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Evaluation of Partition Coefficients to Micelles and Cyclodextrins via Planar Chromatography[1]

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Abstract: The accurate determination of solute partition coefficients or binding constants to micelles and cyclodextrins is essential in kinetic modeling studies that use these substances. These coefficients are also useful in many other fields where it is useful to know the nature and magnitude of a solute–"pseudophase" interaction. In this work an efficient, inexpensive thin-layer chromatographic method is developed and utilized to determine the partition coefficients of several solutes between water and micelle or cyclodextrin. Advantages of this technique over traditional methods are discussed.

Introduction

The study of the catalytic properties of micelles and cyclodextrins as well as their interaction with a variety of solutes continues to be a significant and rapidly evolving field.[2-6] New kinetic schemes and mechanisms are continually proposed, tested, and revised.[5,6] A variety of functionalized surfactants and cyclodextrins have been synthesized and scrutinized. One constant requirement in these studies is the necessity of accurately and efficiently determining solute binding constants and/or partition coefficients. A wide variety of techniques have been used to determine these constants including UV–visible spectroscopy,[5,8] solubility,[8-10] dialysis,[11] gel filtration,[12-14] vapor pressure,[15] NMR[16], potentiometry,[17] polarography,[18] and HPLC.[19] Each method has advantages and disadvantages. UV spectroscopy, for example, is a particularly popular technique. However, it can only be used if the solute being studied absorbs light in the proper spectral region and if a significant spectral change occurs when the solute interacts with a micelle or cyclodextrin molecule. Most other techniques have analogous restrictions and can also be tedious and time consuming. For most of the aforementioned techniques one must make very accurate measurements in order to avoid large errors.

The HPLC method for the determination of solute partition coefficients to micelles[19] was a direct result of the development of pseudophase liquid chromatography (PLC). It was noted that the method was not restricted to any single type of compound provided a refractive index detector was used. While the HPLC method is a good general technique, one must have access to an appropriate unit and have some chromatographic knowledge in order to use the proper column for the solute of concern. We found that modifying the basic PLC equation and determining developmental gradients for a variety of micellar and cyclodextrin solutions result in a rapid, economical TLC technique to determine partition coefficients of many solutes between micelles or cyclodextrins and water. An additional advantage of this method is that several compounds can be run simultaneously, allowing side-by-side comparisons of substances of similar partition coefficients.[20,21]

Experimental Section

Materials. electrophoresis purity sodium dodecyl sulfate (SDS) was obtained from BioRad Laboratories, cetyltrimethylammonium chloride (CTAC) was obtained from Pfaltz & Bauer, cetyltrimethylammonium bromide (CTAB) and α-cyclodextrin were obtained from Sigma. The CTAB was recrystallized three times from ethanol–water before use. Deionized water was used to make all solutions. Polygram polyamide-6 UV_{254} thin-layer chromatographic sheets (Brinkmann) were used for all determinations.

Methods. All TLC developments were done in a 11.75 in. long, 4 in. wide, and 10.75 in. high Chromaflex developing tank. SDS mobile-phases concentrations were 0.10, 0.20, 0.30, and 0.40 M. CTAC mobile-phase concentrations were 0.0125, 0.025, 0.05, and 0.097 M. CTAB mobile-phase concentrations were 0.005, 0.01, 0.025, and 0.0375 M. α-Cyclodextrin mobile-phase concentrations were 0.025, 0.05, 0.075, and 0.10 M. In addition all solutes were developed with a pure water mobile phase. Compounds with small but finite R_f values in the neat aqueous mobile phase yielded particularly accurate and reproducible partition coefficients. In addition, the less a compound tended to streak the more accurate and reproducible were the determined partition coefficients. In general, one should spot the smallest amount of a compound possible on the TLC plate (providing visualization is still possible).

To evaluate the change in "pseudophase" concentration during development, several polyamide plates were individually developed with different concentration micellar and cyclodextrin mobile phases (vide supra). The plates were then dried and scanned (in the direction of development) with a Shimadzu 910 TLC scanning densitometer (reflectance mode). Plates developed with SDS micelles were scanned at 218 nm, plates developed with CTAC or CTAB micelles were scanned at 226 nm, and plates developed with α-cyclodextrin were scanned at 225 nm.

Partial specific volumes ($\bar{v}$) for CTAC and α-cyclodextrin were found to be 0.977 and 1.122 mL/g, respectively, as determined by established techniques.[22] The partial specific volumes for SDS (0.862 mL/g) and CTAB (0.9987 mL/g) have been previously reported.[22,23]

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Results and Discussion

Equation 1 was originally derived to describe the chromato-

$$\frac{V_s}{V_e - V_m} = \frac{\bar{v}(K_{MW} - 1)}{K_{SW}} C_m + \frac{1}{K_{SW}} \quad (1)$$

graphic behavior in separations involving micellar mobile phases in HPLC,[19] where V_s = volume of the stationary phase, V_m = volume of the mobile phase, V_e = elution volume of a solute, C_m = concentration of micelles in the mobile phase, $\bar{v}$ = partial specific volume of the surfactant in the micelle, K_{MW} = partition coefficient of a solute between the micelle and water, and K_{SW} = partition coefficient of a solute between the stationary phase and water.

It is also known that for ideal chromatographic separations

$$k' = (V_e - V_m)/V_m$$

and

$$R_f = 1/(1 + k')$$

where k' = partition ratio (also known as the capacity factor) and R_f = retardation factor, a parameter used in planar chromatography denoting the ratio of the distance the mobile phase travels over the distance traveled by the solute.

Combining the above two relationships it can be seen that

$$V_e = V_m(1 - R_f)/R_f + V_m$$

By substituting the right side of this expression for V_e in eq 1 and rearranging, one obtains a pseudophase partition equation for TLC:

$$\frac{R_f}{1 - R_f} = \left(\frac{V_m}{V_s}\right)\left(\frac{(K_{MW} - 1)\bar{v}}{K_{SW}}\right) C_m + \left(\frac{V_m}{V_s}\right)\left(\frac{1}{K_{SW}}\right) \quad (2)$$

Ideally, a plot of $R_f/(1 - R_f)$ vs. C_m (the concentration of micelles in the mobile phase) would give a straight line of slope $(V_m/V_s)((K_{MW} - 1)\bar{v}/K_{SW})$ and intercept $(V_m/V_s)(1/K_{SW})$. By taking the ratio of slope over intercept, both V_m/V_s and K_{SW} cancel. Thus one can easily determine K_{MW} provided $\bar{v}$ is known. The same equation can be used to calculate partition coefficients of solutes to cyclodextrin. For a cyclodextrin mobile phase, C_c (rather than C_m) = concentration of cyclodextrin molecules and $\bar{v}$ = partial specific volume of the cyclodextrin in the mobile phase.

For a single solute one would expect to obtain identical values of K_{MW} using either TLC and eq 2 or HPLC and eq 1. This, however, is true only if certain conditions are met. Specificially, the wetting of the dry bed (in TLC) must not significantly affect the partition coefficient, the changing phase ratio (in TLC) must not significantly affect K_{MW}, and the concentrations of micelle or cyclodextrin in the mobile phase should not change during development (i.e., decrease in TLC). The first condition is reasonable for polyamide stationary phases if a solute does not travel near the solvent front (i.e., $R_f < 0.8$). Unfortunately one cannot assume that the concentration of micelle or cyclodextrin in the mobile phase is constant during development in view of reports of surfactant binding to stationary phases[24] and related solvent demixing phenomena in TLC. It is apparent that the concentration of the pseudophase along the length of a developed plate must be determined experimentally. If the pseudophase concentration along the TLC plate remains constant and identical with that in the reservior, then eq 2 can be used to calculate K_{MW}. If the pseudophase concentration on the TLC plate remains constant but lower than that in the reservoir, then eq 2 can be used to calculate K_{MW} provided corrected values of C_m are used. If the pseudophase concentration decreases during development, then one must determine whether the "gradient" is linear or nonlinear. One can use eq 2 to approximate K_{MW} if the gradient is linear by simply plotting the average value of C_m. However, if the gradient is nonlinear, eq 2 must be modified to take this into account.

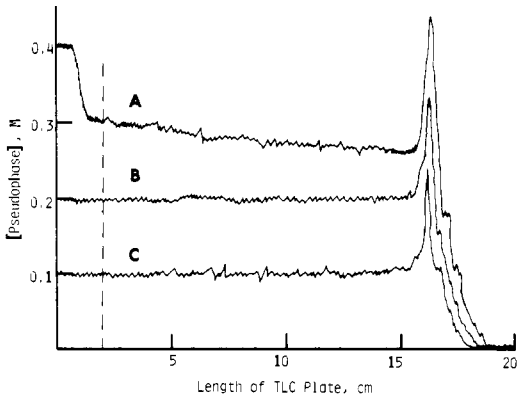

Figure 1. Typical densitometric scans of polyamide TLC plates developed with different micellar or cyclodextrin solutions: (A) scan (218 nm) of a plate developed with 0.4 M SDS; (B) scan (226 nm) of a plate developed with 0.2 M CTAC; (C) scan (226 nm) of a plate developed with 0.1 M α-cyclodextrin. The dotted line at 2.0 cm indicates the point where a compound would be spotted on the TLC plate prior to development. The spike at 16 cm is the discontinuity at the solvent front (impurities often are concentrated there).

Table I. Variation in the Retardation Factor (R_f) of a Series of Compounds with Increasing Concentration (g/ML) of Micelle or α-Cyclodextrin in the Mobile Phase

	concn of CTAC in the micelle[a] (C_m)			
compound	0.004	0.008	0.016	0.031
p-nitrophenol	0.10	0.09	0.17	0.28
p nitroaniline	0.07	0.08	0.14	0.22
DDE[b]	0.04	0.04	0.05	0.08
	concn of CTAB in the micelle[a] (C_m)			
compound	0.0015	0.0034	0.0088	0.0134
p-nitrophenol	0.07	0.09	0.14	0.28
p-nitroaniline	0.07	0.08	0.15	0.21
DDE	0.02	0.03	0.04	0.06
	concn of SDS in the micelle[a] (C_m)			
compound	0.0265	0.0438	0.0524	0.0784
p-nitrophenol	0.24	0.25	0.31	0.35
p-nitroaniline	0.14	0.22	0.19	0.26
DDE	0.12	0.15	0.19	0.26

	concn of α-cyclodextrin (C_c)				
compound	0.0	0.0243	0.0486	0.073	0.0973
p-nitrophenol	0.03	0.13	0.25		0.39
m-nitrophenol	0.04	0.13	0.22		0.34
o-bromobenzoic acid	0.09		0.20	0.25	0.27
m-bromobenzoic acid	0.03		0.22	0.28	0.33
o-aminobenzoic acid	0.09		0.11		0.15
p-hydroxybenzoic acid	0.11	0.27	0.43		0.64

[a] The concentration of surfactant that resides in micelles (excluding free monomer) is $C_m = C - CMC$ where C_m is the concentration of surfactant in the micelle, C is the total concentration of surfactant in the mobile phase, and CMC is the critical micelle concentration (see ref 19). [b] DDE = 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene.

Scanning densitometry of polyamide plates developed with different concentrations of SDS, CTAB, CTAC, and α-cyclodextrin showed two basic types of behavior (Figure 1). Mobile phases containing α-cyclodextrin, CTAB, and CTAC did not appear to change during development. Furthermore the surfactant concentration of the solution impregnating the plate seemed to be identical with that of the reservoir. Mobile phases containing SDS showed a linear decrease in surfactant concentration during development (Figure 1). Consequently, average values for C_m must be used when calculating K_{MW} for SDS micelles. For ex-

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Table II. Least-Squares Analysis and Calculated Partition Coefficients from the Treated Data of Table I[a]

compd	slope	inter-cept	corr. coeff	K_{MW}[c]	log[c] K_{MW}
	CTAC Micelles				
p-nitrophenol	11.40	0.028	0.987	417	2.62
p-nitroaniline	7.90	0.034	0.996	238	2.38
DDE[b]	1.75	0.027	0.966	66	1.82
	CTAB Micelles				
p-nitrophenol	18.06	0.036	0.980	503	2.70
p-nitroaniline	14.71	0.043	0.981	342	2.53
DDE	3.37	0.017	0.982	199	2.30
	SDS Micelles				
p-nitrophenol	4.48	0.186	0.940	28	1.45
p-nitroaniline	4.59	0.061	0.981	87	1.94
DDE	4.28	0.010	0.988	497	2.69
	α-Cyclodextrin				
p-nitrophenol	6.37	0.017	0.998	332	2.52
m-nitrophenol	4.90	0.038	0.999	114	2.05
o-bromobenzoic acid	3.32	0.095	0.970	31	1.49
m-bromobenzoic acid	5.38	0.026	0.994	183	2.26
o-aminobenzoic acid	0.79	0.095	0.980	7	0.86
p-hydroxybenzoic acid	17.36	0.018	0.990	854	2.93

[a] Values of surfactant and cyclodextrin partial specific volumes used to calculate these partition coefficients are given in the Experimental Section. [b] DDE = 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene. [c] K_{MW} values are dimensionless partition coefficients and are different from binding constants (K_b), which have units of M^{-1}. According to Berezin et al.[25] for dilute solutions, these two quantities are related by $K_b = (K_{MW} - 1)V$ where V is the molar volume of the surfactant. Thus values of K_{MW} and K_b for a given solute and micelle or cyclodextrin would not be similar unless they were $\gg 1$ and V approached 1.

ample, when the reservior contains 0.2 M SDS, the average polyamide plate concentration is 0.16 M; for a reservoir of 0.3 M, the plate average is 0.19 M; for a reservoir of 0.4 M, the plate average is 0.28 M.

Retention data for several solutes chromatographed with both micellar and cyclodextrin mobile phases are shown in Table I. Typical plots of these data according to eq 2 are shown in Figure 2. As expected, the plots are linear and when treated according to the method of least squares show a high degree of correlation (Table II). The uncertainty in K_{MW} increases considerably when the intercept is near zero. In these cases small changes in the slope result in relatively large changes in the intercept and therefore in K_{MW}. It is apparent (Table II) that the charge of the micelle plays a major role in solute–micelle interactions. This might be

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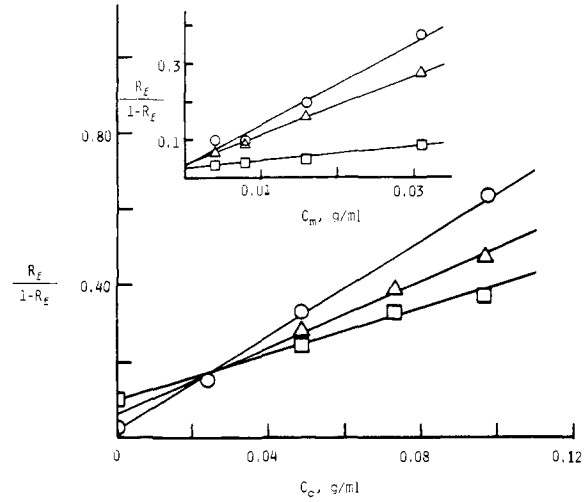

Figure 2. Plots of $R_f/(1 - R_f)$ (for polyamide plates) vs. the concentration of α-cyclodextrin (C_c) or surfactant in the micelle (C_m, insert). In the α-cyclodextrin plot the circles (○) denote *p*-nitrophenol, the triangles (△) *m*-bromobenzoic acid, and the squares (□) *o*-bromobenzoic acid. In the insert plot for CTAB micelles the circles (○) denote *p*-nitrophenol, the triangles (△) *p*-nitroaniline, and the squares (□) DDE. Using eq 2, one can calculate values of K_{CW} or K_{MW} (insert) from such plots by taking the ratio of the slope over intercept.

expected for acidic or basic solutes which can assume a charge opposite to that of the micelle (*p*-nitrophenol or *p*-nitroaniline, for example). However, it is just as true for solutes that are not ionizable (DDE, for example). It is apparent that dipolar molecules and/or molecules with polarizable electrons (e.g., π systems) interact very differently with micelles of opposite charge. Furthermore it is apparent that solutes interact differently with micelles composed of identical surfactants but different counterions (see CTAC and CTAB, Table II). It is well-known that changing the counterions will change the aggregation number, critical micelle concentrations, and fraction of charge of a micelle.[2-4]. These changes are reflected in the K_{MW} values. *It appears, to a large extent, that while the hydrophobic core of the micelle is necessary for solubilizing water-insoluble or weakly soluble compounds, it is often electrostatic effects that determine the degree of interaction.* Hence, electrostatic interactions are largely responsible for the selectivity seen in micellar chromatography.

Planar chromatography provides an efficient, effective way to determine partition coefficients of solutes between water and micelles or cyclodextrin. These K_{MW} values are useful to those involved not only in kinetic studies but also in evaluating the effect of pseudophase structure and type on solute interactions.

Registry No. α-Cyclodextrin, 10016-20-3.