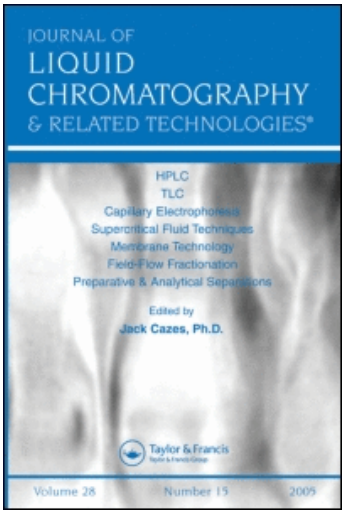

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Ion Interaction: The Energetics and Mechanism of The Competitive Behavior Between Two Similarly Charged Molecules. 1. The Effect of Ionic Strength, Acetonitrile and Surfactant Concentration

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ION INTERACTION: THE ENERGETICS AND MECHANISM OF THE COMPETITIVE BEHAVIOR BETWEEN TWO SIMILARLY CHARGED MOLECULES. 1. THE EFFECT OF IONIC STRENGTH, ACETONITRILE AND SURFACTANT CONCENTRATION

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ABSTRACT

The competition between two molecules of similar polarity for adsorption sites on the stationary phase is discussed in light of the effects of acetonitrile, surfactant (cyclohexylaminopropane sulfonic acid, CAPS) and salt concentrations on the retention of the thyroid hormones (3,5-diiodo-thyronine, T2; 3,3',5-triiiodo-thyronine, T3 and thyroxine, T4). The kinetics of adsorption and desorption for the surfactant are fast which allows for actual competition, otherwise an electrostatic "wall" would be seen by the analyte leading to exclusion. A three-parameter equation relates the surfactant concentration and ionic strength to the retention of the hormones, which suggests that the retention and selectivity arise from the structure and "density" of the outer layer in a Stern-Gouy-Chapman model and confirms that only the outer electrical layer significantly contributes. The data are analyzed with both Langmuir and Freundlich equations which describe an intermediary state where the marginal heat of adsorption begins to be negligible with respect to the surface concentration of the surfactant.

INTRODUCTION

Early investigations (1-3) have demonstrated the strong adsorption of the "ion-pair reagent" on the stationary phase,

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introducing the dynamic ion-exchange theory. Current theories attempt to provide a quantitative understanding of the more generalized retention problem of "ion interaction" (4,5), in particular, "ion visualisation" (6) and "vacancy peaks" (7,8). The later two are related to the same underlying mechanisim, competition between the mobile phase molecules for sites on the stationary phase. This series of two papers will attempt to describe and quantitate the mechanisms and energetics of competitive behaviour between an analyte and a surfactant with a particular attention on the effect of the hydro-organic solvent, the ionic strength and the molecular size of the charged analyte.

The adsorption of charged molecules onto the stationary phase has been described differently by different authors. Kissinger (9) and Kraak and co-workers (10,11) found a strong adsorption of the "ion-pair reagent" on the stationary phase, leading to the creation of the dynamic ion-exchange theory.

The search for an analytical description of the "ion interaction" theory has been marked by attemps that were not entirely satisfactory. The electrical double layer (or Stern-Gouy-Chapman) theory was successfully used by Cantwell and Puon (12) and Iskandarani and Pietrzyk (13,14) to demonstrated that the adsorption of ionized molecules was largely governed by the electrical nature of the double layer at the surface of the chromatographic media. In particular the theory describe the the ionic strength effect on the analyte retention, at a given and constant surface charge density. The surface charge density was experimentally controlled by covalently ataching sulphonic acids on the surface of the chromatographic support. However it should be noted that with surfactants in the mobile phase, an increase in ionic strength, will lead to an increase of the surface

concentration of surfactant, in other words, increasing the surface charge density of the chromatographic particle. Later an "exclusion" phenomena were added by Cantwell and coworkers (15,16); however the analytical description of the ionic strength effect was partially lost with the use of surface chemical potential. In a more classically oriented approach that included interfacial tension and chemical potential arguments Deming and coworkers (17,18) successfully described the competition process. The ionic strength effect which is imbedded in the activity coefficient and other parameters, does not directly contribute by itself to the understanding of the effect. Deelder and van den Berg (19) proposed a modified Langmuir isotherm which described the adsorption as a free energy change involving a transfer from the mobile to the stationary phase, and consisted of two different sets of events. First, the work required for the molecule to transfer from the bulk of the mobile phase to the stationary phase. Second, the work done against the electrical potential located at the surface, lowering the net energy of adsorption. This electrical potential was evaluated using a Stern-Gouy-Chapman approach. The form of the resulting equation was similar to the Freundlich equation, which was not surprising since this equation could be derived form a series of Langmuir isotherms with exponentially decreasing adsorption energy (20). This equation was successfully used by Jandera et al. (21). A generalized model based on Gibbs' description of adsorption was proposed by Kovats et al.(22,23) for chromatographic systems in an attempt to describe concepts that included hold-up volume and "ghost peaks".

As implied above, our experimental model consists of two competing molecules, a surfactant and an analyte, were both

bear charges of the same polarity. At high pH, the thyroid hormone molecules bears two negative charges at each end of the molecule. Similarly, at high pH the CAPS surfactant bears a single negative charge since the amine is not ionized (see fig 1). The chromatographic packing material PRP-1, is made of a poly-(styrene-divinylbenzene) polymer which is not adversely affected by pH values between 1-13 (24-26). This property is different from that of the widely used silica-based particles with covalently bound alkane chains, where the core dissolves in mobile phases of pH 8 and above. Early work demonstrated that polymeric materials like PRP-1 had chromatographic properties similar, or at least parallel to those of alkyl-bound silica (26-29). Since the chromatographic material is made of a single component then the adsorbtion sites sould be all similar and without secondary sites like silanol group, common to "classical reverse phase material.

The Void-Volume Problem

Knowledge of the void volume is essential for the determination of the thermodynamic parameter k'; this problem is particularly acute in the case of a charged interface. For large retention times, the percent error for k' is typically less than 10% and depends only on the relative error of the void time. As the retention time is closer to the void time, the relative error on k' increases.

The void-volume determination for a reverse-phase liquid chromatographic system has been reviewed by Krstulovic et al. (30) and by Berendsen et al. (31). The void-volume problem can be described in terms of the definition for the boundary between the stationary and mobile phase using Gibbs' formalism (32). Yonker et al. (33,34) have shown that the stationary phase is

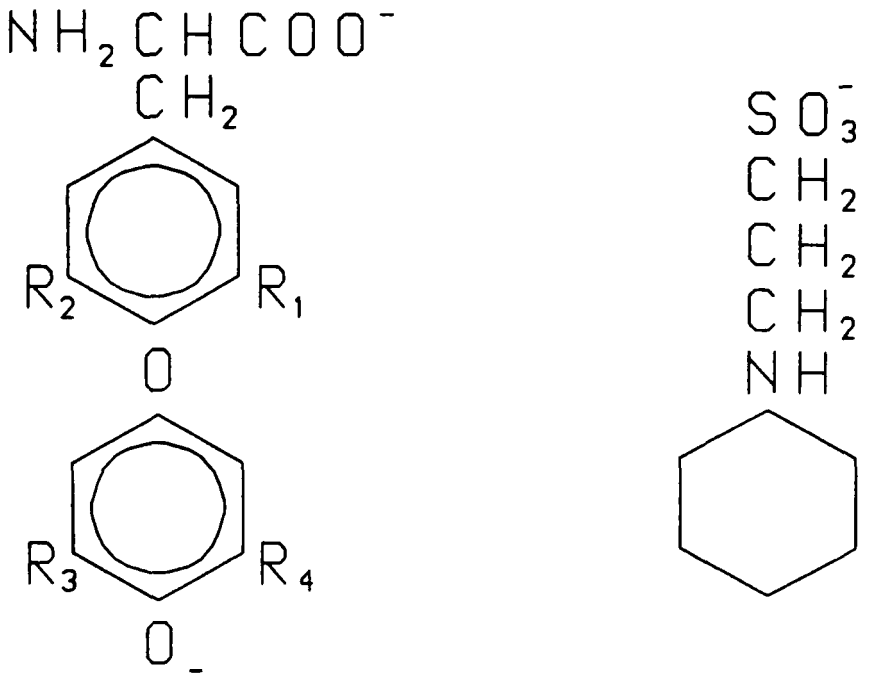

FIGURE 1. Structures of the thyroid hormones and of the surfactant CAPS. T0: R1, R2, R3, R4 = H; T2: R1, R2 = I, R3, R4 = H; T3: R1, R2, R3 = I, R4 = H; T4: R1, R2, R3, R4 = I.

formed from the solvation of both the silanol and alkyl moieties where the amount of organic modifier adsorbed is proportional to the amount present in the mobile phase. This results in a diffuse boundary that approximates a continuum extending toward the mobile phase, from a condensed phase (i.e. 100% organic modifier) at the stationary phase surface to the mobile phase composition. For charged surfactants adsorbed at the interface, the situation is somewhat different. If both the "ion-pair reagent" and the analyte are of the same polarity, as in this work, then the analyte could be excluded from the pores. It is

conceivable that larger solutes would be excluded to a greater extent than smaller ones.

These studies indicate that the void volume can change from 0.2 to 0.6 ml depending on the concentration and the nature of the mobile phase additive and the method used for this evaluation (linearization of homologues; temperature effect; injection of salts or D_2O). There is no widely accepted method in the literature. Typically the methods range from no mention, the use of an unretained component (based sometimes on unknown criteria) to the first change in base line. Other techniques are seldom used.

Hence it is possible to postulate that the closer are the molecular features between the analyte and the void volume markers, the more reliable the measurement (30).

EXPERIMENTAL

Column Preparation

A 15-cm reverse-phase column was packed upward with 10-um PRP-1 particles (Hamilton Co, Reno, NV 89510 USA) using a Sandon HPLC Packing Pump (Shandon Southern Products, Asmoor, Runcorn, Cheshire WA7 1PR, UK). The manufacturer recommends packing PRP-1 in a solution made of 2.5% NaCl and 10% glycerin; however the packing material is not wetted by this solution. Our method simply required a solvent similar to the mobile phase used (0.02 M Phosphate, pH 11.5 and 10% acetonitrile). After sonication for 10 minutes under vacuum, the slurry is poured all at once into the reservoir and packed with a pressure of 5000 psi. The reduced plate height of the column produced was somewhat better than 10.

Chromatographic System

The chromatographic system consisted of the column described above thermostated at 30.0 C. The pumps are Waters M6000 and M6000A (Waters Associates Inc., Milford, Mass. 01757 USA). For the determination of the breakthrough curve the pumps are connected as for gradient work and controlled by a Waters model M660 solvent programmer. A precolumn is located between the pumps and the injector (Rheodyne, Berkley CA. USA).

The amperometric detector and cell were built in this lab (35). The potential between the reference (Ag/AgCl) and working electrodes was set at about 0.6 volts and monitored with an Orion Research model 701 A / digital pH meter (Cambridge, Mass. USA) operating in the millivolt mode. The output of the electrochemical detector was recorded with a Heath-Schlumberger recorder, model SR-204 (Mississauga, Ont. Canada).

Mobile Phases

The batch solutions were produced in 4-liter lots from "all glass distilled" water solutions of K_2HPO_4 (BDH Assured, BDH Chemicals, Montreal, Canada) adjusted to pH 11.5 by the addition of NaOH (Anachemia Chemicals, Montreal, Canada) before the addition of the acetonitrile (Accusolv, Anachemia Chemicals, Montreal, Canada) to make the final volume. The various mobile phases containing various concentrations of CAPS (Sigma Chemical Co, St. Louis, Mo. USA) were made from dilutions of a stock solution with the batch solution. The stock is 0.05 M CAPS made with the batch solution, the "pH" is adjusted with NaOH to the same as that read for the batch solution (which includes acetonitrile). Typically the difference between the "pH" measurement of the stock solution before and after addition of acetonitrile was about 0.2 "pH" units.

CAPS Adsorption Isotherm

The breakthrough curve method was used to determine the adsorption isotherm of CAPS. The mobile phases were the same as those used to determine the retention of the thyroid hormones. The results obtained with the electrochemical detector were identical to those measured with a refractive index detector (model R 401, Waters Associates Inc., Milford, Mass. 01757 USA). The refractive index detector was not used in subsequent experiments due both to the poor sensitivity of the instrument and the impossibility of having both instruments in series (the pressure at the inlet of the electrochemical detector might damage the flow cell of the refractive index detector, or the void volume at the reference electrode precluded the use of another detector following it). A solution of KNO_3 (BDH Assured, BDH Chemicals, Montreal, Canada) was used to determine the breakthrough void volume.

Retention of Thyroid Hormones

The retention times of the thyroid hormones (thyronine, T0;3,5-diiodo-thyronine, T2; 3,3',5-triiodo-thyronine, T3 and thyroxine,T4; all from Sigma Chemical Co, St. Louis, MO, USA) were measured at the peak maximum. The hormone concentration was adjusted to produce a signal of about 100 nA. Iskandarani and Pietrzyk (13,14) have theoretically and experimentally demonstrated a relationship between 1/k' and the amount of analyte using equilibrium arguments and assuming a Langmuir isotherm. For an analyte concentration of less than about 1 ug per injection, the same study shows that the ionic strength effect is negligible. Preliminary experiments have demonstrated the absence of this effect for the conditions described above.

The retention time used in this study is the average of at least three injections.

RESULTS AND DISCUSSION

CAPS Adsorbtion

During the breakthrough experiments it was observed that the rise time for adsorption and decay time for desorption lasted a few minutes (see figure 1), suggesting fast kinetics. In contrast to other surfactants, the straight chain alkyl sulphonic acids are well known for their slow desorption rate. Fast kinetics probably results from the slightly polar amine located at the center of the molecule (see fig 2) that prevents the molecule from "sinking" into the stationary phase.

The data generated from the breakthrough curve were tested for suitability for Langmuir and Freundlich behaviour, first with classical linear regression. Analysis of the residual with respect to both distribution and actual values in conjunction with some explorations on the effect of coefficient variability on residuals brought forth the conclusion that a better set of parameters could be determined. Hence, a Simplex procedure was implemented to find the best set (35). Statistically both the linear regression and the simplex iteration results are equivalent but the latter were selected due to better behaviour of the residuals.

TABLE 1

Results from the Langmuir Isotherm Analysis. The correlation between $\%CH_3CN$ and $Ln(a_c[CAPS])$ is .99. See text and figure 3b.

$\%CH_3CN$	a_c	$[CAPS]_x$	$Ln(a_c[CAPS]_m)$
0	215	0.155	3.509
7.5	26.9	0.257	1.934
10	18.6	0.194	1.284
12.5	21.4	0.143	1.118
15	1.575	0.961	0.414

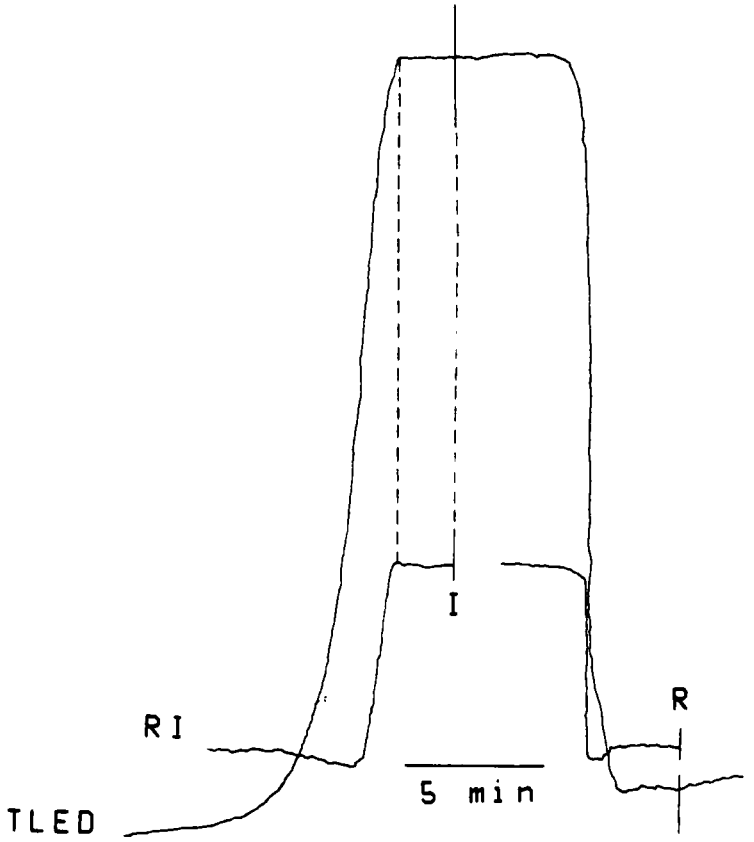

FIGURE 2. Breaktrough curve for CAPS as recorded by a Thin Layer Electrochemical Detector (TLED) and a Refractive Index detector (RI). The initial conditions are 0.02 M Phosphate pH 11.5 and 10% acetonitrile. The final condition are the same but also containing 0.05 M CAPS. The flow rate in 1.5 ml/min. At point "R" the a step gradient is Run to final condition. The point "I" mark a return to initial condition.

On quality of fit alone it was not possible to determine which of the classical isotherms best described the adsorption of CAPS (see fig 3 and table 1). As discussed above, the Freundlich isotherm can be derived as a sum of Langmuir isotherms where the logarithm of the energy of adsorption varies with the concentration of adsorbed surfactants on the stationary phase. Using the Stern-Gouy-Chapman theory, it was demonstrated that the interfacial electrical potential depends on the surface concentration build-up of the surfactant.

Three regions can be recognised in this generalized adsorption isotherm. For low concentrations the distance between the adsorbed surfactant molecules is large enough that the contribution of the electrical field is not significant, resulting in a constant adsorption energy with Langmurian properties. The second region is characterized by a rapid change in the electrical field with adsorption of CAPS, modifying the energy of adsorption. This is effectively analyzed with a Freundlich equation. Finally, at larger concentrations, the marginal change of potential with respect to adsorbed surfactant molecules is so small that the electrical potential at the surface is constant, yielding a constant energy of adsorption as required by a Langmuir isotherm. At intermediate concentrations, both the Freundlich and Langmuir equations would yield a satisfactory description.

The effect of ionic strength was studied with increasing amounts of KNO_3 (.03 and .05 M) added to the mobile phases. The resulting breakthrough curves where not very different from the observed curves that did not contain any added KNO_3 except for a fast rising shoulder caused by the potassium and nitrate ions passing through the detector.

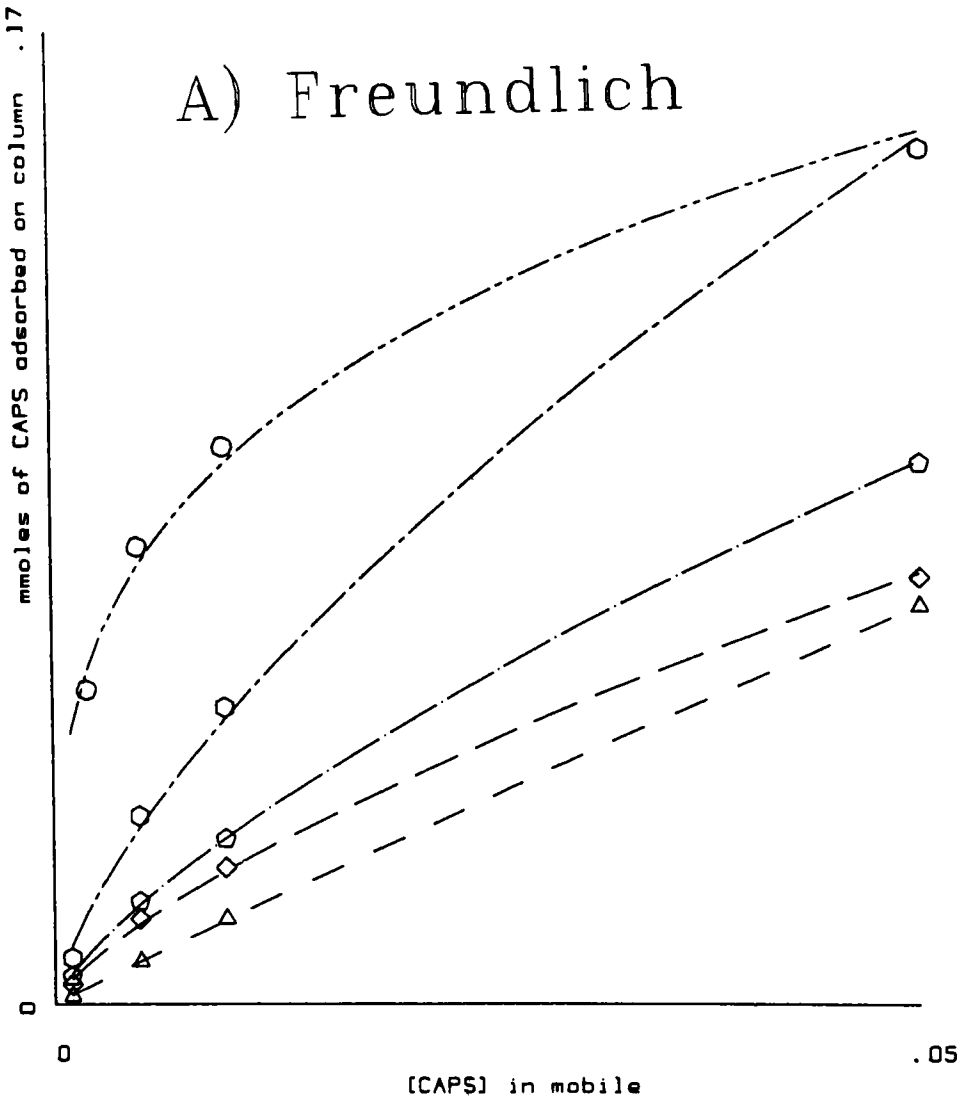

FIGURE 3. CAPS adsorbtion isotherm. The experimental condition are as stated in the experimental section for acetonitrile concentrations: 0%, 7.5%, 10%, 12.5% and 15% for the lines from top to bottom, respectively. The experimental data (polygones) are analysed using A) Freundlich or B) Langmuir isotherm (lines). Note that for the highest concentration of surfactant for the solutions containing 0 and 7.5% acetonitrile the data points are overlapping.

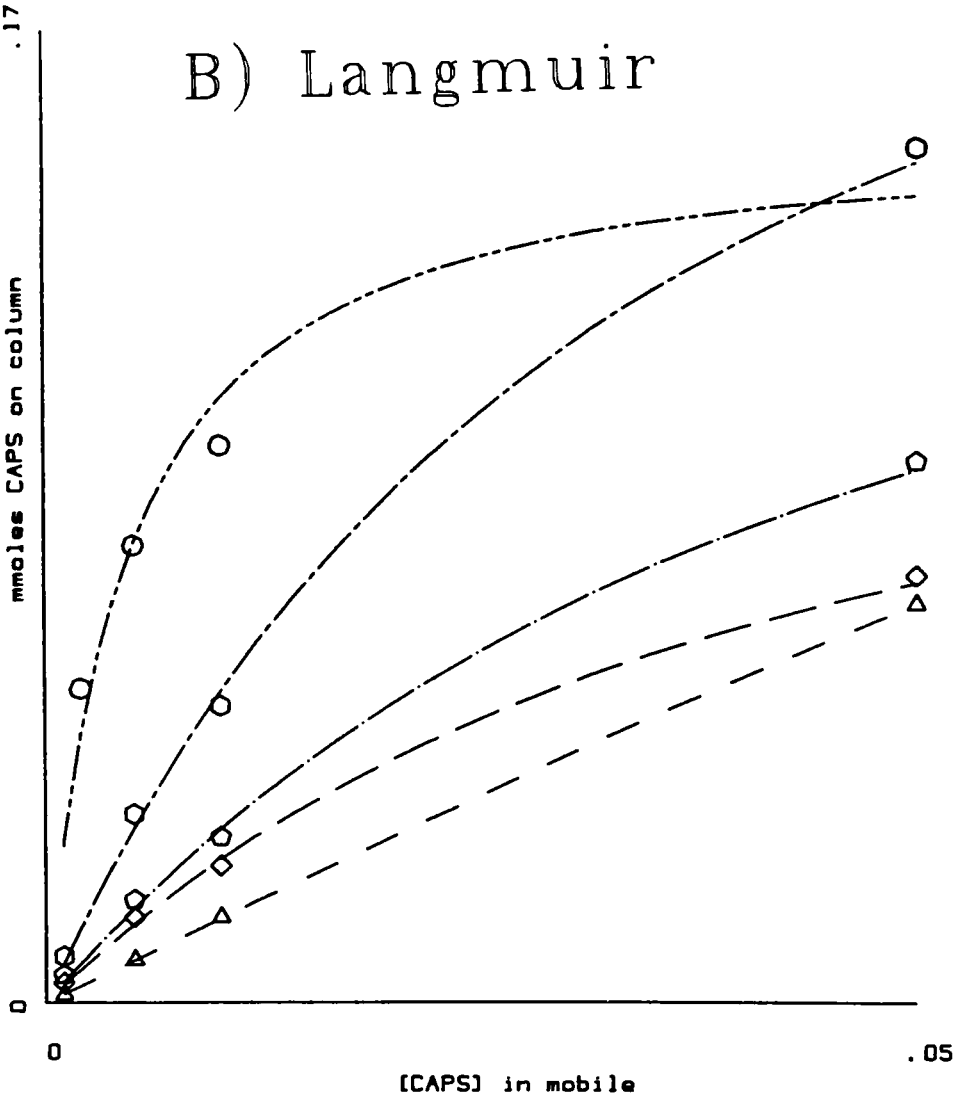

FIGURE 3 (continued)

The effect of temperature on CAPS adsorption was studied assuming a Langmuirian behaviour. Using linear free energy arguments it can be shown that:

$$\text{Ln } (a_c\ [\text{CAPS}]_x) = \text{slope } (\%\ CH_3CN) + \text{intercept}$$

demonstrating that the total energy of adsorption (the "ln"therm) is linearly dependent on the organic content (see table 1). The "a_c" term is an enthalpy term and indicates that the heat of adsorption in the 7.5% to 12.5% acetonitrile range is constant.

From experimental evidence the temperature does not affect the adsorption significantly in the chromatographically useful range of acetonitrile (7.5% to 15%). The heat of adsorption can be evaluated using:

$$1/a_c = C \exp (H_{ads} / R\ T)$$

where C, H_{ads}, R and T represent a proportionality constant, the heat of adsorption, the gas constant and the temperature in degrees Kelvin, respectively. Considering experimental evidence and theoretical extrapolation, the heat of adsorption has been evaluated to be at most 8 KJ/Mol. This value is smaller than was expected for a sulphonic acid of this length and is probably due to the slightly polar amine.

Determination of the Void Volume

From the above discussion, exclusion phenomena were expected to result from repulsive forces between charges of the same polarity located on each of the hormones and the surfactant. Futhermore if exclusion is present then a dependency on the size of the solute and on the surfactant concentration is expected and should be expressed as a reduction in time spent in the column with increasing molecular size.

This hypothesis was tested using a series of negatively charged molecules of different size and of increasing similarity to the thyroid hormones. These molecules are KNO_3, tyrosine and thyronine. No significant dependency on the size of the test solute with respect to the mobile phase composition (acetonitrile and surfactant) was observed.

For the iodinated thyronine molecules we concluded that if exclusion exists (for this system) it is not significant within experimental error. In high pH solution the thyronine backbone is ionized at both ends, the acid part of the amino acid moiety and the phenolic group at opposite ends of the molecule. Addition of iodine does not change the the spatial position of these two charges. Actually the addition of iodine on the outer ring results in a shielding of the phenolic charge. We propose the following: a) the pores of the stationary phase are of dimensions such that the interaction between the exposed charged group of the adsorbed surfactant and the incoming test solutes is minimal due to the large distance and b) a large number of ions are adsorbed, "neutralising" the coulombic interaction with the result that the coulombic forces do not extend significantly into the solution. Hence the sample molecules are not significantly excluded from the pores.

The void volume was determined to be 1.7 $\pm$.3 ml for all conditions used.

A Model for the Competitive Behaviour

The adsorption behaviour of the surfactant was shown to be Langmuirian in nature; the kinetics of adsorption were also demonstrated to be rapid. Assuming that the analyte also follows a Langmuirian adsorption, this system can be described with

a "multiple Langmuir isotherm " equation (20). This concept describes a situation where the molecules compete for sites on the stationary phase and is consistent with the model expressed by Bidlinmeyer (5) as "ion-interaction". Within this model the ions not only interact through coulombic forces for increased or decreased retention, but also displace one another.

The retention time reduction can be rationalised and described using the Multiple Langmuir Isotherm as a starting point. For a two-molecule system, the equation takes the form:

$$\frac{[T]_s}{[T]_m} = \frac{a_t\,[T]_x}{1 + a_c\,[CAPS]_m} \qquad (1)$$

where T and CAPS represent the thyroid hormone and CAPS molecules; s, x and m are subscripts standing for stationary phase, maximum stationary phase and mobile phase, respectively, and where a_t and a_c are the "a" terms in the general Langmuir equation for the thyroid hormones and the CAPS surfactants, respectively. The heat of adsorption on the stationary phase is related to the "a" term. The left-hand side of the equation is related to the chromatographic k' (using the phase ratio Φ), which yields upon rearrangement:

$$\frac{1}{k'} = \frac{(\ \Phi\ )}{(a_t\,[T]_x)} + \frac{(\ \Phi\ )\,a_c\,[CAPS]_m}{(a_t\,[T]_x)} \qquad (2)$$

The intercept (and part of the slope) term can be shown to be the inverse of k' (when $[CAPS]_m=0$), from a Langmuir equation for dilute solutions

$$\Phi\, k' = a_t\,[T]_x \qquad (3)$$

The above data and equations were subjected to the simplex procedure (35) using the relative standard deviation criteria of optimization (see table 2 and figure 4; note that all the data points are within one standard deviation from the line).

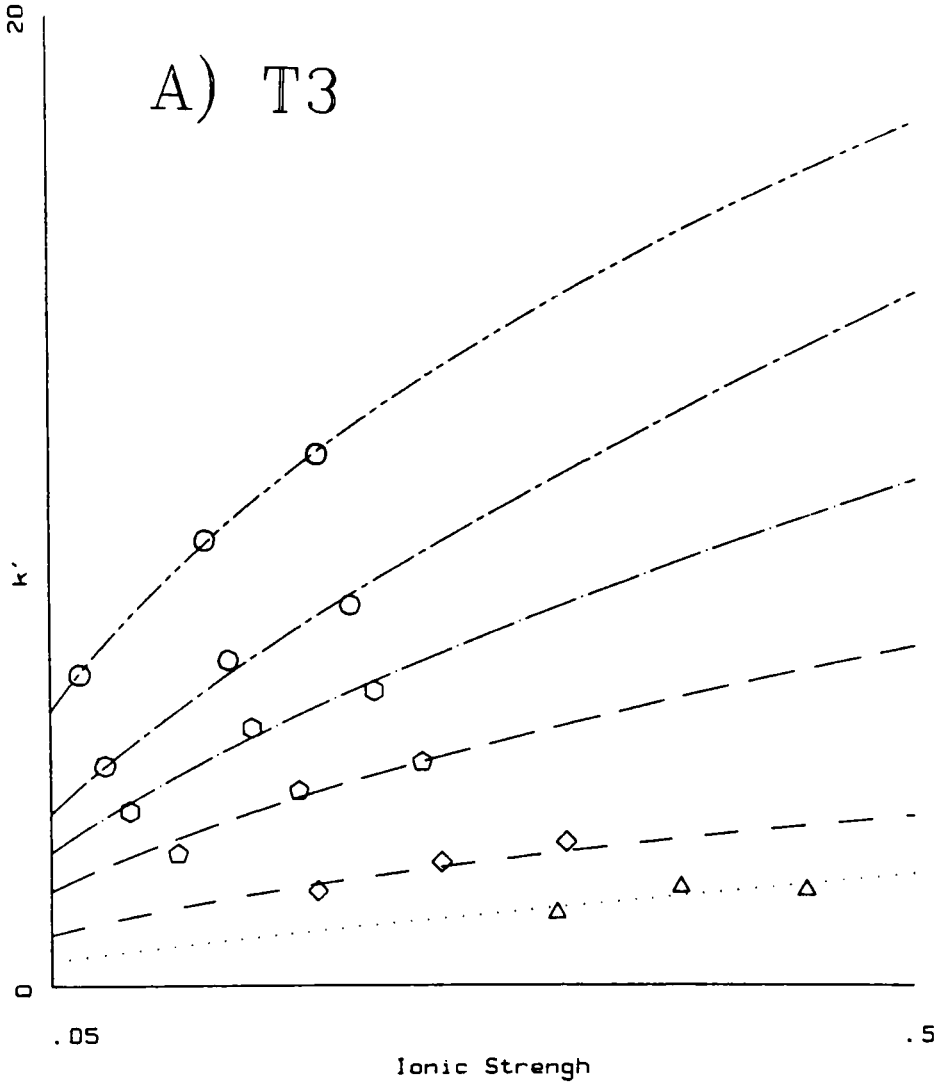

FIGURE 4. Retention of the thyroid hormones as a function of the mobile phases concentration of the surfactant CAPS (molar). The experimental conditions are as stated in the experimental section using : 7.5%, 10%, 12.5% and 15% acetonitrile (V/V) and are represented by the lines going from top to bottom, respectively. The line represents the results derived from the simplex optimisation using eq 2 (also see table I). A) T3 B) T4.

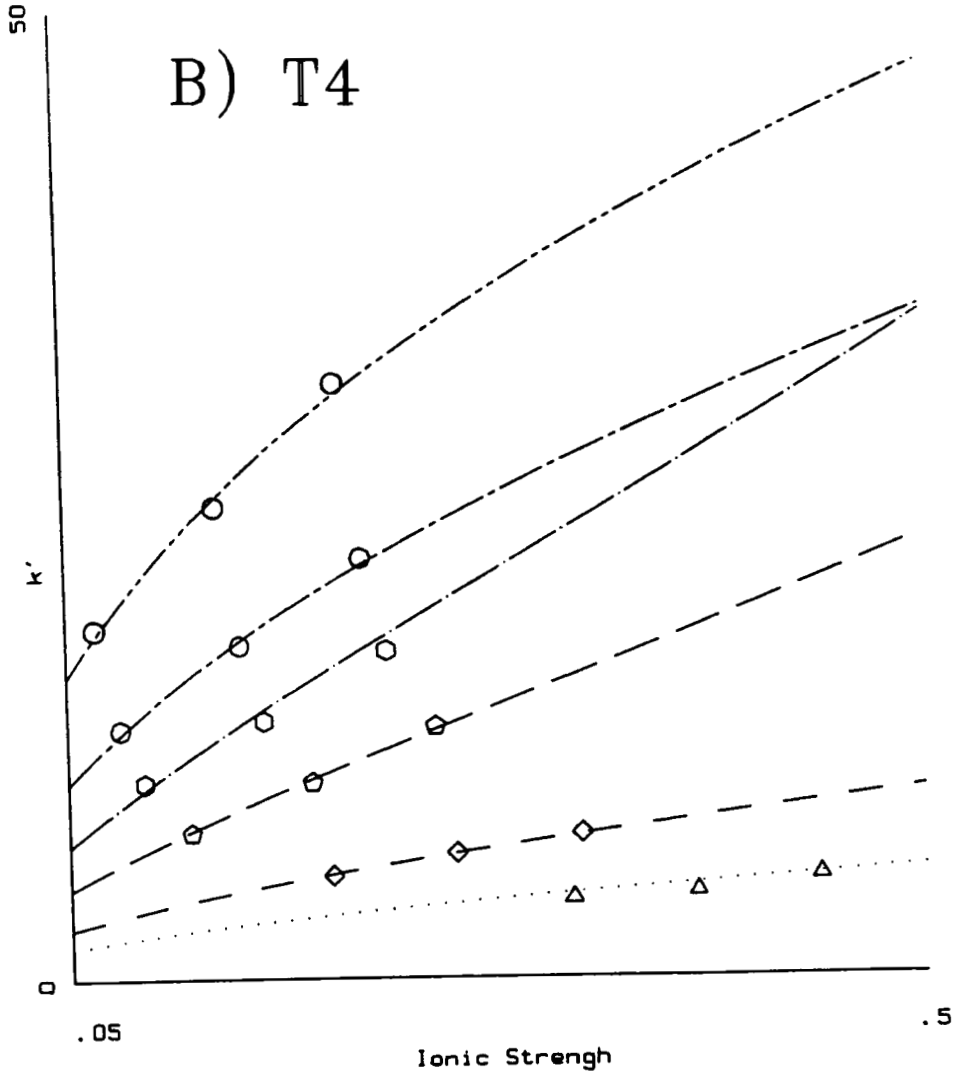

FIGURE 4 (continued)

The multiple Langmuir isotherm describes a situation where the molecules compete for sites on the surface. Within this model the adsorbed ions do not simply interact through coulombic forces to reduce the total energy of adsorption of the sample ion, but actually may displace the surfactant. At first this might contradict the "ion-exclusion" approach. However both can be reconciled by considering the kinetics of adsorption and desorption of the surfactant used. The "ion-exclusion" phenomenon would be observed when the surfactant's kinetics are larger than the time-frame of a chromatographic experiment; on the other hand, competitive behaviour would be observed when the surfactant's kinetic time-frame is smaller than the chromatographic time-frame. It has been demonstrated above that under the conditions employed the kinetics of adsorption and desorption, of the surfactant CAPS, are exceptionally fast.

TABLE 2

Results from the Multiple Langmuir Isotherm Analysis using a simplex (36) algorithm for estimation of the parameters. The suitability of fit is reported the largest relative standard deviation between any experimental point and the computed line (%RSD apply to retention time and the standard deviation is determined from replicates)

	$\%CH_3CN$	7.5	10	12.5	15
T3	$\Phi\ a_c / a_t\ [T]_x$	4.844	5.202	9.520	16.63
	$\Phi / a_t\ [T]_x$	0.02086	0.05757	0.09048	0.3256
	a_c	232	103	105	51.1
	% rel std dev	63	44	21	12
T4	$\Phi\ a_c / a_t\ [T]_x$	1.196	2.364	4.023	7.858
	$\Phi / a_t\ [T]_x$	0.01047	0.01616	0.02868	0.1324
	a_c	114	146	140	59.4
	% rel std dev	41	43	19	21

The "a_c" term measured by the adsorption isotherm (breakthrough curve) is between ten- and five-times smaller than that measured by the chromatographic experiment. The chromatographic experiment is subject to the local energetics of the eluting band. This means that the presence of the thyroid hormones significantly alters the electrostatic potential at the interface leading to a different energy of adsorption for the molecules. The latter effect has been speculated by Deming and Stranahan (8).

Multiple Langmuir Isotherms and the Ionic Strength Effect

From a Stern-Gouy-Chapman perspective, the concentration of salts in the vicinity of the adsorbed charged group affects the electrical potential gradient. Cantwell and Puon (9) have proposed that

$$1/k' = I + S/u \qquad (4)$$

were u, I and S are the square root of the ionic strength, the intercept and the slope, respectively.

The chromatographic data, at different CAPS concentrations, were fitted to equation (4) using the simplex algorithm and the relative standard deviation as criteria of optimization (35). For all the plots and considering each data point, the worst fit was 25% of one standard deviation. As predicted by theory the intercept was independent of the concentration of CAPS for both T3 and T4.

By analogy between equation (4) and equation (3) and with the assumptions that the phase ratio and the $[T]_x$ are independent of the ionic strength it is reasonable to express:

$$\frac{1}{a_c} = I + \frac{S}{u} \qquad (5)$$

and using this relationship with the multiple Langmuir isotherm equation (eq 2) the following is generated:

$$\frac{1}{k'} = \frac{\Phi\, I_t}{[T]_x} + \frac{\Phi\, S_t}{u\,[T]_x} + \frac{\Phi\,(I_t\, u + S_t)\,[CAPS]_m}{(I_c\, u + S_c)} \quad (6)$$

This equation was subjected to a simplex algorithm fit program as a 6-parameter equation using the relative standard deviation criteria of optimization (35) for both T4 and T3 retention data with respect to different ionic-strength and CAPS-concentration combinations (eighteen data points per thyroid hormone). Note that without a simplex method it would not be possible to analyze the data in light of this equation, since this equation can not be linearised. Individual data points were at least within 25% and 42% of one standard deviation of the computed line for T3 and T4, respectively.

It is well known from statistical practice that the quality of a fit increases with the number of parameters used, however, at one point, the improvement will level off. Hence, eq (6) was tested to see which of the parameters were not significant. This process reduced the equation to:

$$\frac{1}{k'} = \frac{\Phi\, I_t}{[T]_x} + \frac{\Phi\, S_t}{u\,[T]_x} + \frac{\Phi\, S_t\,[CAPS]_m}{I_c\, u} \quad (7)$$

which is a three-parameter equation. The resulting lines are within 28% and 26% of one standard deviation from the data points for T3 and T4, respectively (see fig 5 and table 3).

TABLE 3

Results from the Multiple Langmuir Isotherms - Ionic Strength Analysis using a simplex algorithm for estimation of the parameters by minimizing the maximum relative standard deviation (36). All of the individual data points are at a maximum 58% and 52% of one standard deviation from the computed line respectively for T3 and T4.

For T3,

$$1/k' = -4.954E\text{-}3 + 4.554E\text{-}2\ u + 6.553\ [CAPS]_m/u$$

For T4

$$1/\kappa' = -2.120E\text{-}3 + 1.583E\text{-}2\ u + 2.562\ [CAPS]_m/u$$

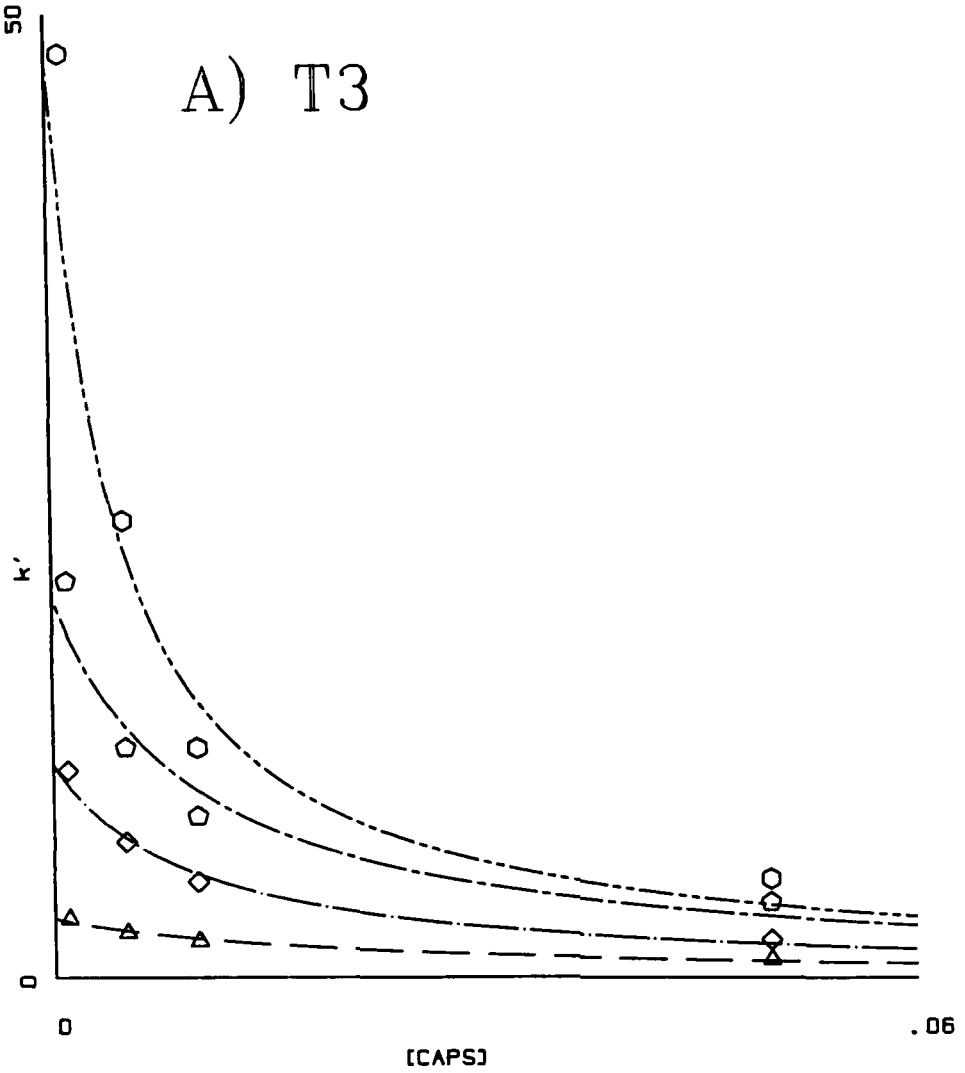

FIGURE 5. Retention of the thyroid hormones as a function of the mobile phases ionic strength. The experimental conditions are as stated in the experimental section execpt that the mobile phase contains 0.0, 0.0025, 0.005, 0.01, 0.025, 0.05 M CAPS and are represented by the lines going from top to bottom, respectively in 12.5% (V/V) acetonitrile. The line represents the results derived from the simplex optimisation using eq 7 (also see table II). A) T3 B) T4. Note that the lines have been extrapolated for a better perspective.

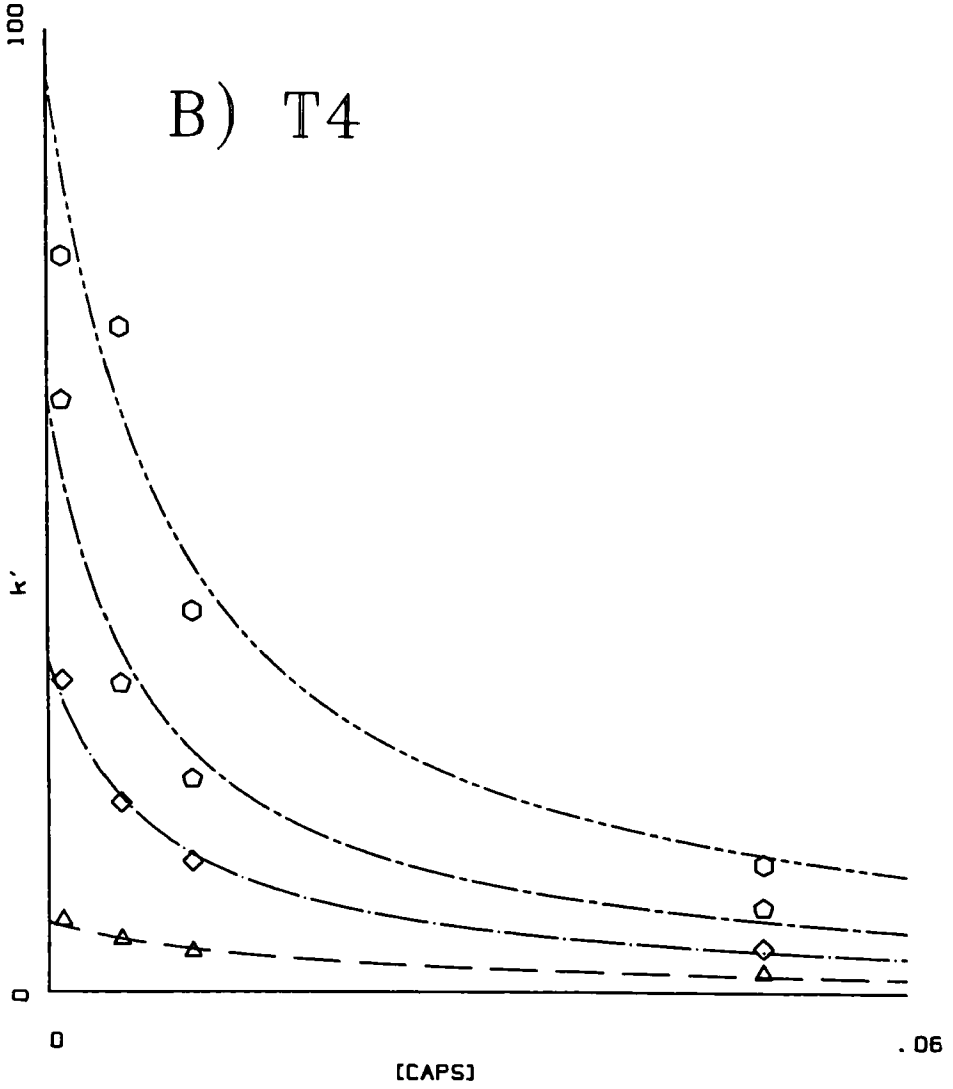

FIGURE 5 (continued)

This final equation is the result of two subsequent assumptions. First, it assumes that S_c is not significant with respect to the overall process with the result that the rate of change of the energy of adsorption of CAPS with respect to "u" is not significantly large. This has already been experimentally observed with the breakthrough experiment. Second, starting from the last assumption the

$$\frac{\Phi\ I_t\ [CAPS]_m}{I_c}$$

term generally contributes less than 10% to the overall result. The importance of this term increases with the combination of very low ionic strength and high CAPS concentration. This combination is not observed in practice since the CAPS contributes significantly to the total ionic strength.

The intercept portion of eq (7) describes, essentially, the thickness and the permitivity of the Stern layer, which is related to the dielectric constant and the ordering of the medium (20) of that region. Both "I_t" and "I_c" are independent of the ionic strength, as demonstrated by theory and experiments. This suggests that the Stern layer does not have a significant effect on the retention behavior of the solute for a given mobile phase concentration of CAPS.

CONCLUSION

The exclusion phenomenon has been explained in light of slow kinetics of desorption of the surfactant. When the kinetics are at least as fast as the chromatographic time-frame then the system is in equilibrium. If this requirement is met, as is the case here, then competitive behaviour is expected.

The interpretation of the "full" equation, in light of the significant parameters and a given $[CAPS]_m$, suggests that the

first layer of charges does not control the selectivity, but that the second does. This is not all that new, since it is well known that the nature of the counter ions has a significant effect on the selectivity of the mobile phase. Furthermore it is reasonable that the real control on retention is related to the "density" (ie $[CAPS]_a$) and "structure" of the adsorbed ions in the outer layer.

Finally both of the adsorbed species interact with one another in such a way that the energy of adsorption of one is dependent of the other. In other words, the energy of adsorption of one species is different when observed in a "neat eluant" than in the presence of the other.

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