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Macromolecular surfactant as a pseudo-stationary phase in micellar electrokinetic capillary chromatography

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Abstract

We examined polymers of sodium 11-acrylamidoundecanoate [poly(Na 11-AAU)] with a very high molecular mass ($>10^6$) for their potential use as a pseudo-stationary phase in micellar electrokinetic capillary chromatography (MEKC). Size-exclusion chromatography and capillary electrophoresis studies reveal that the polymers are highly charged, and have a densely packed chain structure. For aromatic compounds, the polymeric surfactant showed significantly different selectivity than sodium dodecyl sulfate (SDS). It was suggested that one molecule of poly(Na 11-AAU) forms one micelle. The structural stability of this pseudo-stationary phase permitted its use with relatively high percentages of organic modifiers in the buffer medium, allowing the separation of highly hydrophobic compounds which are difficult to analyze by conventional MEKC with SDS.

Keywords: Pseudo-stationary phases; Surfactants; Polynuclear aromatic hydrocarbons; Poly(sodium 11-acrylamidoundecanoate); Benzenes; Naphthalenes

1. Introduction

In 1984, Terabe et al. introduced a new separation mode of capillary electrophoresis (CE) using surfactants [1]. This new CE mode, called micellar electrokinetic capillary chromatography (MEKC), is based on the differential distribution of solutes between a running buffer phase and a pseudo-stationary phase, allowing the separation of uncharged analytes under the influence of an electric field. The nature of the surfactant can have a significant effect on the separation process in MEKC. The first pseudo-stationary phase to be applied was sodium dodecyl sulfate (SDS) [1], and thus far SDS has found the most use in MEKC.

MEKC using monomeric micellar systems, however, has the problems of limited elution range and the difficulty of analyzing hydrophobic analytes: due to the high partition coefficients, hydrophobic analytes tend to elute close to the end of the separation window with very high retention factors. The formation of a micelle, characterized by the critical micellar concentration (CMC) of free surfactant, is affected by several parameters, including the temperature, ionic strength and pH of the medium and the addition of modifiers to the micellar system. Any changes in these parameters can affect the migration behavior of the solutes.

Recently, several research groups have reported the use of the covalently linked surfactants as a pseudo-stationary phases in MEKC [2–13]. Since the oligomers or polymers of surfactant monomers are considered to form a micelle from a single molecule

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(i.e., CMC=0), the concentration of these surfactants remains constant irrespective of the changes in the analytical conditions. The polymerized surfactants can withstand high concentration of organic solvents. Thus, they can be used for separation of hydrophobic analytes in the presence of relatively high contents of organic modifiers. Also, the covalently linked surfactants afford different selectivities as compared with micelles formed from SDS [2–13].

Very little work has been done with high-molecular-mass polymers as pseudo-stationary phases in MEKC. Ozaki et al. reported the use of butyl acrylate–butyl methacrylate–methacrylic copolymer sodium salt of M_r 40 000 for MEKC separations [7]. Wallingford and Ewing mentioned the separation of catechols with sulfonated polymer particles (Eastman AQ55S) and discussed the potential of such monodispersed polymers as pseudo-stationary phases [14].

In this study, sodium 11-acrylamidoundecanoate (Na 11-AAU) is synthesized and polymerized. The polymer is characterized by means of size-exclusion chromatography (SEC)–refractive index (RI) detection, SEC–multiangle laser light scattering detection (MALLS), and CE. The selectivity of poly(Na 11-AAU) for the substituted benzene and naphthalene compounds and polynuclear aromatic hydrocarbons is compared with that of SDS. The advantages of using poly(Na 11-AAU) in MEKC are described.

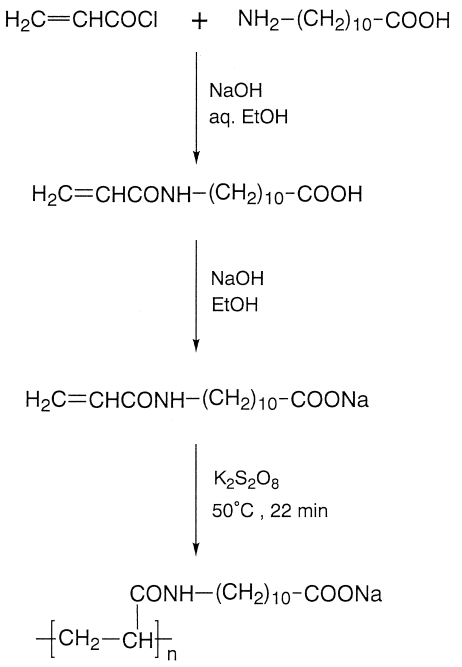

Fig. 1. Synthetic scheme for preparation of sodium 11-acrylamidoundecanoate (Na 11-AAU) and polymer.

2. Experimental

2.1. Synthesis of poly(Na AAU)

The Na 11-AAU monomer was prepared according to the procedure of Yeoh et al. [15]. A synthetic schematic for poly(Na 11-AAU) is presented in Fig. 1. A 6-ml volume (0.072 mol) of acryloyl chloride (Wako, Osaka, Japan) was added dropwise to 10 g (0.05 mol) of 11-aminoundecanoic acid (Sigma, St. Louis, MO, USA) in an aqueous ethanol solution (250 ml of 99.5% ethanol and 35 ml of water) containing 6 g (0.15 mol) of NaOH. The reaction mixture was stirred for a few hours below 10°C and then filtered. The filtrate was acidified with dilute HCl. Adding 4 l of water to the filtrate formed a white precipitate of 11-acrylamidoundecanoic acid (11-AAUA). The crude product was recrystallized from aqueous ethanol. The sodium salt of 11-AAUA was prepared by reacting 1.6 g (0.04 mol) of NaOH with 10 g (0.04 mol) of pure 11-AAUA dissolved in 500 ml of dehydrated ethanol (Wako). The solution was stirred overnight at room temperature. Evaporating the solvent with a rotary evaporator followed by vacuum desiccation resulted in a dry product. The crude Na 11-AAU was recrystallized from dehydrated ethanol at 0°C and dried in a vacuum chamber at room temperature.

The polymer, poly(Na 11-AAU), was prepared by free radical polymerization in water. Pure Na 11-AAU (5.55 g, 0.02 mol) was dissolved in 100 ml of distilled, deionized water. The solution was bubbled with nitrogen for 1 h and then placed in a 50°C bath. The catalyst $K_2S_2O_8$ (0.054 g, 2 mmol) was added, and the solution was stirred under nitrogen for 22 min. The polymer was precipitated in an excess of acetone. The precipitated polymer was filtered and washed repeatedly with methanol. Finally, the product was dried under vacuum.

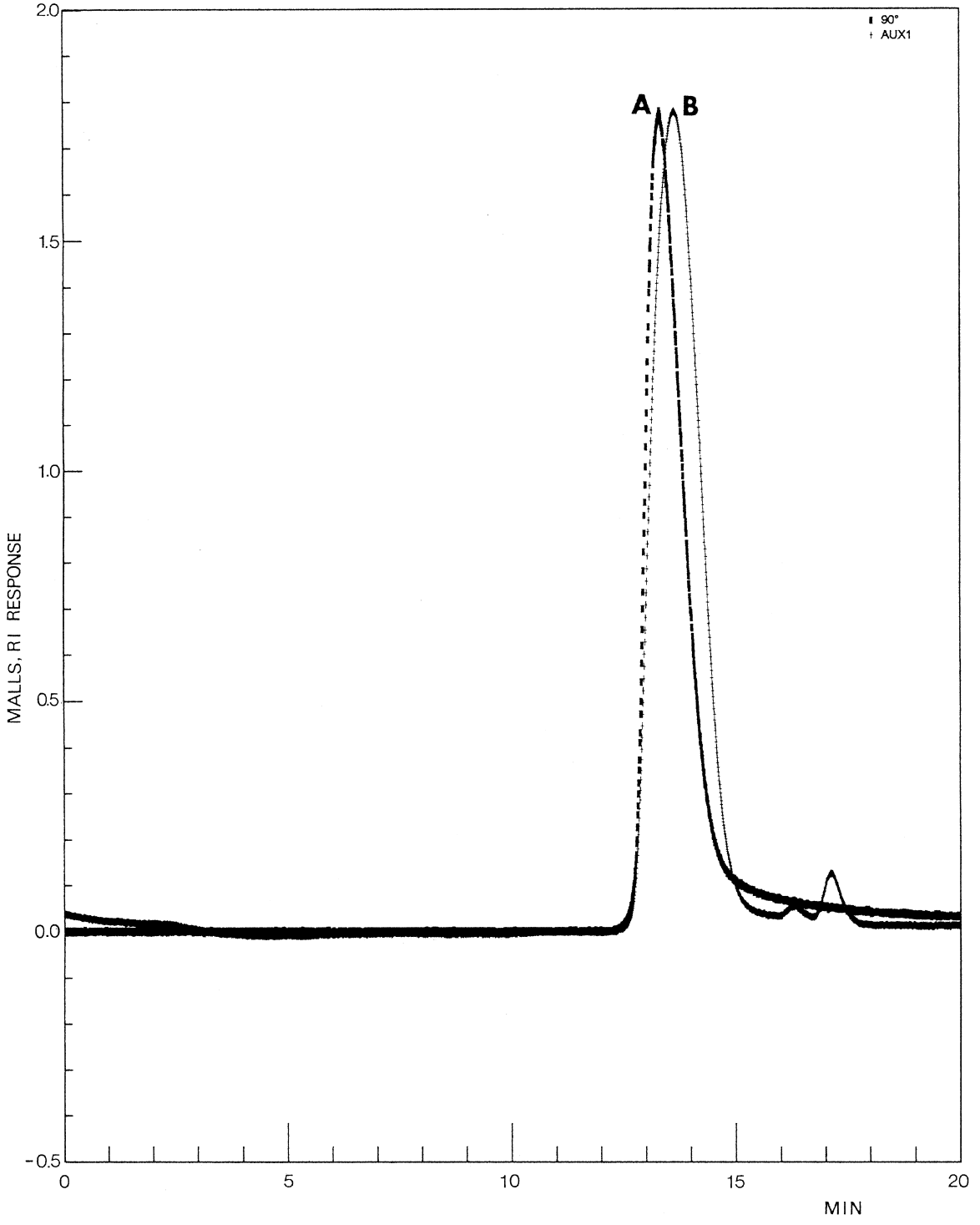

Fig. 2. (A) SEC–MALLS (at 90°) and (B) SEC–RI chromatograms of poly(Na 11-AAU). Column, α-M (Tosoh)×2; mobile phase, 10 m*M* Tris–borate (pH 8.0); sample concentration, 0.047% (w/v); flow-rate, 1 ml/min; temperature, 35°C.

2.2. CE and MEKC apparatus

The CE and MEKC experiments were performed using a laboratory-built instrument. A Matsusada Precision Devices (Kusatsu, Japan) Model HCZE-30PN0.25-LD high-voltage power supply and a Jasco (Tokyo, Japan) Model 870-CE on-column UV detector were employed. The detector was operated at 215 or 254 nm. Data were recorded with a System Instruments (Tokyo, Japan) Model Labchart 180 integrator. Samples were introduced electrokinetically at the anodic side. Separations were accomplished by applying a constant voltage of +20 kV. All experiments were carried out at ambient temperatures.

Fused-silica capillaries (GL Sciences, Tokyo, Japan) of 50 μm I.D. were cut to 65 cm. A small section of the polyimide coating was burned off to make a transparent window for the detector at 15 cm from the end of the capillary. The capillaries were washed with 1 *M* NaOH, deionized, distilled water, and the running buffer before use. The background electrolyte consisted of a 12.5 m*M* mixture of Na_2HPO_4 and $Na_2B_4O_7$ buffered at pH 9.3. For MEKC separations, appropriate percentages of poly(Na AAU) (w/v) and of acetonitrile were added to the background electrolyte. After mixing in a sonicator, the final running buffers were filtered through 0.45-μm hydrophilic-PTFE syringe filters (Advantec, Tokyo, Japan).

2.3. SEC apparatus

SEC analysis was carried out at 35°C using a Tosoh (Tokyo, Japan) modular SEC system, which consisted of a Tosoh Model CCPS pump, a Tosoh Model SD-8022 degasser, a Rheodyne (Cotati, CA, USA) Model 7125 injection valve with a 100-μl loop, a Tosoh Model 8020 RI detector and a Wyatt Technology (Santa Barbara, CA, USA) Model DAWN-DSP MALLS system. The MALLS system was equipped with a 5 mW He–Ne laser (λ_0=632.8 nm) and a ca. 30 μl scattering cell. The SEC columns used were two Tosoh Model α-M columns connected in series before the MALLS system. Cellulose acetate filters with 0.1-μm and 0.45-μm pore diameters were located before the injector and the MALLS system, respectively. A 10 m*M* Tris–borate buffer solution (pH 8.0) was used as the eluent at 1 ml/min. Molecular masses obtained by SEC–RI were calibrated by a series of monodisperse pullulan standards obtained from Showa Denko (Tokyo, Japan). All solutions were filtered through Advantec (Tokyo, Japan) 0.45-μm cellulose acetate membrane filters. The concentrations of samples injected into the columns were 0.047% (w/v). For comparison of particle diameters, laboratory-made linear polyacrylamides were analyzed on the SEC–MALLS apparatus.

3. Results and discussion

3.1. SEC of Poly(Na 11-AAU)

SEC chromatograms of poly(Na 11-AAU), obtained using RI and MALLS detectors, are shown in Fig. 2. The large peak at 13.21 min was assigned to poly(Na 11-AAU). It can be seen that the synthesized polymer has a monomodal size distribution. The assignments of a few small peaks in the chromatogram were not made: possibly, these peaks are due to the oligomers.

The scattered intensity data at angles (θ) obtained by SEC–MALLS were analyzed by the use of the following equations:

$$\frac{Kc}{R(\theta)} = \frac{1}{M_w P(\theta)} + 2A_2 c \tag{1}$$

$$K = \frac{2\pi^2 n_0^2 (\mathrm{d}n/\mathrm{d}c)^2}{N_A \lambda_0^4} \tag{2}$$

$$\frac{1}{P(\theta)} = 1 + \frac{1}{3}\langle S^2 \rangle_z q^2 \tag{3}$$

$$q = \left(\frac{4\pi n_0}{\lambda_0}\right) \sin\left(\frac{\theta}{2}\right) \tag{4}$$

where $R(\theta)$ is the excess absolute time-averaged scattered intensity, known as the Rayleigh ratio of the scattered light, c is the sample concentration, M_w is the weight-average molecular mass, A_2 is the second virial coefficient, n_0 is the solvent refractive index, λ_0 is the wavelength of the incident light, N_A is Avogadro's constant, ($\mathrm{d}n/\mathrm{d}c$) is the refractive index increment, and $\langle S^2 \rangle_z$ is the mean square radius

of gyration of polymers (or *z*-average square radius of gyration). The value of (d*n*/d*c*) was determined by calibrating the response of the refractomer. The value of A_2 was assumed to be zero because the concentration of the polymers eluted from the column was very low. Fig. 3 shows the $Kc/R(\theta)$ vs. $\sin^2(\theta/2)$ plot for poly(Na 11-AAU). The values of M_w and $\langle S^2 \rangle_z$ can be calculated from the intercept and slope of the linear plot.

Table 1 summarizes the molecular masses of poly (Na 11-AAU) measured by the two SEC methods. The values of M_w are remarkably high, and the molecular mass distributions are rather narrow for a radical copolymerization. These results obtained here are in accordance with literature data by Yeoh et al. [15]. From the fact that poly(Na 11-AAU) molecules have narrow distributions, they considered that the polymerization of Na 11-AAU proceeds in a state of aggregation (micelles) [15]. The rapid completion of the polymerization was another evidence for the micellar polymerization mechanism. However, they suggested that the polar heads of the anionic surfactant molecules were distributed over the outer surfaces of the micelles, while the acrylol groups were aggregated in the cores of the micelles. The close proximity of the reactive acrylol groups was believed to accelerate the polymerization.

However, there must be considerable doubt as to the polymerization mechanism, if one considers the aggregation number (AN) of the polymer on the

Table 1
Molecular mass of poly(Na 11-AAU)[a]

	M_w	M_w/M_n
SEC with RI detection	$1.89 \cdot 10^6$	1.54
MALLS–SEC	$3.09 \cdot 10^6$	1.29

[a] Polymerization conditions: concentration, Na 11-AAU (0.2 *M*) and $K_2S_2O_8$ (2 m*M*) in water; temperature, 50°C; reaction time, 22 min. See Section 2.3 for SEC conditions. M_w = Weight-average molecular mass; M_n = number-average molecular mass.

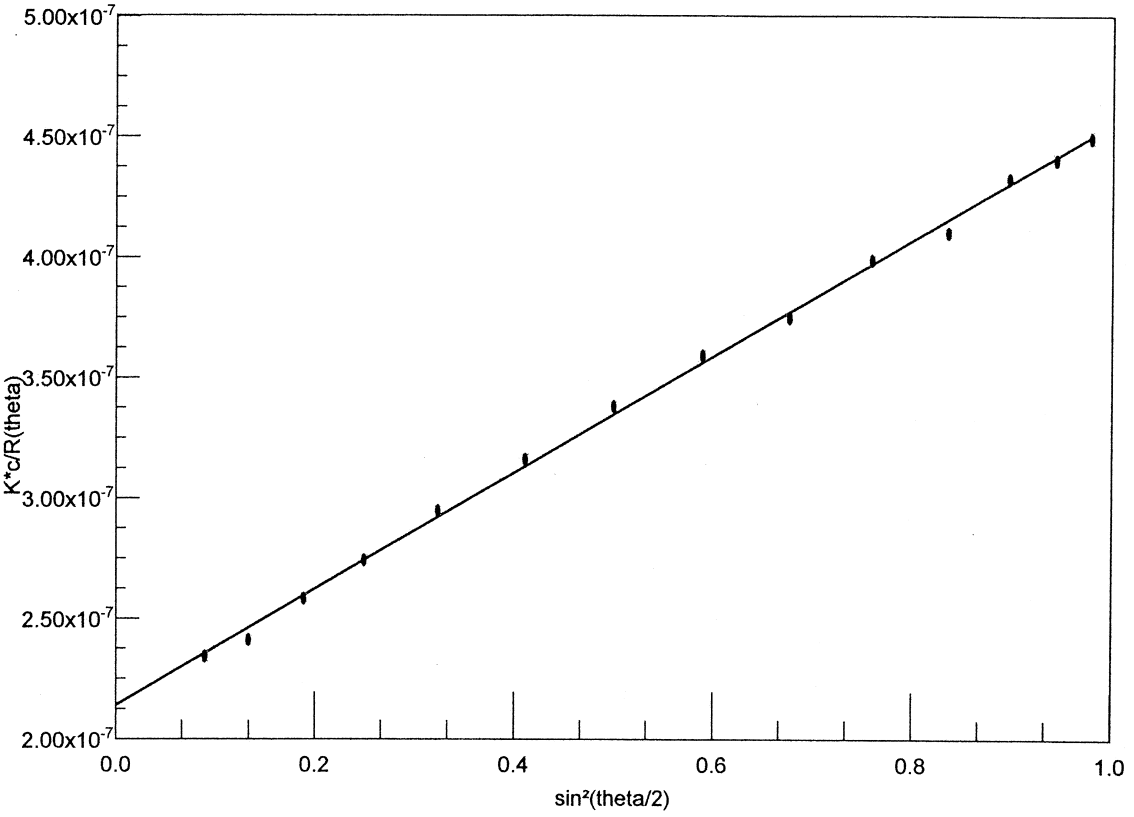

Fig. 3. $Kc/R(\theta)$ plotted as a function of $\sin^2(\theta/2)$ for poly(Na 11-AAU) in 10 m*M* Tris–borate solution (pH 8.0).

basis of the oil droplet model [16]. According to this model, AN is given as

$$AN = \frac{4}{3}\pi l^3 \frac{\rho N_A}{M_0} \quad (5)$$

where l, ρ, M_0 are the chain length, the density, and the molecular mass of the monomer. From Eq. (5), the AN for the polymer aggregation is calculated to be approximately 30: this value is no match for that obtained by dividing the molecular mass of the

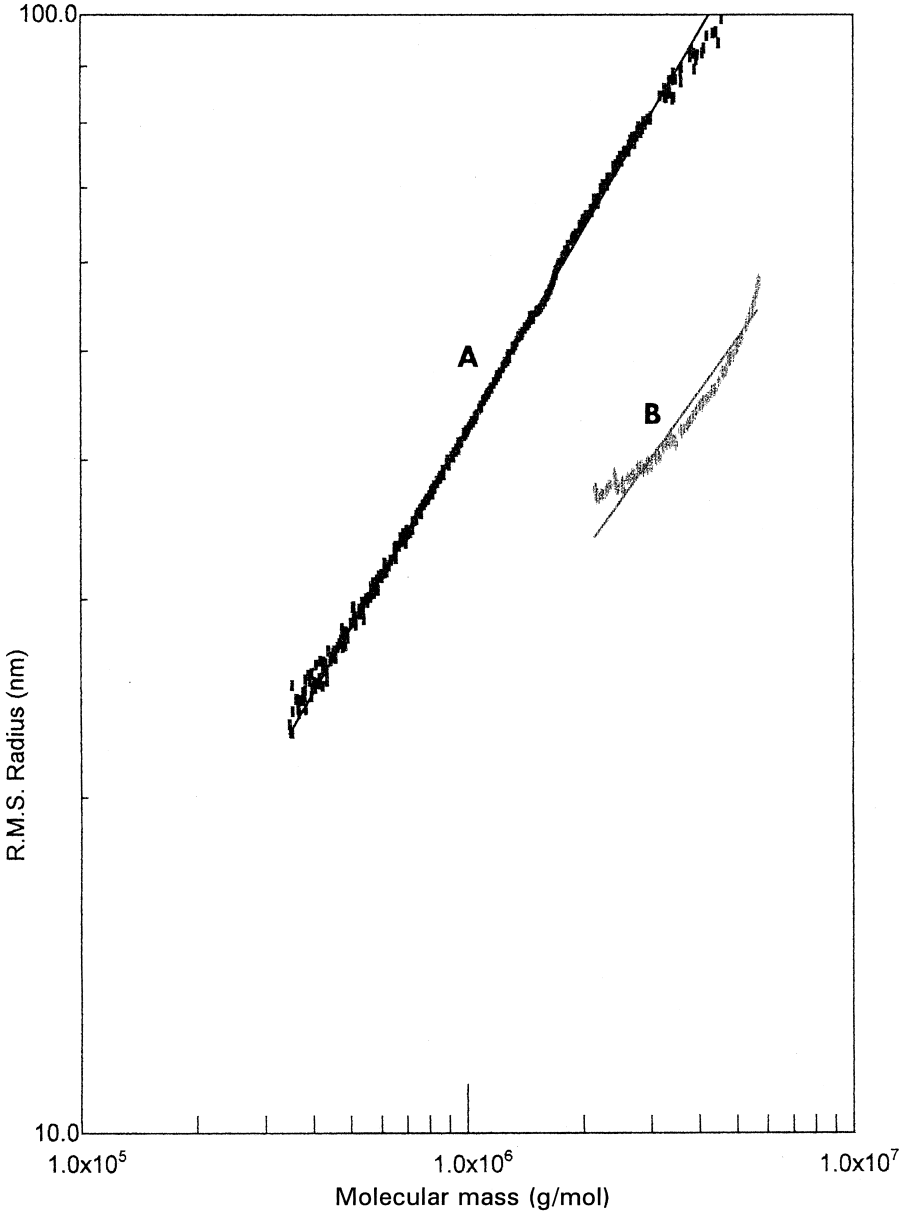

Fig. 4. Plots of RMS radius versus molecular mass for poly(Na 11-AAU) and non-crosslinked polyacrylamides: (A) polyacrylamide; (B) poly(Na 11-AAU).

polymer by M_0 (i.e., >6800). Therefore, it is unlikely that the monomer polymerizes in a fashion that Yeoh et al. [15] envisaged.

Fig. 4 shows the plots of root mean square (RMS) radius of gyration (represented by $\langle S^2\rangle_z^{1/2}$) versus molecular mass for poly(Na 11-AAU) and linear polyacrylamides. It may be of interest to note that the radii of poly(Na AAU) are smaller than those of linear polyacrylamides with the same degree of polymerization. Generally, the following power law holds for homopolymers:

$$\langle S^2\rangle_z^{1/2} \propto M_w^a \quad (6)$$

The power a is 0.6 for typical flexible polymers in good solvents. The values of a calculated from Fig. 4 were 0.59 and 0.48 for polyacrylamides and poly(Na 11-AAU), respectively. Therefore, polyacrylamides assumes an expanded random coil conformation with excluded-volume effects, while poly(Na 11-AAU) is considered to have a Gauss chain conformation [17]. It may be surprising that poly(Na 11-AAU) molecules which have charges over their outer surface can have more compact structures than uncharged linear polyacrylamides. The average radius of poly(Na 11-AAU) is ca. 50 nm.

3.2. CE of monomer and polymer of Na 11-AAU

Fig. 5 shows the electropherograms of Na 11-AAU and poly(Na 11-AAU) molecules. Both molecules are charged under the experimental conditions and travel in the opposite direction to the electroosmotic flow (EOF). Dimethyl sulfoxide was used as

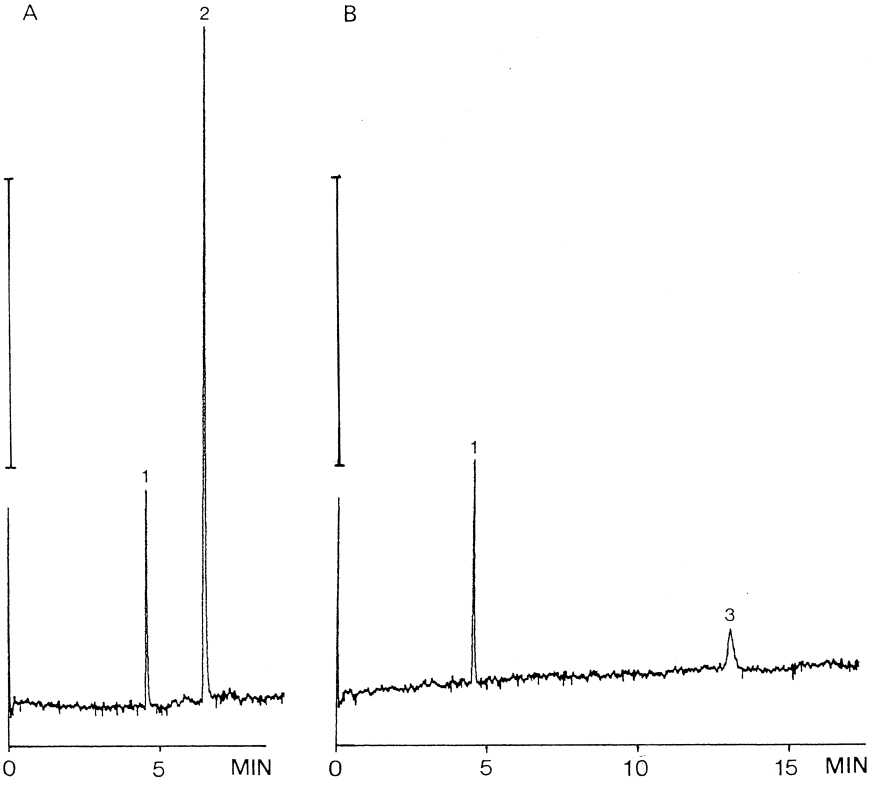

Fig. 5. Electrophoresis of Na 11-AAU monomer (A) and polymer (B). Solutes: (1) dimethyl sulfoxide, (2) Na 11-AAU monomer, and (3) poly(Na 11-AAU). Conditions: capillary, 65.0 cm (50.0 cm to the detector)×50 μm I.D.; buffer, 12.5 m*M* of each of Na_2HPO_4 and $Na_2B_4O_7$ (pH 9.3); applied voltage, 18.0 kV; detection wavelength, 215 nm. The scale bars attached to the chromatogram indicate 0.001 AU.

Table 2
Electrophoretic mobilities (μ) of the monomer and polymer of Na 11-AAU[a]

	μ ($cm^2\ V^{-1}\ s^{-1}$)
Na 11-AAU monomer	$-1.899 \cdot 10^{-4} \pm 0.008$
Na 11-AAU polymer	$-4.189 \cdot 10^{-4} \pm 0.005$

[a] Conditions: capillary, 65.0 cm (50.0 cm to the detector) × 50 μm I.D.; buffer, 12.5 m*M* of each of Na_2HPO_4 and $Na_2B_4O_7$ (pH 9.3); applied voltage, 18.0 kV; detection wavelength, 215 nm.

an EOF marker. Obviously, the net charge density of the polymer is higher than that of the monomer. The electroosmotic mobilities obtained from these experiments are listed in Table 2. The mobility of poly(Na 11-AAU) is comparable to that of SDS [5].

3.3. MEKC in aqueous solution

Fig. 6 shows the MEKC separations of alkyl phenyl ketones obtained using SDS and poly(Na 11-AAU) as a pseudo-stationary phase. Relatively narrow band spacings are observed between peaks 4–8 for the SDS-MEKC separation. This behavior is commonly seen in SDS-MEKC, where hydrophobic compounds elute near the elution time of the micelle due to the complete incorporation of the hydrophobic compounds into the micelle phase [9]. By contrast, the band spacings are wider between the late-eluting peaks in poly(Na 11-AAU) MEKC. Probably, the less hydrophobic structure of the polymer than SDS seems to contribute the smaller partition coefficients for the hydrophobic compounds.

Fig. 7 shows the separations of benzene and naphthalene derivatives. Clearly, the separation selectivity obtained from poly(Na 11-AAU) is considerably different from that from SDS.

Shown in Fig. 8 is a plot of the retention factors, k', for some aromatic compounds as a function of the concentration of poly(Na 11-AAU). The retention factors are proportional to the polymer concentration and the intercepts for these plots are almost zero. This suggests that the micelle of poly(Na 11-AAU) is formed from one molecule.

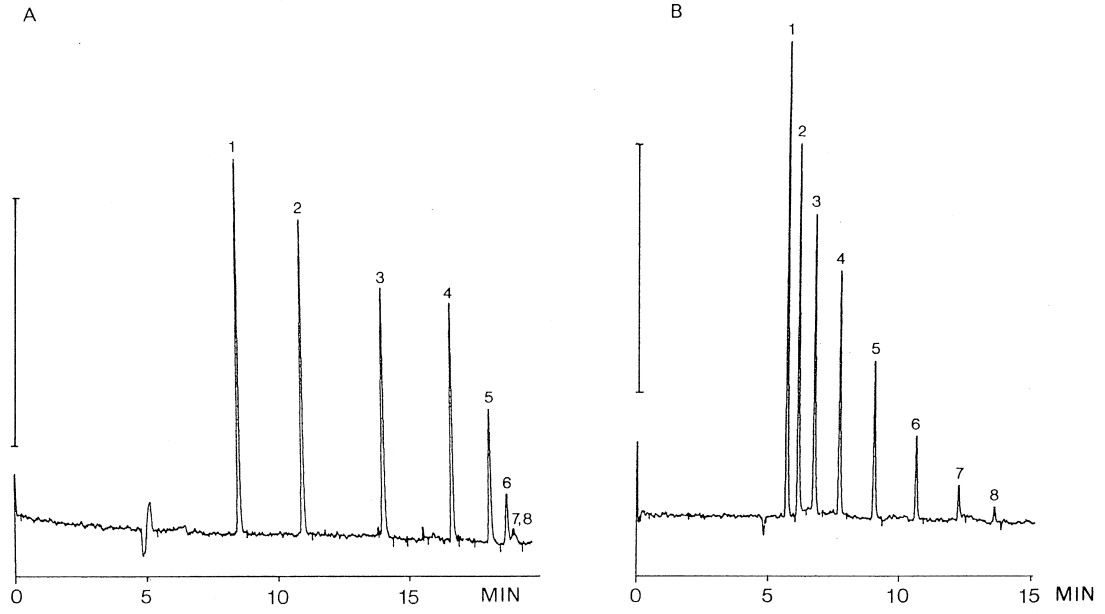

Fig. 6. Separation of alkyl phenyl ketones: (A) 41.6 m*M* SDS; (B) 1.20% (w/v) poly(Na 11-AAU). Conditions: capillary, 65.0 cm (50.0 cm to the detector) × 50 μm I.D.; buffer, 12.5 m*M* of each of Na_2HPO_4 and $Na_2B_4O_7$ (pH 9.3); applied voltage, 18.0 kV; detection wavelength, 254 nm. Solutes: (1) methyl phenyl ketone, (2) ethyl phenyl ketone, (3) *n*-propyl phenyl ketone, (4) *n*-butyl phenyl ketone, (5) *n*-pentyl phenyl ketone, (6) *n*-hexyl phenyl ketone, (7) *n*-heptyl phenyl ketone, and (8) *n*-octyl phenyl ketone. The scale bars attached to the chromatogram indicate 0.001 AU.

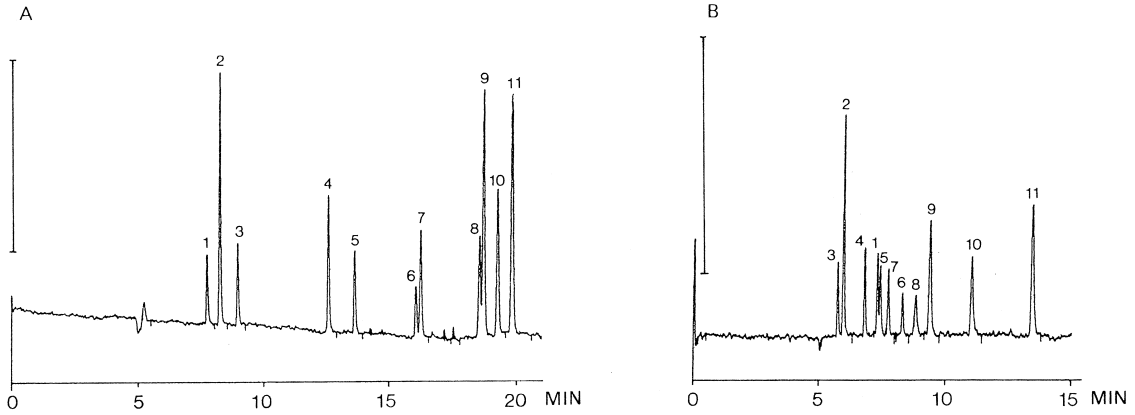

Fig. 7. Separation of benzene and naphthalene derivatives: (A) 41.6 m*M* SDS; (B) 1.20% (w/v) poly(Na 11-AAU). Solutes: (1) *p*-nitroaniline, (2) nitrobenzene, (3) anisole, (4) α-naphthylamine, (5) naphthalenemethanol, (6) naphthaleneethanol, (7) naphthalene, (8) diphenyl ether, (9) biphenyl, (10) fluorene, and (11) phenanthrene. Conditions as in Fig. 6. The scale bars attached to the chromatogram indicate 0.001 AU.

3.4. MEKC in aqueous acetonitrile solution

Fig. 9 shows the separations of polynuclear aromatic hydrocarbons (PAHs) in 30% acetonitrile with SDS and poly(Na 11-AAU) as a pseudo-stationary phase. The comparison clearly shows the advantages

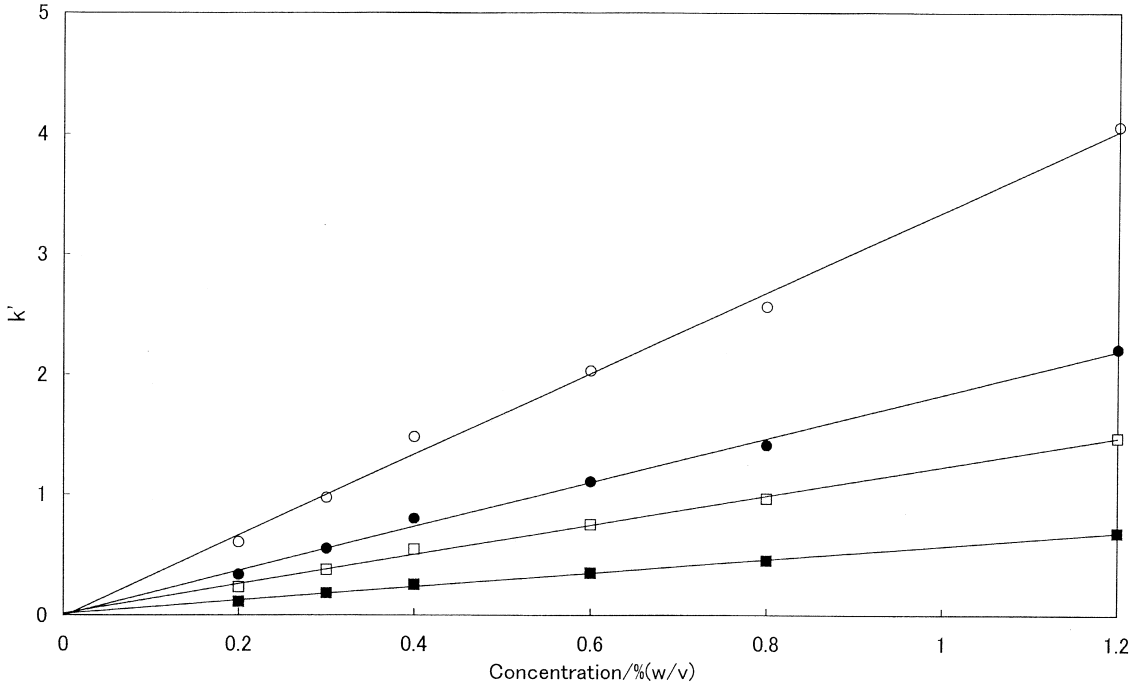

Fig. 8. Retention factors of aromatic compounds as a function of poly(Na 11-AAU) concentration. Solutes: (○) fluorene, (●) biphenyl, (□) naphthaleneethanol, (■) α-naphthylamine. Conditions as in Fig. 7.

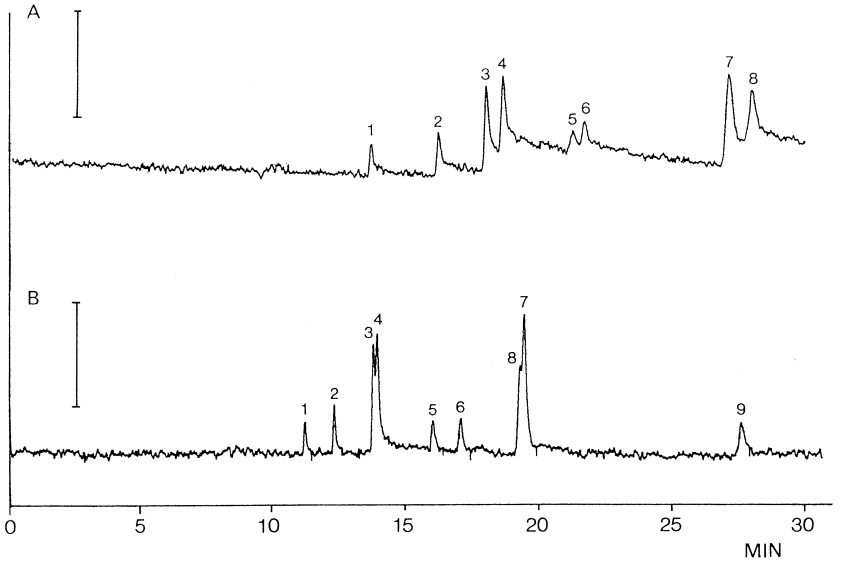

Fig. 9. Separation of PAHs in acetonitrile–12.5 m*M* phosphate buffer, pH 9.3 (30:70): (A) 34.5 m*M* SDS, (B) 1.00% (w/v) poly(Na 11-AAU). Conditions: capillary, 65.0 cm (50.0 cm to the detector)×50 μm I.D.; applied voltage, 18.0 kV; detection wavelength, 254 nm. Solutes: (1) napthalene, (2) fluorene, (3) phenanthrene, (4) anthracene, (5) fluoranthene, (6) pyrene, (7) chrysene, (8) benz[*a*]anthracene, and (9) benzo[*a*]pyrene. The scale bars attached to the chromatogram indicate 0.0005 AU.

of using molecular micelles as a pseudo-stationary phase in MEKC that is very stable in the presence of large amounts of organic solvents. Good chromatograms were not obtained for PAHs in 30% acetonitrile with SDS. Using SDS as a pseudo-stationary phase, benzo[*a*]pyrene eluted at ca. 45 min. It can be seen that chrysene elutes before benz[*a*]anthracene with SDS, but the elution order is reversed with poly(Na 11-AAU).

4. Conclusion

The polymer reported represents a new alternative to standard micelles for use as a pseudo-stationary phase in MEKC. The polymer affords a different selectivity from SDS and high stability in the presence of organic modifier. As can be seen in the chromatograms, the separation efficiencies observed are nearly as good as those observed with standard micelles, in spite of the fact that the size distribution of the polymer surfactant contributes to band broadening in MEKC [18]. Additionally, the use of the polymer is advantageous over conventional micelles for on-line coupling of MEKC with electrospray ionization mass spectrometry because the high molecular mass of the polymer is well beyond the mass range of the spectrometer. Further experiments are in progress to characterize more thoroughly the micelle structure of poly(Na 11-AAU): the information on the micelle structure would give a clue to the explanation of the unique selectivity obtained with the polymer.

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