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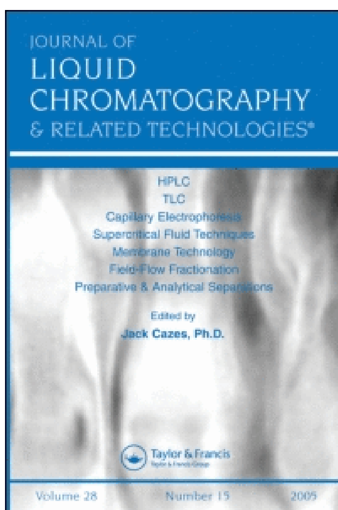

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DETERMINATION OF ASSOCIATION CONSTANTS IN CYCLODEXTRIN OR VANCOMYCIN-MODIFIED MICELLAR CAPILLARY ELECTROPHORESIS

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ABSTRACT

The estimation of association constants of chiral analytes to chiral selectors is often useful in determining the nature and extent of interactions that exist between them. These determinations also provide insight into the mechanisms that are involved in the enantiorecognition of these analytes. Simple models have been developed previously to estimate association constants of analytes binding through a 1:1 stoichiometry with a ligand. These models have also been extrapolated to explain the binding behavior of analytes in systems containing two ligands. In the present study, the binding behavior of some chiral analytes was studied in micellar capillary electrophoresis (CE) systems modified with hydroxypropyl-β-cyclodextrin (HPBCD) or vancomycin as the chiral selectors. The pseudophase or three phase model was used as the basis for the determination of the absolute binding constants of the solutes to the selector and to the micellar phases.

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However, these systems are complicated due to the interactions that exist not only between the analyte and the chiral selector/micellar phase but also between the ligands themselves. In the case of the hydroxypropyl-β-cyclodextrin/sodium dodecyl sulfate (SDS) system, the SDS monomers are included in the cyclodextrin cavity. Similarly, in the vancomycin/SDS system, vancomycin partitions itself into micelles. The limitations associated with the use of ideal mathematical models in these complex systems, are also discussed.

INTRODUCTION

Cyclodextrins are one of the more widely used selectors for chiral separations in CE. Although native cyclodextrins are used for specific applications,[1,2] there has been a constant search for better chiral selectors compatible to CE systems. Recently, several derivatized cyclodextrins have been introduced for use in CE.[3-5] These derivatized cyclodextrins are effective because they frequently have higher solubility, somewhat different enantioselectivities and many of them are charged. Another class of selectors that have proved to be very useful in chiral CE are the macrocyclic antibiotics.[6-8] The application range of commonly used chiral selectors was further expanded when they were used in conjunction with micellar media.[9-11] Enantiomeric separations based upon the selective partitioning of solutes between a chiral selector and a micellar pseudophase has been proven to have numerous applications in CE, particularly in the separation of neutral and hydrophobic compounds in aqueous run buffers.[12,13]

Several approaches have been utilized to measure the extent of binding of analytes to chiral selectors and/or micellar media and to help to understand some of the mechanistic features underlying enantioseparations. Many theoretical models (often derived from chromatographic systems) have been developed to determine the association or binding constants of solutes to commonly used chiral selectors or pseudophases.[3,10,14] Most of these studies have been done on simple systems involving a 1:1 association of a solute to a single ligand. Shibukawa et al.[15] studied the extent of binding of leucovorin and related compounds to native γ-cyclodextrin. Gahm and Stalcup[4] determined the extent of binding of dinitrobenzoylated amino acids to naphthylethylcarbamate-derivatized cyclodextrin, and Penn et al. measured the binding of tioconazole enantiomers to hydroxypropyl-β-cyclodextrin.[16] Similarly, the binding of solutes to micellar systems has been evaluated.[17] A variety of experimental, mathematical and graphical approaches have been used to determine these binding constants.[17] Equations have been developed

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that allow the determination of equilibrium constants, with reasonable accuracy, for solutes binding in a 1:1 stoichiometry with a ligand. However, very few binding studies have been done for more complex systems such as those containing a polyvalent ligand[18] or systems where a solute is capable of binding to two different ligands.[10,19] In these cases, modified equations have been developed to determine the respective binding constants of the solute to each ligand.

In the present study, the binding constants of analytes to hydroxypropyl-β-cyclodextrin in sodium dodecyl sulfate (SDS)-modified run buffers were determined using the modified equation proposed for the vancomycin-SDS system.[10] Also, an attempt was made to recalculate some of the binding constants determined for solutes to the vancomycin-SDS system, using the simpler graphical plotting methods outlined in an earlier work,[17] instead of the more involved method adopted in a previous vancomycin-SDS study.[10] The difficulties and inconsistencies encountered in the accurate determination of binding constants in a multicomponent/multipseudophase system using these methods are discussed.

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EXPERIMENTAL

The hydroxypropyl-β-cyclodextrin (average molar substitution = 0.6) used in this study was obtained from Astec (Whippany, NJ). Vancomycin hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO) and SDS was obtained from Bio-Rad (Richmond, CA).

The monosodium hydrogen phosphate used to prepare the run buffer was purchased from Aldrich (Milwaukee, WI). All the analytes evaluated were obtained from Aldrich (Milwaukee, WI) or from Sigma Chemical Co. (St. Louis, MO) .

Capillary electrophoresis was performed using a 50 μm x 50 cm (57 cm to the detector) capillary at 15 kV, using a PACE 2100 CE unit (Beckman Instruments, CA). All analytes were detected using on-line UV detection at 254 nm. All experiments were carried out at 25° C. A 0.05 M phosphate buffer (pH = 7) was used as the run buffer.

The fused silica capillaries used in this study were purged for 10 minutes with a 0.1M KOH solution, followed by a water rinse for 5 minutes, at the beginning of each day.

Subsequent to each run, the capillary was rinsed with KOH, water and the run buffer for two minutes each. Methanol (Fisher Scientific , NJ) was used as the electroosmotic flow (eof) marker and quinine hydrochloride (Aldrich, WI) was used as the micelle marker. All buffers and analyte solutions were filtered using 0.45μm filters (Alltech, IL), before introduction into the capillary.

Statistical treatment of the data obtained was performed using the weighted linearized least square software.[20] A surface tensiometer (model 20, Fisher, St.Louis, MO) was used to perform surface tension measurements.

RESULTS

Simple pseudophase models can be used to describe the association of an analyte with various components in solution (e.g., chiral selectors, micelles, etc.).[10,17,21] Each distinct molecular or aggregational component in solution that participates in the separation (in an associative manner) can be treated as a distinct phase or more accurately, as a pseudophase. This includes the bulk solution (that can contain salts, buffer, etc., but not other pseudophase components) which is treated as a pseudophase. This terminology and approach was developed in the early days of micelles and cyclodextrin-based separations.[22-25] For example, when both micelles and cyclodextrins are present in the run buffer, the three pseudophases would be: a) the micelle b) the cyclodextrin, and c) the rest of the bulk solution. If two different cyclodextrins (one charged and one uncharged, for example) were used,[26] then each would constitute a distinct pseudophase.

In evaluating the binding of analytes to the selector and the micelle in the hydroxypropyl-β-cyclodextrin / SDS and vancomycin / SDS systems, the pseudophase model was used. Since it is expected that the mobility of the free analyte would be different from its mobility when bound to the selector or the micelle pseudophase, it is envisaged that the experimentally determined mobility of the solute (μ_i) is a weighted average of its mobilities in each of the three pseudophases.[2,10] Based on the three phase model, an equation was previously derived to determine the binding constants of a solute to the chiral selector and the micelle.[10] A general form of this equation is:

$$K_{mic} = \frac{1}{[M]_f}\left(\frac{\mu_f - \mu_i}{\mu_i - \mu_m}\right) + \frac{K_{sel}[S]_f}{[M]_f}\left(\frac{\mu_s - \mu_i}{\mu_i - \mu_m}\right) \qquad (1)$$

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where K_{mic} and K_{sel} are, respectively, the equilibrium constants for binding of the solute to the micelle and of the solute bound to the selector. $[M]_f$ refers to the equilibrium concentration of the free micelle, $[S]_f$ is the equilibrium concentration of the free selector existing in solution . Also, μ_f represents the mobility of the free solute in the aqueous phase, μ_s is the mobility of the solute-selector complex and μ_m is the mobility of the solute-micelle complex. μ_i is the experimentally measured mobility of the solute at a given concentration of the selector and the micelle. This equation requires the independent determination of μ_s and K_{sel} in order to determine K_{mic}. The value of μ_s was estimated using the relationship $\mu \propto f/M^{2/3}$ where μ is the mobility of the solute, f its fraction of charge and M is its molecular weight. By plotting mobility versus $f/M^{2/3}$ for standards of known charge and molecular weight, the mobility μ_s for the appropriate selector was determined.[10] This value of μ_s was then substituted into the equation, $K_{sel}[S]_f = (\mu_f - \mu_i)/(\mu_i - \mu_s)$ to determine K_{sel}.[10,16] The K_{sel} value, thus determined, was substituted into equation (1) to obtain K_{mic}. In order to circumvent the use of this procedure in this study, μ_s was determined by the graphical method outlined earlier.[17] The double reciprocal method was used, in which $1/(\mu_I - \mu_f)$ was plotted as a function of change in selector concentration. By measuring the slope and intercept of the linear plot obtained, μ_s and K_{sel} were calculated. The advantage of this method is that actual mobility of the solute-ligand complex, which is usually measured as the limiting mobility of the solute at saturating ligand concentrations, is not required to be determined for calculation of K_{sel} and hence a range of inconveniences and errors associated with its measurement can be avoided.[17] In an attempt to develop a method to simultaneously determine K_{sel} and K_{mic}, equation (1) was rearranged as follows :

$$\frac{K_{mic}}{K_{sel}} = \frac{1}{[M]_f K_{sel}}\left(\frac{\mu_f - \mu_i}{\mu_i - \mu_m}\right) + \frac{[S]_f}{[M]_f}\left(\frac{\mu_s - \mu_i}{\mu_i - \mu_m}\right) \quad (2)$$

Equation (2) can be re-expressed as :

$$\frac{[S]_f}{[M]_f}\left(\frac{\mu_s - \mu_i}{\mu_i - \mu_m}\right) = \frac{K_{mic}}{K_{sel}} + \frac{1}{[M]_f K_{sel}}\left(\frac{\mu_i - \mu_f}{\mu_i - \mu_m}\right) \quad (3)$$

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By plotting $\frac{[S]_f}{[M]_f}\left(\frac{\mu_s - \mu_i}{\mu_i - \mu_m}\right)$ on the Y axis and $\frac{1}{[M]_f}\left(\frac{\mu_i - \mu_f}{\mu_i - \mu_m}\right)$ on the X axis for the linear equation (3), K_{sel} and K_{mic} can be conveniently and simultaneously determined using the relationships

$$K_{sel} = \frac{1}{\text{slope}} \text{ and } K_{mic} = \frac{\text{Intercept}}{\text{Slope}}.$$

In determining K_{mic} using equation (3), K_{sel} and μ_s were proposed to be determined simultaneously using the double reciprocal plot.

Determination of Binding Constants of Analytes in the Hydroxypropyl-β-Cyclodextrin System

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The mobility of an analyte bound to hydroxypropyl-β-cyclodextrin (μ_{cd}) was determined by measuring the mobility of the analyte at various concentrations of hydroxypropyl-β-cyclodextrin [HPBCD] and plotting $1/(\mu_i - \mu_f)$ versus 1/[HPBCD].[17] Figure 1 shows the double reciprocal plots obtained for the first eluted enantiomer of some of the analytes studied. It must be noted that in the calculation of μ_i and μ_f the migration times were corrected in order to minimize effects of changes in the mobility of the solute due to changes in the viscosity of the solutions at various hydroxypropyl-β-cyclodextrin concentrations.[15,17] From the slopes and intercepts of these lines, K_{sel} was calculated as the ratio of intercept to slope and μ_{cd} was calculated from the intercept.[17]

The binding constants obtained for various analytes to hydroxypropyl-β-cyclodextrin using this method are shown in Table 1. After having determined μ_{cd}, the concentration of SDS in the run buffer was varied while maintaining a constant concentration of hydroxypropyl-β-cyclodextrin. The mobility of the analyte was determined at each of these concentrations. The mobilities thus measured were then substituted into equation (3) in order to directly and simultaneously determine K_{sel} and K_{mic} as mentioned above. The K_{sel} measured in this manner could be expected to be compared to K_{sel} previously calculated for the binding of the analyte to hydroxypropyl-β-cyclodextrin using the double reciprocal plot. However, it was found that this approach yielded negative values of K_{sel}.

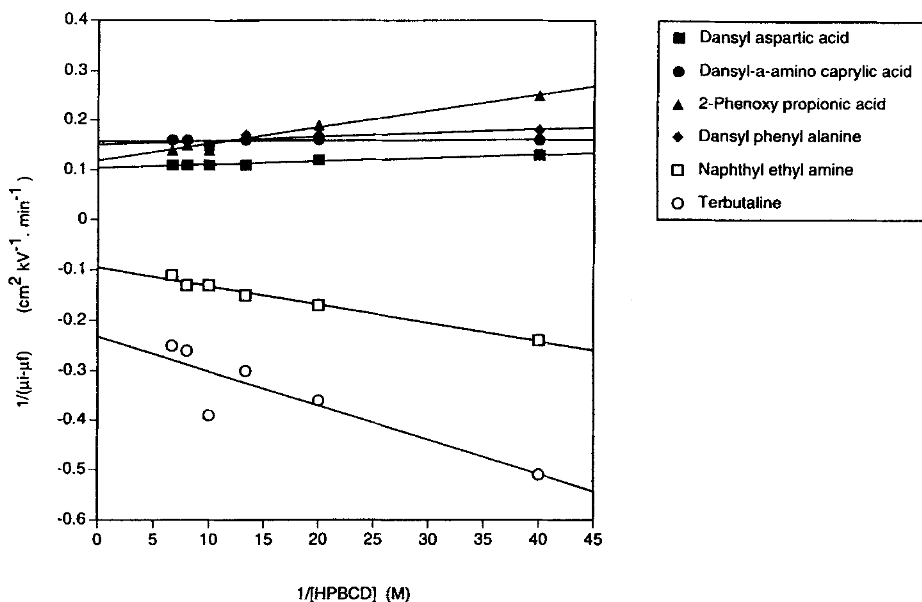

Figure 1. Double reciprocal plots obtained for solutes at various concentrations of hydroxypropyl-β-cyclodextrin.

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Table 1

Binding Constants of Chiral Solutes to Hydroxypropyl-β-Cyclodextrin

Compound	$\mu_{cd}1$	$\mu_{cd}2$	$K_{cd}1$ (M^{-1})	$K_{cd}2$ (M^{-1})	$K_{mic}1$ (M^{-1})	$K_{mic}2$ (M^{-1})
Dansyl aspartic acid	-7.26	-7.29	76±10	68±8	5.3±0.6	5±0.6
Dansyl-α-amino caprylic acid	-2.79	-2.95	236±22	197±16	242±72	221±66
2-Phenoxypropionic acid	-5.12	-5.39	26±2	24±3	2.4±0.1	2.3±0.3
Dansyl phenylalanine	-3.52	-3.83	255±39	210±39	87±14	77±13
Naphthylethylamine	0.52	0.05	22±2	21±2	327±50	326±50
Terbutaline	3.94	3.50	46±12	42±16	196±38	193±39

These values of K_{sel}, which did not have any physical meaning, could be attributed to the fact that the rearranged equation (3) involves the use of a (μ_i - μ_f) term. In the SDS micellar systems, the mobility of the solute (μ_i) tended toward more negative values as compared to m_f of the solute in phosphate buffer. Therefore, (μ_i -μ_f) is always a negative quantity. Thus, the values plotted on the X-axis were always negative values while those plotted on the Y-axis were positive values. Such a plot yielded a line that always had a negative slope and usually a positive intercept, which when used in further calculation of K_{sel} yielded negative numbers. Thus, equation (3) could not be used for further determinations of K_{mic}. The method for determining K_{mic} in this multipseudophase system had to be restricted to the mathematical substitution of K_{sel} and μ_{cd} (obtained from the double reciprocal plots) into the original equation (1) rather than from the graphical estimation from equation (3). The K_{mic} values obtained in this manner are shown in Table 1.

It might be expected that the binding constants determined in the mixed micelle/cyclodextrin system should correlate well with the individual binding constants determined separately for the solutes binding 1:1 to hydroxypropyl-β-cyclodextrin or to SDS micelles. However, this may not be the case if interactions exist between hydroxypropyl-β-cyclodextrin and SDS. In order to ascertain if such an interaction existed between hydroxypropyl-β-cyclodextrin and SDS, surface tension measurements were carried out to determine the critical micelle concentration (cmc) of SDS at various concentrations of hydroxypropyl-β-cyclodextrin dissolved in 0.05 M phosphate buffer (pH 7). The cmc data obtained are compiled in Table 2. Clearly, the cmc increases almost linearly with the concentration of hydroxypropyl-β-cyclodextrin. This sort of phenomenon occurs when the solution concentration of free surfactant is reduced thereby increasing the apparent cmc.

It can be deduced from these data that there is considerable interaction (inclusion) of SDS monomers with the hydrophobic cavity of hydroxypropyl-β-cyclodextrin. Hence, in systems containing both micelles and cyclodextrins, the analyte would have to compete with monomeric SDS (which exists in equilibrium with micellar SDS) to form an inclusion complex with the hydroxypropyl-β-cyclodextrin. The competitive analyte/cyclodextrin binding constants in this more complex system could be very different from those measured in the absence of SDS. Obviously, competitive binding effects could affect the resolution and the separation mechanism of these analytes in capillary electrophoresis as well.

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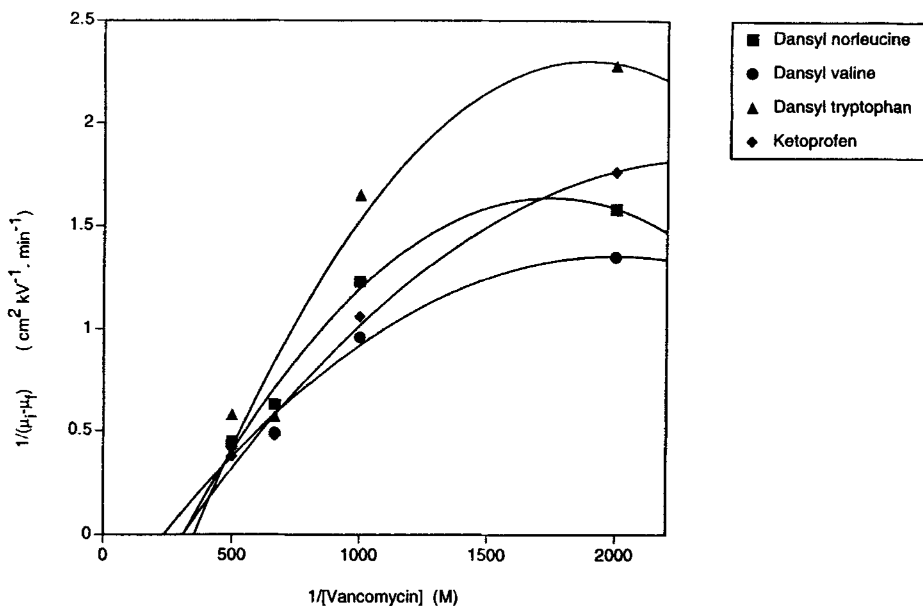

Figure 2. Double reciprocal plots obtained for solutes at various concentrations of vancomycin.

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Table 2

Variation of the Critical Micelle Concentration of Sodium Dodecyl Sulfate with Hydroxypropyl-β-Cyclodextrin Concentration

Concentration of of HPBCD (mM)	CMC of SDS (mM)
3	3.8
5	5.8
10	10.6
25	23.2
50	45.9
75	59.8
100	86.2

Determination of Binding Constants in the Vancomycin System

As in the case of the hydroxypropyl-β-cyclodextrin/SDS system, the first step in the determination of binding constants of analytes to the vancomycin and micelle respectively, was to determine μ_s and K_{sel}. The graphical procedure adopted in the hydroxypropyl-β-cyclodextrin system was used to determine μ_{vanco} so as to estimate the extent of binding of solutes to the vancomycin selector. In order to do this, each solute was injected at various concentrations of vancomycin and their mobilities were determined from their migration times. Uncorrected migration times were used in the calculation of mobilities of the solutes in the vancomycin system.[10]

Figure 2 shows the double reciprocal plots obtained for the first eluted enantiomer of some of the solutes evaluated in the vancomycin system. The plot of $1/(\mu_i-\mu_f)$ versus 1/[vancomycin] made for these solutes was unlike the plots in the hydroxypropyl-β-cyclodextrin system. It is evident that the reciprocal of the mobility difference varied non-linearly with the reciprocal of the vancomycin concentration (Fig. 2). The values of μ_{vanco} and K_{vanco} cannot be determined from these plots (in the same manner as was done in the determination of μ_{cd} and K_{cd}) since the slope and intercept of a linear plot are required for determining these constants. It is envisaged that the non-linearity of these plots is a manifestation of the wall binding property of vancomycin.[6,10] It is evident that the simpler graphical procedure cannot be used in the direct determination of μ_{vanco} and K_{vanco} for further mathematical substitution into equation (1) to determine K_{mic}. Hence, the indirect estimation of μ_{vanco}, as was done in the previous study,[10] may be the only method suitable for the estimation of this parameter. For this reason, further recalculations of binding constants of the analyte to vancomycin and micelles in the vancomycin/SDS system were not repeated in this study.

DISCUSSION

The association of an analyte to two different ligands or pseudophase components can best be evaluated by equation (1) when there is little or no interaction between the ligands themselves. However, in the case of the hydroxypropyl-β-cyclodextrin/SDS system, the SDS monomer interacts with the cyclodextrin cavity. Since the interactions between hydroxypropyl-β-cyclodextrin and SDS do not significantly alter the binding behavior of the solute to each ligand, equation (1) can still be used to quantitate these interactions. However, it must be understood that the binding constant of an

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analyte to cyclodextrin may be different in the presence of surfactant as compared to the identical measurement in the absence of the surfactant. The vancomycin/SDS system is more complex. In this case, the vancomycin substantially partitions into SDS micelles. There are several other factors that contribute to the difficulties in the accurate determination of absolute binding constants of solutes in vancomycin-modified micellar capillary electrophoresis. Firstly, the wall adsorption of vancomycin allows it to exist as a "fourth phase" besides the micelle pseudophase, the unadsorbed vancomycin phase and the bulk solution phase. Obviously, a three phase model cannot accurately account for such a "four phase" or pseudophase system.

Secondly, the partitioning of vancomycin into the micellar pseudophase may alter the physico-chemical nature of the mixed micelles. Also, at sufficiently high surfactant concentrations, the mixed micelles may be distinguished as a different phase from the neat SDS micelles. Further, in varying the concentration of SDS in the micellar system at constant vancomycin concentration, the nature and ratio of the micelle/mixed micelle may itself change.

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The problem of wall binding of vancomycin may be overcome in the determination of binding constants of analytes to vancomycin, by studying the mobility of vancomycin in solutions containing various concentrations of the analyte.[27] The application of this method would, however, be restricted to measurements in systems where the selectors have large UV absorbance while the analytes have low UV absorbance and high solubility in the media being studied. For example, the absence of a UV chromophore on hydroxypropyl-β-cyclodextrin precludes the use of this method from being applied to that system. Another approach to minimize the effects of wall binding in the determination of absolute association constants would be to make these evaluations using coated capillaries.

CONCLUSIONS

In determining the binding constants of solutes to SDS micelles and the chiral selector phase, it became evident that the pseudophase model was effective in quantitating the binding of solutes to a simple system such as those containing hydroxypropyl-β-cyclodextrin and SDS. Its use was restricted in the complex vancomycin/SDS system wherein the existence of multiple equilibria made it impossible to accurately determine the absolute binding constants of solutes to the vancomycin and the micellar phases. However, since the major contribution to the total binding came from the partitioning of the solute to the vancomycin and the SDS phases, it was possible to determine the approximate

binding constants of solutes in these phases based on the three phase model. In making the determination of absolute binding constants, additional interactions from wall binding of vancomycin, its partitioning into SDS as well as changes in the nature of the micelle would have to be accounted for.

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