Selectivity of alkylamide bonded-phases with respect to organic acids under reversed-phase conditions

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Abstract

It was shown previously by Czajkowska et al. [J. Chromatogr. A, 691 (1995) 217] that alkylamide phases are better for separating pyridinecarboxylic acids than conventional alkyl packings. They allow separation of these compounds from hydrophobic substances over a wide range of mobile phase compositions. In the current work the retention data for pyridinedicarboxylic acids were measured on monomeric and polymeric alkylamide phases at 35°C by using the acetonitrile–water eluent and utilized to calculate the nonspecific (methylene) and specific (carboxylic and amine) selectivities. Subsequently, the selectivity data were plotted against the pH of the mobile phase. It was shown that while the methylene selectivity does not change much at the pH range from 2 to 5, the specific selectivites studied depend strongly on the pH of the mobile phase. The magnitude of this dependence is determined by the type of the bonded phase and silica support as well as the length of the terminal alkyl groups.

Keywords: Alkylamide-bonded stationary phases; Stationary phases, LC; Selectivity; Organic acids; Pyridinecarboxylic acids

1. Introduction

It was shown previously [1–3] that alkylamide (AA) phases exhibit a combination of specific and nonspecific interactions with respect to the solutes of various polarities and that these properties make them suitable for separating pyridinedicarboxylic acids even in the presence of hydrophobic compounds. These bonded phases appear to have interesting physicochemical and chromatographic properties [4–10]. Their sorption affinity to acetonitrile, methanol and water, which are typical components of the hydro-organic mobile phase, differ signifi-

cantly from that observed for conventional alkyl phases [4]. The presence of the internal amide group in the bonded ligand seems to have a positive influence on the structural ordering of the alkylamide stationary phases, and consequently on their chromatographic performance [5].

Alkylamide phases are usually synthesized by a two-step process, in which first a aminopropyl phase is formed and subsequently reacted with a suitable alkanoyl chloride [1–10]. These phases contain some fraction of unreacted aminopropyl groups in addition to the alkylamide bonded ligands and residual silanols. It is noteworthy that commercial analogues of these phases have been recently introduced [11]. In addition, this type of bonded phases has been also synthesized by using one-step bonding chemistry,

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i.e., first the amide functional group was incorporated into a suitable silane and subsequently the entire ligand was directly attached to the silica surface [11]. In comparison to the alkylamide phases prepared by a two-step process, the AA phases obtained by one-step bonding chemistry do not have aminopropyl groups. Also, one-step bonding chemistry was used to prepare monomeric phases with the internal carbamate group [12].

In contrast to the previous articles [1–3], the current work concentrates on the selectivity of alkylamide phases with respect to organic acids under reversed-phase conditions. This work is an extension of previous study reported in [2]. The polymeric and monomeric alkylamide phases reported previously [2] were studied and compared to the commercially available amide functionalized Supelcosil ABZ and ABZ Plus columns. The retention data for pyridinedicarboxylic acids were measured on these phases at 35°C by using the acetonitrile–water eluent and utilized to calculate the nonspecific (methylene) and specific (carboxylic and amine) selectivities. Subsequently, the selectivity values were plotted against the pH of the mobile phase and analyzed in order to provide a quantitative comparison of different types of alkylamide phases.
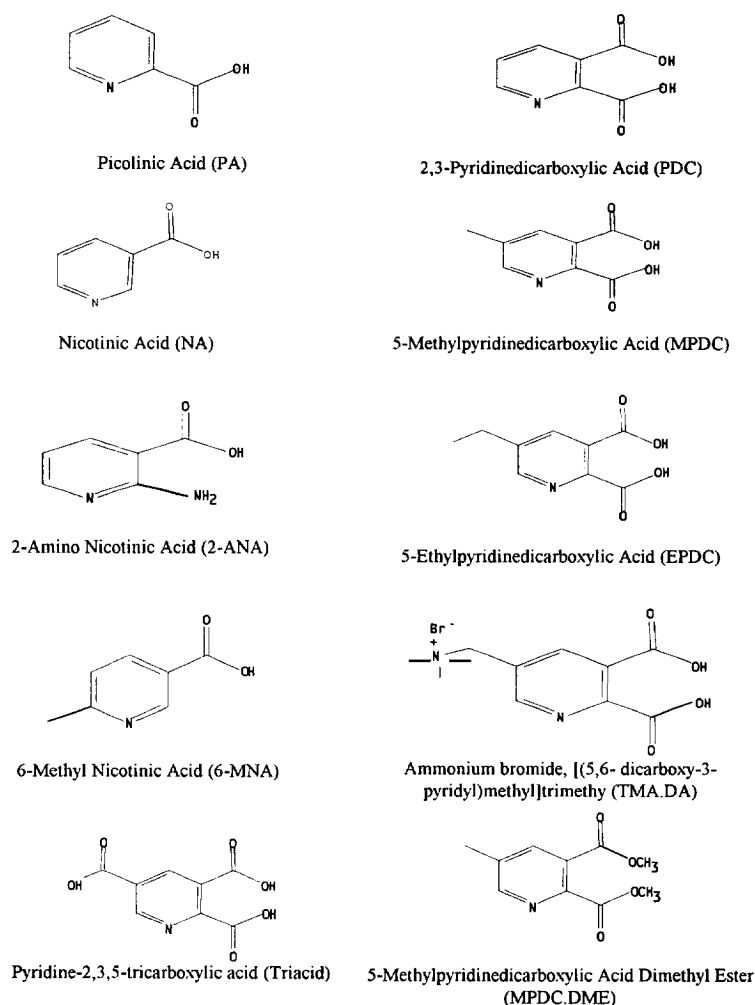

Fig. 1. Chemical structures of the acids.

2. Experimental

2.1. Chromatographic measurements

The major portion of retention data was collected for a series of pyridinecarboxylic acids (see Fig. 1) using the acetonitrile–water (5:95, v/v) mobile phase at different pH values (2.0–7.0). Some data were measured for the same solutes at fixed pH (=2.3) of the mobile phase and different concentrations of acetonitrile (from 0 to 20%). All measurements were performed as previously [2] at 35°C using a 1 ml/min flow-rate. Two Hewlett-Packard HP 1050 and HP 1090 liquid chromatographs were employed to collect the retention data, which were

acquired and processed using a model Hewlett-Packard 3350 Laboratory Data System.

2.2. Alkylamide columns

The same (15 cm×4.6 mm I.D.) columns as previously [2], packed using mono- and polymeric alkylamide phases with terminal C_6 and C_{12} ligands, were used in the current study. The AA phases were prepared by a two-step process in which an initial aminopropyl phase was synthesized and subsequently reacted with suitable alkanoyl chloride [2]. While the second step (i.e., reaction with alkanoyl chloride) was the same for both types of phases, the first step was different. In the case of monomeric phases, the
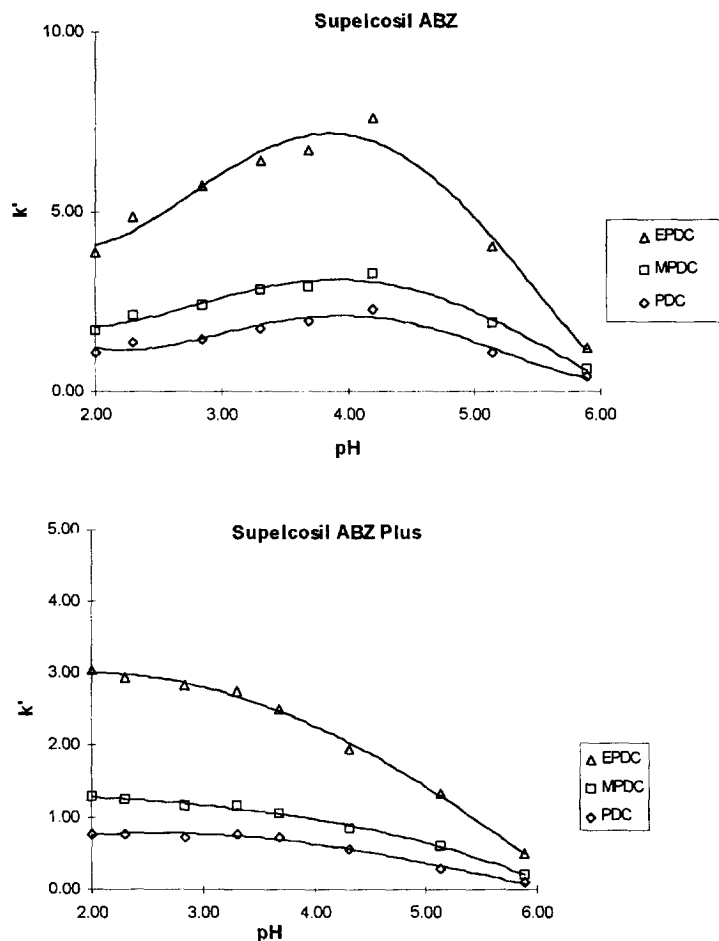

Fig. 2. Values of k' vs. pH measured for selected alkylpyridinedicarboxylic acids on the Supelcosil ABZ and ABZ Plus columns at 35°C using the acetonitrile–water (5:95, v/v) eluent.

Table 1
Elemental analysis data for the alkylamide phases studied [2]

Column Code	Packing type	Amine groups (μmol/m^2)	Amide groups (μmol/m^2)
AA-MC-6	Monomeric, C_6	0.07	0.88
AA-MC-12	Monomeric, C_{12}	0.63	1.46
AA-PC-6	Polymeric, C_6	1.28	1.55
AA-PC-12	Polymeric, C_{12}	0.73	2.10

silica was initially reacted with amino-propyldimethylethoxysilane, however aminopropyl-triethoxysilane was used to prepare polymeric phases. Both, monomeric and polymeric packings were obtained using a 5-mm Eka Nobel Kromasil silica. The coverage densities of amine and amide groups for these phases are given in Table 1. However, surface and structural properties of the silica were discussed in the previous work [2].

For comparative purposes two Supelcosil ABZ and ABZ Plus columns purchased from Supelco (Bellafonte, PA, USA) were studied. According to the manufacture information [11] the ABZ column was prepared in an analogous way as the polymeric AA columns, i.e., using a two-step process. However, the ABZ plus column was synthesized by a single-step surface attachment procedure. In both cases the terminal chains consisted of 16 carbon atoms. Some comparative measurements were performed on two 15 cm 5-μm RP C_8 Eka Nobel Kromasil and C_8 Inertsil columns. The latter was packed with a octyl phase bonded to 5-μm Inertsil silica, which had 4.25 μmol/m^2 coverage density [1].

3. Results and discussion

A comparison of the chromatographic properties of all alkylamide packings studied was performed by using the retention data of pyridine carboxylic acids and its derivatives (see Fig. 1) collected at different pH values of the mobile phases containing 5% of acetonitrile. Some measurements were carried out also at a constant pH and different levels of acetonitrile in the mobile phase. Exemplary retention data for the RPLC systems studied are presented in Figs. 2–6. As shown in Fig. 2, the dependencies of the

capacity factor k' on the pH of the mobile phase for three homologues of pyridine 2,3-diacids (PDC, MPDC and EPDC) on the ABZ columns are similar to those reported previously on the AA phases [2]. The retention of diacids on the ABZ columns initially increases with increasing pH over the range from 2 to 4, and later decreases as it was observed previously for the polymeric AA phases [2]. In the case of the ABZ Plus column the retention of diacids decreases with increasing pH in a similar manner as for the monomeric AA phases and conventional alkyl bonded packings [2].
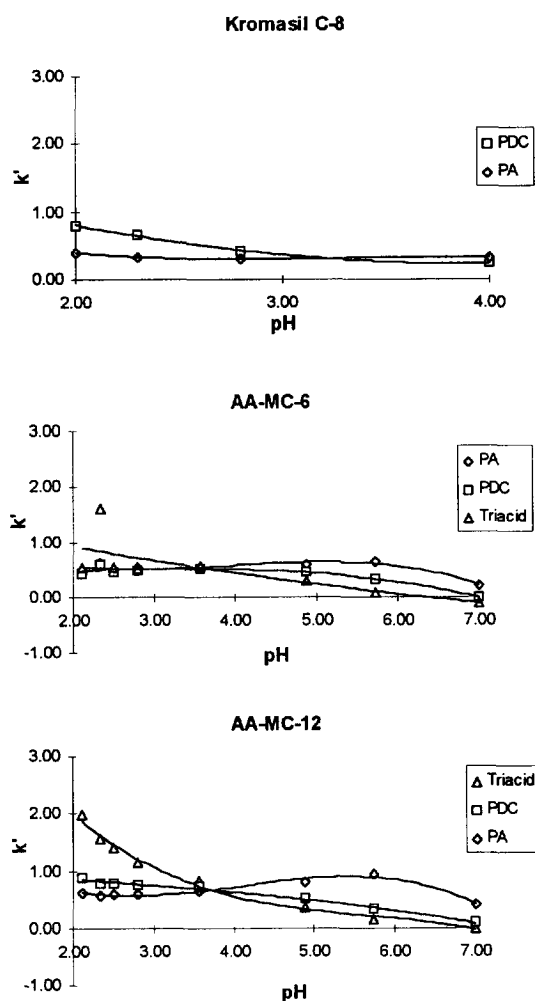

Fig. 3. Values of k' vs. pH measured for selected mono-, di- and triacids on the Kromasil C_8, AA-MC-6 and AA-MC-12 columns at 35°C using the acetonitrile–water (5:95, v/v) eluent.

Another exemplary dependencies of k' on the pH are shown in Figs. 3–5 for pyridine mono-, di- and triacids. As can be seen from Figs. 3–5, the retention of these solutes has a tendency to increase in the following order: Kromasil C_8<AA-MC-6<AA-MC-12<Supelcosil ABZ Plus<Supelcosil ABZ<AA-PC-6<AA-PC-12. The strongest dependence of k' on the pH is observed for triacids and it becomes less pronounced for diacids and monoacids. In the case of polymeric columns (i.e., AA-PC-12, AA-PC-6, Supelcosil ABZ and Supelcosil ABZ Plus), the k'(pH) curves for tri-, di- and monoacids have a maximum, which lies at an intermediate pH value

located between the pK values for acidic (pK_a~2) and basic (pK_b~5–6) forms of a given solute (see the pK values summarized in Table 2). This maximum lies about pH=3 for triacids and shifts towards higher pH for diacids and monoacids. Also, the k'(pH) curves for triacids differ significantly from those for di- and monoacids, especially in the range of lower pH. In this range the k' values for triacids are much higher than the corresponding values for other acids. In contrast, the differences between the k'(pH) curves for tri-, di- and monoacids on alkyl and monomeric alkylamide phases are much smaller (see Fig. 3). It is noteworthy that the k' vs. pH data
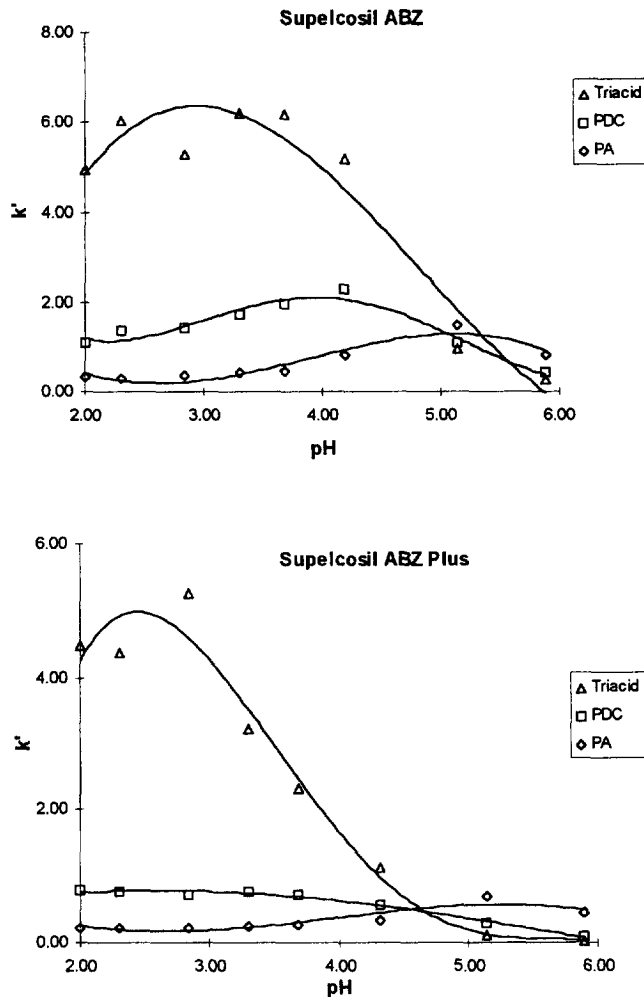

Fig. 4. Values of k' vs. pH measured for selected mono-, di- and triacids on the Supelcosil ABZ and ABZ Plus columns at 35°C using the acetonitrile–water (5:95, v/v) eluent.
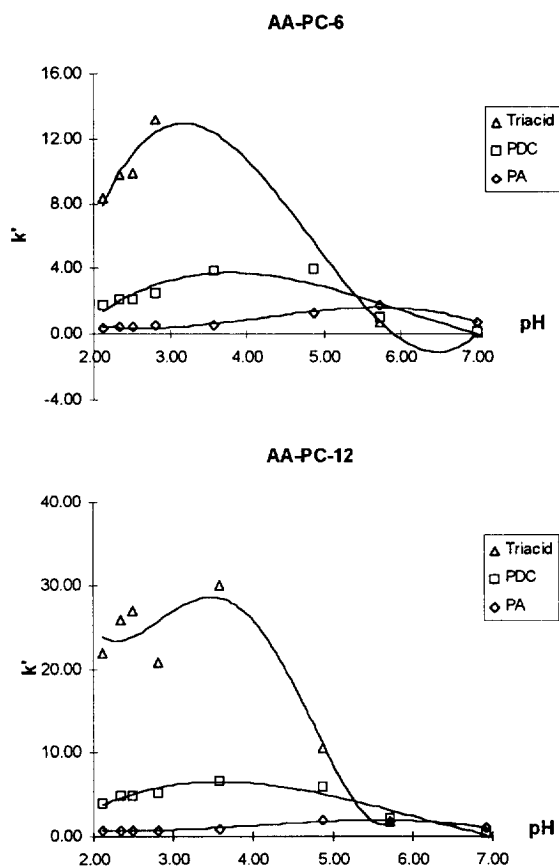
AA-PC-6

AA-PC-12

Fig. 5. Values of k' vs. pH measured for selected mono-, di- and triacids on the AA-PC-6 and AA-PC-12 columns at 35°C using the acetonitrile–water (5:95, v/v) eluent.

[13] to describe the retention of amino acids in a solvent generated ion-exchange chromatography.

All k'(pH) curves shown in Figs. 3–5 intersect at a certain pH value, characteristic for each column studied. For conventional RP and monomeric AA columns this characteristic pH value is about 3.5. For the remaining AA columns it is shifted towards the higher pH values accordingly to their increasing ion-exchange ability (i.e., 4.5 for the ABZ Plus column, 5.2 for the ABZ column and 5.5 for the AA-PC-6 and AA-PC-12 columns). As can be seen this characteristic value is higher for all polymeric AA phases synthesized via a two-step process than for the ABZ Plus packing, which does not contain residual amine groups. This result suggests that the presence of aminopropyl ligands in the polymeric AA stationary phase increases its ability towards ionic interactions.

An illustration of the dependence of k' on the concentration of acetonitrile is shown in Fig. 6. While the retention of a nonpolar solute (e.g., pyridinecarboxylic acid diester) decreases rapidly, this decrease is very small for diacids (e.g., MPDC). A similar behavior was previously observed for the AA phases [1,2].

An interesting comparison of the packings studied was performed by means of the functional selectivity expressed as the natural logarithm of the relative retention ($\alpha = k'_2/k'_1$) of two compounds differing by one functional group, e.g., carboxylic, amine or methylene group. Acidic selectivities were calculated for the carboxylic groups located at the 2nd (PDC/NA) and 3rd (PDC/PA) positions of the pyridine ring. In addition, for the ABZ columns, the specific selectivity for two carboxylic groups located at the

can be fitted by a polynomial function of type $y = ax^3 + bx^2 + cx + d$ with the correlation coefficient $R^2 > 0.96$. A similar expression was used by Kraak et al.

Table 2
Values of pK_a for the compounds studied[a]

Compound	Acid step 1	Acid step 2	Acid step 3	Basic (pyridinum)
Picolinic acid (PA)	0.99			5.55
Nicotinic acid (NA)	2.02			4.72
2-ANA	2.02			6.31
6-MNA	2.16			5.49
2,3-PDC	1.07	1.90		5.01
Triacid	2.17	3.43	4.11	–
MPDC	1.13	2.04		5.37
EPDC	1.14	2.05		5.43

[a] PKALC Program, Version 1.0, 1992 (CompuDrug Chemistry, Compudrug NA, Rochester, NY, USA).

Supelcosil ABZ (Solid Line)
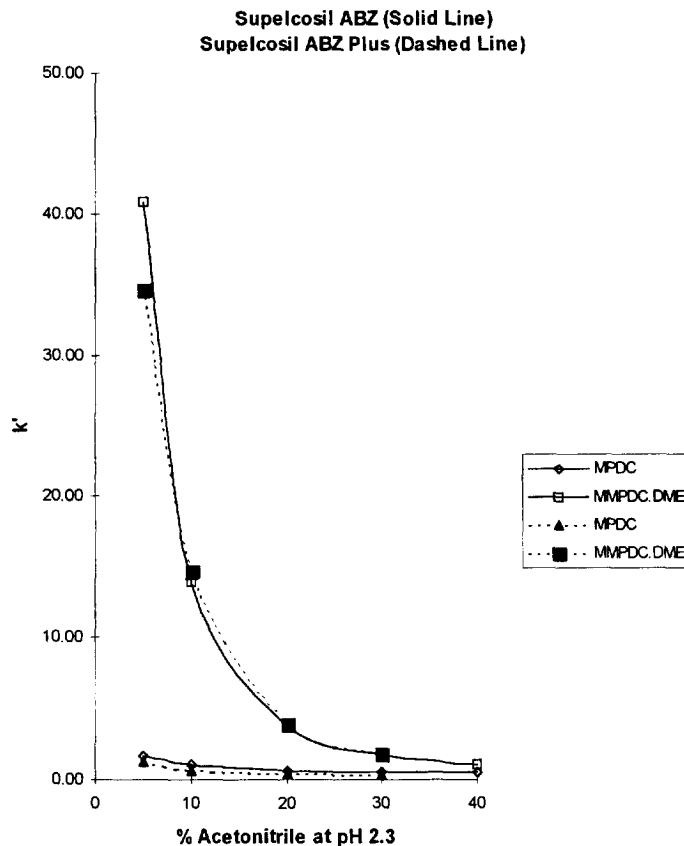**Supelcosil ABZ Plus (Dashed Line)**

Fig. 6. Values of k' vs. acetonitrile concentration measured for selected solutes on the Supelcosil ABZ and ABZ Plus columns at pH=2.3 and 35°C.

2nd and 3rd positions was calculated (PDC/pyridine). The amine selectivity was calculated using retention data for 2- and 6-amino-pyridinenicotinic acids versus nicotinic acid (6-ANA/NA, 2-ANA/NA). An additional specific selectivity was evaluated for 5-methylene-trimethylammonium bromide of 2,3-pyridine-dicarboxylic acid versus 5-methylpyridine-dicarboxylic acid (TMA.DA/MPDC). The non-specific (methylene) selectivity was evaluated as the slope of the linear dependence of ln k' vs. the number of carbon atoms in the alkyl chain using three 5-alkyl substituents of 2,3-pyridine diacids (i.e., PDC, MPDC and EPDC).

The selectivity data, expressed as the natural logarithm of the methylene selectivity, were plotted against pH and the concentration of acetonitrile in the mobile phase (see Fig. 7). For all columns studied, the methylene selectivity remains constant

over the examined pH range. The absolute values of the methylene selectivity change accordingly to the length of the terminal alkyl chain and these values for all amide columns studied are lower than for a conventional RP column Inertsil C_8. The values of the methylene selectivity decrease with the increasing level of acetonitrile for both alkylamide and conventional phases. As can be seen from the top panel in Fig. 7, the hydrophilicity of the packings studied increases in the following order: Inertsil C_8<Supelcosil ABZ Plus<Supelcosil ABZ<AA-PC-12<AA-MC-12<AA-MC-6<AA-PC-6.

A comparison of the natural logarithm of the functional selectivities plotted as a function of the pH of the mobile phase is presented in Figs. 8–11. As can be seen from Fig. 8, the carboxylic selectivity depends strongly on the pH of the mobile phase, especially for polymeric AA packings. The sequence
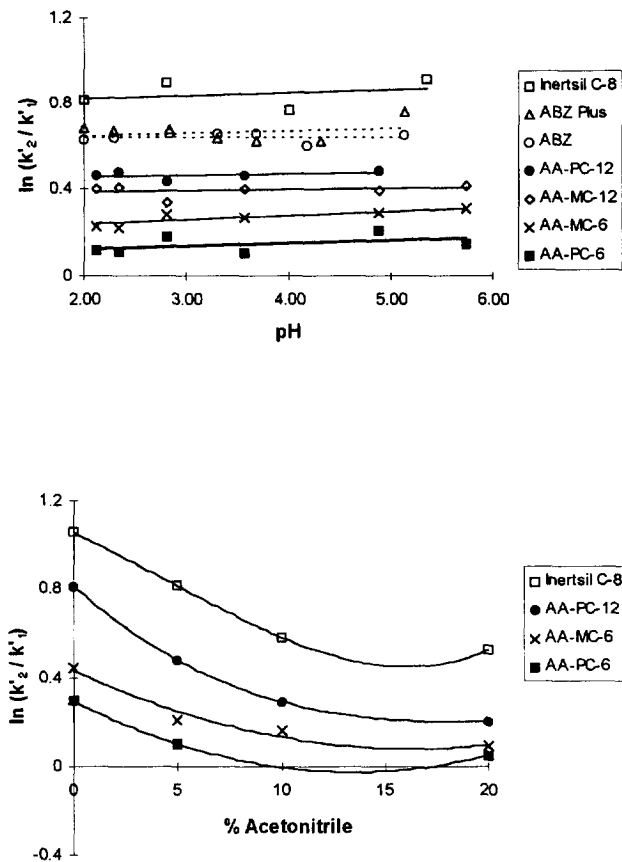
Fig. 7. Comparison of the logarithm of the methylene selectivity, $s=\ln(k'_2/k'_1)$, plotted as a function of pH (top panel) and acetonitrile concentration (bottom panel) for different stationary phases at 35°C. Data at different pH were collected using the acetonitrile–water (5:95, v/v) eluent, whereas data at different acetonitrile concentrations were measured at pH=2.3 (pH=2.8 was used in the case of Inertsil C_8 column). Each s-value was evaluated as the slope of the linear dependence of $\ln k'$ vs. the number of carbon atoms in the alkyl chain using PDC, MPDC and EPDC.

of the carboxylic selectivity curves is the following: the top curves for the AA-PC-12 and AA-PC-6 columns are followed by the curves for the ABZ and ABZ Plus columns, and finally by the curves of the conventional and AA-MC columns. Surprisingly, the Kromasil C_8 column in the pH range below 2.8 exhibits greater carboxylic capacity in comparison to both monomeric AA columns. It is probably due to the different concentrations of residual silanols. In contrast to the later columns, the carboxylic selectivity curves for the polymeric columns exhibit a distinct maximum in the pH range from 2.5 to 3.5 and they decrease rapidly at higher pH values. A high selectivity towards diacids in that pH range does not reflect a good resolution, which depends

greatly on the peak width. Unfortunately, in this region the chromatographic peaks of diacids and especially triacids are very broad. Also, the carboxylic selectivity depends on the position of carboxylic group in the pyridine ring as well as the type of head molecule. Although, as shown on the top panel in Fig. 9 the selectivity curves are analogous for different head molecules, some differences are visible especially for acidic ones. As regards the position of carboxylic group, the pH effect is more pronounced when that group is located on 3rd position (see the bottom panel in Fig. 9). This effect is even a lot more visible for two carboxylic groups (see top curves on both panels in Fig. 10). The retention behavior at lower pH values and the
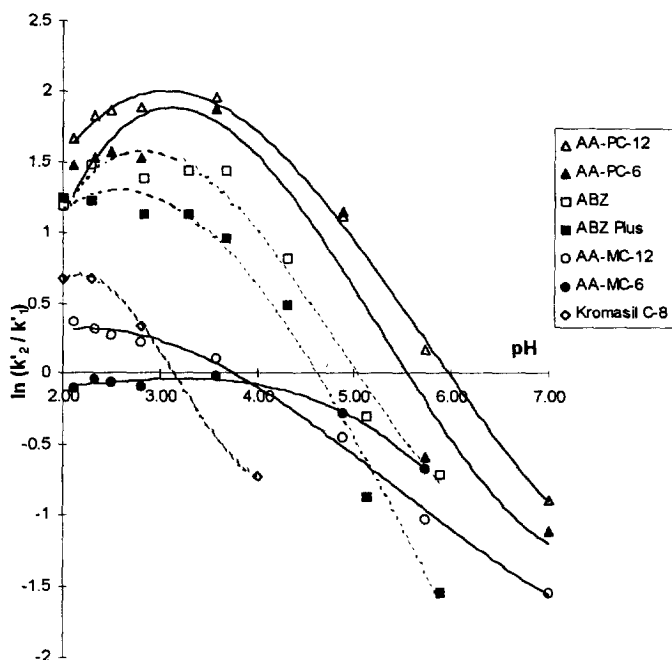
Fig. 8. Comparison of the logarithm of the carboxylic selectivity (PDC/PA) plotted as a function of pH of the acetonitrile–water (5:95, v/v) eluent at 35°C for different columns.
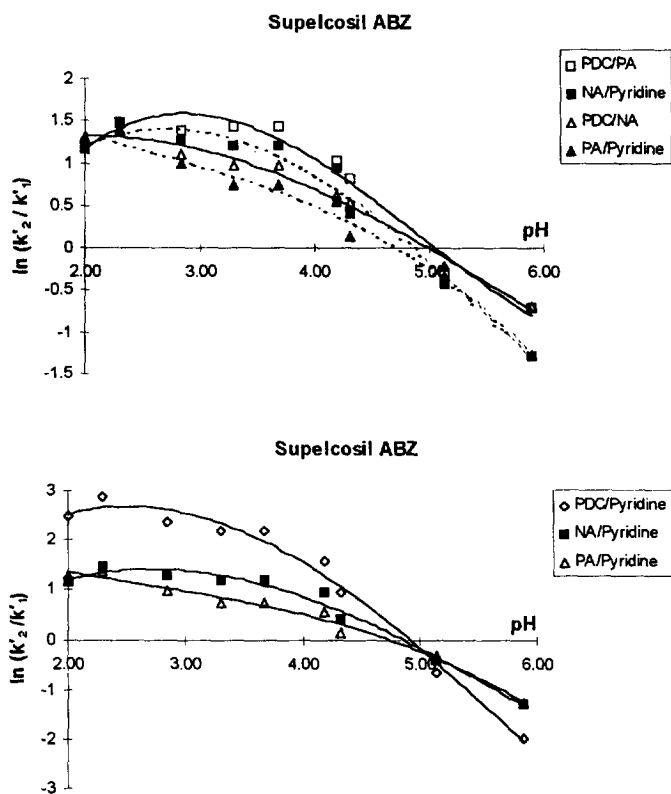

Fig. 9. Effect of the head molecule (top panel) and the position of the carboxylic group (bottom panel) on the logarithm of the carboxylic selectivity plotted as a function of pH of the acetonitrile–water (5:95, v/v) eluent at 35°C for the Supelcosil ABZ and ABZ Plus columns.
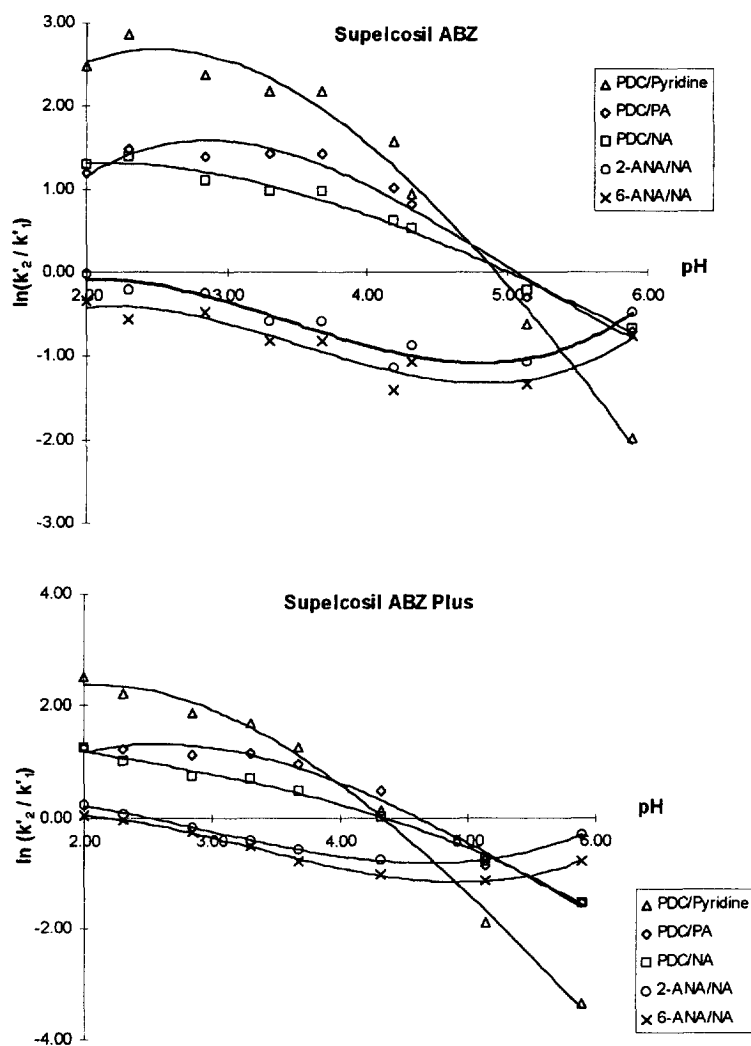
Fig. 10. Comparison of the logarithm of the carboxylic and amine selectivites plotted as a function of pH of the acetonitrile–water (5:95, v/v) eluent at 35°C for the Supelcosil ABZ and ABZ Plus columns.

corresponding changes in the carboxylic selectivity can be attributed to the ionic type of interactions of the ionized carboxylic group with protonated amide and/or amine functionalities of the stationary phase. Since these changes are greater for columns prepared via a two-step process (see Figs. 3, 4 and 8), the residual amine groups seem to be a dominant factor in controlling ionic interactions in the polymeric AA phases. Nevertheless, the absolute retention of acids on all types of the AA phases is always higher than on a conventional alkyl bonded phase. Even the phase prepared via one-step reaction (e.g., ABZ Plus

phase), which does not contain residual amine functionalities, and its amide functionality is the only possible site for ionic interactions, retains acids longer than conventional C_8 columns.

Bottom curves on both panels in Fig. 10 show the amine selectivity data. As can be seen from this figure, the logarithmic selectivity is negative and slowly decreases (i.e., the separation ability of these solutes increases) and finally slightly increases with increasing pH probably due to a greater contribution of nonspecific interactions because of reduction of the positive charge which amine could have at lower
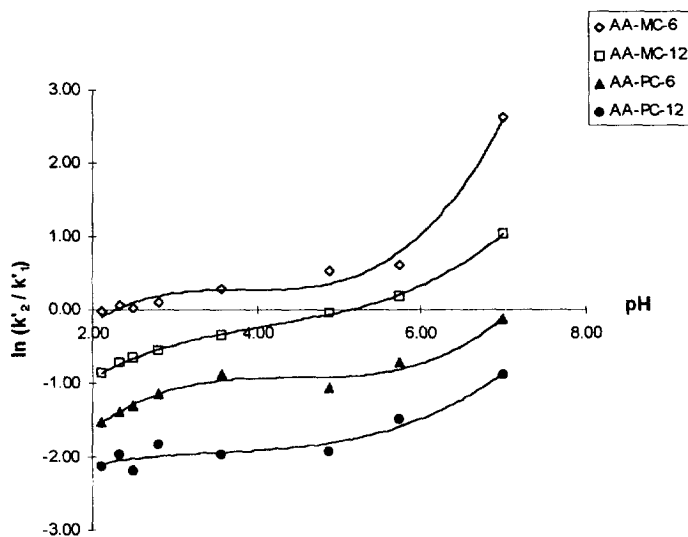
Fig. 11. Comparison of the logarithmic selectivity data for TMA.DA/MPDC plotted as a function of pH of the acetonitrile–water (5:95, v/v) eluent at 35°C for different AA phases.

pH values. A more accurate interpretation of the amine selectivity is difficult since the head molecule had acidic properties, which can compensate partially the effect of amine group. Also, the existence of negatively charged sites can be considered. For instance, both the cation and anion-exchange properties of chromatographic columns with bonded amino acids were related to the amine groups [14,15]. It was noted also that their physicochemical properties were altered by the silica surface. The test solutes used in the current study (i.e., pyridine carboxylic acids, especially those with acidic functionality at the position closest to the ring nitrogen) are zwitterionic molecules and if the AA phases exhibit similar properties, their cationic and anionic sites are able to interact strongly with these zwitterionic species.

A comparison of the TMA.DA/MPDC selectivities is shown in Fig. 11 for four AA phases studied. In this case, the selectivity curves increase with increasing pH of the mobile phase. The selectivity curves for monomeric phases lie above those for polymeric phases, i.e., the top curve is for the AA-MC-6 phase, followed by curves for the AA-MC-12, AA-PC-6 and AA-PC-12 phases. Since the highest absolute values of the TMA.DA/MPDC selectivity are for polymeric phases, these phases

seem to have the strongest ability for ionic interactions.

4. Conclusion

It was shown that the selectivity data calculated for various functional groups such as methylene, carboxylic and amine allowed a quantitative comparison of different alkylamide phases according to their ability for nonspecific and ionic interactions. The retention of organic acids on these phases is controlled to a high extent by ionic interactions, which are especially important in the case of polymeric AA phases. It appeared that the monomeric AA phases, especially those prepared via one-step reaction, can be considered as intermediate phases between polymeric AA and conventional alkyl packings.

Acknowledgments

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