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Control of migration time window and selectivity in electrokinetic chromatography with mixed polymeric pseudostationary phases

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Abstract

Control of migration time windows and the improvement of separation were achieved by utilizing mixed polymeric pseudostationary phases in electrokinetic chromatography (EKC) in water–organic solvent mixtures. Polyallylamine (PAA)-supported pseudostationary phases, one with hexadecyl groups (PAA-C_{16}) with the shorter migration times and the other with decyl groups (PAA-C_{10}) with the longer migration times, enabled the control of migration time windows when used as a mixture. PAA-C_{16} provided better separation for a region with relatively small k' values as in typical micellar EKC, while PAA-C_{10} gave better separation for a region with relatively large k' values as in reversed-phase liquid chromatography, the mixture of the two PAA derivatives resulting in better overall separations for a wide range of hydrophobic compounds. The migration time of a solute is determined by the relative contribution of each pseudostationary phase to the partition of the solute in a mixed carrier system. A theoretical explanation was provided by assuming the contribution of the two polymeric pseudostationary phases working independently. The migration time of the effective (imaginary) pseudostationary phase was shown to be in between the actual migration times of the two carriers, and can be different for each solute depending upon the relative contribution of the two pseudostationary phases to the solute partition. © 1998 Elsevier Science B.V.

Keywords: Selectivity; Pseudostationary phases; Migration time; Polynuclear aromatic hydrocarbons

1. Introduction

The primary function of a pseudostationary phase in electrokinetic chromatography (EKC) is to provide a medium for a solute to partition against the bulk aqueous phase like a stationary phase in reversed-phase liquid chromatography (RPLC). The way they function, however, is different from each other in that a pseudostationary phase limits separation times, when the electrophoretic mobility of the carrier is smaller than the opposing electroosmotic flow of a bulk aqueous phase [1,2]. This is the case with a typical EKC system with sodium dodecyl sulfate (SDS) micelles in water. In a sense this is an advantage, because it guarantees a short separation time, while it could also be a disadvantage, if one is interested in the separation of relatively hydrophobic compounds. Hydrophobic compounds which mostly partition into the micelle phase are hard to separate in micellar EKC (MEKC). The addition of an organic solvent or cyclodextrin to a MEKC system has been reported to be successful [3–13], but the approach is accompanied by some difficulties. The addition of an organic solvent causes the instability

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of micelle structures, while the addition of cyclodextrins adds complexities to the separation conditions and the understanding of the resulting separations. It is important to control t_C, or the migration time window, to optimize EKC separations [13].

We have been studying the performance of polymer-supported pseudostationary phases in EKC, including alkylated polyallylamine (PAA) [14] and alkylated starburst dendrimers (SBDs) [15,16]. They can provide high efficiency separations for a wide range of hydrophobic compounds in a full range of water–organic solvent (0–90%) mixtures. The polymeric carriers showed the expansion of migration time windows above certain organic solvent concentrations depending upon the alkyl chain length of the pseudostationary phase [14,16–18]. At 30–50% methanol, PAA with decyl groups (PAA-C_{10}) provided separations similar to RPLC with wide migration time windows, while PAA with hexadecyl groups (PAA-C_{16}) provided typical EKC-like separations with narrow migration time windows. The results prompted us to study the use of a mixture of these pseudostationary phases for controlling the migration time windows.

Previous examples of the use of mixed pseudostationary phases in EKC include the addition of cyclodextrin to SDS-MEKC systems to effect chiral separations [3,11,19] and to increase the partition of hydrophobic compounds into the aqueous phase [9,10,12], and the addition of another surfactant to SDS-MEKC systems for varying selectivity and a migration range [20–24]. This report is to provide successful examples where the mixtures of PAA-supported pseudostationary phases with different alkyl chain lengths and different migration times allowed easy control of migration time windows and the improvement of separations in buffer–organic solvent mixtures.

2. Experimental

2.1. Materials

2.1.1. Alkylated PAA with carboxylate groups (PAA-C_nH_{2n+1})

The pseudostationary phases [PAA-C_{10}(20) and PAA-C_{16}(10)] prepared in a previous study [14] were used. The numbers in the parentheses indicate the degree of alkylation of PAA, and will be omitted in the following section.

2.1.2. Samples

Polynuclear aromatic hydrocarbons (PAHs) were purchased from AccuStandard (New Haven, CT, USA).

2.1.3. Equipment and measurement

The same equipment was used as in previous studies [14–16]. Detection was carried out at 254 nm. The capillary (50 μm I.D., 375 μm O.D.) length was 48 cm with the effective length of 33 cm. PAA-supported pseudostationary phase was used at a concentration of 20 mg/ml in 20 m*M* borate buffer (pH 9.3)–organic solvent mixtures, unless noted otherwise. The solutions were filtered with a membrane filter (0.2 μm) before use. EKC was carried out at ambient temperatures under air circulation. The t_0 value was obtained by the injection of methanol or acetonitrile, and t_C values by the iteration method [25,26] using a series of alkyl phenyl ketones.

3. Results and discussion

The chromatograms in Fig. 1 show the characteristics of PAA-supported polymeric pseudostationary phases in EKC. In 20% methanol both PAA-C_{10} and PAA-C_{16} carriers provided migration profiles similar to that with a SDS-MEKC system in aqueous buffer. Hydrophobic compounds migrated close to the migration time of the carrier (t_C). This is due to the predominant partition of the hydrophobic solutes into the pseudostationary phase.

The migration range of these compounds, however, expanded at 40% methanol with PAA-C_{10}, followed by the compression of the migration range with a further increase in methanol content to 60%. The latter observation, the decrease in retention with the increase in the organic solvent content is similar to that in RPLC, and is caused by the decrease in partition coefficients (k' values). At 60% methanol, the migration times of alkyl phenyl ketones become much shorter with PAA-C_{10} than in 40% methanol, while PAA-C_{16} resulted in a wider migration range

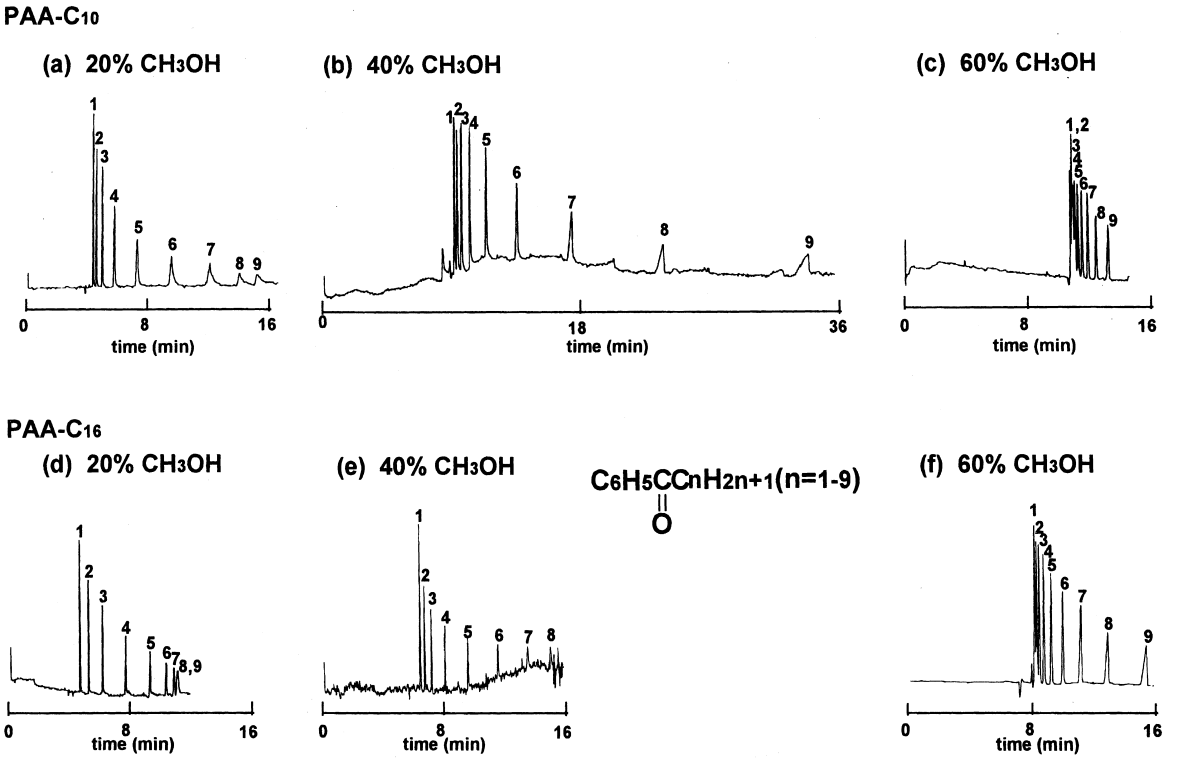

Fig. 1. Effect of methanol contents on the separation of alkyl phenyl ketones (C_6H_5–CO–C_nH_{2n+1}, $n=1$–9) in 20–60% methanol with PAA-C_{10} (a to c) and PAA-C_{16} (d to f); field strength: 400 V/cm; carrier: 20 mg/ml; separation solution: 20 m*M* borate buffer (pH 9.3)–methanol mixture. Peak numbers indicate the number of carbon atoms in the alkyl group.

and better separation with the larger k' values than PAA-C_{10}.

Similar results were obtained for aromatic hydrocarbons as shown in Fig. 2. Long migration times were observed for the aromatic hydrocarbons at 40% methanol with PAA-C_{10}, followed by the compression of the migration range with the increase in methanol content to 60%. PAA-C_{16} showed a narrow migration range in 40% methanol, and a much wider migration range and better separation than PAA-C_{10} in 60% methanol. The results indicate that a migration range of solutes is limited by t_C below a certain methanol content as in a typical MEKC system, and by k' values at a high methanol content as in a RPLC system. The expansion of the migration time window was observed between the two regions. Above a certain methanol content, 30% for PAA-C_{10} and 60% for PAA-C_{16}, the migration range of the solutes is no longer limited by t_C.

For samples containing a small number of compounds, separation is feasible by using any combination of the alkyl-chain length of the pseudostationary phase and the organic solvent content, as shown in Fig. 2. However, the separation of a large number of compounds with a wide range of hydrophobic property needs further optimization. As shown in Fig. 3a, PAA-C_{16} showed poor resolution in 50% methanol for the late-migrating solutes among the 16 PAHs designated as priority pollutants by EPA. The eight compounds (Nos. 9–16) migrated as three groups in 50% methanol presumably due to the small t_C value. In 60% methanol, the separation became much better with the wider migration time window, showing 15 peaks for the 16 PAHs. Slight compression of the earlier part and the expansion of the later part can be seen compared to the separation in 50% methanol. The results compares favorably with those in capillary electrochromatography (CEC) [27,28]. At 65% as shown in Fig. 3c, the migration range was compressed by the decrease in k' values resulting in

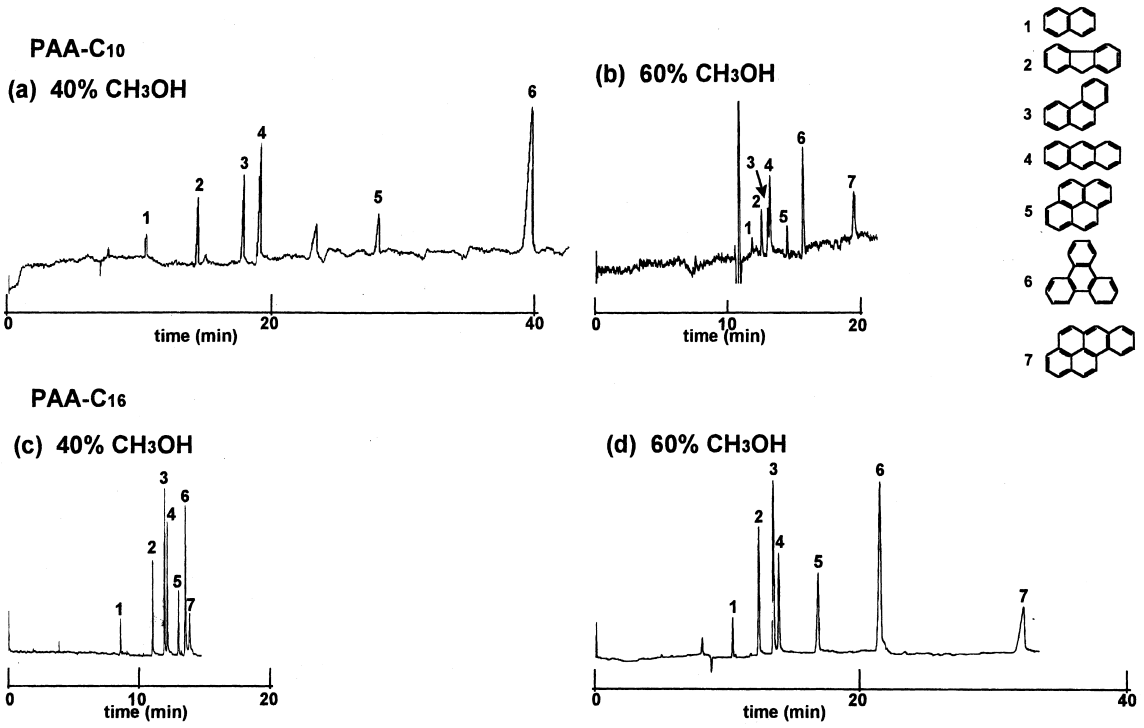

Fig. 2. Effect of methanol contents on the separation of aromatic hydrocarbons with PAA-C_{10} (a, b) and PAA-C_{16} (c, d) in 20 m*M* borate buffer–methanol mixtures. Field strength; 400 V/cm: carrier; 20 mg/ml: solutes; (1) naphthalene (2) fluorene (3) phenanthrene (4) anthracene (5) pyrene (6) triphenylene (7) benzo[*a*]pyrene.

the loss of resolution at the earlier part, with dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene (peak 14 and 15) still overlapping in spite of the sign of partial separation.

In buffer–acetonitrile mixtures, optimum separation of the 16 PAHs was obtained at 50% acetonitrile, resulting in the separation into 15 peaks, as shown in Fig. 4c. It was impossible to obtain a complete separation, because acenaphthene and fluorene (peaks 3 and 4) overlap in the early part. At 40–45% acetonitrile, the migration time window was limited by t_C, resulting in poor separation of the late-migrating species. Thus with a single polymeric pseudostationary phase, migration time windows can be expanded by the addition of an organic solvent, but the complete separation of a wide range of closely-related compounds is still hard to achieve. In RPLC, gradient elution is commonly employed for the separation of such mixtures so that one can obtain the greater resolution at the beginning and to narrow the peak spacings at the later part of the chromatogram [29,30].

As shown in Fig. 5, PAA-C_{10} and PAA-C_{16} gave very different results between 30 and 60% methanol in terms of the migration range and relative peak spacings at the earlier and the later parts of the chromatograms. In 30–40% methanol, PAA-C_{10} provided wide peak spacings for the more hydrophobic aromatic compounds while PAA-C_{16} gave short t_C and narrow peak spacings for such compounds. At 60% methanol, PAA-C_{10} provided a wide migration time window and small k' values resulting in the better separation of the larger aromatic hydrocarbons, whereas PAA-C_{16} gave a finite migration range and provided separation for a wide range of PAHs. We examined the use of mixtures of PAA-C_{10} and PAA-C_{16} to control the migration time window and to improve separations.

A mixture of PAA-C_{10} and PAA-C_{16} at 1:1 ratio gave a migration profile in between those with single

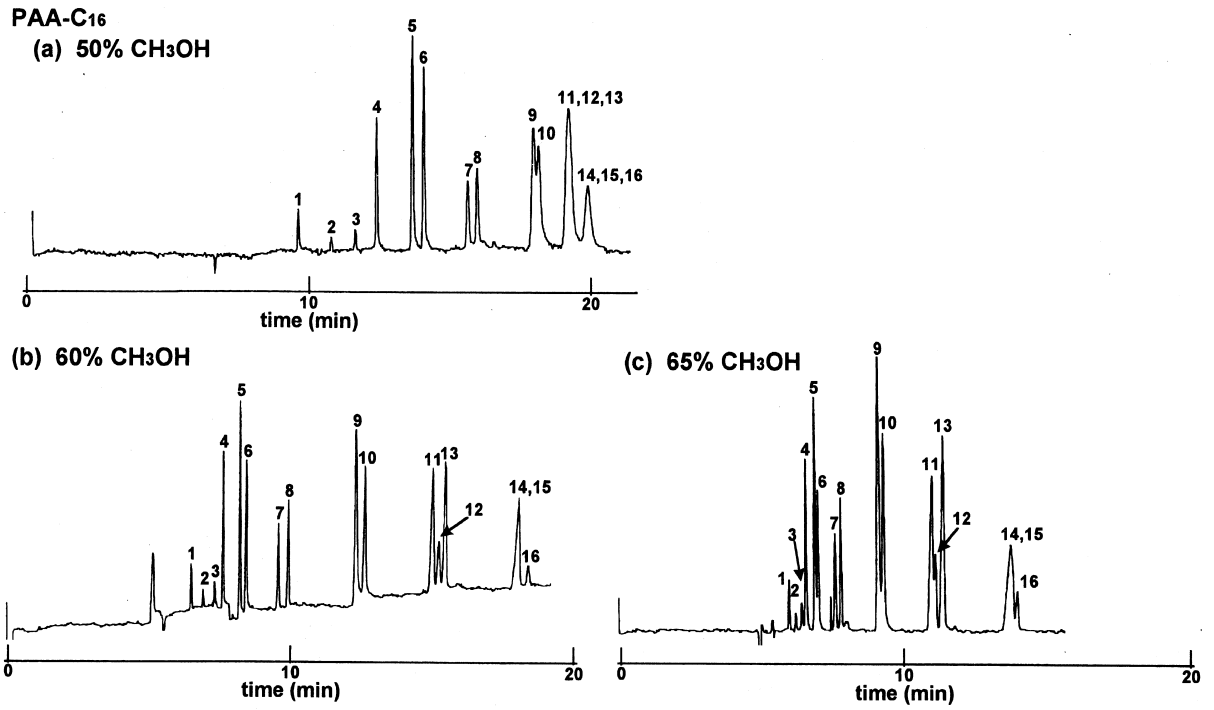

Fig. 3. Separation of 16 PAHs, priority pollutants designated by EPA, with PAA-C_{16}. Borate buffer (20 m*M*)–methanol, (a) (50:50), field strength; 400 V/cm, (b) (40:60, v/v), field strength; 600 V/cm, (c) (35:65, v/v), field strength; 600 V/cm, carrier; 20 mg/ml. Solutes: (1) naphthalene (2) acenaphthylene (3) acenaphthene (4) fluorene (5) phenanthrene (6) anthracene (7) fluoranthene (8) pyrene (9) chrysene (10) benz[*a*]anthracene (11) benzo[*b*]fluoranthene (12) benzo[*k*]fluoranthene (13) benzo[*a*]pyrene (14) dibenz[*a,h*]anthracene (15) indeno[1,2,3-*cd*]pyrene and (16) benzo[*ghi*]perylene.

pseudostationary phases, resulting in similar peak spacings between naphthalene and fluorene (peaks 1 and 2) and between triphenylene and benzo[*a*]pyrene (peaks 6 and 7), while either single pseudostationary phase resulted in very different peak spacings for the two pairs (Fig. 6). Total separation time with the mixed pseudostationary phases is much shorter than that with PAA-C_{10} and the peak capacity much greater than that with PAA-C_{16}. The results suggest that the use of a mixed pseudostationary phase can control t_C, and in turn the migration time window, as well as the total separation.

Fig. 7 shows an example of the variation of selectivity by the use of mixed pseudostationary phases. Benzo[*k*]fluoranthene (peak 12) overlaps with benzo[*b*]fluoranthene (peak 11) with PAA-C_{10} as a carrier, and with benzo[*a*]pyrene (peak 13) with PAA-C_{16} in 35% acetonitrile. By using a mixture, one can separate peak 12 from peaks 11 and 13, resulting in a complete separation for the PAHs (peaks 1–13), although the separation of the last three compounds was not possible under the conditions. Acenaphthene and fluorene (peaks 3 and 4) were separated by the contribution of PAA-C_{16}, while benzo[*k*]fluoranthene (peak 12) was more separated from benzo[*a*]pyrene (peak 13) by employing the greater [PAA-C_{10}]/[PAA-C_{16}] ratio in the mixture.

Fig. 8b shows the complete separation of the 16 PAHs with a 1:1 mixture of PAA-C_{10} and PAA-C_{16}, while either carrier could not provide a complete separation in the single carrier system. In 60% methanol (Fig. 8a), PAA-C_{10} did not provide much separation for the PAHs except for peaks 13–16, while PAA-C_{16} provided the separation of most PAHs except dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene (peaks 14 and 15). It is clear that PAA-C_{16} made the major contribution to the

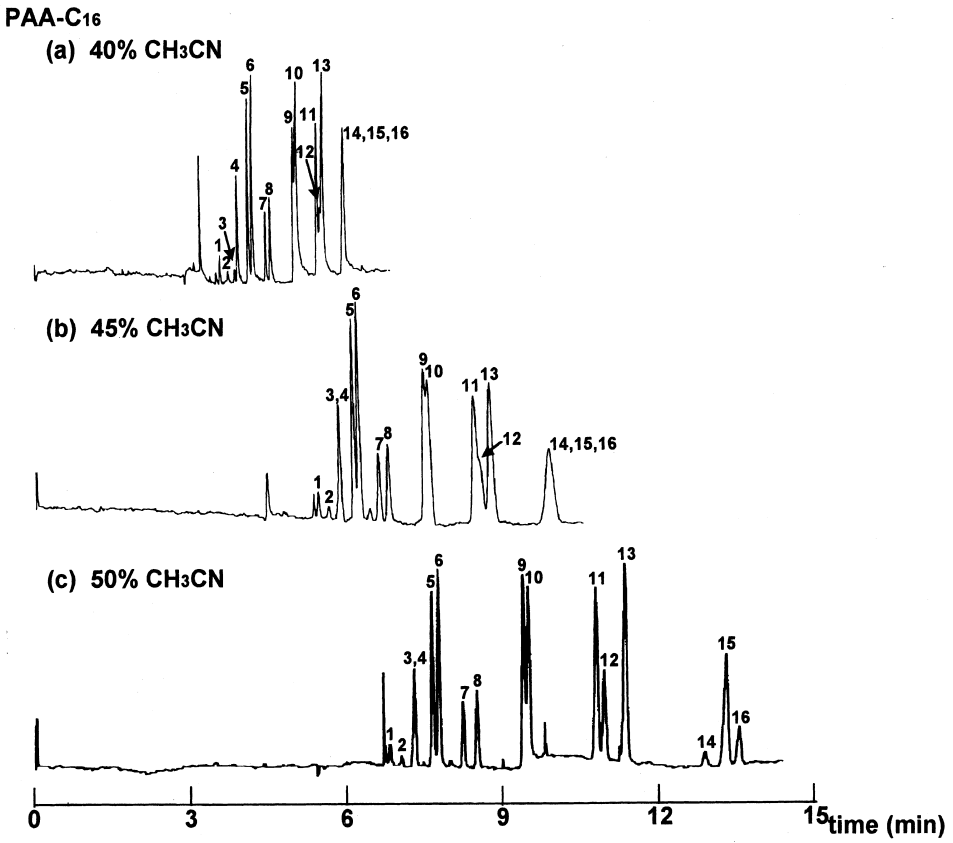

Fig. 4. Separation of 16 PAHs with PAA-C_{16}. Borate buffer (20 m*M*)–acetonitrile, (a) (60:40), Field strength; 400 V/cm, (b) (55:45, v/v), field strength; 400 V/cm, (c) (50:50, v/v), field strength; 400 V/cm. Carrier: 20 mg/ml. Separation solution: 20 m*M* borate buffer–acetonitrile mixture. See Fig. 3 for the identification of the solutes.

separation of compounds 1–13, while PAA-C_{10} contributed significantly to the separation of late-migrating compounds, especially 14–16, in the mixed carrier system.

The contribution of each pseudostationary phase in a mixed carrier system can be explained by the following equations. In an EKC system containing two (polymeric) pseudostationary phases (PS1 and PS2) working independently, the partition coefficients of a solute with the two pseudostationary phases, $k'_{(PS1)}$ and $k'_{(PS2)}$, are given by Eqs. (1a) and (1b), where C_{PS1}, C_{PS2}, and C_m stand for the amount of the solute present in PS1, in PS2, and in the aqueous phase. The effective k' value of the whole system (k'_{eff}) is the sum of the k' values with the two pseudostationary phases.

$$k'_{PS1} = C_{PS1}/C_m \quad (1a)$$

$$k'_{PS2} = C_{PS2}/C_m \quad (1b)$$

$$k'_{eff} = (C_{PS1} + C_{PS2})/C_m = k'_{PS1} + k'_{PS2} \quad (2)$$

The total mobility of a solute in a mixed pseudostationary phase system can be calculated from the k' value and the electrophoretic mobility associated with each pseudostationary phase by Eq. (3), where $\mu_{ep(PS1)}$ and $\mu_{ep(PS2)}$ are the electrophoretic mobilities of the two individual pseudostationary phases,

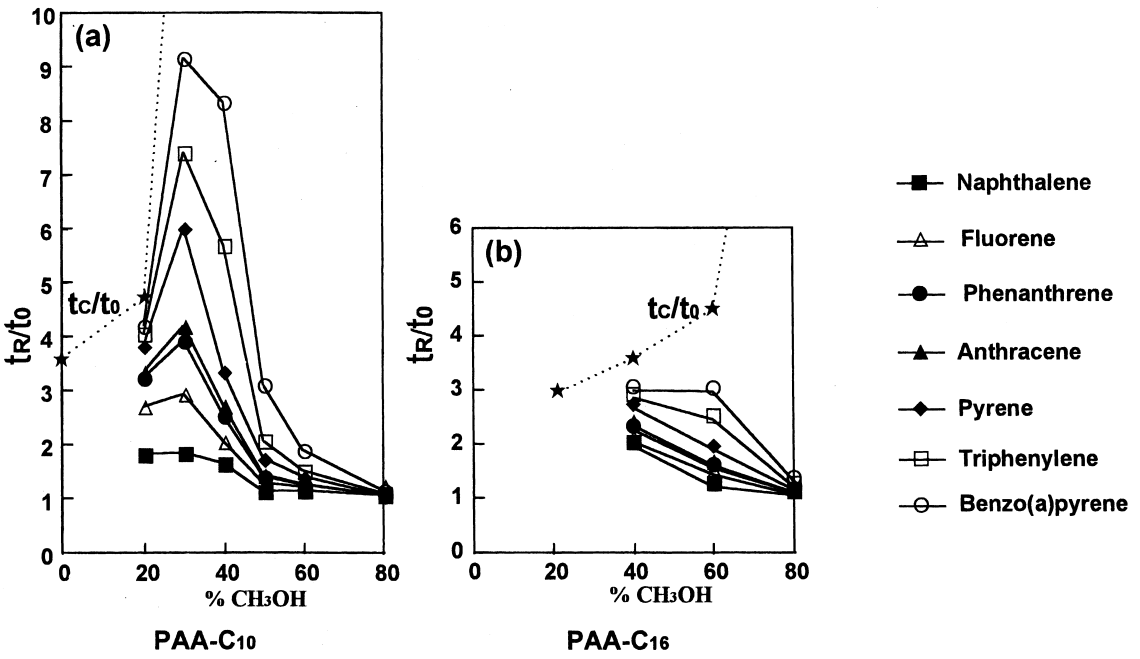

Fig. 5. Variation of migration times of solutes (t_R/t_0) and the PAA-C_n (t_C/t_0) with methanol content. For experimental conditions, see Fig. 2. Solutes: PAHs.

μ_{eo} the electroosmotic mobility of a mixed carrier system, and C_0 the total amount of the solute

$$\mu_{calc} = \mu_{eo} + \mu_{ep(PS1)}C_{PS1}/C_0 + \mu_{ep(PS2)}C_{PS2}/C_0$$
$$= \mu_{eo} + (C_m/C_0)(\mu_{ep(PS1)}C_{PS1}/C_m + \mu_{ep(PS2)}C_{PS2}/C_m)$$
$$= \mu_{eo} + [1/(1 + k'_{PS1} + k'_{PS2})](\mu_{ep(PS1)}k'_{PS1} + \mu_{ep(PS2)}k'_{PS2}) \quad (3)$$

The total mobility of a solute can also be described by Eq. (4), using the electrophoretic mobility of an effective (imaginary) pseudostationary phase, $\mu_{ep(PS\text{-}eff)}$, in the mixed system.

$$\mu_{obs} = \mu_{eo} + \mu_{ep(PS-eff)}(C_{PS1} + C_{PS2})/C_0$$
$$= \mu_{eo} + \mu_{ep(PS-eff)}k'_{eff}/(1 + k'_{eff}) \quad (4)$$

$$\mu_{ep(PS-eff)}k'_{eff} = \mu_{ep(PS1)}k'_{PS1} + \mu_{ep(PS2)}k'_{PS2}$$
$$\mu_{ep(PS-eff)} = \mu_{ep(PS1)}k'_{PS1}/(k'_{PS1} + k'_{PS2}) + \mu_{ep(PS2)}k'_{PS2}/(k'_{PS1} + k'_{PS2}) \quad (5)$$

Eq. (5) obtained from Eqs. (2)–(4), indicates that the imaginary pseudostationary phase migrates between PS1 and PS2. The effective migration time window in a mixed carrier system is determined by the effective migration time of the imaginary carrier which can be described by the relative magnitude of the k' values with respect to the two pseudostationary phases, and can be different for each solute, according to Eq. (5). In the case of the mixture of PAA-C_{10} and PAA-C_{16}, the latter made a major contribution to the separation of the PAHs except for a few compounds migrating later, as shown in Fig. 9. Separations of peaks 11–13 and 14–16 in the mixed carrier system were assisted by the contribution of PAA-C_{10} which should give much larger t_C than PAA-C_{16}. In this way the mixture of the two polymeric pseudostationary phases provided adequate peak capacities for both earlier and later parts of chromatograms and allow the control of the migration time window, or the separation time.

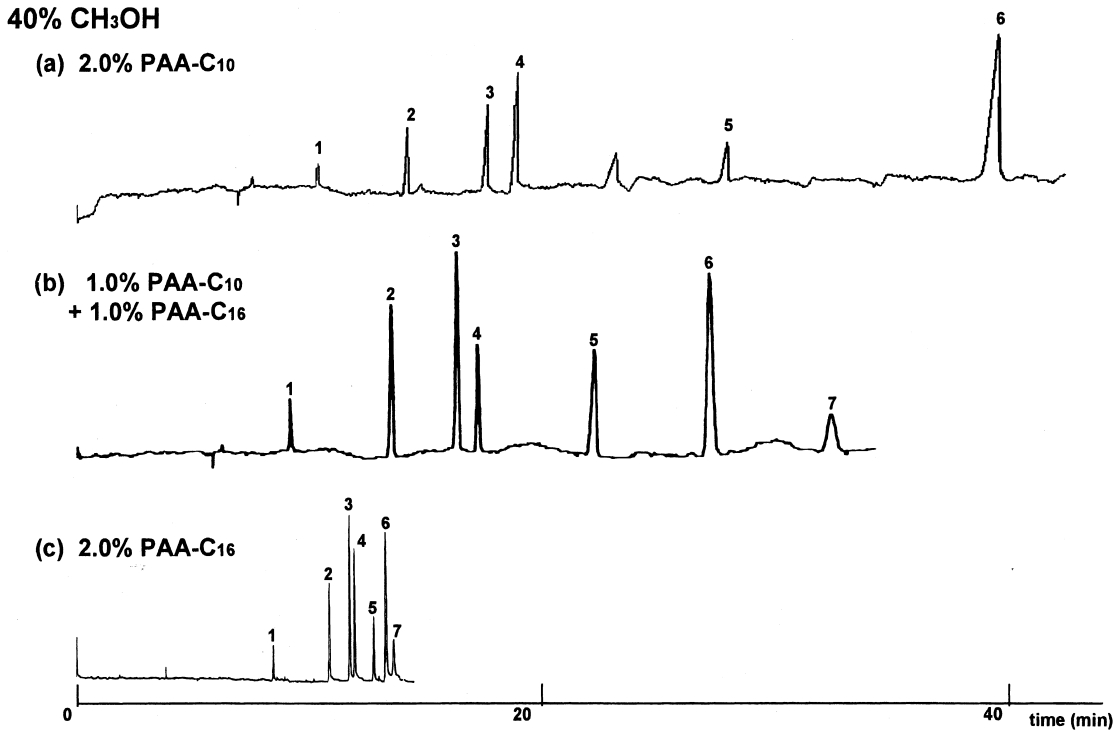

Fig. 6. Separation of aromatic hydrocarbons with (a) PAA-C_{10}, 2% (w/v), (b) PAA-C_{10}, 1% (w/v)+PAA-C_{16}, 1% (w/v), and (c) PAA-C_{16} 2% (w/v), in 20 m*M* borate buffer–methanol (60:40, v/v) mixtures. Field strength=400 V/cm. Solutes; (1) naphthalene (2) fluorene (3) phenanthrene (4) anthracene (5) pyrene (6) triphenylene (7) benzo[*a*]pyrene.

EKC has an inherent advantage, generally showing wider peak spacings at the beginning and narrower peak spacings at the later part of chromatograms, similar to a gradient elution in RPLC. The present results suggest that one can expand the later part by adding a second pseudostationary phase having a larger t_C value. A structural change of the carrier to increase the exposure of ionic groups was assumed to take place with the increase in an organic solvent content in order to explain the increased contribution of the electrophoretic mobility resulting in an abrupt increase in t_C at a certain and different organic solvent concentration for each alkyl chain length of the PAA-supported pseudostationary phase [14].

The present interpretation of the results with mixed pseudostationary phases is based on the assumption that the two pseudostationary phases should work independently. Micelle polymers (polysoaps) commonly form a local micelle structure [31] by intramolecular aggregation of hydrophobic groups to support the present interpretation, while intermolecular aggregation is also possible. Either intramolecular or intermolecular aggregation of hydrophobic groups of alkylated PAAs is compatible with the observation in single carrier EKC systems. With the carriers used in the present study, the density of alkyl groups per ionized head group, $[C_nH_{2n+1}]$/[COONa], is much smaller than unity, yet greater than a critical alkyl group content (CAC) [31,32] for each alkyl chain length, presumably leading to the major contribution of intramolecular aggregation (local micelle structure) of individual carriers rather than intermolecular aggregation, although the contribution of the latter cannot be ruled out at high concentrations.

Physical characterization of the alkylated PAAs, including the molecular size and the solution viscosity will provide clearer pictures. Validity of Eqs. (3)–(5) should be examined in a well defined system

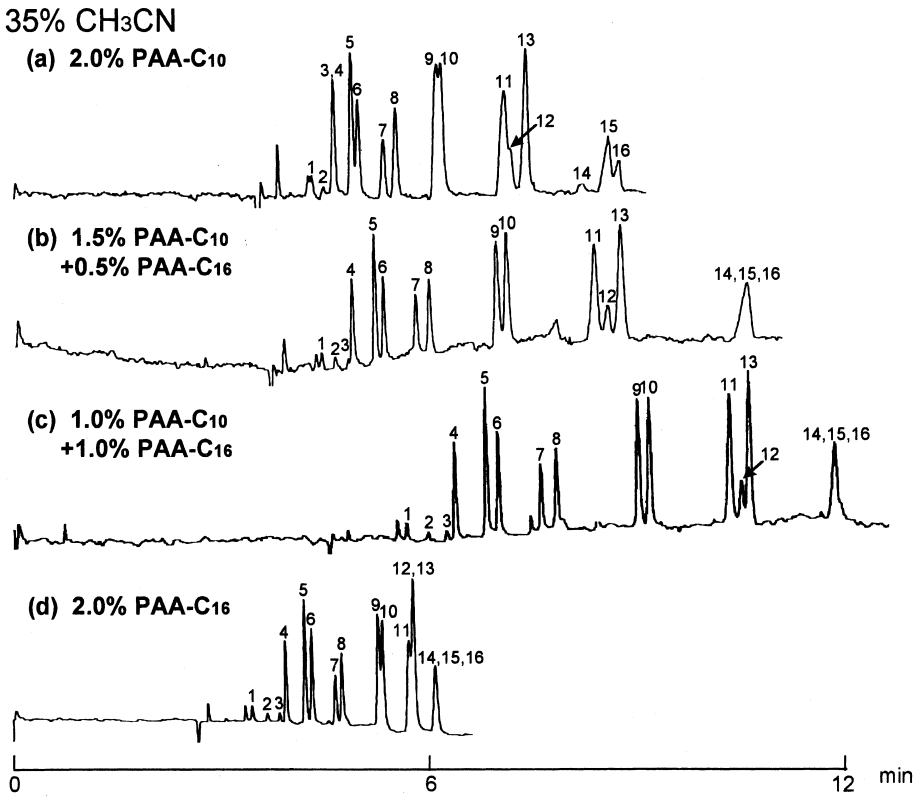

Fig. 7. Separation of 16 PAHs with (a) PAA-C_{10}, 2% (w/v), (b) PAA-C_{10}, 1.5% (w/v)+PAA-C_{16}, 0.5% (w/v), (c) PAA-C_{10}, 1% (w/v)+PAA-C_{16}, 1% (w/v), and (d) PAA-C_{16} 2% (w/v), in 20 m*M* borate buffer–acetonitrile (65:35, v/v) mixtures. Field strength=400 V/cm. See Fig. 3 for the identification of the solutes.

under temperature control, by employing a carrier with a proven unimolecular micelle structure. In the present system, the simulation based on Eqs. (3)–(5) assuming the constant electrophoretic mobility and binding constants of the pseudostationary phase at different concentrations, resulted in consistent selectivity but up to 20–40% difference in t_R/t_0 values for the late-migrating solutes from the observed values in the mixed carrier systems.

In this work, mixtures of polymeric pseudostationary phases having similar structures was shown to be effective for modifying the selectivity. The approach corresponds to the use of multiple stationary phases in RPLC. Practical applications of this approach may be found in EKC separations in totally aqueous systems and with the use of a selective pseudostationary phase having donor–acceptor functionality, known to provide high selectivity in RPLC, in combination with a hydrophobic pseudostationary phase. EKC has a definite advantage in this approach in terms of the freedom in the mixing ratios and the cost of pseudostationary phases.

4. Conclusion

Facile control of a migration time window and selectivity was achieved by using mixtures of PAA-supported pseudostationary phases with C_{10} and C_{16} alkyl groups which provided long and short migration times (t_C values), respectively, in the presence of an organic solvent. An explanation was provided assuming the independent contribution of the two pseudostationary phases to solute partition.

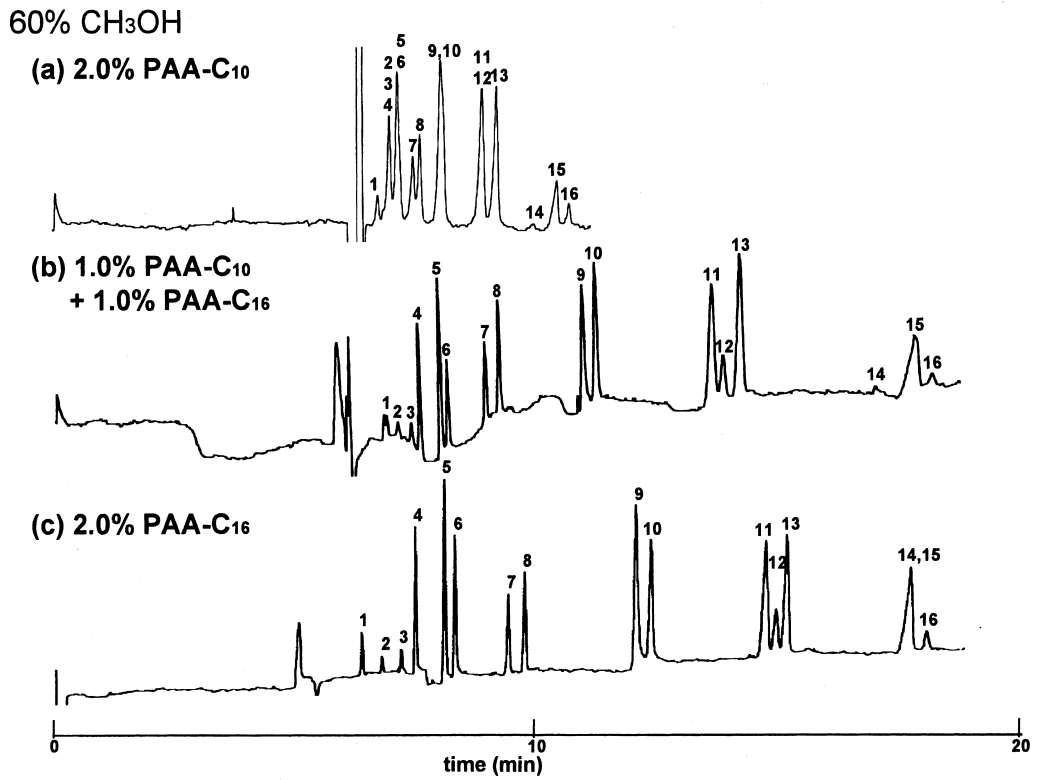

Fig. 8. Separation of 16 PAHs with (a) PAA-C_{10}, 2% (w/v), (b) PAA-C_{10}, 1% (w/v)+PAA-C_{16}, 1% (w/v), and (c) PAA-C_{16} 2% (w/v), in 20 m*M* borate buffer–methanol (40:60) mixtures. Field strength=600 V/cm. See Fig. 3 for the identification of the solutes.

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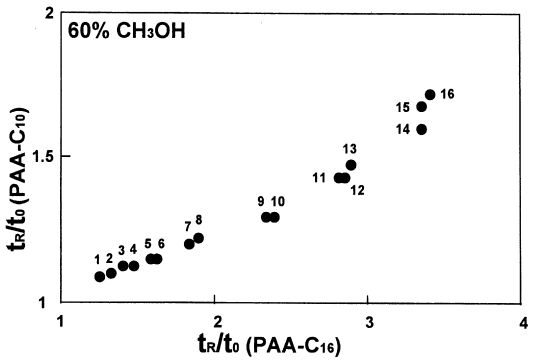

Fig. 9. Plots of relative migration times (t_R/t_0 values) with PAA-C_{10} against t_R/t_0 values with PAA-C_{16}. See Figs. 3 and 8 for the experimental conditions and the identification of the solutes.

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