

Influence of eluent composition on retention and selectivity of alkylamide phases under reversed-phase conditions

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Abstract

The sorption properties of alkylamide phases have been studied under reversed-phase conditions using different compositions of methanol–water as eluents. These studies have revealed that the specific structural properties of the alkylamide phases have a greater influence on solute retention and selectivity than is observed for conventional alkyl bonded phases. Also, it has been demonstrated that the composition of solvents in the stationary phase can be changed significantly by the presence of a specific interaction site in the bonded ligands.

1. Introduction

A variety of chemically bonded phases (CBP) have been used as packing materials in reversed-phase high performance liquid chromatography (RP-HPLC) [1–3]. Their selectivity is determined by many factors, such as: chemical nature of bonded ligands, their coverage density, and surface properties of the solid support. These factors influence strongly the retention mechanism and selectivity of chemically bonded phases under RP-HPLC conditions [4–6].

In many of the earlier RP-HPLC models it was assumed that the retention mechanism was controlled by the specific and nonspecific solute–solvent interactions in the mobile phase, whereas the bonded alkyl chains were passive acceptors

of the solute [7,8]. Recent studies of chemically bonded phases, especially phases of specific functionality and structural properties [9–11], have shown that the type, structure and density of the CBP are important factors which control the solute's retention. Moreover, it has been suggested that alkylamide (AA) phases with incorporated solvent molecules have a more ordered structure than conventional alkyl phases [11].

Homologs are often used as test solutes to evaluate the relative hydrophobicity of different CBPs under RP-HPLC conditions [12–14]. The nonspecific or hydrophobic selectivity for adjacent members in a homologous series is defined as follows:

$$\alpha_c = k'_{n+1}/k'_n \quad (1)$$

where k'_{n+1} and k'_n are respectively the capacity factors for solutes with $n + 1$ and n carbon atoms in their alkyl chains [12]. In many cases the natural logarithm of α_c is nearly constant over a range of homolog pairs were steric effects do not

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play a role [12]. Further, the $\ln \alpha_c$ is proportional to the free energy of transfer of a methylene group ($-\text{CH}_2-$) between the mobile and stationary phases [6,13] and it can be determined from the slope of the plot of $\ln k'_n$ vs. n_c measured at a constant composition of hydro-organic mobile phase:

$$s = d(\ln k'_n) / dn_c = \ln \alpha_c \quad (2)$$

For binary mixtures of water-methanol the dependence of s on the volume fraction of water (φ_w^1) is nearly linear over the entire range of solvent compositions [12,13,15–22]. However, for other hydro-organic mobile phases (e.g. water-acetonitrile, water-tetrahydrofuran, water-isopropanol, etc.) the plots of s vs. φ_w^1 are non-linear [15–22].

In the current work, chemically bonded alkylamide phases were studied under reversed-phase conditions using different compositions of methanol-water as the eluent. These studies have revealed that the specific and structural properties of the alkylamide phases have a greater affect on the solute's retention and selectivity than is observed for conventional alkyl bonded phases. The sorption properties of the alkylamide phases were evaluated on the basis of retention measurements for alkylbenzene homologs. It has been shown that the composition of solvents in the stationary phase can be changed significantly by the presence of a specific interaction site in the bonded ligands.

2. Experimental

2.1. Materials

LiChrospher Select B (Merck, Darmstadt, Germany) with a mean particle diameter of 5 μm and with a BET specific surface area (S_{BET}) of 570 m^2/g has been used to prepare the chemically bonded phases. Two types of packings were synthesized: conventional C_{18} phases and alkylamide (AA) phases with alkyl chains of different length ($-\text{CH}_3$, $-\text{C}_5\text{H}_{11}$ and $-\text{C}_{17}\text{H}_{35}$). The AA phases were synthesized in a sealed glass reactor by a two-step process [9–11], in

which an initial aminopropyltrimethylsilyl phase was prepared under environmentally isolated conditions and subsequently reacted with either acetyl, hexanoyl and stearoyl chlorides. For instance, the amino phase modified with acetyl chloride contain the following bonded ligands: $-\text{Si}(\text{CH}_3)_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}-\text{CO}-\text{CH}_3$ (methylamide chains) and $-\text{Si}(\text{CH}_3)_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_2$ (aminopropyl residuals). In addition, each silica-based chemically bonded phase contains unreacted surface silanols. Listed in Table 1 are the coverage data for the materials based on elemental carbon and nitrogen analysis. Additional information (obtained from solid-state NMR, porosimetry and secondary-ion mass spectrometry) about these packings as well as the unmodified silica have been reported elsewhere [10,11].

Solvents

Methanol and acetonitrile (HPLC grade purity) were purchased from Fisher Scientific (Fairlawn, NJ, USA). The hydro-organic mobile phases were prepared from deionized water purified in-house using a Milli-Q reagent water system (Millipore, El Paso, TX, USA).

Table 1
Surface coverages of the aminopropyl (SG-NH₂), alkylamide (AA) and octadecyl (C₁₈) phases

Phase	R ^a	Total %C	c_{RP}^b	$c_{\text{NH}_2}^c$
SG-NH ₂	—	6.9	—	2.33
AA ₁	—CH ₃	8.3	1.38	0.95
AA ₂	—C ₅ H ₁₁	9.9	0.98	1.35
AA ₃	—C ₁₇ H ₃₅	19.7	1.59	0.74
C ₁₈	—C ₁₈ H ₃₇	23.2	2.42	—

For the AA phases c_{RP} denotes only the concentration of alkylamide ligands and c_{NH_2} represents the concentration of unreacted (residual) aminopropyl groups; the total coverage density is the sum of the surface concentrations of alkylamide ligands and aminopropyl residuals. Note that for the aminopropyl phase c_{NH_2} denotes the total coverage density because this phase has only one type of bonded ligands.

^a R-alkyl chain attached to the amide group.

^b c_{RP} -concentration of bonded alkyl-ended ligands expressed in [$\mu\text{mol}/\text{m}^2$].

^c c_{NH_2} -concentration of aminopropyl groups expressed in [$\mu\text{mol}/\text{m}^2$].

The modified materials were packed into 60×2.1 mm I.D. stainless-steel tubes purchased from Supelco (Bellefonte, PA, USA) according to procedures described elsewhere [3,4].

2.2. Chromatographic studies

Retention measurements were carried out at 298 K using a liquid chromatograph which consisted of a Spectra-Physics (San Jose, CA, USA) Model SP-8810 precision isocratic pump, a Model Spectra 100 variable UV detector ($\lambda = 254$ nm), and a Chromjet integrator. Solutes were injected using a Rheodyne (Berkeley, CA, USA) Model 7125 sampling valve with $20\text{-}\mu\text{l}$ sample loop. In all chromatographic investigations the flow rate (1 ml/min) was monitored with a Phase Separations (Queensberry, Clwyd, UK) Model FLOSO A1 flow meter connected to the detector outlet.

3. Results and discussion

Shown in Figs. 1 and 2 are the retention data for the alkylbenzenes plotted as the natural logarithm of the capacity ratio (k') vs. the number of carbon atoms (n_c) in the chain segment of the solute. These data were measured on both the alkylamide and C_{18} phases (cf., Table 1) using different mixtures of water-methanol as the mobile phase. The ($\ln k'$ vs. n_c) dependencies for the AA_1 , AA_2 and AA_3 phases are presented in Figs. 1a, 1b and 1c, respectively, and the data for the C_{18} phase are plotted in Fig. 2. Except for the data shown in Fig. 1a for the totally aqueous conditions, the remaining Figs. contain a series of ($\ln k'$ vs. n_c) plots measured for different compositions of the binary mobile phase.

A comparison of the plots presented in Figs. 1 and 2 shows that their slopes change systematically with the composition of the mobile phase. In all cases the dependence of $\ln k'$ on the number of carbon atoms in alkyl chain of the successive homologs is linear as has been reported [12,13,15–22]. For the longer alkylamide phases the retention data could be measured

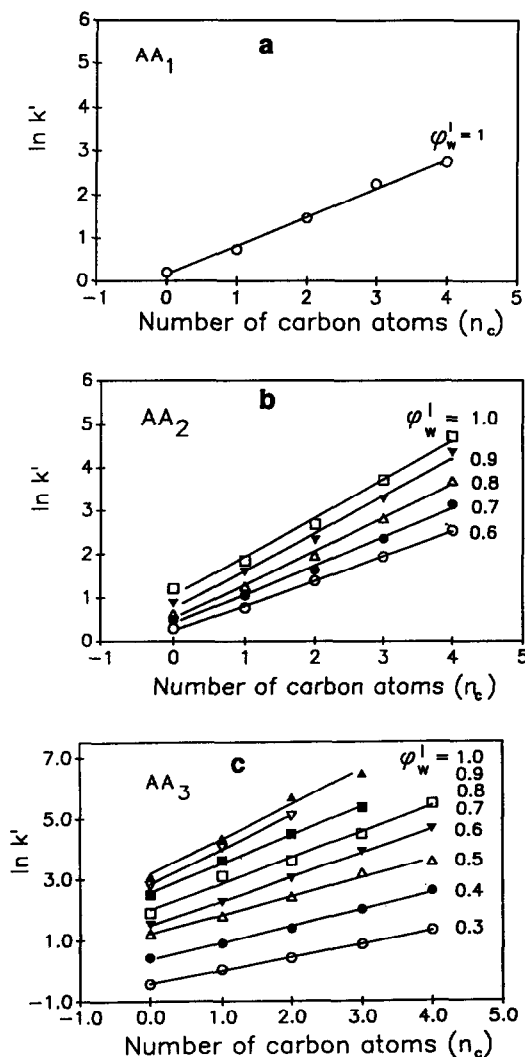


Fig. 1. Experimental dependencies of the natural logarithm of the capacity ratio ($\ln k'$) on the number n_c of carbon atoms in the alkyl chain of homologous alkylbenzenes in the water-methanol mobile phase on (a) AA_1 , (b) AA_2 and (c) AA_3 packings at 298 K measured at different volume fractions of water in the mobile phase.

over a wider range of the mobile phase concentrations. For example, a satisfactory separation of alkylbenzenes could be achieved on the methylamide phase with almost totally aqueous eluents, whereas in the case of the longer AA phases the same solutes were separable over a wide range of the mobile phase concentrations. The length of alkyl chain in the bonded

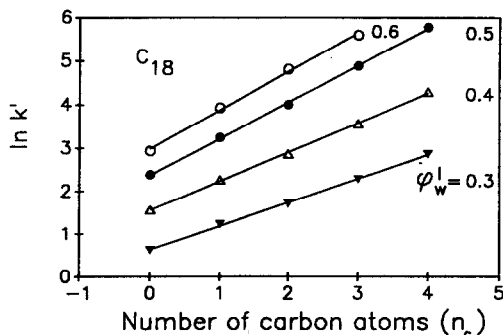


Fig. 2. Experimental dependencies of the natural logarithm of the capacity ratio ($\ln k'$) on the number n_c of carbon atoms in the alkyl chain of homologous alkylbenzenes in the water-methanol mobile phase on the C_{18} packing at 298 K measured at different volume fractions of water in the mobile phase.

alkylamide ligands had a significant influence on the methylene selectivity (defined by the slope of the $\ln k'$ vs. n_c plot, which characterizes quantitatively the separation of homologue solutes).

The slopes of the linear dependencies shown in Figs. 1 and 2 were plotted against the volume fraction of water in the mobile phase, φ_w^1 (cf., Fig. 3). Unfortunately, construction of the (s vs. φ_w^1) plot for the AA_1 packing was impossible because the alkylbenzenes could be separated only over a very limited concentration range of the mobile phases (i.e., $\varphi_w^1 \sim 1$). It can be seen from Fig. 3 that the relationships between s and φ_w^1 were linear for both the AA and C_{18} phases when water-methanol mobile phases were used. These results agree with earlier reports on the

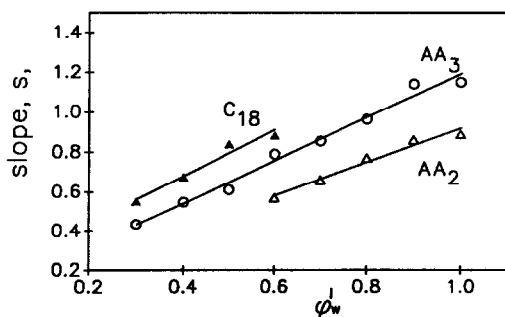


Fig. 3. The natural logarithm of the methylene selectivity plotted against the volume fraction of water in water-methanol eluent for the AA and C_{18} phases at 298 K.

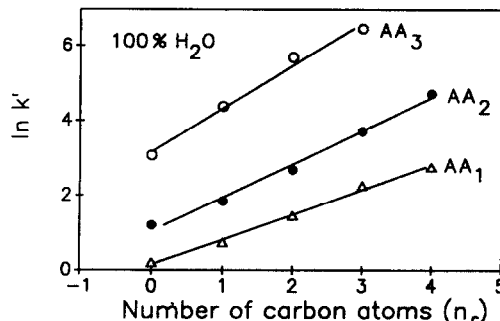


Fig. 4. Experimental dependencies of the natural logarithm of the capacity ratio ($\ln k'$) on the number n_c of carbon atoms in the alkyl chain of homologous alkylbenzenes for 100% water on the AA packings at 298 K.

concentration dependence of the methylene selectivity [12,13,15–22].

The (s vs. φ_w^1) plots shown in Fig. 3 were used to estimate the s value under fully aqueous conditions (i.e., the s_w value). The quantity s_w was determined graphically by linear extrapolation of the (s vs. φ_w^1) plot to the volume fraction $\varphi_w^1 = 1.0$. In contrast to the C_{18} phase, the s_w values for the AA phases could be evaluated from the ($\ln k'$ vs. n_c) plots measured under fully aqueous conditions (cf., Fig. 4). The extrapolated values of s_w are summarized in Table 2 as well as the s_w values measured using 100% water. As can be seen from Table 2, the extrapolated and measured values of s_w for the AA phases differ only about 3%.

A comparison of the data summarized in Table 2 shows that the s_w values for the AA phases with short alkyl chains are smaller than those for the C_{18} packings. Moreover, the s_w

Table 2
Values of K_{wo} and s_w for the systems studied

Phase	K_{wo}	$s_{w(ext)}^a$	$s_{w(me)}^b$
AA_1	—	—	0.66
AA_2	4.13	0.92	0.89
AA_3	0.87	1.19	1.15
C_{18}	0.81	1.38	—
C_{18}^c	1.08	1.19	—

^a $s_{w(ext)}$ denotes extrapolated s_w -values.

^b $s_{w(me)}$ denotes measured s_w -values.

^c Data from ref. 21.

values for the AA phases increase with increasing length of the alkyl chain attached to the amide group. Thus, either an extension of the bonded ligands hydrophobic segments and/or an increase in the coverage density of the alkyl ligands increases the probability of the non-specific methylene–ligand interactions, which results in an increase in the s_w value. In addition, differences in the conformational behavior of the bonded ligands and their ability to screen the residual silanols and aminopropyl groups alter the chain–chain interactions. For instance, an increase in the concentration of alkyl bonded ligands (compare the C₁₈ phases in Table 2) reduces the total number of residual silanols which causes an increase in the s_w value. In the case of AA phases an increase in the concentration of alkylamide ligands reduces the total number of residual aminopropyl groups (c_{NH_2}), however, the amount of residual silanols is not changed. A change in the ratio of alkylamide ligands to residual aminopropyl groups can change both the structure and composition of the stationary phase. Alkylamide and aminopropyl ligands as well as residual silanols control the intercalation (competitive sorption) process of solvent molecules into the chemically bonded phase, which alter their physicochemical properties.

If the s_w value is known, the methylene selectivity data shown in Fig. 3 can be plotted against the ratio of the volume fractions of water and methanol in the mobile phase [19,21]:

$$(s_w - s)^{-1} = (s_w - s_o)^{-1} + K_{w_o} (s_w - s_o)^{-1} \varphi_w^1 / \varphi_o^1 \quad (3)$$

where K_{w_o} is the equilibrium constant that describes the displacement process between molecules of water and organic solvent for ideal mobile and surface phases, and s_w represents a hypothetical methylene increment which characterizes retention for the pure aqueous mobile phase. According to Eq. 3, which was derived by assuming a partition–displacement model for the solute's retention [22,23], the dependence of $(s_w - s)^{-1}$ on the $(\varphi_w^1 / \varphi_o^1)$ ratio should be linear. Fig. 5 demonstrates that Eq. 3 is a good repre-

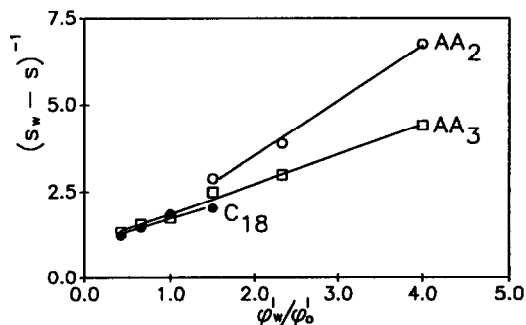


Fig. 5. Dependence of $(s_w - s)^{-1}$ on $\varphi_w^1 / \varphi_o^1$ for alkylbenzenes chromatographed in the water–methanol mobile phases on the AA and C₁₈ columns at 298 K.

sentation of the selectivity data measured on the AA and C₁₈ phases. From the slopes of the linear dependencies shown in Fig. 5 the K_{w_o} equilibrium constants can be calculated for the phases studied; K_{w_o} characterizes the competitive sorption of water and methanol into the chemically bonded phase. The K_{w_o} values for the systems studied are summarized in Table 2. For purposes of comparison, the values of K_{w_o} and s_w reported in ref. 21 for the LiChrosorb Si-60 with a specific surface area of 550 m²/g and modified with the low-density C₁₈ phase (the carbon loading was 11.1%) are included.

The K_{w_o} values summarized in Table 2 provide information about the composition of solvents that are incorporated into the stationary phase. In the case of the octadecyl phases the K_{w_o} values decrease with increasing carbon loading, e.g., $K_{w_o} = 1.08$ for the C₁₈ phase with 11.1% C and 0.81 for the phase with 23.2% C. Based on the model of competitive adsorption for ideal solutions on energetically homogeneous solids [23], when $K_{w_o} > 1$ water is preferentially intercalated into the bonded phase, whereas for $K_{w_o} < 1$ organic solvent is preferentially sorbed. Thus, since for the high coverage density C₁₈ packing the K_{w_o} value is smaller than unity, the data indicate that methanol is preferentially intercalated into the chemically bonded phase. However, the preferential sorption of water can occur for octadecyl phases with lower coverage densities.

A comparison of the K_{w_o} values for the

alkylamide and octadecyl phases with similar carbon loadings show that although methanol is preferentially intercalated into both these phases, its sorption is slightly smaller for the AA₃ packing. The later effect is probably due to the presence of the specific interaction site in alkylamide phases, which has a stronger interaction with water molecules. It is logical to expect that this effect should be greater in the case of the AA₂ phase, which contains a shorter (C₅H₁₁) alkyl chain attached to the amide group; for this phase $K_{wo} = 4.13$.

The K_{wo} values calculated using Eq. 3 refer to an ideal stationary phase, the composition of which is described by the following relationship [20,21]:

$$\varphi_w^\sigma = \frac{K_{wo}\varphi_w^1}{\varphi_o^1 + K_{wo}\varphi_w^1} \quad (4)$$

Eqn. 4 expresses the volume fraction (φ_w^σ) of water in the stationary phase in terms of the mobile phase's composition (*i.e.*, the volume fraction of water in the mobile phase, φ_w^1). Since Eq. 4 was derived for ideal phases, it does not predict an azeotropic point on the excess sorption isotherm (*i.e.*, the value of the volume fraction, φ_w^1 , at which $\varphi_w^\sigma = \varphi_w^1$). The probability for azeotropic behavior is greater for systems with K_{wo} close to unity and has been reported for alkyl bonded phases in contact with water-methanol eluents [18,21].

Shown in Fig. 6 are the individual sorption

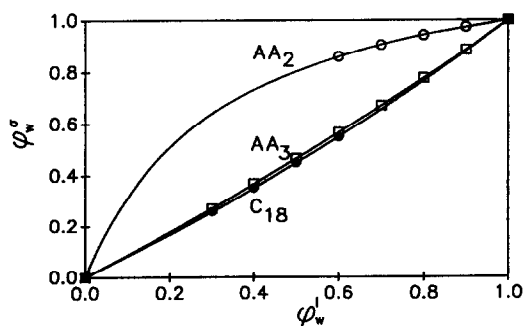


Fig. 6. Dependence between the volume fractions of water in the stationary and mobile phases for the AA and C₁₈ packings at 298 K.

isotherms of water on the AA₂, AA₃ and C₁₈ phases calculated using Eq. 4. In the case of the C₁₈ and AA₃ phases, the sorption excesses of water in the stationary phase (which are proportional to $\varphi_w^\sigma - \varphi_w^1$), are similar and both are negative. Thus, the excess of sorbed methanol in the stationary phase must be positive because the sum of the excesses for all compounds must be equal to zero [23]. In contrast to the AA₃ phase, the sorption excess of methanol for the AA₂ packing was negative. In addition, for the AA₂ packing the difference in the compositions of solvents in the stationary and mobile phases is much greater than that for the C₁₈ and AA₃ phases.

A significant excess of water in the AA phases with short alkyl chains strongly affects their chromatographic selectivity (*cf.*, Fig. 7). Shown in Fig. 7 are separations of derivatives of aniline under totally aqueous conditions on the AA phases. The best resolution between individual peaks was obtained for the AA phases with longer alkyl chains, *i.e.*, for the AA₃ and AA₂ packings. However, an extension of the alkyl chain resulted in an increase in the analysis time. Thus, the alkylamide phases with medium length alkyl chains seem to be especially attractive for separation of polar compounds such as amines and amides. An advantage of the AA phases in comparison to the conventional alkyl packings is

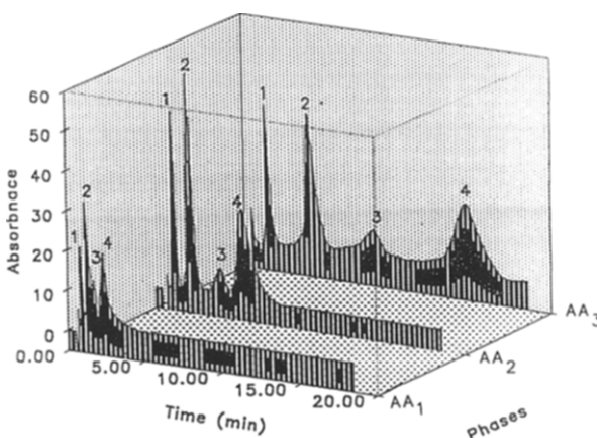


Fig. 7. Separations of alkylnilines under fully aqueous conditions on the AA phases at 298 K. Peaks: 1 = aniline, 2 = methylaniline, 3 = dimethylaniline and 4 = diethylaniline.

that good resolution can be achieved under fully aqueous conditions. A possible important use for such packings is to separate biomolecules such as: peptides, polypeptides and proteins.

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5. References

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