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A Weak Cation-Exchange Phase for the Separation of Biogenic Amines by Open Tubular Liquid Chromatography

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Key Words

Column liquid chromatography
Open tubular columns
Weak cation-exchange stationary phase
Biogenic amines
LIF detection

Summary

The preparation and performance of a weak cation-exchange stationary phase for Open Tubular Liquid Chromatography (OT-LC) was investigated. The stationary phase was prepared in 5.4 μm I.D. fused silica capillaries by *in situ* photopolymerization of a mixture of silicon acrylate and acrylic acid. The influence of pH, counter ion concentration and organic modifier concentration of the mobile phase on the retention was studied with catecholamines as test solutes using LIF detection. Other biological amines like amino acids, small peptides and nucleic acid derivatives could be separated on this stationary phase as well. The kinetic performance of the stationary phase was studied with several cations and neutral solutes.

Introduction

In the last years there is an increasing interest in miniaturization of separation techniques. An increase in separation speed and efficiency and the ability to analyze very small samples are generally regarded as the greatest reasons to study micro separation techniques. During the last two decades several miniaturized liquid chromatographic methods have been developed like microcolumn LC [1], packed capillary LC [2], OT-LC [3], electrochromatography [4] and microchip LC [5].

Among them, so far only microcolumn LC and packed capillary LC, also called microcapillary LC, have ob-

tained acceptance as routine techniques [2]. The other techniques are very promising but are still in the research stage.

The open geometry of the capillary column in OT-LC offers an increase in separation efficiency compared to packed columns, provided that the internal diameter of the capillary is small (1–10 μm). Although the great potentials of OT-LC, which were e.g. demonstrated by the group of Jorgenson with the determination of the amino acid content of a single cell [6], the technique has hardly been applied yet.

The development of OT-LC has i.a. been delayed by the lack of techniques to create a thick retentive layer in small I.D. capillaries with sufficient sample capacity necessary to avoid column overloading. The last couple of years we reported on the results of an *in situ* photopolymerization technique to create polymeric acrylate stationary phases in fused silica capillaries [3, 7–9]. This technique is different from other coating techniques in that the polymer is formed inside the capillary. These retentive layers are build up from a bifunctional silicone-acrylate and an acrylate monomer. The retentive and kinetic properties of the stationary phase could be varied by selection of the type of acrylate monomer. Various acrylic monomers e.g. with alkyl chains [3], an ethoxy group [7] and a hydroxy group [9] have been used. These stationary phases exhibited excellent separation performance in both reversed- and normal phase chromatography but had one major limitation: only neutral compounds could be retained. Charged solutes were invariably unretained; in fact they were used for the determination of the dead volume of the column. This limitation seriously restricts the applicability of OT-LC. Biologically active compounds like amino acids and catecholamines are charged and have important functions in health and disease states [10, 11]. An OT-LC separation procedure for these compounds could be of considerable interest, because the extremely small sample size needed is often of great importance in biological studies [12–14]. In RP OT-LC, using polyacrylate stationary phases, these amines could only be

separated in combination with pre-column derivatization [8]. The use of a cation-exchange layer in OT-LC has been demonstrated before by Müller et al. [15, 16]. However, serious problems were encountered during the coating of the columns. Preparation of the stationary phases by means of static coating took at least 6 weeks [15] and a dynamic coating procedure [16] was found difficult to reproduce.

In this study we report on an investigation to prepare a weak cation-exchange stationary phase (SiA-AA) for OT-LC. The retentive properties of the layer as function of the pH, counter ion concentration and acetonitrile concentration of the mobile phase were tested with catecholamines. The retention of aromatic amino acids, peptides and nucleic acid derivatives was studied as function of the pH of the mobile phase. The kinetic performance of the stationary phase was investigated with cations and neutral solutes.

Although we successfully applied UV detection on 5–10 μm I.D. capillaries in the past [3, 7–9], in this study we used laser induced fluorescence (LIF) detection to increase the detectability of the solutes. Native fluorescence of the biogenic amines was measured after excitation with 257 nm radiation from a frequency doubled argon ion laser.

Experimental

Apparatus

The OT-LC system was similar to the one described previously [7].

The frequency doubled output (257 nm) from an argon ion laser (model INNOVA 300 Fred, Coherent Laser group, Santa Clara, CA, USA) was used as the excitation source for LIF detection. A double monochromator, (model MCG 990, Macam, Livingston, Scotland) with two holographic gratings was used to select the emission wavelength. Fused silica lenses (Newport Corporation, Irvine, CA, USA) were used for focusing the laser beam and collecting the fluorescence. The emission light was measured with a photomultiplier tube (model PM 102 S, Macam, Livingston, Scotland) operated at -1000 V . The amplifier included a RC-filter with a time constant of 0.5 seconds. A detailed description of the UV LIF detection set up can be found elsewhere [17].

Materials

Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) with inner diameters of approximately 5.4 μm were used. The outside UV transmitting 'buffer' coating of the capillaries allowed in situ photopolymerization.

HPLC-grade acetonitrile was obtained from Biosolvent (Amsterdam, The Netherlands); acrylic acid (AA) was purchased from Fluka (Buchs, Switzerland). Silicone acrylate (SiA) (Tegomer V-Si2150) was obtained from Goldschmidt (Essen, Germany). Catecholamines and nucleotides were obtained from Sigma (St. Louis, MO, USA), peptides were obtained from Nutritional Biochemicals Corporation (Cleveland, Ohio, USA).

Coating Procedure

The photopolymerization of acrylates to immobilize a polymer film in fused silica capillaries has been described before [7]. Briefly the procedure consists of the following successive steps:

- Etching of the fused silica surface to enlarge the number of silanol groups.
- Silylation of the surface with a silyl reagent containing an acrylate group. The acrylate group on the surface is needed to anchor the polymer layer.
- Filling the capillary with a solution of acrylates and the photoinitiator in acetone after which the capillary is irradiated with UV light to initiate the polymerization.
- Evaporation of the acetone.
- Thermal curing of the stationary phase at elevated temperatures.

The coating conditions are described in Table I.

Chromatography

The solutes were eluted with various buffered mobile phases. Buffers in the low pH range (3.3–5.8) were prepared from acetic acid, intermediate pH buffers (6–7.5) were prepared from sodium dihydrogen phosphate while borax was used to prepare high pH buffers (8.6–10.0). All buffers were used in a concentration of 20 mmol L^{-1} unless stated otherwise. Sodium hydroxide was used to adjust the pH. Sodium chloride was added to adjust the counter ion (Na^+) concentration to 60 mmol L^{-1} , unless stated otherwise.

Table I. Experimental coating conditions and dimensions of the prepared fused silica columns¹.

Cap	$L(\text{cm})$	monomer (% v/v)	$d_c (\mu\text{m})$	$d_f (\mu\text{m})$	V_s/V_m
1	80	7.5 %SiA – 7.5%AA	4.64	0.38	0.35
2	51	7.5 %SiA – 7.5%AA	4.68	0.36	0.33

1: DMPA concentration: 3.0 mg mL^{-1} for both monomer solutions; polymerization solvent: acetone; light intensity: 0.14 mW ; irradiation time: 730 s, d_c : column diameter, d_f : film thickness, V_s/V_m : phase ratio.

Figure 1

Length of the emptied part of the capillary versus the elapsed evaporation time. Temperature: □ 34 °C (column 1), ○ 22 °C (column 2). Solid lines represent the theoretical square root dependence between the capillary length and evaporation time.

Results and Discussion

Column Preparation

The dimensions of the coated capillaries can be found in Table I. As can be seen a reasonably thick cation-exchange stationary phase can be immobilized in 5.4 µm i.d. capillaries by *in situ* photopolymerization of acrylates.

In the coating procedure the evaporation of the solvent from the capillary is the most time consuming step. Previously we have used acetone-pentane mixtures as the solvent, which turned out to allow the fastest evaporation [3]. However, this solvent mixture appears not applicable for the preparation of the present stationary phase, as no polymer gel was formed. Most probably the difference in polarity of the pentane/acetone mixture and the acrylate polymer is too large. These problems were not encountered with pure acetone and this solvent was therefore used accepting a slower evaporation speed.

The coating speed can also be increased by elevation of the temperature at which the solvent is evaporated from the capillary. The effect of the temperature on the speed of solvent evaporation from the polyacrylate coated columns was studied by following the evaporation process in time at different temperatures. Evaporation of acetone from column 1 and 2 was performed under vacuum at respectively 34 °C and 21 °C. Figure 1 shows the length of the emptied part of the capillary (solvent evaporated) versus the elapsed time. As can be seen a significant reduction of the evaporation time can easily be realized by increasing the temperature from 21 °C to 34 °C.

From static coating in GC [18] it is known that the length of the empty part of the capillary increases with the square root of the time:

$$L = c \sqrt{t}$$

where L is the length of the emptied part of the capillary, c is an empirical constant and t is the time. The experimental data points were fitted with this function. At 21 °C and 34 °C, the values of c were 1.20 ms^{-1/2} and 1.65 ms^{-1/2} respectively. At 34 °C the empty part of the capillary increases by a factor 1.38 faster than at 21 °C, or alternatively, to yield a certain capillary length the required evaporation time is reduced by a factor of 1.89 (= (c₃₄/c₂₁)²) at 34 °C. The relationships can be used to estimate the evaporation time for any capillary length under these coating conditions.

Characterization of the Stationary Phase

The retention behaviour of column 1 and 2 appeared to be similar, but to allow a fair and consistent characterization of the stationary phase and to speed up the measurements, the retention experiments were done on column 2 only.

Influence of the pH of the Mobile Phase on the Retention of Catecholamines

The SiA-AA stationary phase is built up from a silicon acrylate and acrylic acid, the latter has a pK_a of 4.25 in free solution [19]. Retention of the positively charged amines can be assumed to occur mainly by electrostatic interactions with the carboxylic groups present in the layer. Therefore the SiA-AA stationary phase can be considered as a weak cation-exchanger.

Since the pH of the mobile phase determines the charge of the ionizable solute and that of the carboxylic group in the stationary phase, the pH will be an important parameter for adjusting the retention and selectivity of cations. Figure 2 shows the retention of adrenaline and noradrenaline on the SiA-AA stationary phase as function of pH.

The mobile phase consisted of the required buffer, with an added amount of NaCl such that the Na⁺ concentration was 60 mmol L⁻¹. At pH 3.2 the catecholamines are positively charged. However the carboxylic groups are probably for only about 10 % ionized and hence the ion-exchange capacity is small. This means that the retention of the catecholamines by ion-exchange will be small.

With increasing pH the carboxylic groups are more deprotonated and the retention of adrenaline and noradrenaline increases. Remarkable is the change in elution order between the two catecholamines at around pH 4.8. This reversal of the elution order between adrenaline and noradrenaline is usually not

Figure 2

Capacity factors of adrenaline and noradrenaline 2 as function of the pH of the mobile phase with $[Na^+] = 60 \text{ mmol L}^{-1}$. Symbols: ■) noradrenaline, ○) adrenaline

found in conventional cation-exchange [20] and RP ion-pair chromatography [21].

Possibly, hydrophobic interactions contribute to the overall retention at low pH. As adrenaline has one methyl group more in its structure compared to noradrenaline stronger hydrophobic interactions and larger retention can be expected for adrenaline at low pH.

At high pH the retention of the catecholamines is strongly reduced. This is caused by deprotonation of the amine and hydroxyl group of the catecholamines resulting eventually in a negative charge causing expulsion from the stationary phase. At pH 10 all retention is lost.

Influence of the Counter Ion Concentration in the Mobile Phase on the Retention of Catecholamines

The ion-exchange process involves competition between solute ions and counter ions, to pair with the oppositely charged functional groups in the stationary phase. According to ion-exchange theory the capacity factor of a solute should be inversely proportional to the counter ion concentration in the mobile phase. A linear relation between $\log k'$ and $\log [\text{counter ion}]$ can therefore be expected. The slope should be equal to the charge of the solute, in this case equal to one. The retention of adrenaline and noradrenaline was studied as function of the Na^+ concentration with an aqueous mobile phase of pH 5.4. Figure 3 shows such a log-log plot.

Although the observed relationship is not perfectly linear the slopes of -0.95 and -0.92 for noradrenaline and adrenaline respectively indicate that retention of

Figure 3

Capacity factors of adrenaline and noradrenaline as function of the Na^+ concentration in the mobile phase. The pH of the mobile phase is 5.4. Symbols as in Figure 2.

the amines on the SiA-AA stationary phase at pH 5.4 is largely governed by an ion-exchange mechanism and that hydrophobic interactions only play a minor role.

The retention of the catecholamines can easily be adjusted over one order of magnitude by increasing the Na^+ concentration in the mobile phase, whereas selectivity is only slightly affected. The Na^+ concentration is therefore a valuable parameter for changing the absolute retention of the catecholamines.

Influence of Acetonitrile Concentration in the Mobile Phase on the Retention of Catecholamines

The retention of the catecholamines can be influenced by the addition of an organic modifier to the mobile phase. This is illustrated in Figure 4 showing the capacity factor of noradrenaline and adrenaline versus the acetonitrile concentration in the mobile phase. The pH (measured before the addition of acetonitrile) and the total counter ion concentration were kept constant for all mobile phase compositions. As can be seen the capacity factor of noradrenaline decreases with increasing concentration of acetonitrile and becomes constant at about 50 %. The same behaviour is found for adrenaline but the decrease in capacity factor still continues after addition of 50 % acetonitrile. The difference in retention between adrenaline and noradrenaline, whose structures differ only by one methyl group, becomes larger with increasing acetonitrile concentration. As a result of this the resolution between noradrenaline and adrenaline is substantially improved. These observations are in contrast with findings in RP LC, where the difference in retention caused by a substituted methyl

Figure 4
Effect of the acetonitrile concentration in the mobile phase on the capacity factors of adrenaline and noradrenaline. The Na^+ concentration is 18 mmol L^{-1} , the pH of the buffer is 5.4. Symbols as in Figure 2.

Figure 5
Separation of adrenaline and noradrenaline on column 2 with 68 % acetonitrile in the mobile phase. Peaks: 1) adrenaline ($k' = 2.9$); 2) noradrenaline ($k' = 7.2$).

Figure 6
Experimental and theoretical plate height curves for several compounds on column 2. Compounds: ●: noradrenaline ($k' = 2.85$), ○: adrenaline ($k' = 2.94$), △: 2,3-dimethylphenol ($k' = 3.60$), □: 4-ethylphenol ($k' = 3.60$). Mobile phase for noradrenaline: acetonitrile/buffer (75:25 v/v %) and for other compounds: pure aqueous buffers. Theoretical plate height curves: (—) adrenaline, (.....) 2,3-dimethylphenol and (---) 4-ethylphenol.

group decreases with increasing percentage of organic modifier in the mobile phase.

Figure 5 illustrates the high resolution between the two catecholamines eluted with 68 % (v/v) acetonitrile in the mobile phase. In this chromatogram capacity factors of adrenaline and noradrenaline are smaller compared to those in Figure 4 at corresponding acetonitrile concentration since the counter-ion concentration is higher, 30 mmol L^{-1} .

Effect of the Mobile Phase on the Film Thickness of the Stationary Phase

Polymeric ion-exchange resins can swell by uptake of water to an extent that depends on the crosslinking [22]. Since swelling of polyacrylate stationary phases was found to have a large effect on the column performance [9], the swelling of the SiA-AA stationary phase was determined with various mobile phase compositions. The film thickness of the stationary phase was calculated by measuring the actual internal diameter and the original internal diameter of the capillary before preparing the stationary phase. This was done by measuring the holdup time of an unretained solute or solvent as described in [3].

Column 2 was consecutively flushed with i) a hydrochloric acid solution of pH 2 (fully protonated acrylic acid groups), ii) deionized water (fully deprotonated acrylic acid groups), iii) a phosphate buffer, $[\text{Na}^+] = 60 \text{ mmol L}^{-1}$, pH = 7.2, and mixtures of methanol (75 % v/v) and acetonitrile (75 % v/v) with iii. The film thickness was virtually constant under all these conditions. This indicates that this phase does not swell neither in aqueous solutions nor in aqueous-organic solvent mixtures. These results are in contrast with previous swelling experiments of polyacrylate stationary phases in aqueous-organic solvents [9].

Column Performance and Stability

The performance of the SiA-AA stationary phase was determined by measuring plate heights of several test solutes at different velocities of the mobile phase. The cation adrenaline and two neutral compounds, 4-ethylphenol and 2,3-dimethylphenol, were selected for these experiments to reveal possible differences in band broadening between charged and neutral solutes. Figure 6 shows the experimental plate heights versus the mobile phase velocity. By fitting the plate height values with the extended Golay equation, using the diffusion coefficient of the solute in the mobile phase calculated with the Wilke-Chang equation [23], the diffusion coefficient of a solute in the stationary phase, D_s , can be estimated. By using an aqueous buffered mobile phase for adrenaline, 4-ethylphenol and 2,3-dimethylphenol, D_s values of respectively 1.3 , 1.4 and $1.2 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ were estimated from Figure 6. These diffusion coefficients are comparable to a previous reported D_s value of $1.2 \times 10^{-12} \text{ m}^2 \text{ s}^{-1}$ obtained for 1,3-dihydroxybenzene on the

Figure 7

Separation of catecholamines and related compounds on column 1. Mobile phase: 1 % acetic acid, $[Na^+] = 60 \text{ mmol L}^{-1}$, pH = 3.6/acetonitrile (80:20 v/v %). LIF detection: excitation: 2 mW 257 nm, emission: 320 nm. All compounds are present in a concentration of $6.6 \times 10^{-5} \text{ mol L}^{-1}$: 1 = DOPAC (3,4-Dihydroxyphenylacetic acid); 2 = Homovanillic acid; 3 = Noradrenaline; 4 = Adrenaline; 5 = Normethanephine, Dopamine; 6 = Methanephine; 7 = Tyramine; 8 = 3-Methoxytyramine.

SiA-EEA stationary phase with water as the mobile phase [9].

In both systems the efficiency is seriously impaired by such small diffusion coefficients.

The estimated diffusion coefficient of adrenaline, retained by ion-exchange, is quite similar to the diffusion coefficients of the neutral compounds 2,3-dimethylphenol and 4-ethylphenol, which are merely retained by hydrophobic interactions. As also the size of the solutes is almost similar, it can be concluded that the kinetics of the ion-exchange process involved in the retention of adrenaline does not impair separation efficiency.

The plate heights of the test solutes on the SiA-AA stationary phase are high compared to previously found values in polyacrylate coated open tubular columns [8]. Comparing plate heights observed here (120–150 μm) and in the study [8] at the same linear velocity of 10 mm s^{-1} (12–25 μm) a loss in efficiency of nearly a factor 10 is observed. The favourable H-values in [8] were obtained exclusively with swollen stationary phases. Swelling was obtained with the addition of an organic solvent to the mobile phase.

To study the effect of the mobile phase composition on the performance of the SiA-AA stationary phase, plate heights of noradrenaline were measured with a mobile phase containing 75 % (v/v) acetonitrile. These measurements are included in Figure 6 (solid circles). As can be seen the plate heights of noradrenaline ($k' = 2.9$) almost fully coincide with the plate height values of adrenaline ($k' = 2.9$), eluted with a pure aqueous buffer. A direct comparison of these measurements is justified since the important parameters like capacity factor, film thickness and molecular size are very similar. No improvement of the separation efficiency of the SiA-AA stationary phase is achieved by increasing the organic

Figure 8

Capacity factors of Gly-Trp (■), Leu-Phe (●), Gly-Phe (▲) and Tyr (□) versus the pH of the mobile phase.

modifier concentration in the mobile phase. Most probably no improvement of the efficiency occurs because the stationary phase does not swell.

The column performance appeared to be constant during several months of operation. A separation of several catecholamines and related compounds on the cation-exchange column is shown in Figure 7.

The first two compounds, DOPAC (3,4-dihydroxyphenylacetic acid) and homovanillic acid, are negatively charged. Despite the presence of a negatively charged group, retention and selectivity are just sufficient to allow the separation of these two solutes. The other amines are retained by ionic interactions. Normethanephine and dopamine coelute under these conditions.

Separation of Biogenic Amines

The SiA-AA stationary phase can be used for the separation of several other classes of amines than catecholamines. The retention behaviour of amino acids and dipeptides on the cation-exchange column has been studied as function of the pH of the mobile phase. Figure 8 shows the capacity factor of the dipeptides Gly-Trp, Leu-Phe, Gly-Phe and the amino acid tyrosine. Increasing the pH of the mobile phase from 3.3 to 5.8 leads to a decrease in retention of these amines. The retention behaviour of the peptides as function of pH is the reverse of that of the catecholamines. The amino acids and dipeptides have, unlike the catecholamines, a carboxyl group in their structure. Depending on the pH of the mobile phase the carboxyl group is partly or totally dissociated and the peptide is amphoteric. No explanation is yet found for the increase in retention of Leu-Phe and tyrosine at pH 6.7.

The retention behaviour of several nucleic acid derivatives was studied in relation to the pH of the mobile phase. Figure 9 shows the capacity factors of adenine,

Figure 9
Capacity factors of adenine (▲), adenosine (■) and guanosine (●) versus the pH of the mobile phase.

Figure 10
Separation of adenine-monophosphate (1), guanosine (2), adenosine (3) and adenine (4) on column 2. Chromatographic conditions as in Figure 3. LIF detection: excitation: 20 mW 257 nm, emission: 390 nm.

adenosine and guanosine in the pH range 3.3 to 6.7. A maximum in the capacity factor of both adenine and adenosine was observed around pH 4.5. This elution behaviour is caused by two counteracting effects influencing the retention. The amino groups of adenosine and adenine have pK_a values of 3.5 and 4.1 respectively [24] and the compounds become less positively charged with increasing pH. On the other hand in the same pH region the stationary phase obtains more charge by deprotonation of the carboxylic groups. This explains the occurrence of a maximum. The remaining retention of the compounds at higher pH must be ascribed to hydrophobic interactions.

Figure 10 shows a separation of adenosine-monophosphate (AMP), guanosine, adenosine and adenine (Ade). The injected amounts ranged from 8×10^{-15} mol for AMP to 2×10^{-14} mol for Ade. The nucleotide

adenosine-monophosphate is not retained on the cation-exchange phase since the acidic phosphate group bears a negative charge under present separation conditions.

Conclusions

The SiA-AA polyacrylate stationary phase is stable and suited for the separation of several classes of biogenic amines. The film thickness of the cation-exchange stationary phase is independent of pH and counter ion concentration of the mobile phase.

The cations are retained by an ion-exchange mechanism. Since neutral compounds can be retained as well on the SiA-AA stationary phase, hydrophobic interactions are assumed to contribute to the overall retention of cations. The retention behaviour of catecholamines, dipeptides and nucleobase derivatives as function of the pH is significantly different. A negative charge on the molecule strongly reduces the retention on the ion-exchange phase. Furthermore the addition of an organic modifier to the mobile phase and alteration of the counter ion concentration in the mobile phase can be used to vary retention.

The column efficiency is yet unsatisfactory and future research is planned in order to improve the performance of the cation-exchange stationary phase. The separation efficiency might be increased by 1) fabricating less cross-linked stationary phases by which the extent of swelling can be increased, 2) changing the chemical composition of the stationary phase, 3) elevation of the temperature or by 4) applying thinner stationary phase films.

References

- [1] H. J. Cortes, J. R. Larson, G. M. McGowan, *J. Chromatogr.* **607**, 131 (1992).
- [2] J. P. Chervet, M. Ursem, J. P. Salzmann, *Anal. Chem.* **68**, 1507 (1996).
- [3] R. Swart, J. C. Kraak, H. Poppe, *J. Chromatogr.* **670**, 25 (1994).
- [4] J. H. Knox, I. H. Grant, *Chromatographia* **24**, 135 (1987).
- [5] S. C. Jacobson, R. Hergenröder, L. B. Koutny, J. M. Ramsey, *Anal. Chem.* **66**, 2369 (1994).
- [6] M. D. Oates, B. R. Cooper, J. W. Jorgenson, *Anal. Chem.* **62**, 1573 (1990).
- [7] R. Swart, J. C. Kraak, H. Poppe, *J. Chromatogr.* **689**, 177 (1995).
- [8] R. Swart, J. C. Kraak, H. Poppe, *Chromatographia* **40**, 587 (1995).
- [9] R. Swart, S. Brouwer, J. C. Kraak, H. Poppe, *J. Chromatogr.* **723**, 203 (1996).
- [10] R. M. Wightman, J. M. Finnegan, K. Pihel, *Trac* **14**, 154 (1995).
- [11] T. Nagatsu, *J. Chromatogr.* **566**, 287 (1991).
- [12] L. A. Dawson, J. M. Stow, C. T. Dourish, C. Routledge, *J. Chromatogr.* **700**, 81 (1996).
- [13] L. Hernandez, S. Tucci, N. Guzman, X. Paez, *J. Chromatogr.* **652**, 393 (1993).
- [14] M. W. Lada, R. T. Kennedy, *Anal. Chem.* **68**, 2790 (1996).

- [15] S. R. Müller, W. Simon, H. M. Widmer, K. Grolimund, G. Schomburg, P. Kolla, *Anal. Chem.* **61**, 2747 (1989).
- [16] S. Müller, D. Scheidegger, C. Haber, W. Simon, *HRC&CC* **14**, 174 (1991).
- [17] R. Swart, J. W. Elgersma, J. C. Kraak, H. Poppe, submitted to *J. Microcol. Sep.*
- [18] C. P. M. Schutjes, Ph. D. Thesis, Technical University, Eindhoven (1983) p 95.
- [19] R. C. Weast, *Handbook of Chemistry and Physics*, 64th edition, CRC Press, FL, USA, D-167.
- [20] P. Moleman, J. J. M. Borstrok, *J. Chromatogr.* **232**, 418 (1982).
- [21] T. P. Moyer, N. Jiang, *J. Chromatogr.* **153**, 365 (1978).
- [22] H. F. Walton, *J. Chromatogr.*, Library Volume, 51B, chap. 5, p 227, 5th edition, E. Heftmann, ed., Elsevier, Amsterdam, 1992.
- [23] R. C. Reid, "The properties of gases and liquids" McGraw-Hill, New York, 1987.
- [24] D. D. Perrin, "Dissociation constants of organic bases in aqueous solution", Butterworths, London, 1965.

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