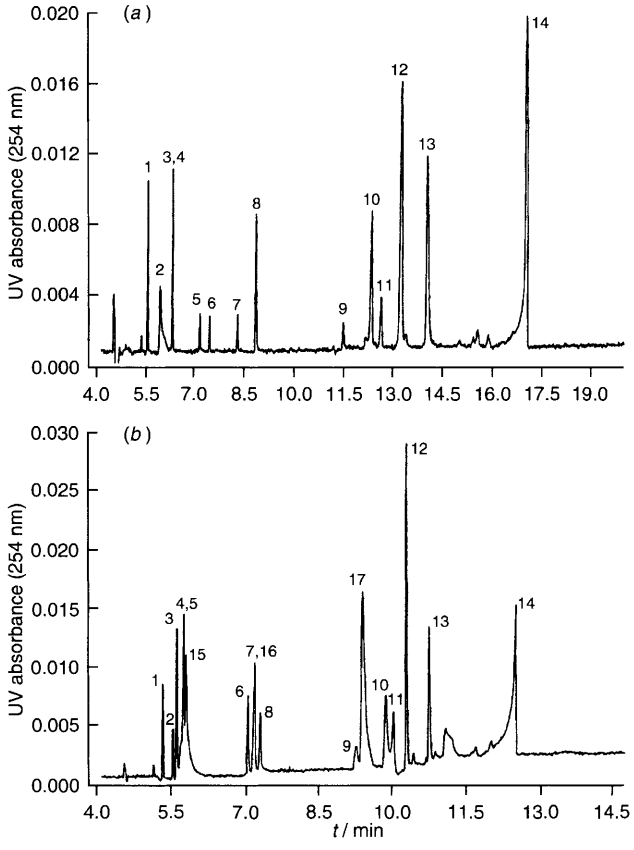


**Fig. 2** Electropherograms of a number drug substances analysed using micelles made up of (*a*) DC and (*b*) GDC: (1) acetaminophen, (2) mephenytoin, (3) lidocaine, (4) cromakalim (5) tolbutamide, (6) warfarin, (7) dexamethasone, (8) ropinirole, (9) testosterone, (10) nabumetone, (11) prazepam, (12) fluoxetine, (13) progesterone, (14) halofantrine, (15) sumatriptan, (16) fenoldopam and (17) propranolol. Buffer consisted of 50 mmol dm$^{-3}$ boric acid adjusted to pH 9 with potassium hydroxide and containing 40 mmol dm$^{-3}$ bile salt; capillary, 50 mm i.d. × 57 cm (50 cm to detector); voltage, 22 kV; detection, 214 nm; 25 °C. Samples (10$^{-3}$–10$^{-5}$ mol dm$^{-3}$), dissolved in aqueous methanol or a 4% aqueous solution of bile salt, were applied by pressure injection for 1 s.

**Table 2** Comparison of measured or calculated log $P$ values with estimates obtained with deoxycholate micelles

| Drug | Biological indication | MECC at pH 8.0 log $P$ | MECC at pH 9.0 log $P$ | Octanol–Water log $P$ |
|---|---|---|---|---|
| acetaminophen | analgesic | 1.6 | 1.7 | 0.510 |
| mephenytoin | anticonvulsant | 1.9 | 1.9 | 1.74 |
| sumatriptan | antimigraine | 2.4 | 2.3 | 0.93 |
| warfarin | anticoagulant | 2.4 | 2.4 | 2.52 |
| dexamethazone | antiinflammatory | 2.6 | 2.6 | 2.58 |
| ropinirole | antiparkinsonian | 2.6 | 2.8 | 2.51 |
| nabumetone | antiinflammatory | 3.5 | 3.4 | 3.08 |
| tolbutamide | antidiabetic | 2.4 | 2.4 | 2.34 |
| testosterone | androgen | 3.3 | 3.3 | 3.32 |
| lidocaine | anesthetic | 2.1 | 2.1 | 2.26 |
| prazepam | antidepressant | 3.5 | 3.5 | 3.73 |
| fluoxetine | antidepressant | 3.7 | 3.6 | 4.05 |
| progesterone | progestogen | 3.8 | 3.8 | 3.87 |
| cromakalim | antihypertensive | 2.1 | 2.1 | 2.32 |
| fenoldapam | renal blood flow enhancer | 2.5 | 2.2 | 2.39 |
| propranolol | antihypertensive | 3.4 | 3.3 | 3.56 |

interaction. Linear regression analysis of log $k'$ values obtained at pH 8 gives eqn. (2).

$$\log P = 0.766\ (\pm 0.027) \log k' - 1.793\ (\pm 0.072) \quad (n = 18, r = 0.988, s = 0.0898) \qquad (2)$$

Essentially the same correlation coefficient ($r = 0.990$) was obtained with the data at pH 9. Using this calibration we tested the applicability of this technique for the estimation of log $P$ values of a number of drug substances with a variety of therapeutic indications. Results are shown in Table 2. For the majority of drugs estimation of log $P$ is acceptably accurate. Two noticeable exceptions are acetaminophen (paracetamol) and sumatriptan. As expected from its low log $P$ value, the migration time of the former analyte is close to that of methanol. However, as $t_r$ approaches $t_o$ estimation of log $P$ becomes less precise. The case of sumatriptan is more difficult to explain. This molecule is highly polar containing a benzimidazole moiety in addition to a tertiary amine and a sulfonamide group and, unlike the other drugs, appears to interact with the micelles by more than one predominant mechanism. Excluding the results for these two drugs we have plotted the remaining data in Tables 1 and 2. The line shown in Fig. 1 is best fit and linear regression gives eqn. (3), similar to that obtained for the standard analytes.

$$\log P = 0.692\ (\pm 0.030) \log k' - 1.610\ (\pm 0.084) \quad (n = 32, r = 0.973, s = 0.130) \qquad (3)$$

Log $P$ values in the range 1–4.5 are suitable for estimation by this MECC method. The partitioning characteristics of molecules containing acidic groups with a p$K$a below 7 cannot be determined as their net negative charge reduces the migration rate of these analytes which travel mainly *via* free-zone electrophoresis.

In Table 1 we have also included the capacity factors of the standard compounds obtained with mono-glucosylated deoxycholic acid.[13] It is interesting to note that increasing the hydrogen acceptor and hydrogen donor capability of the micelles has altered the migration of a number of analytes. Thus when this surfactant is used nitro-substituted compounds now migrate slower than the corresponding parent molecules, whereas the reverse is true for the non-glucosylated deoxycholate. Fig. 2(*b*) shows the migration of a number of drug substances obtained with the glucosylated bile salt. In comparison to data obtained with deoxycholic acid itself [Fig. 2(*a*)] major changes in relative migration times are observed for the more hydrophilic compounds, corresponding to peaks in the first part of the electropherogram.

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