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Retention of pyridinecarboxylic acids on monomeric and polymeric alkylamide phases

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Abstract

Retention of pyridinecarboxylic acids was studied on monomeric and polymeric alkylamide phases with C₆ and C₁₂ terminal alkyl chains at different pH values and various concentrations of acetonitrile in the mobile phase. It was shown that the retention of these acids on both types of alkylamide phases is significantly influenced by the pH of the mobile phase. The pH effect was more pronounced for polymeric phases, which for this reason appeared to be better for the simultaneous separation of ionic and non-ionic solutes.

Keywords: Stationary phases, LC; Mobile phase composition; Pyridine carboxylic acids; Carboxylic acids

1. Introduction

The separation of organic solutes of complex functionality on conventional chemically bonded phases is often difficult under reversed-phase conditions [1]. An alternative for improving this separation is the use of chemically bonded phases with specific properties such as alkylamide phases, which contain terminal alkyl chains attached to alkylamide groups [2]. Alkylamide phases appear to have interesting chromatographic properties [3–8]. In addition to residual silanols and alkylamide ligands, these phases contain unreacted aminopropyl groups, which are suspected to increase their structural stability [6]. Also, the sorption affinity of these phases with respect to solvent molecules differs

significantly from that observed for conventional alkyl phases [3].

In previous work [1], it was shown that alkylamide phases (AA) exhibit a combination of specific and non-specific interactions with respect to solutes of various polarities and that they are much more suitable for the separation of pyridine mono- and dicarboxylic acids than conventional chemically bonded phases. Pyridine-based acids, especially those with a carboxylic group on the α -position to the ring nitrogen, are difficult to analyze owing to poor peak shape, irreversible adsorption or complete lack of retention.

In this work, the separation properties of two types of alkylamide phases, monomeric and polymeric with C₆ and C₁₂ terminal alkyl chains, were evaluated on the basis of retention measurements of pyridinedicarboxylic acids under

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reversed-phase conditions at different pH values, ionic strength and organic solvent content in the mobile phase. It was shown that for both types of alkylamide phases the retention of pyridinedicarboxylic acids was influenced by the pH of the mobile phase. The pH dependence of the retention data was more pronounced for polymeric phases, and therefore these phases seemed to be more promising for simultaneous separations of ionic and non-ionic solutes since the retention of the latter compounds is mainly controlled by the concentration of organic modifier.

2. Experimental

2.1. Introductory remarks

Extensive retention measurements were carried out for pyridinecarboxylic acids on monomeric and polymeric alkylamide phases with C₆ and C₁₂ terminal alkyl chains at different pH values (2.0–7.0) and various concentrations of organic modifiers (acetonitrile, methanol and 2-propanol). For comparative purposes a few compounds, which do not form internal hydrogen bonds, e.g., benzoic acid, 5-methylpyridinedicarboxylic acid dimethyl ester (MPDCDME) and 4-picoline, were also used.

2.2. Reagents and materials

The spherical silica used to prepare alkylamide-bonded phases was 5- μ m Kromasil (Eka Nobel, Bohus, Sweden). Reagents utilized for silica modification such as 3-aminopropyltriethoxysilane and suitable alkanyl chlorides were purchased from Aldrich (Milwaukee, WI, USA). The remaining modifier, 3-aminopropyltrimethylethoxysilane, was obtained from Pertrarch Systems (Levittown, PA, USA). The other chemicals used in the synthesis of alkylamide phases, such as methanol and toluene, were purchased from Aldrich. The organic solvents used in retention measurements

were obtained from Baxter (acetonitrile, methanol) and Baker (2-propanol). Aldrich, Fluka and American Cyanamid were sources of the chromatographic solutes used (see Fig. 1). Dionized water was purified using a Millipore (El Paso, TX, USA) Milli-Q system.

2.3. Alkylamide columns

Mono- and polymeric alkylamide phases with attached C₆ and C₁₂ ligands were prepared by a two-step process, in which an initial aminopropyl phase was synthesized and subsequently reacted with a suitable alkanoyl chloride [2]. Monomeric phases were synthesized by initially modifying the silica surface with aminopropyltrimethylsilane; however, aminopropyltriethoxysilane was used for preparing polymeric phases. Monomeric and polymeric packings were obtained using 5- μ m Eka Nobel Kromasil silica. Four different alkylamide phases were synthesized: two monomeric phases with C₆ and C₁₂ terminal alkyl chains and two analogous polymeric phases. All these materials were packed into 15 cm \times 4.6 mm I.D. stainless-steel columns from Supelco (Bellefonte, PA, USA). These columns were filled with a given phase using a Haskel (Burbank, CA, USA) Model DST-52 pump as described previously [1].

2.4. Characterization of silica and bonded phases

Surface and structural properties of the Kromasil silica were determined by high-resolution thermogravimetric and sorption measurements. First, thermodesorption of physically adsorbed water from Kromasil and dehydroxylation of its surface were measured in a nitrogen atmosphere using a TA Instruments (New Castle, DE, USA) TGA 2950 high-resolution thermogravimetric analyzer. The instrument was equipped with an open platinum pan and an automatically programmed temperature controller. The thermogravimetric (TGA) curve, which

gives the dependence of the mass loss of a sample as a function of temperature or time, was measured over the temperature range 20–1000°C. The heating rate was regulated automatically by the instrument in order to maintain a constant temperature during a given thermal event. The maximum heating rate between thermal events was set a 10°C/min.

In addition, a complete nitrogen adsorption isotherm was measured at –195.7°C by using a Micromeritics (Norcross, GA, USA) ASAP 2010 volumetric sorption analyzer. This instrument is capable of automatically measuring the adsorbed amount as a function of the equilibrium pressure for a given pressure table. Its special feature is the possibility of measuring the incremental adsorption data at very low pressures. Prior to making adsorption measurements, the silica sample was outgassed under vacuum at 200°C for 3 h. Next, the nitrogen adsorption isotherm was recorded over the entire pressure range. In addition, low-pressure data were collected by using a small incremental dose of the adsorbed amount (e.g., 3 cm³ STP/g). Finally, these data were used to extract some information about surface heterogeneity and porosity of the silica studied.

The carbon and nitrogen loadings of alkylamide phases were determined by elemental analysis, which was done by Robertson Microлит Laboratories (Madison, NJ, USA). The C and N contents were determined for both the initial aminopropyl phases and the final alkylamide phases. These values were used to calculate surface concentrations of aminopropyl and alkylamide groups.

2.5. Chromatographic measurements

A Hewlett-Packard HP 1050 and a modular liquid chromatograph consisting of a Spectra-Physics SP 8800 pump, an LKB Model 2125 column oven, a variable-wavelength UV detector (ABI 785A) and an HP 1050 autosampler were used to measure retention data on the alkylamide phases studied. These data were acquired and processed using a Hewlett-Packard 3350 Laboratory Data System.

The retention data for a series of pyridinecarboxylic acids (see Fig. 1) were measured at different acetonitrile concentrations and pH values of the mobile phase. The major portion of the chromatographic data was collected in the pH range 2–7.0 (50 mM sodium phosphate buffer) and at acetonitrile concentrations varying from 0 to 20%. The influence of pH on the solute retention was examined for a mobile phase containing 5% of acetonitrile. The effect of acetonitrile content in the mobile phase on the solute retention was studied at pH 2.3 for all the compounds on four columns. Separate experiments were carried out to study the selectivity for MPDC and MPDCDME on the AA-PC-12 column using different compositions of methanol–buffer and 2-propanol–buffer eluents. The concentration of phosphate buffer in these experiments ranged from 50 to 150 mM. All chromatographic experiments were carried out at 35°C and a flow-rate of 1 ml/min.

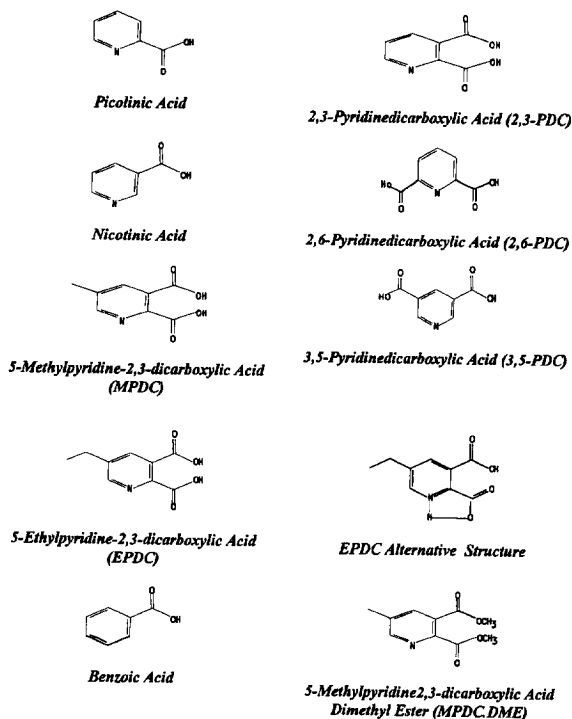


Fig. 1. Structures of acids.

3. Results and discussion

3.1. Surface and sorption properties of silica

A complete nitrogen adsorption–desorption isotherm is shown in Fig. 2. Its shape is typical of mesoporous silicas with relatively uniform pore-size distributions. This type of distribution was confirmed by transforming adsorption isotherm data to the incremental pore-volume distribution, which is shown in Fig. 3. The pore-volume distribution was calculated by employing an advanced numerical method, which combines the density functional theory description of the local adsorption with a regularization method for solving the integral equation of adsorption [9]. As can be seen in Fig. 3, the pore-volume distribution for the silica studied is uniform with an average pore diameter of about 10 nm. There is an indication of the existence of a small amount of fine pores; see the two small bars in the distribution curve around 2 nm. The comparative plot shown in Fig. 4 for the dependence of the volume adsorbed on the surface film thickness on a non-porous reference solid [10]

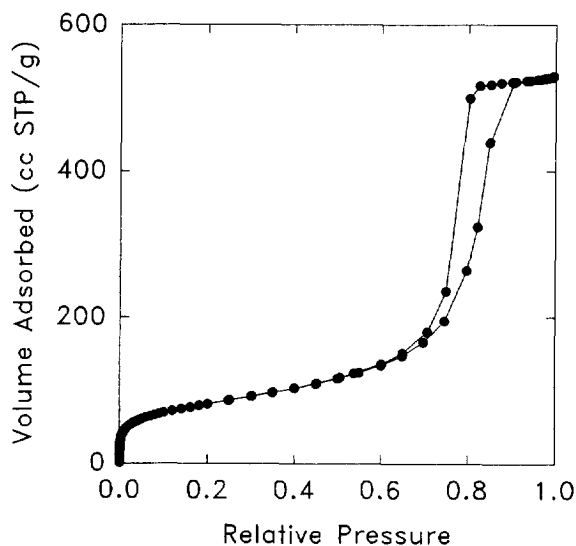


Fig. 2. Complete nitrogen adsorption–desorption isotherm on Kromasil at -195.7°C .

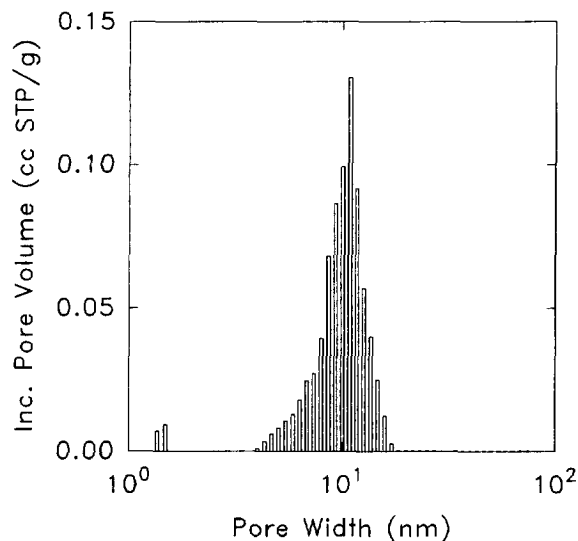


Fig. 3. Incremental pore-volume distribution for Kromasil calculated from nitrogen adsorption isotherm.

was used to estimate the volume of these fine pores. This volume was slightly greater than $0.01 \text{ cm}^3/\text{g}$, whereas the total pore volume was 0.82

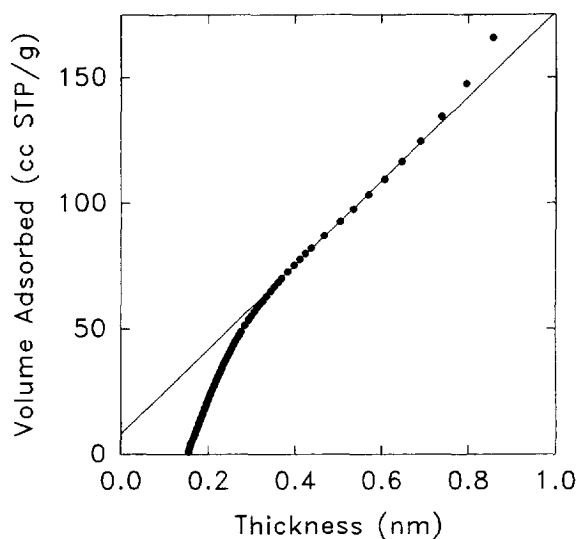


Fig. 4. t -Plot for nitrogen adsorption data on Kromasil.

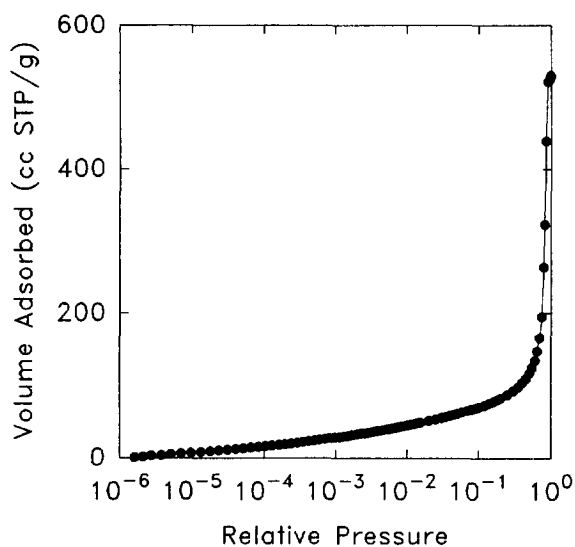


Fig. 5. Low-pressure nitrogen adsorption data on Kromasil at -195.7°C .

cm^3/g . Thus, the silica studied can be considered as a mesoporous solid. Its BET specific surface area was $311 \text{ m}^2/\text{g}$. These values are close to those provided by the manufacturer: BET spe-

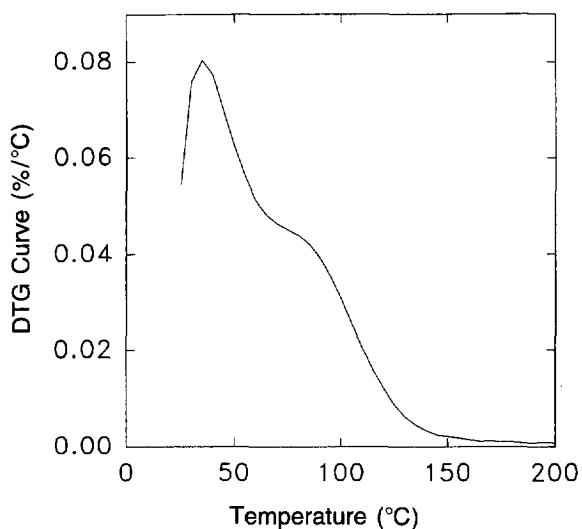


Fig. 7. DTG curve for thermodesorption of physically adsorbed water on the Kromasil surface.

cific surface area $340 \text{ m}^2/\text{g}$, total pore volume $0.9 \text{ cm}^3/\text{g}$ and average pore size 10 nm .

The low-pressure part of the nitrogen adsorption isotherm, which is shown in Fig. 5, was

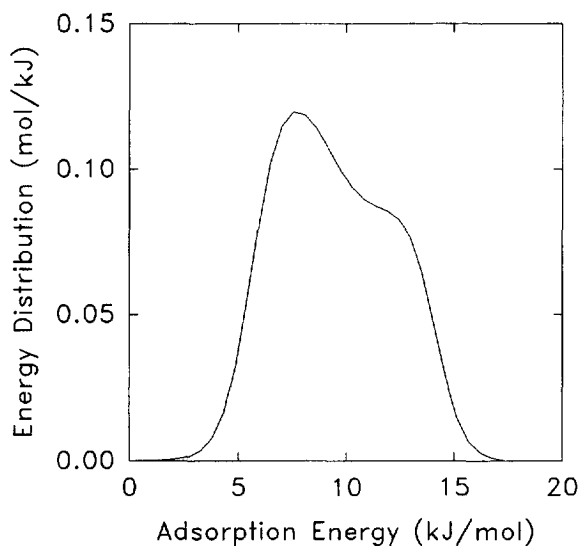


Fig. 6. Adsorption energy distribution calculated from the low-pressure nitrogen isotherm data shown in Fig. 5.

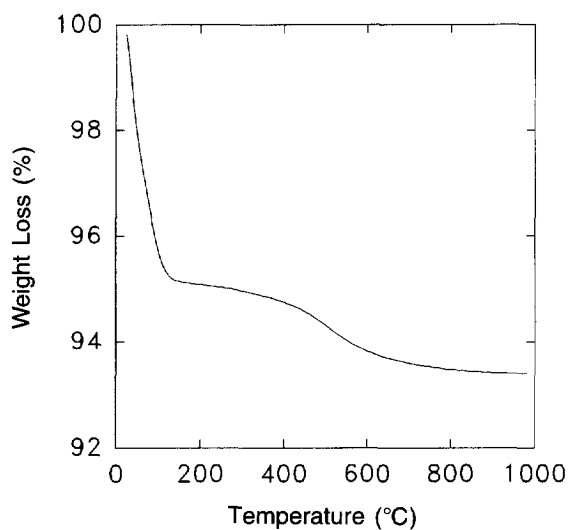


Fig. 8. Thermogravimetric mass-loss curve for the Kromasil sample with adsorbed water.

Table 1
Elemental analysis data for the alkylamide phases studied

Column code	Packing type	Carbon load (wt.%)	Nitrogen load (wt.%)
AA-MC-6	Monomeric, C ₆	3.84	0.36
AA-MC-12	Monomeric, C ₁₂	9.41	0.63
AA-PC-6	Polymeric, C ₆	7.90	1.06
AA-PC-12	Polymeric, C ₁₂	12.27	1.00

utilized to calculate the adsorption energy distribution by using a numerical procedure described elsewhere [11]. This distribution is a measure of surface heterogeneity of the silica studied. Its complex shape indicates that at least two types of adsorption sites can be distinguished on the silica surface (see Fig. 6). Similar energy distributions for silica surfaces have been reported previously [12]. Also, the differential thermogravimetric (DTG) curve for thermodesorption of water molecules from the silica surface (see Fig. 7) provides additional confirmation for the calculated energy distribution.

The overall mass loss for the silica sample, shown in Fig. 8, contains two steps, related to thermodesorption of physically adsorbed water and dehydroxylation of the silica surface [13]. The total amount of all silanols for the silica studied, estimated on the basis of the mass-loss

shown in Fig. 8 in the temperature range 200–1000°C, is about 7.1 $\mu\text{mol/g}$. This value converts to 4.25 OH groups/nm² and is not much below the maximum value of 4.6 OH groups nm² [13].

3.2. Coverage densities of alkylamide phases

Elemental analysis data are given in Table 1. These data were used to calculate surface concentrations of alkylamide ligands and unreacted aminopropyl groups by employing a method described previously [4,5]. The coverage density of the initial polymeric aminopropyl phase was 2.83 $\mu\text{mol/m}^2$ and was much higher than the corresponding densities of initial monomeric phases. The coverage density data are presented in Table 2. As can be seen, the amount of unreacted aminopropyl groups varies for the phases studied. In the case of the polymeric

Table 2
Coverage densities for the alkylamide phases studied

Column code	Packing type	Amine groups ($\mu\text{mol/m}^2$)	Amide groups ($\mu\text{mol/m}^2$)
M-NH ₂	Monomeric, amino phase	0.95	–
AA-MC-6	Monomeric, C ₆	0.07	0.88
M-NH ₂	Monomeric, amino phase	2.09	–
AA-MC-12	Monomeric, C ₁₂	0.63	1.46
P-NH ₂	Polymeric, amino phase	2.83	–
AA-PC-6	Polymeric, C ₆	1.28	1.55
P-NH ₂	Polymeric, amino phase	2.83	–
AA-PC-12	Polymeric, C ₁₂	0.73	2.10

phases this amount is greater than that for the corresponding monomeric phases.

3.3. Discussion of chromatographic measurements

A comparison of the chromatographic properties of monomeric and polymeric alkylamide phases with C_6 and C_{12} terminal alkyl chains was performed by using the retention data of two series of pyridinedicarboxylic acids; 2,3-pyridinedicarboxylic acid homologs (2,3-PDC, MDC, EPDC) and pyridinedicarboxylic acid substitutional isomers (2,3-PDC, 2,6-PDC, 3,5-PDC). The experimental dependences of the capacity factor k' on pH for all these diacids on four columns studied are shown in Figs. 9–12. They indicate a mixed ion-exchange and parti-

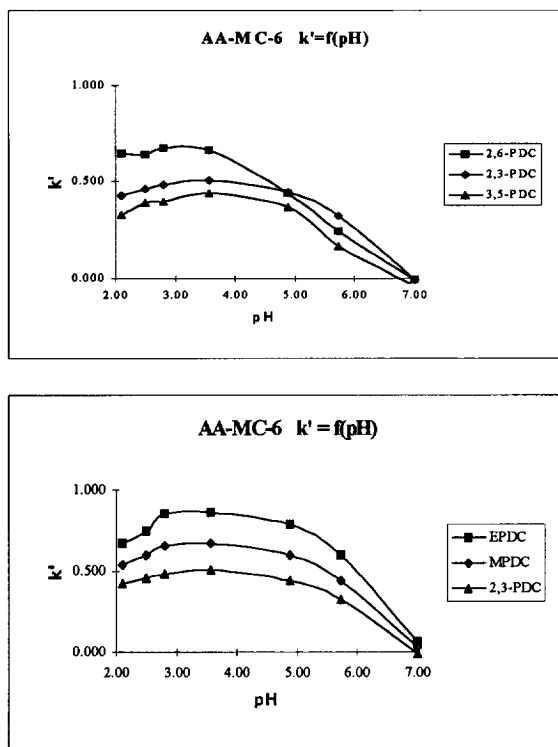


Fig. 9. Comparison of k' vs. pH for pyridinedicarboxylic acids on the AA-MC-6 column at 35°C. The aqueous–organic mobile phase contained 5% acetonitrile.

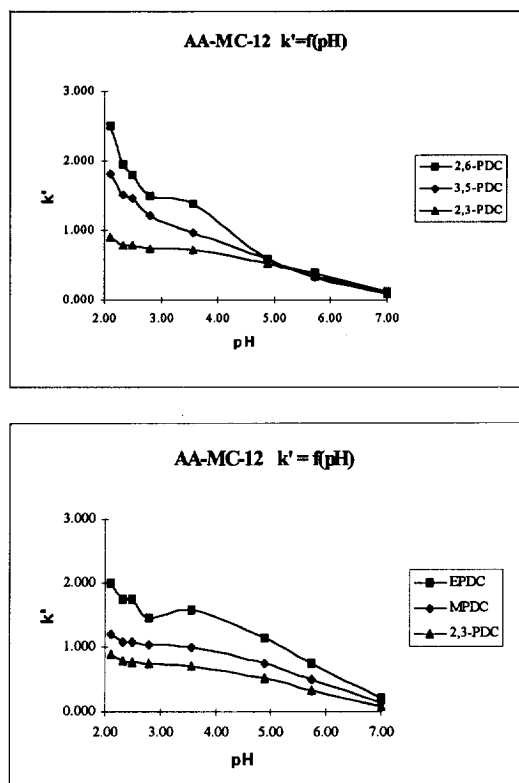


Fig. 10. Comparison of k' vs. pH for pyridinedicarboxylic acids on the AA-MC-12 column at 35°C. The aqueous–organic mobile phase contained 5% acetonitrile.

tion–displacement retention mechanism [1]. Although the ionic type of interaction plays a substantial role in the retention mechanism, especially for the polymeric columns, the retention times of all the compounds increase with increasing length of the terminal alkyl chain.

Pyridinedicarboxylic acids are much more strongly retained on polymeric columns and their retention increases with increase in pH over the range 2–5. However, the pH-related increase in the retention is connected with peak broadening and/or tailing. It was observed previously [1,14] that this effect is much more pronounced for acids with a carboxylic group in the α -position to the basic nitrogen atom in the pyridine ring than for other acids. 2,6-Pyridinedicarboxylic acid was the most strongly retained compound of all the acids examined on the alkylamide columns

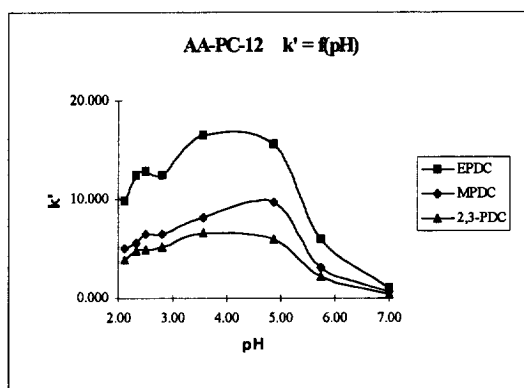
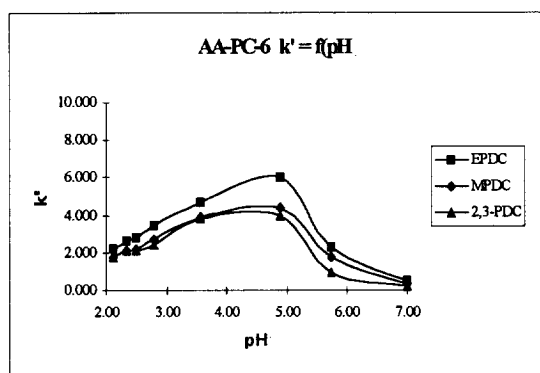
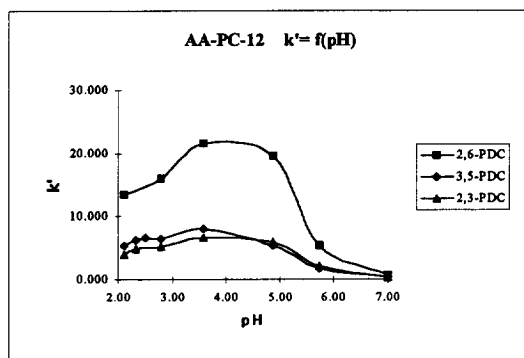
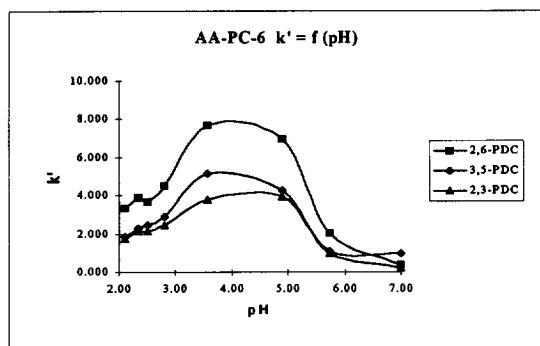


Fig. 11. Comparison of k' vs. pH for pyridinedicarboxylic acids on the AA-PC-6 column at 35°C. The aqueous–organic mobile phase contained 5% acetonitrile.

Fig. 12. Comparison of k' vs. pH for pyridinedicarboxylic acids on the AA-PC-12 column at 35°C. The aqueous–organic mobile phase contained 5% acetonitrile.

studied. Its peak on the polymeric AA-PC-12 column in the pH-range 3–5 (see Fig. 12, $k' \approx 20$) was unacceptably broad. The rapid decrease in the retention time above pH 5 is related probably to the already complete ionization of both carboxylic groups and the ring nitrogen (basic pyridinium $pK_A > 5$, see Table 3), which promotes solubility of solutes in aqueous mobile phase (95% aqueous–5% acetonitrile), and possibly to the reversed (negative) charge of alkylamide phases. The observed decrease in the retention for nicotinic and 3,5-pyridinedicarboxylic acids at pH > 3.5 (Fig. 13), and its starting increase at the same pH for 4-picoline (Fig. 14) seem to confirm the above interpretation. Basic pK_A values for these compounds are 4.72, 4.19 and 6.0, respectively.

For monomeric columns, an increase in the length of the terminal alkyl chain (AA-MC-12, Fig. 10) reduces the pH effect. However, these columns are more selective (the largest observed differences between k' values) for the separation of substitutional isomers of diacids than the polymeric columns, particularly at lower pH between 2 and 3. Monomeric columns provide considerably more symmetrical peaks for all diacids, including 2,6-PDC.

Plots of the capacity factors against pH for selected pyridineacids, benzoic acid and MPDCDME on all alkylamide columns are shown in Figs. 13, 15 and 16. They demonstrate differences between the monomeric and polymeric columns. For 5-ethylpyridine-2,3-dicarboxylic acid (EPDC), the pH dependence on the

Table 3
 pK_A values for the compounds studied

Compound	Acid step 1	Acid step 2	Basic (pyridinium)
Picolinic acid	0.99		5.55
Nicotinic acid	2.02		4.72
2,3-PDC	1.07	1.90	5.01
2,6-PDC	0.34	1.20	5.84
3,5-PDC	1.37	2.23	4.19
MPDC	1.13	2.04	5.37
EPDC	1.14	2.05	5.43
4-Picoline			6.00
Benzoic acid	4.20		

Calculated using PKALC Program, Version 1.0, 1992 (CompuDrug, Chemistry, CompuDrug NA, Rochester, NY).

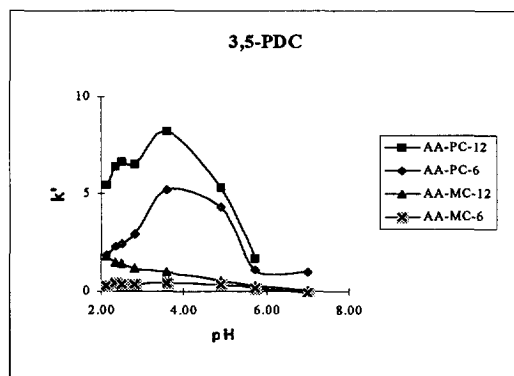
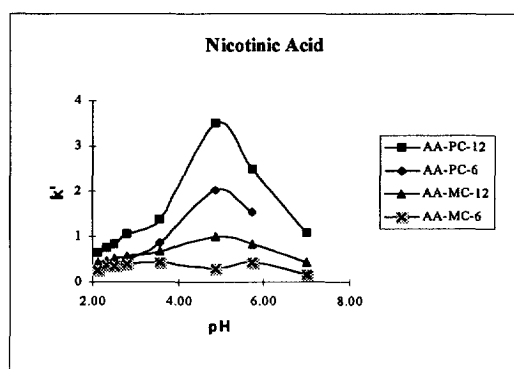


Fig. 13. Comparison of k' vs. pH dependences for nicotinic acid and 3,5-pyridinedicarboxylic acid on all the alkylamide phases studied at 35°C. The aqueous-organic mobile phase contained 5% acetonitrile.

conventional Kromasil C_8 column is also plotted. In some instances the pH dependences on monomeric AA columns (EPDC and 2,6-PDC on AA-MC-12) resemble those on conventional alkyl-bonded columns. However, a direct comparison between monomeric and polymeric types of columns cannot be derived from the presented data, since unfortunately both monomeric columns contained a lower degree of amidization (see Table 2).

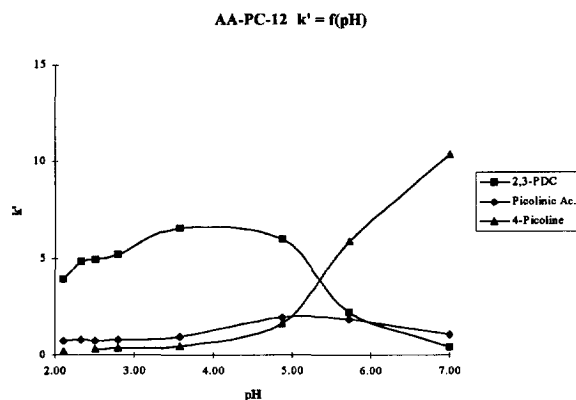


Fig. 14. Comparison of k' vs. pH dependences for 2,3-pyridinedicarboxylic acid, picolinic acid and 4-picoline on the AA-PC-12 phase at 35°C. The aqueous-organic mobile phase contained 5% acetonitrile.

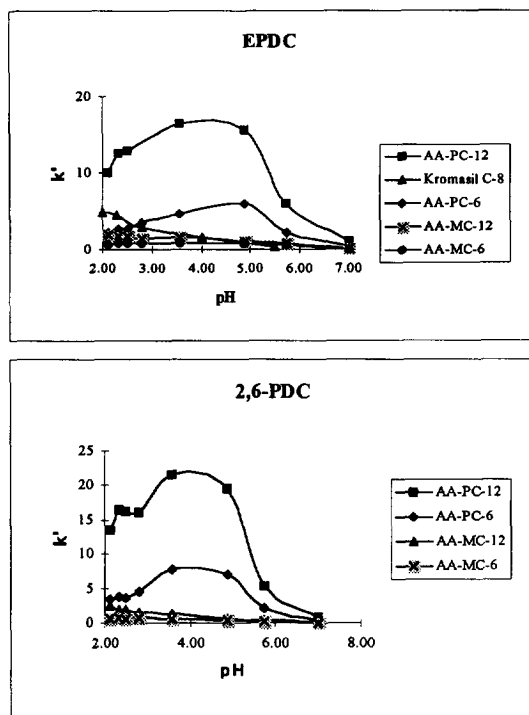


Fig. 15. Comparison of k' vs. pH dependences for 5-ethylpyridine-2,3-dicarboxylic acid and 2,6-pyridinedicarboxylic acid on all the alkylamide phases studied at 35°C. The aqueous-organic mobile phase contained 5% acetonitrile.

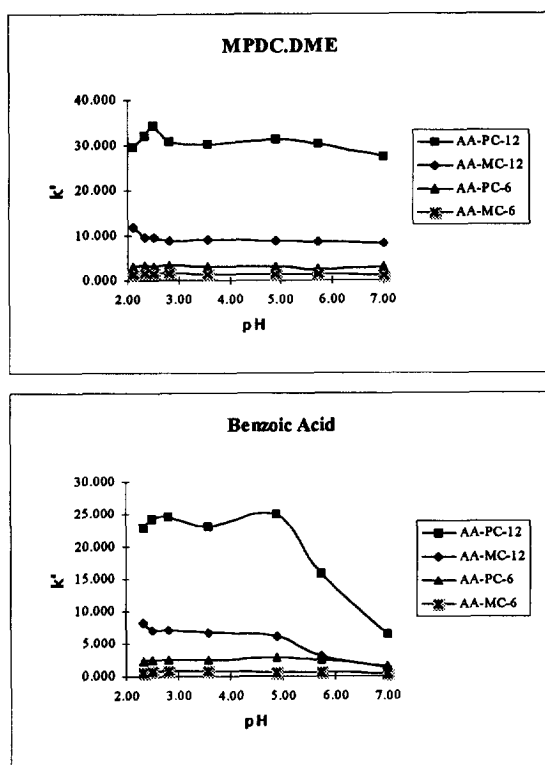


Fig. 16. Comparison of k' vs. pH dependences for 5-methylpyridine-2,3-dicarboxylic acid dimethyl ester and benzoic acid on all the alkylamide phases studied at 35°C. The aqueous-organic mobile phase contained 5% acetonitrile.

As shown previously [1] and in Fig. 17, the acetonitrile concentration in the mobile phase had a different influence on the retention of pyridine acids and neutral compounds. Retention of MPDCDME and even an ionized benzoic acid decreases more rapidly than that of pyridine-related acids. This observation and the complete lack of sensitivity of neutral compounds on the ionic strength of the applied buffer (Fig. 18) create broad possibilities for optimizing conditions for the simultaneous separation of ionic and non-polar compounds. Figs. 19 and 20 show how the peak shape and elution order may be altered by using different solvents and changing the concentration of the phosphate buffer. In comparison with conventional alkyl phases, the AA phases gave better resolution and peak shapes for the solutes studied (see Figs. 21 and 22). Shown in these figures is a comparison of select-

ed separations on the AA-MC-12 column and a commercial 150-mm column packed with 5- μ m Kromasil RP C₈.

4. Conclusions

Comparative studies of the retention of pyridinecarboxylic acids on monomeric and polymeric alkylamide phases have shown that the specific interaction sites in alkylamide phases have a significant influence on the solute retention and selectivity under reversed-phase conditions. The retention of multifunctional organic acids on these columns is governed by a mixed ion-exchange and partition-displacement mechanism. The contribution of ion-type interactions to the overall retention is greater for polymeric

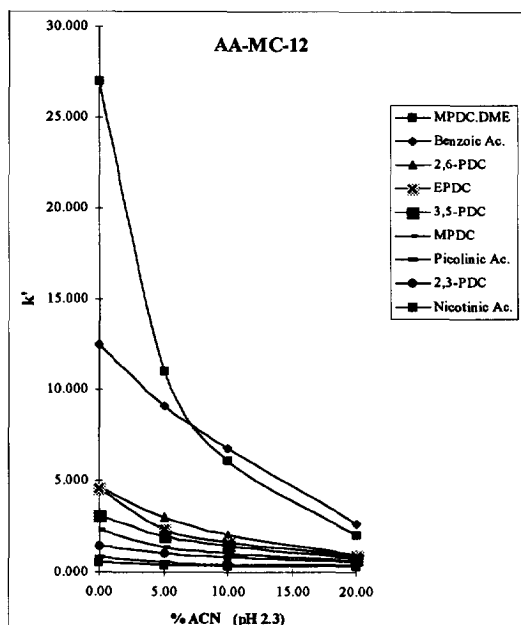


Fig. 17. Comparison of k' vs. acetonitrile concentration dependences for all the solutes listed in Table 3 on the AA-MC-12 column at 35°C. The mobile phase contained 95% of 50 mM phosphate buffer (pH 2.3) and 5% acetonitrile.

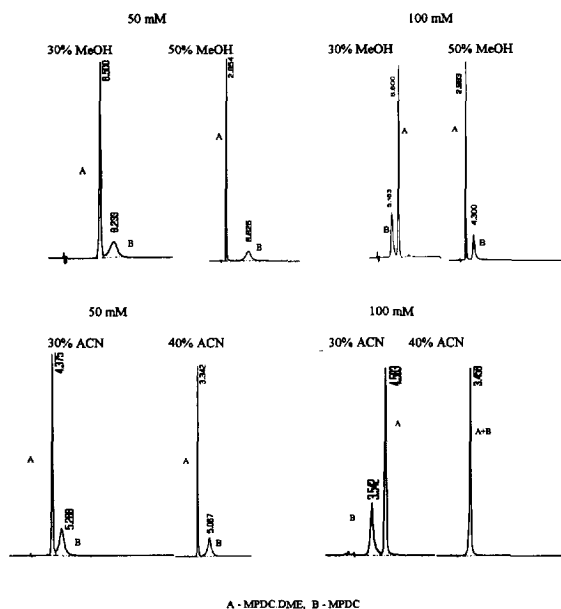


Fig. 19. Comparison of elution order and peak shapes of 5-methylpyridine-2,3-dicarboxylic acid (MPDC) and its dimethyl ester (MPDCDME) at different concentrations of phosphate buffer (pH 2.8) and various solvent levels on the AA-PC-12 column. Conditions: 35°C, 1 ml/min, 254 μ m.

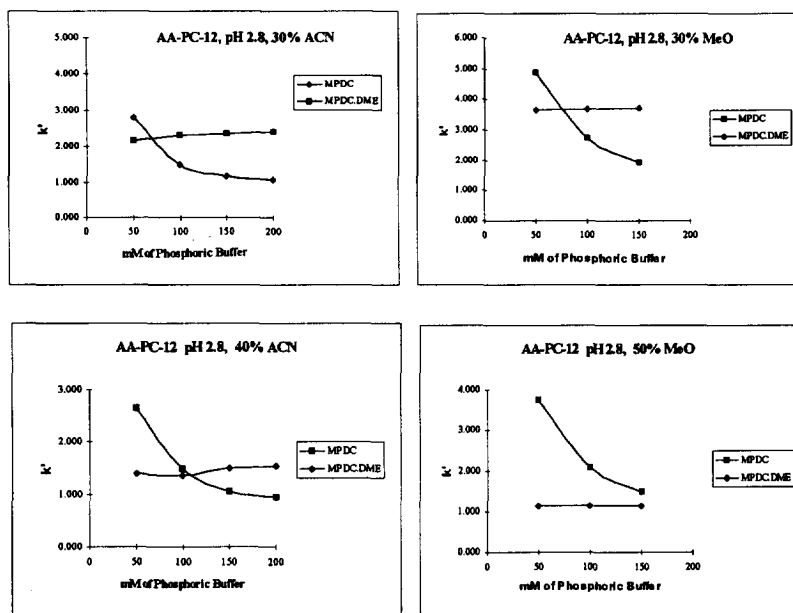


Fig. 18. Comparison of k' vs. phosphate buffer concentration for 5-methylpyridine-2,3-dicarboxylic acid and its dimethyl ester on the AA-PC-12 column at different concentrations of acetonitrile and methanol.

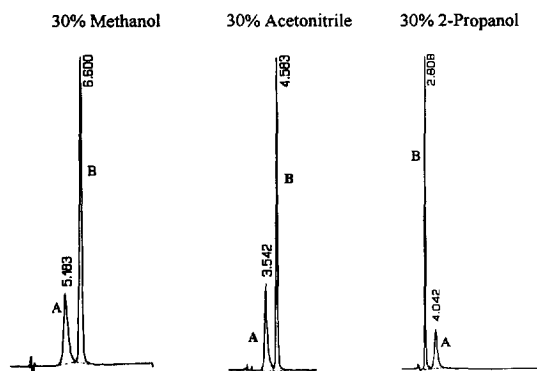


Fig. 20. Comparison of elution order and peak shapes of 5-methylpyridine-2,3-dicarboxylic acid (MPDC, A) and its dimethyl ester (MPDCDME, B) with different solvents and 100 mM phosphate buffer (pH 2.8) on the AA-PC-12 column. Conditions: 35°C, 1 ml/min, 254 μ m.

alkylamide phases and consequently these phases are more suitable for the simultaneous separation of non-polar and ionic compounds. Monomeric phases demonstrate higher selectivity than polymeric phases for the separation of substitutional isomers of pyridinedicarboxylic acids.

Kromasil C-8

AA-MC-12

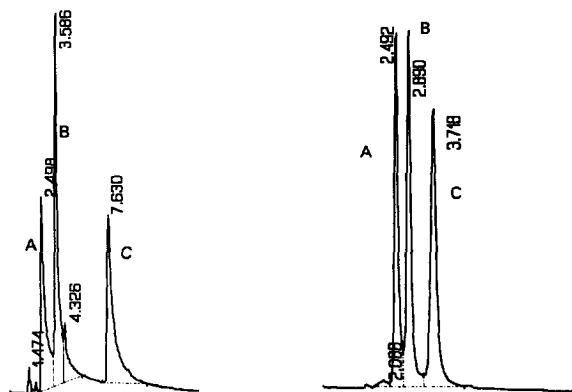


Fig. 21. Comparison of separation of 2,3-PDC (A), MPDC (B) and the EPDC (C) on 15-cm Kromasil C₈ and AA-MC-12 columns. The mobile phase was 50 mM phosphate buffer (pH 2.11)–acetonitrile (95:5). Conditions: 35°C, 1 ml/min, 254 μ m.

Kromasil C-8

AA-MC-12

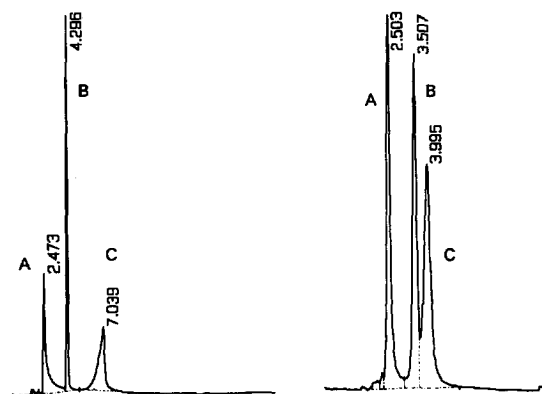


Fig. 22. Comparison of separation of 2,3-PDC (A), 3,5-PDC (B) and 2,6-PDC (C) on 15-cm Kromasil C₈ and AA-MC-12 columns. The mobile phase was 50 mM phosphate buffer (pH 2.11)–acetonitrile (95:5). Conditions: 35°C, 1 ml/min, 254 μ m.

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