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Journal of Chromatography A, 836 (1999) 295–303

JOURNAL OF CHROMATOGRAPHY A

Effects of electrokinetic chromatography conditions on the structure and properties of polyallylamine-supported pseudo-stationary phase A study by dynamic light scattering

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Received 5 October 1998; received in revised form 31 December 1998; accepted 31 December 1998

Abstract

The hydrophobic selectivity and electrophoretic mobility of polymeric pseudo-stationary phases supported by polyallylamine (PAA) were found to be affected by the degree of alkylation of PAA, the organic solvent content of the separation medium, and also by the concentration of the pseudo-stationary phase. The results obtained with dynamic light scattering, fluorescence spectroscopy, viscosity measurement and electrokinetic chromatography indicate that the PAA-supported pseudo-stationary phases assume swollen structures to expose more ionic groups to the aqueous medium at a lower concentration and at a higher organic solvent content, leading to the lower hydrophobic property and the greater electrophoretic mobility, which in turn result in the wider migration time window. Inadequate solvation of alkyl groups causes intermolecular aggregation of the polymeric carriers in aqueous buffer solutions, especially at high carrier concentrations. Dynamic light scattering measurement was shown to be particularly useful to elucidate the conformational change of the polymeric pseudo-stationary phase under various conditions.

Keywords: Electrokinetic chromatography; Polyallylamine pseudo-stationary phases; Pseudo-stationary phases; Dynamic light scattering

1. Introduction

Polymeric pseudo-stationary phase can provide separations under the conditions that preclude the use of micelles formed by low-molecular-mass surfactants [1–3]. Polymeric carriers can be used in a full range (0–95%) of organic solvent concentrations, broadening the scope of application of electrokinetic chromatography (EKC) [1–8]. Consistent structures of polymeric pseudo-stationary phases were assumed in most cases, while Palmer and co-workers [6,7] and Palmer and Terabe [9] suggested the possibility of structural change of polymeric carriers with the addition of organic solvents. Polyallylamine (PAA)-supported pseudo-stationary phase also gave results that can be explained by taking into account the structural change of a carrier caused by the change in organic solvent content [5,10]. A greater migration time window [t_C (a migration time of a carrier)

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0021-9673/99/$ – see front matter
PII: S0021-9673(99)00039-4

divided by t_0 (a migration time with an electroosmotic flow)] was observed on the basis of the greater electrophoretic mobility of the carrier relative to electroosmotic flow at a higher organic solvent concentration. The concentration of PAA-supported carriers also affected the solute-binding properties (k'), the k' values being non-linear with the change in carrier concentrations, although linear relations have been reported with some polymeric pseudo-stationary phases [7,11–13].

It is known that the size of alkyl substituents, the degree of alkylation, and the solvent composition can influence the conformation of polyelectrolytes [14,15]. Dynamic light scattering (DLS) is an excellent means to provide information on the conformation of polymeric materials [16–19]. We report here the effects of an organic solvent content and a carrier concentration on the conformation of PAA-supported pseudo-stationary phase studied by DLS and on the chromatographic properties of the carrier, including the electroosmotic mobility, electrophoretic mobility and hydrophobic selectivity.

2. Experimental

2.1. Pseudo-stationary phase

PAA-supported pseudo-stationary phases having carboxylate groups and alkyl groups, dodecyl (PAA-C_{12}) or hexadecyl groups (PAA-C_{16}), listed in Table 1 were prepared, as reported previously [5]. The degree of alkylation was controlled by the feed ratios of the reagents indicated in the parentheses, and determined by nuclear magnetic resonance (NMR) (XL-200, Varian, Sunnyvale, CA, USA) by measuring the intensity of the terminal methyl signal of the alkyl groups and that of the methoxycarbonyl group prior to the hydrolysis of the ester groups of PAA-(C_nH_{2n+1})($CH_2CH_2CO_2CH_3$) prepared by the addition of methyl acrylate to the alkylated PAA [5].

2.2. Samples

Polynuclear aromatic hydrocarbons (PAHs) were purchased from AccuStandard (New Haven, CT, USA), and alkyl phenyl ketones ($C_6H_5COC_nH_{2n+1}$, $n=1$–9) from Nacalai Tesque (Kyoto, Japan).

2.3. Equipment and EKC measurement

The same equipment was used as in the previous study [4,5,10]. Detection was carried out at 254 nm. The capillary (50 μm I.D.×375 μm O.D.) length was 48 cm with an effective length of 33 cm. PAA-supported pseudo-stationary phase was used at a concentration of 20 mg/ml in 20 m*M* borate buffer (pH 9.3) or buffer–organic solvent mixtures, unless

Table 1
Hydrophobic properties of pseudo-stationary phase as measured by the contribution of one methylene group to the k' values of alkyl phenyl ketones (concentration of the pseudo-stationary phase: 20 mg/ml)

Pseudo-stationary phase	Degree of alkylation[a]	$\alpha(CH_2)$, solvent		
		Buffer[b]	40% CH_3OH[c]	20% CH_3CN[c]
PAA-C_{12} (10)[d]	0.12	2.14	1.20	1.17
PAA-C_{12} (15)[d]	0.19	2.34	1.75	1.43
PAA-C_{12} (20)[d]	0.22	2.66	1.66	1.97
PAA-C_{16} (5)[d]	0.11	2.23	1.78	1.80
PAA-C_{16} (10)[d]	0.15	2.52	1.91	1.84
PAA-C_{16} (15)[d]	0.20	–[e]	2.10	2.04
PAA-C_{16} (25)[d]	0.23	–[e]	2.03	1.92

[a] Degree of alkylation, [C_nH_{2n+1}]/[Amino group in PAA], determined by NMR measurement of the precursor of the carrier, PAA-(C_nH_{2n+1})($CH_2CH_2CO_2CH_3$), before the hydrolysis of the methoxycarbonyl group.
[b] 20 m*M* borate buffer, pH 9.3.
[c] 20 m*M* borate buffer (pH 9.3)–organic solvent (v/v).
[d] Degree of alkylation (%) based on the feed ratios.
[e] Not completely soluble.

noted otherwise. The solutions were filtered with a membrane filter (0.2 μm) before use. The t_0 value was obtained by the injection of methanol, and t_C by the iteration method [20,21] using a series of alkyl phenyl ketones. EKC measurement was carried out at ambient temperature, using a siphoning method for sample injection.

2.4. Fluorescence measurement

Fluorescence spectra of pyrene were measured by using F4500 fluorospectrophotometer (Hitachi, Tokyo, Japan) with an excitation wavelength of 320 nm. The concentration of pyrene was varied with the concentration of the pseudo-stationary phase.

2.5. Dynamic light scattering measurement

DLS measurement was carried out at 25°C by using ALV-5000E apparatus (ALV, Langen, Germany) with 35 mW He–Ne laser (632.8 nm) light source with the scattering angle of 90°.

2.6. Viscosity measurement

An Ubbelohde viscometer was used to measure the viscosity of the separation solution in the presence of pseudo-stationary phase at 30°C.

3. Results and discussion

3.1. Effect of the degree of alkylation of PAA

Fig. 1 shows the separation of alkyl phenyl ketones and PAHs. The results were obtained with PAA-supported pseudo-stationary phase with dodecyl groups (PAA-C_{12}) with 10–20% alkylation or PAA-C_{16} with 5–25% alkylation as a pseudo-stationary phase. With the increase in the degree of alkylation, a shorter migration time of a carrier (t_C), and a narrower migration time window (t_C/t_0) were observed. The increase in the degree of alkylation resulted in a decrease in electrophoretic mobility of the pseudo-stationary phase. With the pseudo-stationary phase having a higher degree of alkylation, the peaks of alkyl phenyl ketones are more spaced at the earlier part of the chromatogram, while those of the later part migrated close to each other. The pseudo-stationary phases of lower degree of alkylation showed lower sample loading capacity that needed the detection at higher sensitivity, resulting in the less stable baseline.

In aqueous buffer solutions, the greater hydrophobic selectivity, $\alpha(CH_2)$, was observed for alkyl phenyl ketones with the higher degree of alkylation and the longer alkyl groups, as shown in Table 1. This is presumably due to the formation of the more hydrophobic binding sites consisting of alkyl groups associating with each other by hydrophobic interactions. This also explains the reduced accessibility of the ionic groups to the aqueous medium with the higher degree of alkylation, leading to the lower electrophoretic mobility, hence the smaller t_C/t_0 or the narrower migration time window.

3.2. Effect of organic solvent content of the separation solutions

With the increase in organic solvent content of the medium, the smaller hydrophobic selectivity, $\alpha(CH_2)$, was observed, presumably based on the better solvation of the solutes in the aqueous phase and of the alkyl groups of the pseudo-stationary phase, as in reversed-phase liquid chromatography. Fig. 2 shows the variation of relative migration times of solutes with the increase in methanol concentration.

Fig. 2 indicates that larger t_C/t_0 values were observed with the increase in methanol content, indicating the greater electrophoretic mobility of the carrier relative to the electroosmotic flow at a higher methanol concentration. The variation of t_0 is smaller than that of t_C. Very large t_C values were observed at high methanol concentrations due to the higher electrophoretic mobility of the carrier. Optimum separation for hydrophobic solutes and the increase in t_C/t_0 were observed at a higher methanol concentration with the pseudo-stationary phase with a higher degree of alkylation. The results suggest that the polymeric pseudo-stationary phase undergoes a solvent-dependent structural change to increase electrophoretic mobility by exposing the more ionic groups to the aqueous medium due to the better solvation at a higher organic solvent content.

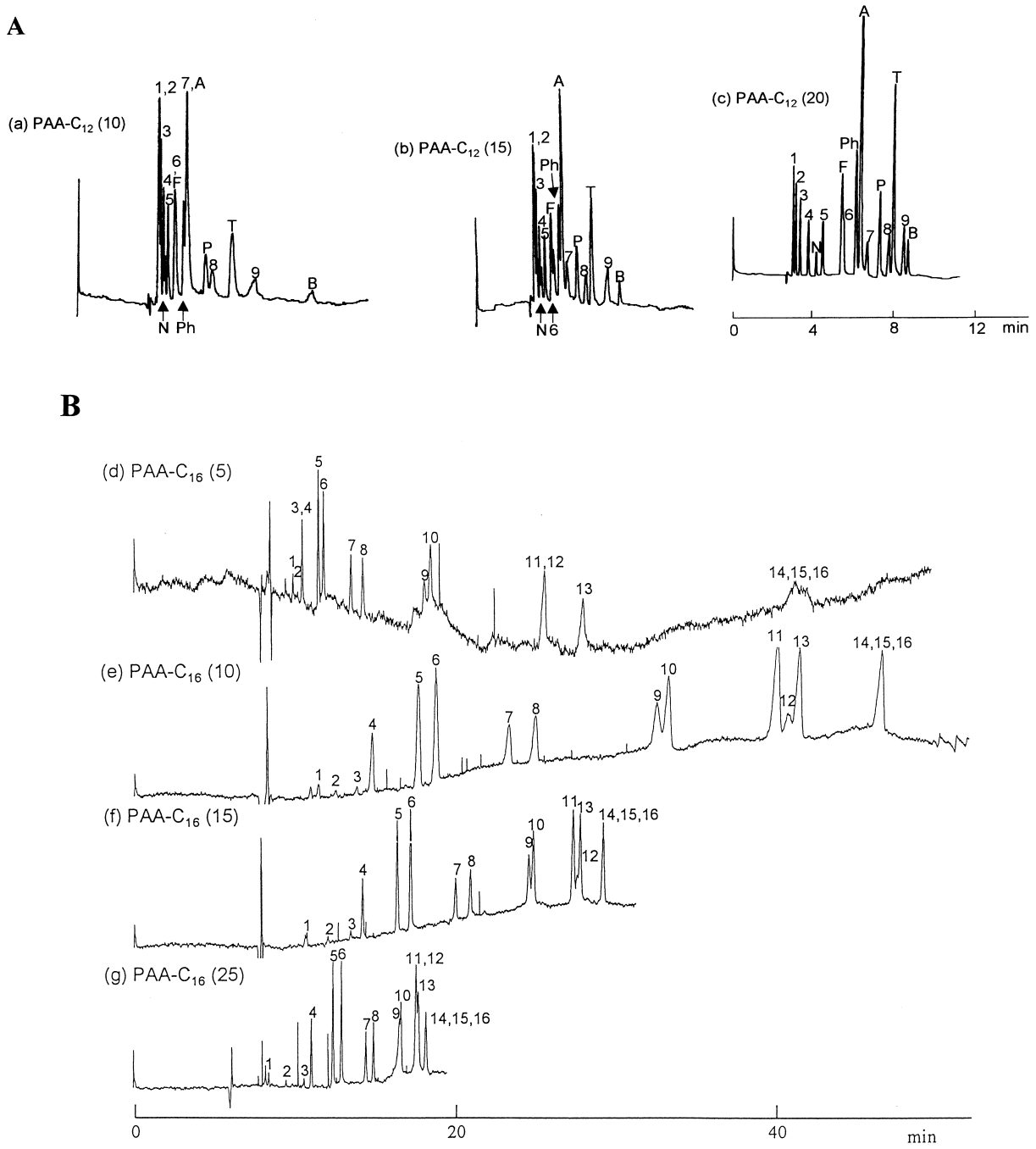

Fig. 1. Effect of degree of alkylation on the separation with PAA-C_{12} and PAA-C_{16}. Effective length of capillary tubing: 33 cm (total length: 48 cm). Field strength: 400 V/cm. Carrier concentration: 20 mg/ml. Separation solution: acetonitrile–buffer (20 m*M* borate, pH 9.3) (2:8) (A), and methanol–buffer (4:6) (B). Solutes, 1–9 = alkyl phenyl ketone ($C_6H_5COC_nH_{2n+1}$, n = 1–9), and N = naphthalene, F = fluorene, Ph = phenanthrene, A = anthracene, P = pyrene, T = triphenylene, B = benzo[*a*]pyrene for (A). PAHs designated as priority pollutants by the US Environmental Protection Agency (EPA), naphthalene (1), acenaphtylene (2), acenaphthene (3), fluorene (4), phenanthrene (5), anthracene (6), fluoranthene (7), pyrene (8), benz[*a*]anthracene (9), chrysene (10), benzo[*b*]fluoranthene (11), benzo[*k*]fluoranthene(12), benzo[*a*]pyrene (13), dibenz[*a*,*h*]anthracene (14), indeno[1,2,3-*cd*]pyrene (15), benzo[*ghi*]perylene (16) for (B).

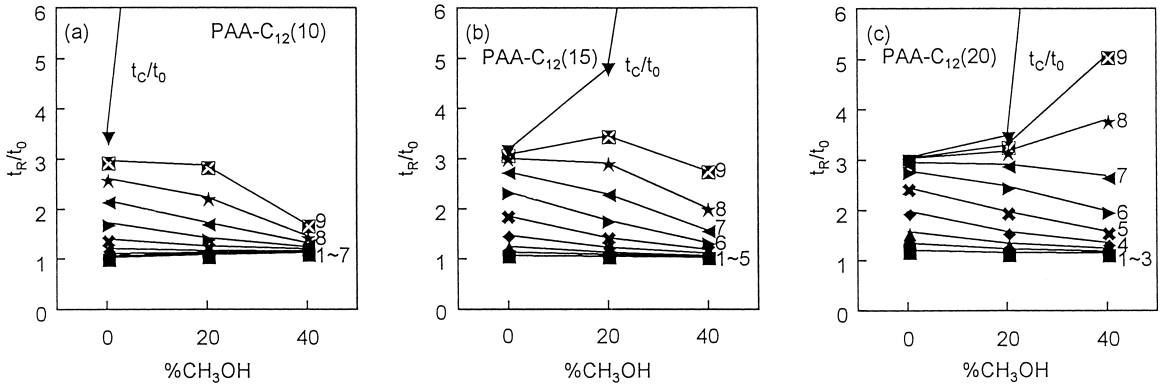

Fig. 2. Variation of migration times of solutes (t_R/t_0) and PAA-C_{12} (t_C/t_0) with methanol content. For experimental conditions, see Fig. 1. Solute: alkyl phenyl ketones.

3.3. Effect of methanol content on the DLS relaxation times of pseudo-stationary phase

The size distribution of the polymer chains comprising the pseudo-stationary phase can be evaluated by DLS [16–19]. If the polymer chains have a size distribution, $P(\Gamma^{-1})$ with the characteristic relaxation time Γ^{-1}, the time-intensity correlation function, $g^{(2)}(\tau)$, is given by Eq. 1 [19]

$$g^{(2)}(\tau) - 1 = \left[\int_0^\infty G(\Gamma) \exp(-\Gamma\tau)\, d\tau \right]^2 \quad (1)$$

In Eq. 1, $G(\Gamma)$ is the characteristic relaxation rate distribution function and Γ is the characteristic relaxation rate. By taking inverse Laplace transform of Eq. 1, one obtains $G(\Gamma)$. Since the characteristic relaxation time, Γ^{-1} $(=1/\Gamma)$, is related to the hydrodynamic radius of the individual polymer chain, R_H, via the Stokes–Einstein relationship, Eq. 2 [19],

$$R_H = \frac{kTq^2}{6\pi\eta}\Gamma^{-1} \quad (2)$$

Γ^{-1} is proportional to R_H, where k is Boltzmann constant, q is the magnitude of the scattering vector, and η is the solvent viscosity. Therefore, one can regard $P(\Gamma^{-1}) = G(\Gamma)$ as the size distribution of the solute polymers with $R_H \sim \Gamma^{-1}$. Hence, by analyzing $P(\Gamma^{-1})$, one obtains useful information about the size distribution of the pseudo-stationary phase in various environments.

Fig. 3 shows the effect of methanol content on $P(\Gamma^{-1})$ of PAA-C_{12}(15) and PAA-C_{16}(10). In an aqueous buffer solution, the carrier showed a bimodal distribution of the relaxation time indicating the presence of much larger structural units than a unimolecular carrier. The result can be explained on the basis of the aggregation of the carriers caused by the intermolecular association of hydrophobic side chains due to poor solvation in the aqueous buffer [14]. In 40% methanol, a narrower distribution was observed in each case, indicating that the better solvation of alkyl groups caused the disappearance of intermolecularly aggregated structures. This is compatible with the observation of the higher electrophoretic mobility of the carrier in 40% methanol, where the pseudo-stationary phase is supposed to exist as a unimolecular carrier, exposing more ionic groups as well as alkyl groups to the aqueous medium due to better solvation.

It should be noted that solvent viscosity, η, changes considerably by changing the solvent from water to a mixture of water and methanol (the viscosity increases about 50% in the mixture of water–methanol; 60:40, v/v). However, this effect does not influence the discussion on the distribution of Γ^{-1}, since it simply results in a horizontal shift of $P(\Gamma^{-1})$.

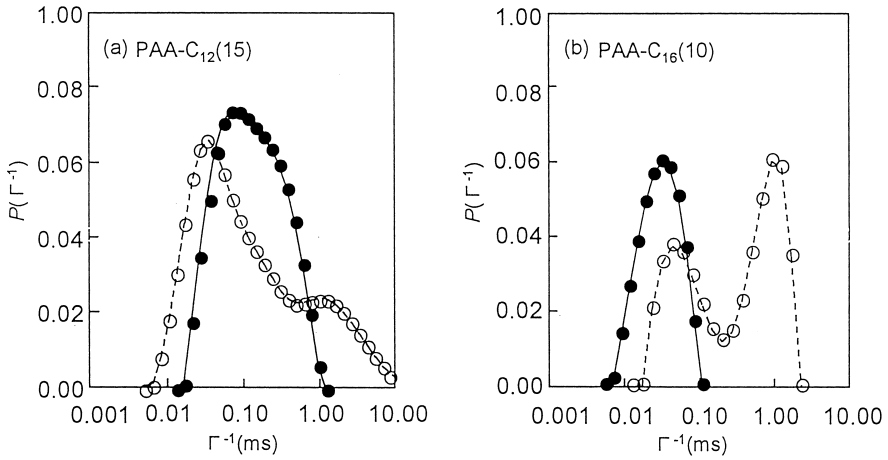

Fig. 3. Effect of methanol content on the characteristic relaxation time distribution function, $P(\Gamma^{-1})$. (a) PAA-C_{12} (15), (b) PAA-C_{16} (10). Pseudo-stationary phase: 2% (w/v). Temperature: 25°C. Solvent: borate buffer (20 m*M* borate, pH 9.3) (– ○ –) and methanol–borate buffer (6:4) (– ● –).

3.4. Effect of concentration of pseudo-stationary phase

Interesting results were obtained with the change in concentration of the pseudo-stationary phase. Fig. 4 shows the separation of alkyl phenyl ketones in borate buffer with 0.2–2.0% carrier concentration. The results have similarity to those in Fig. 1 which shows the effect of the degree of alkylation. (Note here that a slower electroosmotic flow was observed with the increase in carrier concentration due to the increase in viscosity of the separation solution in Fig. 4, while a small variation in t_0 was seen in Fig. 1 at constant carrier concentration). At higher concentration of PAA-C_{16} (10), the peaks of alkyl phenyl ketones are more spaced at the earlier part of the chromatogram, while the peak spacing with the more hydrophobic ketones became narrower than at lower concentration. The maximum peak spacing was observed between alkyl phenyl ketones with $n=4$ and 5 at 2.0% carrier concentration, and between the alkyl phenyl ketones having the larger alkyl groups at the lower carrier concentrations.

The greater hydrophobic selectivity, $\alpha(CH_2)$ value, was observed with the increase in the concentration of pseudo-stationary phase (Fig. 5). The increase in carrier concentration also resulted in the greater band intensity ratio (III/I) of the fluorescence spectrum of pyrene, as shown in Fig. 5. The latter indicates that pyrene molecules are bound to the more hydrophobic binding sites [22] in the presence of the higher concentration of the carrier. Figs. 4 and 5 indicate that the increase in the carrier concentration resulted in the increase in the hydrophobic property of the binding sites of the carrier.

Fig. 6a shows the effect of carrier concentration on DLS relaxation time, and indicates that the increase in concentration leads to intermolecular aggregation of the carriers in an aqueous buffer solution, while such aggregation was not observed in 40% methanol (Fig. 6b). The results can be explained by taking into account the intermolecular association of the polymeric carrier at a high concentration in an aqueous buffer [14]. In 40% methanol, an increase in carrier concentration resulted in slight reduction in size of the carrier. Viscosity measurement provided a support to the present interpretation. An increase in reduced viscosity was observed in 40% methanol at a lower carrier concentration, while a slight increase in viscosity was observed at a higher concentration in 20% methanol, as shown in Fig. 7. Such tendency is well known with polyelectrolytes, and can be explained on the basis of the more extended structure of the polymer leading to the higher viscosity at a lower concentration due to the increase in coulombic repulsion

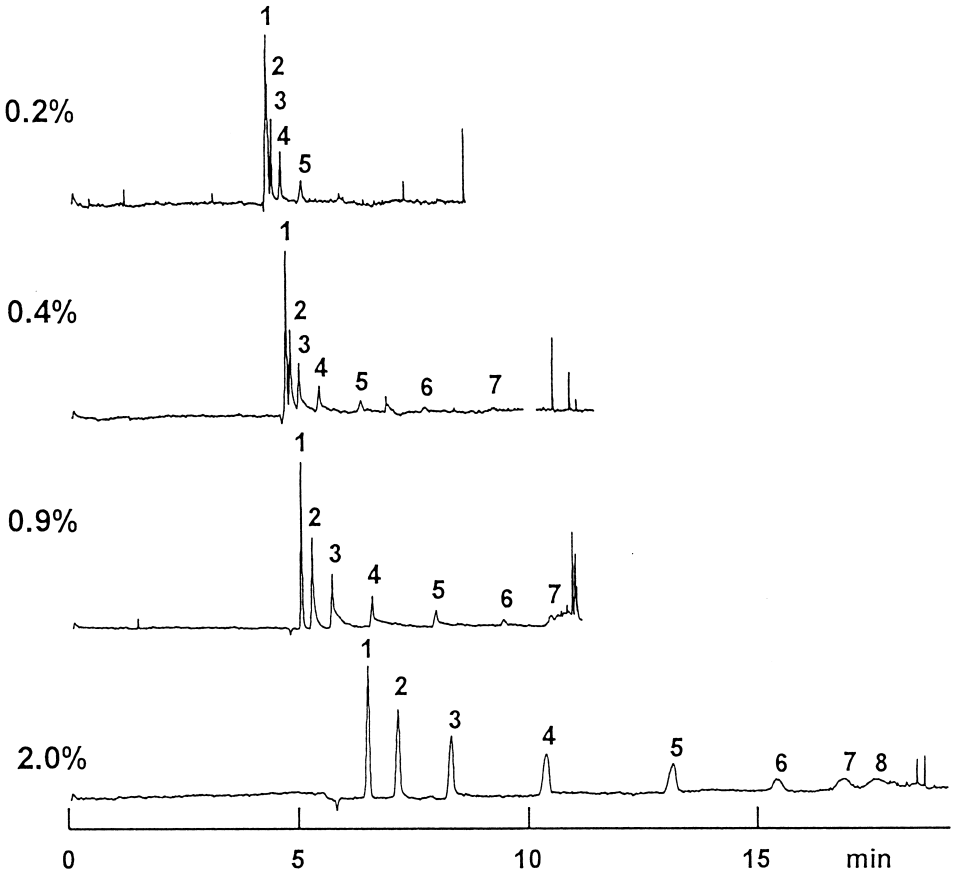

Fig. 4. Effect of concentration of pseudo-stationary phase on the separation of alkyl phenyl ketones. Carrier: PAA-C_{16} (10), solutes: alkyl phenyl ketones ($C_6H_5COC_nH_{2n+1}$), separation solution: 20 m*M* borate buffer, pH=9.3, other conditions as in Fig. 1.

between the ionic groups of the polymer at low ionic strength [23,24]. The tendency appears clearly with polyelectrolytes under well solvated conditions. Under poorly solvated conditions, intermolecular aggregation of the carriers can result in an increase in viscosity at a higher concentration.

Fig. 8 shows the effects of temperature and ionic strength on DLS of PAA-C_{16} (10). Low temperature and high ionic strength facilitated the intermolecular aggregation of the carriers as indicated by DLS measurement. This is compatible with the mechanism of intermolecular association of alkyl groups based on hydrophobic interaction in aqueous buffer solutions. The present results indicate the importance of precise control of temperature and mobile phase composition for reproducible EKC measurement with polymeric carriers. DLS measurement is shown to be an excellent means for studying the conformational change of polymeric pseudo-stationary phases.

4. Conclusions

Hydrophobic selectivity and electrophoretic mobility of PAA-supported pseudo-stationary phase in EKC were affected by the degree of alkylation of the carrier, organic solvent content of the separation medium, and the carrier concentration. PAA-supported pseudo-stationary phase was shown to undergo intermolecular aggregation by hydrophobic interaction under poorly solvated conditions, affecting the

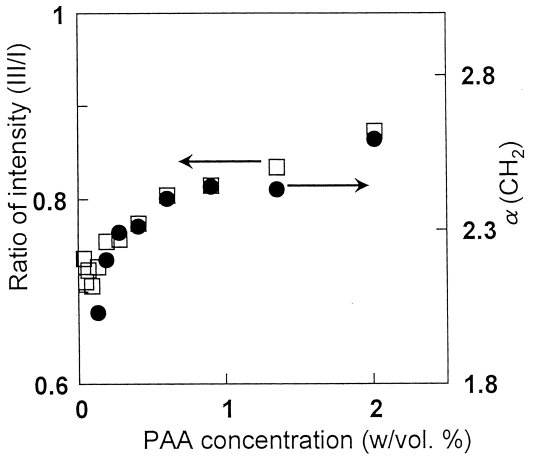

Fig. 5. Effect of concentration of pseudo-stationary phase on hydrophobic selectivity, $\alpha(CH_2)$ (●: scale shown at the right-hand side axis), and the relative peak intensity between band III and band I in fluorescence spectrum of pyrene (□: scale shown at the left-hand side axis). Carrier: PAA-C_{16} (10), solvent: 20 m*M* borate buffer, pH = 9.3. The ratio, [PAA-C_{16} (10)]/[Pyrene] was kept constant in the fluorescence measurement. Relative peak intensity = intensity (band III)/intensity (band I) in fluorescence spectrum of pyrene.

chromatographic properties. DLS measurement was shown to be very effective for the study of a conformational change of the polymeric pseudo-stationary phase.

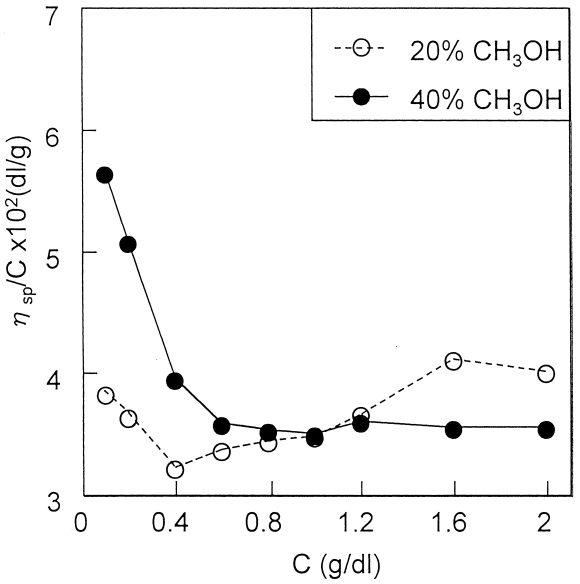

Fig. 7. Effect of carrier concentration on the viscosity of the separation solution. Carrier: PAA-C_{16} (10), solvent: 20% methanol (– ○ –) and 40% methanol (– ● –), temperature: 30°C.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture.

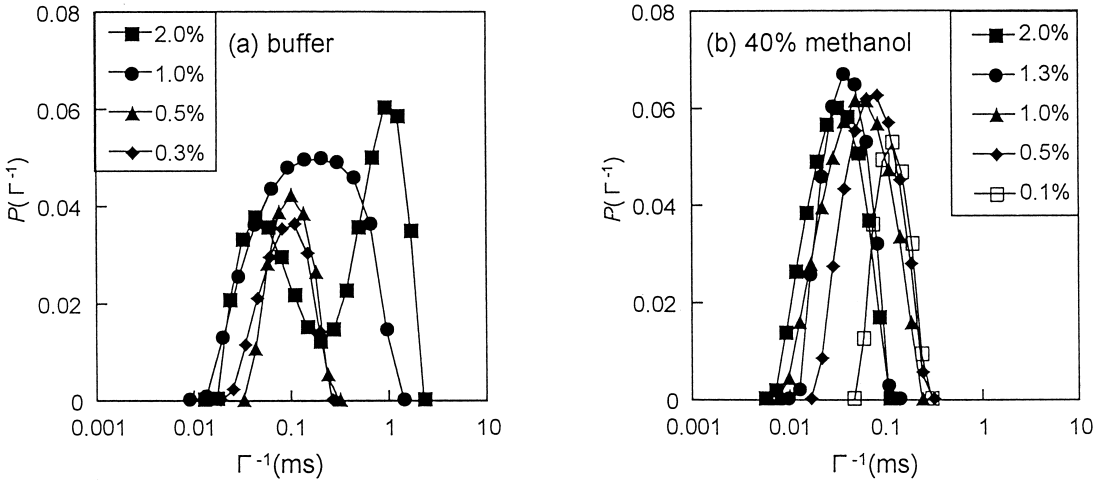

Fig. 6. Effect of concentration of PAA-C_{16} (10) on the characteristic relaxation time distribution function, $P(\Gamma^{-1})$. Solvent: (a) aqueous buffer, (b) 40% methanol. Temperature: 25°C. Carrier concentrations as indicated.

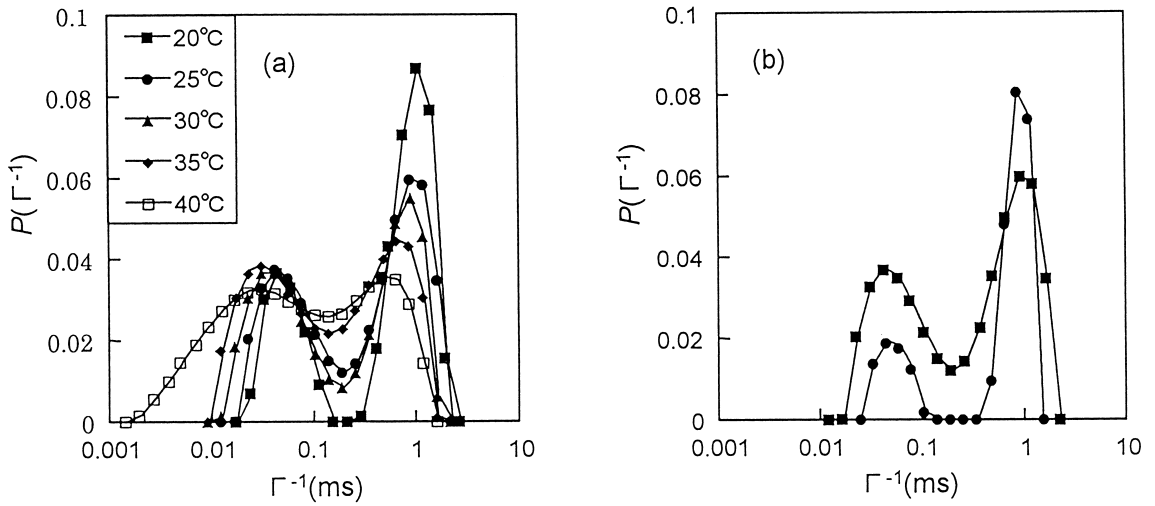

Fig. 8. Effect of temperature (a) and ionic strength (b) on the characteristic relaxation time distribution function, $P(\Gamma^{-1})$, of PAA-C_{16} (10). Carrier concentration: 2% (w/v). Solvent: (a) 20 m*M* borate buffer (pH=9.3), (b) 20 m*M* borate buffer (pH=9.3) (■) and 20 m*M* borate buffer (pH=9.3), 0.1 *M* NaCl (●). Temperature: 25°C.

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