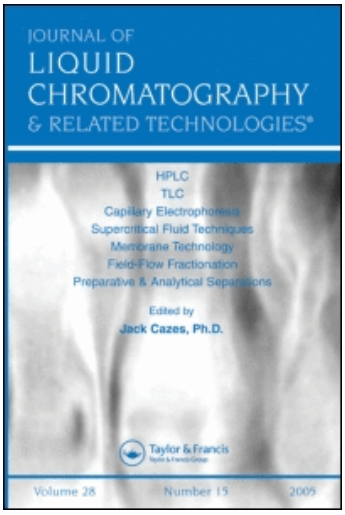


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# Retention in Reversed-Phase Ion-Pair Chromatography of Amines On Alkyl-Bonded Phases

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# RETENTION IN REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF AMINES ON ALKYL-BONDED PHASES.

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## ABSTRACT

Reversed phase ion-pair chromatography of phenethylamine derivatives (noradrenaline, adrenaline, dopamine, synephrine, tyramine and pholedrine) and lower alkylamines has been performed with octyl sulfate as counter ion in an aqueous eluent with a low content of 1-pentanol. LiChrosorb RP-18 was used as the solid phase. The retention of lithium and potassium in the system has also been studied.

On the basis of adsorption and retention studies a model for the chromatographic behaviour of the amines is proposed that includes interaction with two sites with different binding ability in the stationary phase. The adsorption capacity of the sites has been calculated as well as adsorption constants for the octyl sulfate ion pairs.

## INTRODUCTION

Reversed phase ion-pair chromatography has been widely used during the last years for separation of cationic and anionic compounds (e.g. [1-4]). Most separations are performed on a

n-alkyl bonded phase with a solution of the counter ion in mixtures of aqueous buffers and an organic solvent as eluents.

Different models have been proposed for the chromatographic process in ion-pair chromatographic systems with a retaining solid phase. Horvath and co-workers have used a retention model based on the so called solvophobic theory [5,6]. Others have measured the adsorption of hydrophobic counter ions like cetrimide [7], octyl sulfonate [8] and n-alkyl sulfonates [9] on the solid phase and claimed that the chromatographic system can be characterized as ion exhange chromatography with the adsorbed counter ions acting as the stationary phase. The retention mechanism has also been discussed by Bidlingmeyer et al. in a study of sulfonates both as samples and counter ions [10] and Cantwell et al. have applied the Stern-Gouy-Chapman theory of electrical double layer to explain the retention [11].

Tilly-Melin et al. have studied the influence of tetrabutyl-ammonium on the retention of anionic and basic compounds and proposed a retention model based on competition for sites on the adsorbing solid phase between ion pairs formed by components in the eluent and the sample [12,13]. This model have also been used in studies of hydrophobic amines where ions with the same charge as the sample was used to regulate the retention on alkyl-bonded solid phases [14,15], as well as on underivatized silica [16].

In the present study the model of Tilly-Melin et al. has been applied on the chromatographic behaviour of hydrophilic amines as ion pairs with octyl sulfate in a system with 1.15% 1-pentanol in the eluent.

It has been found that the retaining phase is composed of two kinds of sites with different binding ability for the ion pairs. The adsorption of octyl sulfate on the solid phase has been measured and the adsorbing capacity of the solid phase has been calculated as well as constants for the adsorption of the ion-pairs.

## EXPERIMENTAL

### Apparatus

The liquid chromatograph comprised an Altex Model 100 solvent metering pump, an LDC UV-detector Model 1205 with 8 µl cell volume, measuring at 254 nm wavelength and a Valco high pressure valve injector with a sample loop of 30 µl. The chromatographic equipment was thermostated in a water bath, model HETO 02 PT 923 TC (Birkeröd, Denmark). The chromatographic columns were made of 316 stainless steel with a polished inner surface. They were equipped with modified Swagelok connectors and 2 µm stainless steel frits from Altex. The dimensions of the separation columns were (150 x 4.5) mm.

The equipment for post-column reaction with phthaldialdehyd included a second Altex Model 100 solvent metering pump and an LDC fluorometric detector Model 1311 with 30 µl cell volume, excitation wavelength at 340-380 nm and emission cut off at 418 nm. The reactor column, (300 x 4.6) mm, was drypacked with glass beads 16 $\pm$ 2.3 µm.

A Corning 400 Flame Photometer (Halstead, Essex, England) was used as detector in the chromatographic studies on lithium and potassium using the wavelengths 671 nm and 767 nm respectively.

The determination of 1-pentanol was performed with a Varian 1400 gas chromatograh equipped with a flame ionization detector and pH was measured with an Orion 801 Research pH meter equipped with an Ingold combined electrode.

### Chemical and Reagents

Sodium octyl sulfate was obtained from E. Merck (Darmstadt, GFR) and from Eastman Kodak (Rochester, N.Y., USA). 1-Pentanol was

of A.C.S. quality from Fisher Scientific (Pittsburg, Pa., USA). Phthaldialdehyd (für die Fluoreszenzanalyse) were obtained from E. Merck and 2-mercapto-ethanol from Serva (Heidelberg, GFR). The cation exchanger was Amberlite$^{R}$ IR-120 p.a.

All amines used as chromatographic samples were of pharmacopeial or equivalent grade. The structures of the phenethylamine derivatives are given in Table 1.

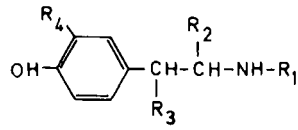
All other substances used were of analytical or reagent grade and used without further purification.

Phosphate buffers were prepared from phosphoric acid, sodium dihydrogen phosphate and disodium hydrogen phosphate while borate buffers were prepared from boric acid and sodium hydroxide. All buffers were prepared with an ionic strength of 0.1 using water, purified by ion-exchange and filtered through a Milli-Q Reagent-Grade Water system.



TABLE 1

List of Phenethylamine Derivatives



| Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Pholedrine | $CH_3$ | $CH_3$ | H | H |
| Tyramine | H | H | H | H |
| Synephrine | $CH_3$ | H | OH | H |
| Dopamine | H | H | H | OH |
| Adrenaline | $CH_3$ | H | OH | OH |
| Noradrenaline | H | H | OH | OH |

## Column Preparation

LiChrosorb RP-18, 10 μm, obtained from E. Merck was used as solid phase. The same batch was used through the study.

The separation columns were packed at 400 bar by a balanced-density slurry technique according to Majors [17]. The solid phase was suspended in tetrachloroethane 0.1 g/ml and treated in ultrasonic bath for 30 sec immediately before packing. After packing 300 ml hexane and 80 ml acetone was passed through the column.

The quality of the columns was tested with methanol-water (6+4) as eluent. A test solution of phenol, 2-phenylethanol, 2,6-dimethylphenol and 2,3,5-trimethylphenol was injected. The accepted columns had a reduced plate height of less than 6 for capacity ratios above 2. The flow rate was 0.6 mm/s.



## Preparation of Eluents

Sodium octyl sulfate was freed from lower alkyl sulfates by the following procedure. An aqueous solution of sodium octyl sulfate was passed through a cation exchanger in hydrogen form. Phosphoric acid was added to the eluate to a content of 0.1 M. The octyl sulfuric acid was then extracted to pentanol using equal phase volumes and re-extracted to aqueous phase by repeated extractions with phosphate buffer pH 3. The concentration of octyl sulfate in the aqueous phase was measured with the extraction method given in [18].

The eluents containing 1.15% pentanol were prepared by mixing solutions equilibrated with pentanol at 25.0°C with equal volumes of pentanol-free buffers.

## Chromatographic Technique

The separation column, the reservoir and the injector were thermostated at 25.0 ± 0.1°C by immersion in a water-bath.

The columns were conditioned by passing 50 ml of buffer with the same content of pentanol as the eluent [19,20] followed by eluent until test samples had a constant retention. The hold-up volume of the column, $V_m$, was determined by the peak obtained when potassium nitrate was injected. The determination was made before the passage of eluent had started.

All samples were injected dissolved in the eluent. All chromatographic results reported are the means of triplicate injections.

## Post-column Derivatisation

Primary amines without UV-adsorbance were detected fluorometrically after on-line post-column derivatisation with phthaldialdehyd [21-23].

The reagent was prepared by mixing 0.4 g phthaldialdehyd, 1 ml 2-mercapto-ethanol, 5 ml ethanol and boric buffer pH 10.4 to 500 ml. The solution was protected from light.

The post-column reactor system with a packed bed reactor described by Deelder et al was used [23]. The flow rate in the separation column was set to 0.6 ml/min and the reagent was pumped at a flow rate of 1.4 ml/min which gave a reaction time of about 1 min.

## Flame Photometer as Detector

The separation column and the ordinary intake of the flame photometer was connected by a capillary tube, 500 mm in length and with an inner diameter of 0.25 mm. The flame photometer was adjusted to aspirate 1.3 ml/min which was equal to the flow rate used in the separation column.

## Determination of Octyl Sulfate and 1-Pentanol Adsorbed on the Solid Phase

Octyl sulfate and 1-pentanol were stripped off by eluting the column with 5 ml ethanol-water (1+4) followed by 10 ml

ethanol-water (4+1) and ethanol to 100 ml. The amount of adsorbed species was obtained as the difference between the amount found in the eluate and in the mobile phase.

The amount of pentanol in the eluate was determined by gas chromatography on a 1.5 meter silanized glass column with an inner diameter of 2 mm packed with 15% OV 225 on Gas Chrom Q 100/200 mesh using 1-butanol as internal standard.

Octyl sulfate in the eluate was determined by the extraction method given in [18]. Control by further elution with water showed that > 99% of adsorbed octyl sulfate had been eluted.

At concentrations of octyl sulfate in the eluent lower than $2 \times 10^{-3}$ M the adsorption of octyl sulfate was measured by break through curves [24].



## RESULT AND DISCUSSION

### Purification of Octyl Sulfate

Commercially available sodium octyl sulfate contains an ionic impurity, that can be detected and quantified in a straight phase chromatographic system with N-metylprotriptyline as counter ion [25]. A chromatogram is shown in Fig. 1. The quantitation was based on peak height measurements and external standardisation.

The degree of impurity was found to vary between 1 and 20 % in different batches of sodium octyl sulfate. The exact nature of the impurity is not known but it has the same retention in the chromatographic system in Fig.1 as propyl sulfate and hexyl sulfonate.

Sodium octyl sulfate was purified by a procedure that included a transformation to octyl sulfuric acid by ion exchange followed by an extraction of the acid from an aqueous phase of pH 2 (i.e. 0.1 M phosphoric acid) into pentanol. Octyl sulfuric acid has in this system a distribution ratio of more than 10 while the

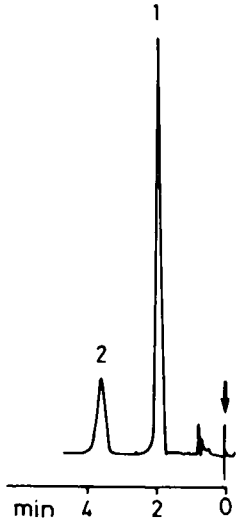



FIGURE 1. Chromatogram of octyl sulfate. Stationary phase: $2.7 \times 10^{-2}$ M N-metylprotriptyline (MPT) in phosphate buffer pH 8.0 on LiChrosorb-diol, 5 µm; Eluent: chloroform + 1-propanol (92+8); Flow rate: 1.1 ml/min; Peaks: 1 = octyl sulfate; 2 = impurity.



extraction of the impurity was insignificant as controlled by the chromatographic system given in Fig. 1. The octyl sulfate was re-extracted to an aqueous buffer of pH 3.0. Repeated extractions were required [18].

## Ion-pair Chromatography with Adsorbing Stationary Phase

Previous studies with aqueous eluents containing 1-pentanol and LiChrosorb RP-18 as solid phase have indicated that adsorption has a decisive influence on the retention of octyl sulfate ion pairs when the content of 1-pentanol is as low as 1.15% [20].

Studies of Tilly-Melin et al and others [12-15] have shown that the retention of ion pairs in adsorbing systems can be

expressed by a model based on competition for a limited number of sites on the adsorbing surface. The principe can be illustrated by the following example.

The distribution of a cation, $Q^+$, to an adsorbing surface as ion pair with the counter ion, $X^-$, can be expressed by the formula

$$Q^+_m + X^-_m + A_s = QX.A_s$$

where $Q^+_m$ and $X^-_m$ represent the ions in the mobile phase, $A_s$ the available adsorption sites and $QX.A_s$ the adsorbed ion pair. A quantitative expression is given by the adsorption constant, $K_{QX}$, defined by

$$K_{QX} = \frac{[QX.A]_s}{[Q^+]_m \cdot [X^-]_m \cdot [A]_s} \qquad (1)$$

The brackets signify concentrations in mol/l in the mobile phase and in mol/g in the solid phase. Analogous equilibrium expressions are valid for each adsorbed ion pair.

The surface can accomodate a limited number of mol of adsorbed species per g. If the ion pair, QX, is the only adsorbed species, the capacity of the surface, $K_0$, is given by the expression

$$K_0 = [A]_s + [QX.A]_s \qquad (2)$$

The concentration of the adsorbed ion pair in the stationary phase is then given by the equation

$$[QX.A]_s = \frac{K_0 \cdot K_{QX} \cdot [Q^+]_m \cdot [X^-]_m}{1 + K_{QX} \cdot [Q^+]_m \cdot [X^-]_m} \qquad (3)$$

obtained by combination of eqs. (1) and (2).

If a sample ion, $B^+$, is injected on the column and $Q^+$ and $X^-$ are components of the mobile phase, the ion pairs BX and QX will compete for the available adsorption sites.
The expression of the adsorption capacity is then

$$K_0 = [A]_s + [QX.A]_s + [BX.A]_s \qquad (4)$$

An expression for the capacity ratio of $B^+$, $k'_{BX}$, can be obtained by combining eq. (4) with the equations for adsorption of the two ion pairs (eq. (1))

$$k'_{BX} = \frac{q.[BX.A]_s}{[B^+]_m} = \frac{q.K_0.K_{BX}.[X^-]_m}{1 + K_{QX}.[Q^+]_m.[X^-]_m + K_{BX}.[B^+]_m.[X^-]_m} \qquad (5)$$

where q is the phase volume ratio expressed in g of solid phase per l of mobile phase present in the column.

Symmetric chromatographic peaks are obtained under such conditions that the concentration of the sample has an insignificant influence on the retention as shown by the relationship

$$(1 + K_{QX}.[Q^+]_m.[X^-]_m) >> K_{BX}.[B^+]_m.[X^-]_m \qquad (6)$$

From eq. (5) follows that the retention can be regulated by the nature and the monomeric concentration in the mobile phase of the counter ion, $X^-$, and the competing ion, $Q^+$, with the same charge as the sample. The eq. is valid for an adsorbing stationary phase that has one kind of adsorption site. Tilly-Melin et al [12,13], Sokolowski et al [14] and others [15] have, however, given examples of systems where the retention of ion pairs on alkyl-bonded phases can be described by a model with two sites of different binding ability. The equation for the capacity

ratio will then in analogy with eq. (5) have the following form when the retention is independent of the sample concentration.

$$k'_{BX} = \frac{q \cdot K_0 \cdot K_{BX} \cdot [X^-]_m}{1 + K_{QX} \cdot [Q^+]_m \cdot [X^-]_m} + \frac{q \cdot K_0^x \cdot K_{BX}^x \cdot [X^-]_m}{1 + K_{QX}^x \cdot [Q^+]_m \cdot [X^-]_m} \quad (7)$$

$K_{QX}^x$ and $K_{BX}^x$ are the adsorption constants of the ion pairs to the second site while $K_0^x$ is its adsorption capacity. The total concentration of QX adsorbed to these two sites in the stationary phase is given by

$$C_{QX,s} = \frac{K_0 \cdot K_{QX} \cdot [Q^+]_m \cdot [X^-]_m}{1 + K_{QX} \cdot [Q^+]_m \cdot [X^-]_m} + \frac{K_0^x \cdot K_{QX}^x \cdot [Q^+]_m \cdot [X^-]_m}{1 + K_{QX}^x \cdot [Q^+]_m \cdot [X^-]_m} \quad (8)$$

The influence of a third species, e.g. 1-pentanol adsorbed to the same sites as QX and BX, can be expressed by eqs. analogous to (3), (5), (7) and (8) by introducing a term in the denominator that expresses the concentration of 1-pentanol in the stationary phase (cf. [15,16]). The magnitude of this term will be constant if the concentration of 1-pentanol in the mobile phase is constant as in the present study.

## Adsorption of Mobile Phase Components

In a previous study it was established that a LiChrosorb RP-18 column in equilibrium with a mobile phase of phosphate buffer pH 3 with 1.15% 1-pentanol was coated with a monolayer of 1-pentanol [20].

If octyl sulfate is added to the mobile phase there is a change of the composition of the adsorbed layer as demonstrated in Fig. 2. The amount of 1-pentanol on the solid phase decreases with

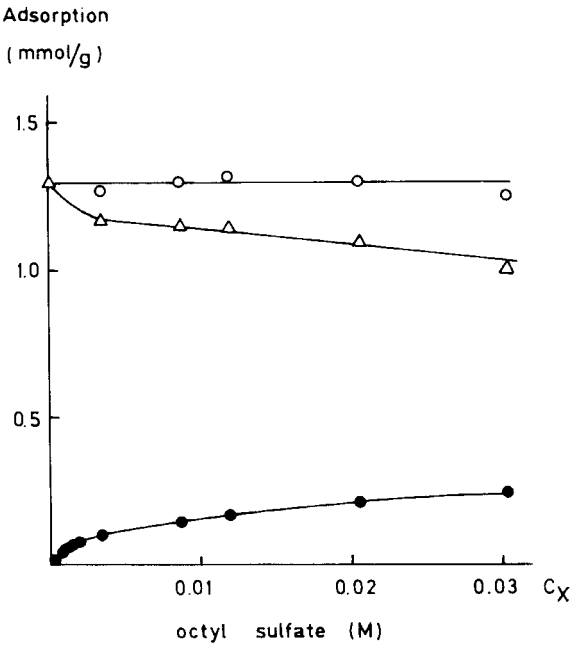



FIGURE 2. Adsorption of 1-pentanol and octyl sulfate to the solid phase. Solid phase: LiChrosorb RP-18, 10 µm; Eluent: octyl sulfate in phosphate buffer pH 3.0 with 1.15% 1-pentanol; ● = octyl sulfate; △ = 1-pentanol; ○ = total adsorption.



increasing adsorption of octyl sulfate, but the total number of moles of the two species is almost constant. This indicates that the two species compete for the same binding sites on the solid phase.

Octyl sulfate can be adsorbed both as ion pair with sodium and in acidic form but it has not been possible to distinguish between the two forms of adsorption by the experimental technique used. The eluent used in the adsorption studies had pH 3.0 and a concentration of sodium between 0.10 and 0.13 mol/l. Distribution studies of octyl sulfate between this aqueous phase and 1-pentanol in batch have shown that the extraction as ion pair with sodium dominates [18], and all the calculations below have been based on

the simplified assumption that octyl sulfate is distributed to the stationary phase as ion pair with sodium only.

The adsorption of sodium octyl sulfate could not be described by a single site model as indicated by an inversed plot based on eq. (3). The two site model given in eq. (8) was then applied as follows.

The two sites were assumed to have highly different binding ability, $K_O$ and $K_{QX}$ being the constants of the stronger site and $K_O^x$ and $K_{QX}^x$ those of the weaker binding site. A computation of the constants was based on eq. (8) transformed to

$$\frac{b}{C_{QX,s} - \frac{K_O^x \cdot K_{QX}^x \cdot b}{1 + K_{QX}^x \cdot b}} = \frac{1}{K_O \cdot K_{QX}} + \frac{b}{K_O} \qquad (9)$$

where $b = [Q^+]_m \cdot [X^-]_m$

A preliminary estimation was made with values of $C_{QX,s}$ obtained at low concentrations of octyl sulfate, $[X^-] < 2 \times 10^{-3}$ M, where the weak adsorption site, $A_s$, should be only slightly covered and the approximation $K_{QX}^x \cdot b < 1$ could be applied. A value of $K_O^x \cdot K_{QX}^x$ was found that gave a straight linear relationship between $b/(C_{QX,s} - K_O^x \cdot K_{QX}^x \cdot b)$ and b by way of trial. Preliminary $K_O$ and $K_{QX}$ were calculated from the slope and the intercept of the line. A small dimerization of octyl sulfate in the aqueous phase was taken into account when calculating the constants [18,20].

The found value of $K_{QX}$ indicated that the strong adsorption site, $A_s$, should be covered to ca 95% with $8 \times 10^{-3}$ M octyl sulfate in the mobile phase. The preliminary $K_O$ and $K_{QX}$ and values of $C_{QX,s}$ obtained at higher concentration of octyl sulfate, $[X^-]_m = (8\text{-}30) \times 10^{-3}$ M, were used in the next computation which was based on the eq.

$$\frac{b}{C_{QX,s} - \frac{K_0 \cdot K_{QX} \cdot b}{1 + K_{QX} \cdot b}} = \frac{1}{K_0^x \cdot K_{QX}^x} + \frac{b}{K_0^x} \qquad (10)$$

where $b = [Q^+]_m \cdot [X^-]_m$

A plot of $b/(C_{QX,s} - K_0 \cdot K_{QX} \cdot b/(1+K_{QX} \cdot b))$ versus b gave a linear relationship. The values of $K_{QX}^x$ and $K_0^x$ obtained from the slope and intercept of the line indicated that the assumption of $K_{QX}^x \cdot b < 1$ was not valid in the entire range it had been applied.

The premliminary values were then used in repeated calculations by successive use of eqs. (9) and (10) until the values of the constants remained unchanged. The results are given in Table 2.

## Retention of Alkali Metal Ion

In the calculation of adsorption constants in Table 2 it was assumed that octyl sulfat is retarded as ion pair with sodium. If the

TABLE 2

Equilibrium Constants for Octyl Sulfate

Solid phase: LiChrosorb RP-18
Eluent: octyl sulfate, $X^-$, in phosphate buffer pH 3.0 with 1.15% 1-pentanol

| Site | $C_X \times 10^3$ (M) | $K_0 \times 10^4$ (mol/g) | $K_{QX} \times 10^{-3}$ | site coverage (%) $A_s$ | site coverage (%) $A_s^x$ |
|---|---|---|---|---|---|
| $A_s$ | 0.23- 1.9 | 0.52 | 25 | 36-83 | 1- 9 |
| $A_s^x$ | 8.6 -31 | 3.1 | 0.50 | 96-99 | 30-62 |

assumption is valid, it should also be possible to retain other alkali metals, e.g. potassium and lithium, as ion pairs with octyl sulfate.

The experiments were performed with on line flame photometric detection (see EXPERIMENTAL). A typical chromatogram is given in Fig. 3. The relation between the capacity ratio and the octyl sulfate concentration is demonstrated in Fig. 4. The corresponding relationship for sodium, calculated by use of eq. (7) and the constants from Table 2, is included in the Figure.

The results indicate that lithium, as expected, is less hydrophobic than sodium while potassium has a slight higher hydrophobicity. Fig. 4 illustrates that all ionic compounds have a specific influence on the retention in ion-pair chromatographic systems with adsorbing stationary phases. An exchange of sodium for lithium in the eluent would thus give rise to an increase of the retention of other cationic samples.



## Retention of Amines as Ion Pair with Octyl Sulfate

All retention studies were performed at pH 3.0 where the alkyl- and arylalkylamines are completely ionized and will be

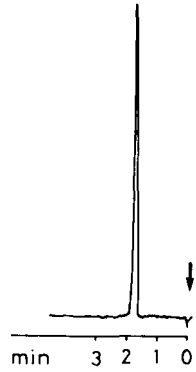



FIGURE 3. Chromatogram of lithium, retained as ion pair with octyl sulfate. Solid phase: LiChrosorb RP-18, 10 μm; Eluent: 0.005 M octyl sulfate in phosphate buffer pH 3.0 with 1.15% 1-pentanol; Flow rate: 1.3 ml/min; Detector: flame photometer; Sample: lithium (0.2 μg).

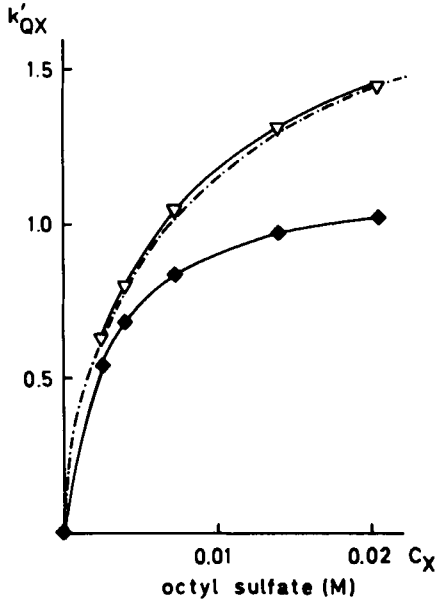



FIGURE 4. Retention of alkali metals as ion pairs with octyl sulfate. Solid phase: LiChrosorb RP-18, 10 µm; Eluent: octyl sulfate in phosphate buffer pH 3.0 with 1.15% 1-pentanol; Sample: ▽ = potassium; ◆ = lithium; -·-·- = sodium, calculated by use of eq. (7), q=822 and constants from Table 2.



retained as ion pairs only. The influence of the concentration of octyl sulfate on the retention of some phenethylamine derivatives (Table 1) is illustrated in Fig. 5.

Since the adsorption of sodium octyl sulfate follows a two site model, it is obvious that an analogous model should be applied to the retention of amines as ion pairs with octyl sulfate. The retention model used is given in eq. (7). The validity of the model was tested by computation of constants for ion pair adsorption and adsorption capacity by successive approximation as demonstrated above.

Eg. (7) can be transformed to

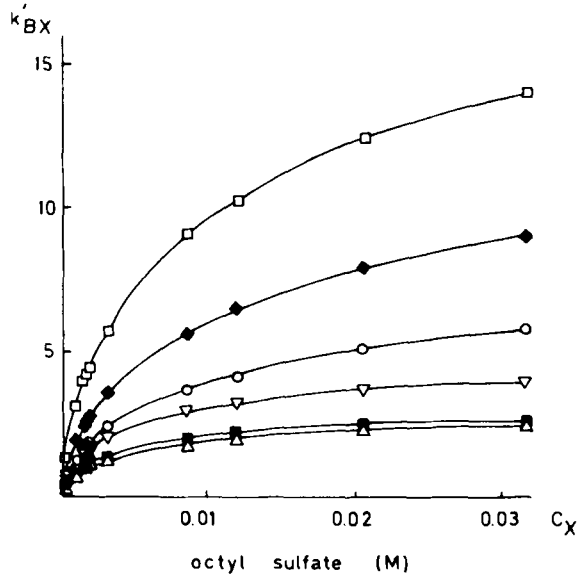



FIGURE 5. Retention of phenethylamine derivatives as ion pairs with octyl sulfate. Conditions as in FIGURE 2.
Samples: △ = noradrenaline; ■ = adrenaline; ▽ = synephrine; ◔ = dopamine; ● = tyramine; □ = pholedrine.

$$\frac{[X^-]_m}{k'_{BX} - \dfrac{c}{1 + K^x_{QX}.b}} = \frac{1}{q.K_0.K_{BX}} + \frac{K_{QX}.b}{q.K_0.K_{BX}} \qquad (11)$$

where $b = [Q^+]_m.[X^-]_m$ and $c = q.K^x_0.K^x_{BX}.[X^-]_m$

A preliminary computation of $q.K_0.K_{BX}$ and $K_{QX}$ was made by use of capacity ratios obtained at $[X^-]_m < 2 \times 10^{-3}$ M and the approximation $K^x_{QX}.b < 1$. The found value were used in a graphical computation of $q.K^x_0.K^x_{BX}$ and $K^x_{QX}$ by use of capacity

ratios obtained at higher concentration of octyl sulfate, $[X^-]_m$ = (8-30) x $10^{-3}$ M, and the eq.

$$\frac{[X^-]_m}{k'_{BX} - \frac{d}{1 + K_{QX}.b}} = \frac{1}{q.K_0^x.K_{BX}^x} + \frac{K_{QX}.b}{q.K_0^x.K_{BX}^x} \qquad (12)$$

where $b = [Q^+]_m.[X^-]_m$ and $d = q.K_0.K_{BX}.[X^-]_m$

The preliminary values were finally corrected by repeated calculations with successive use of eqs. (11) and (12). It should be notified that the retention of the amines in the absence of octyl sulfate was very low and disregarded in the calculations above. The results are given in Table 3.



TABLE 3

Equilibrium Constants for Phenethylamine Derivatives

Solid phase: LiChrosorb RP-18
Eluent: octyl sulfate, $X^-$, in phosphate buffer pH 3.0 with 1.15% 1-pentanol

| | $C_X$=(0.23-1.9) x $10^{-3}$M | | $C_X$=(8.6-31) x $10^{-3}$M | |
|---|---|---|---|---|
| Sample | $K_{BX}$ x $10^{-3}$ [a] | $K_{QX}$ x $10^{-3}$ | $K_{BX}^x$ x $10^{-3}$ [a] | $K_{QX}^x$ x $10^{-3}$ |
| Noradrenaline | 39 | 24 | 0.89 | 0.57 |
| Adrenaline | 37 | 18 | 0.93 | 0.60 |
| Synephrine | 51 | 15 | 1.2 | 0.51 |
| Dopamine | 61 | 20 | 1.6 | 0.35 |
| Tyramine | 97 | 22 | 2.5 | 0.36 |
| Pholedrine | 155 | 20 | 4.0 | 0.38 |

a) calculated by use of q = 822, $K_0$ = 5.2 x $10^{-5}$ and $K_0^x$ = 3.1 x $10^{-4}$ (Table 2)

The found values of $K_{QX}$ and $K^{x}_{QX}$ are in good agreement with the results from the adsorption studies (Table 2) which confirms the validity of the model. The stronger site has about 40 times higher adsorption constant than the weaker site for all the phenethylamine derivatives studied. The effect of the two sites on the retention is illustrated in Fig. 6 with dopamine as object. The retention by the weaker site dominates at higher concentrations of octyl sulfate because the weaker site has about six times higher capacity than the stronger one.

Retention studies were also performed with some lower alkylamines, using fluorimetric detection after post-column derivatisation with phthaldialdehyd (see EXPERIMENTAL). The relation between the capacity ratio and the concentration of octyl sulfate in the eluent is demonstrated in Fig. 7.

The retention data were applied to the two site model and adsorption constants were calculated by eqs. (11) and (12) using the same method as for the phenethylamine derivatives. Straight



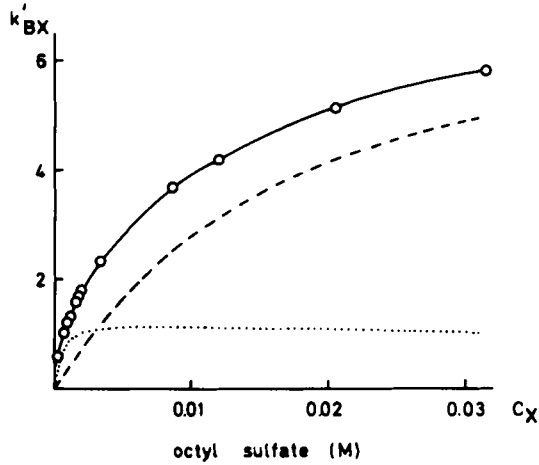



FIGURE 6. Retention of dopamine as ion pair with octyl sulfate. Conditions as in FIGURE 2.; ○ = total retention; ----- = retention to the weaker adsorption site; ..... = retention to the stronger adsorption site.

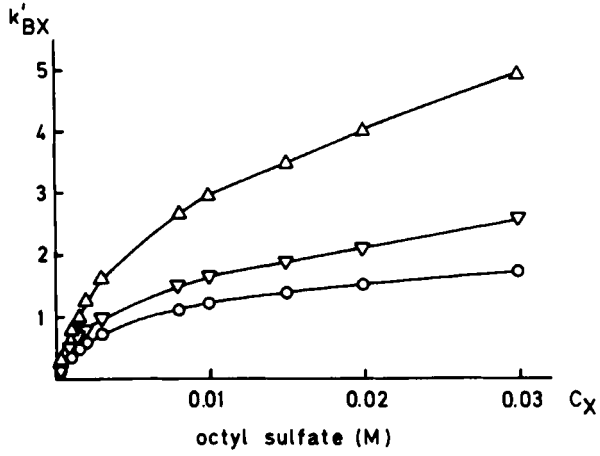



FIGURE 7. Retention of aliphatic amines as ion pairs with octyl sulfate. Conditions as in FIGURE 2 with fluorometric detection after post-column derivatisation with phthaldialdehyd; Samples: ○ = methylamine; ▽ = ethylamine; △ = propylamine.



linear plots were obtained in the graphical computation of the constants and the found values of $K_{QX}$ and $K^{x}_{QX}$ were in good agreement with those found for noradrenaline and dopamine in the same chromatographic system. The alkylamines seems to be adsorbed to the same sites as the phenethylamine derivatives.

The nature of bonded phases has been discussed by several authors (cf. [26-29]). This study has indicated the presence of two sites on the solid phase with different ability to retain ion pairs. It is not possible however to state the nature of the individual site, since the samples used have similar structure and show the same relative binding to both sites.

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