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Ion-exclusion chromatographic behavior of aliphatic carboxylic acids and benzenecarboxylic acids on a sulfonated styrene–divinylbenzene co-polymer resin column with sulfuric acid containing various alcohols as eluent

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Abstract

The addition of C_1–C_7 alcohols (methanol, ethanol, propanol, butanol, heptanol, hexanol and heptanol) to dilute sulfuric acid as eluent in ion-exclusion chromatography using a highly sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) in the H^+ form as the stationary phase was carried out for the simultaneous separations of both (a) C_1–C_7 aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, 2-methylvaleric, isocaproic, caproic, 2,2-dimethyl-*n*-valeric, 2-methylhexanoic, 5-methylhexanoic and heptanoic acids) and (b) benzenecarboxylic acids (pyromellitic, hemimellitic, trimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic and salicylic acids and phenol). Heptanol was the most effective modifier in ion-exclusion chromatography for the improvement of peak shapes and a reduction in retention volumes for higher aliphatic carboxylic acids and benzenecarboxylic acids. Excellent simultaneous separation and relatively highly sensitive conductimetric detection for these C_1–C_7 aliphatic carboxylic acids were achieved on the TSKgel SCX column (150×6 mm I.D.) in 30 min using 0.5 m*M* sulfuric acid containing 0.025% heptanol as eluent. Excellent simultaneous separation and highly sensitive UV detection at 200 nm for these benzenecarboxylic acids were also achieved on the TSKgel SCX column in 30 min using 5 m*M* sulfuric acid containing 0.075% heptanol as eluent.

Keywords: Mobile phase composition; Carboxylic acids; Benzenecarboxylic acids; Alcohols; Heptanol

1. Introduction

Ion-exclusion chromatography, developed by Wheaton and Baumann [1], is widely employed as a simple and convenient analytical tool for the determination of various carboxylic acids. The combination of a highly sulfonated styrene–divinylbenzene co-polymer resins (sulfonated PS–DVB resin) in the H^+ form as the stationary phase and a dilute strong acid, such as hydrochloric acid and sulfuric acid, as the eluent is commonly utilized in ion-exclusion chromatography for carboxylic acids [2]. The use of these dilute strong acids as the eluent is a very effective way of improving the peak shapes of hydrophilic carboxylic acids [3]. However, since the use of an acidic eluent results in suppression of the dissociation of carboxylic acids, a strongly hydrophobic interaction between the carboxylic acids and the surface of the stationary phase occurs. As a result, both strongly tailed peaks and extremely large

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retention volumes for hydrophobic carboxylic acids, such as higher aliphatic carboxylic acids and benzenecarboxylic acids, are obtained on the sulfonated PS–DVB resin column.

The addition of various organic compounds, such as alcohols [4–6], acetonitrile [7–9], sugar alcohols and sugars [10] and polyvinyl alcohols [11], to an acidic eluent has been carried out in order to improve peak shapes and to reduce the retention volumes for hydrophobic carboxylic acids. However, the use of higher concentrations of organic modifiers is limited, because shrinking of the PS–DVB resin occurs. A higher alcohol is one of the most effective organic modifiers in ion-exclusion chromatography for hydrophobic carboxylic acids. Morris and Fritz have demonstrated the effectiveness of an acidic eluent containing a very low concentration of butanol in ion-exclusion chromatography using a sulfonated styrene–divinylbenzene co-polymer resin column for C_1–C_5 aliphatic carboxylic acids (formic, acetic, propionic, butyric and valeric acids) [12]. The authors have also reported the effectiveness of a higher alcohol (heptanol) as organic modifier in ion-exclusion chromatography using an unmodified silica gel column [13], an aluminum-modified silica gel column [14] and a zirconium-modified silica gel column [15] for various aliphatic carboxylic acids. However, the application of a dilute strong acid containing higher alcohols as the eluent in ion-exclusion chromatography using a highly sulfonated PS–DVB resin column for the separation of various aliphatic carboxylic acids and benzenecarboxylic acid has not been attempted.

The aim of this study was to demonstrate the effectiveness of a highly sulfonated PS–DVB resin (TSKgel SCX) as the stationary phase in ion-exclusion chromatography for various carboxylic acids. Due to its large hydrophobicity, TSKgel SCX has been exclusively applied as the stationary phase in the ion-exclusion chromatography of hydrophilic carboxylic acids and common aliphatic carboxylic acids (C_1–C_5 aliphatic carboxylic acids). Therefore, in this study, for the expansion of the utility of TSKgel SCX in ion-exclusion chromatography, the application of a TSKgel SCX column (150×6 mm I.D.) for the ion-exclusion chromatographic separation of (a) C_1–C_7 aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, 2-methylvaleric, isocaproic, caproic, 2,2-dimethyl-*n*-valeric, 2-methylhexanoic, 5-methylhexanoic and heptanoic acids) and (b) benzenecarboxylic acids (pyromellitic, hemimellitic, trimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic and salicylic acids and phenol) was carried out using dilute sulfuric acid containing C_1–C_7 alcohols (methanol, ethanol, propanol, butanol, heptanol, hexanol and heptanol) as the eluent. Heptanol (C_7 alcohol) was the most effective organic modifier for the ion-exclusion chromatographic separation of both these C_1–C_7 aliphatic carboxylic acids and benzenecarboxylic acids. When using 0.05 m*M* sulfuric acid containing 0.025% heptanol as the eluent, symmetric peaks and excellent simultaneous separation of these C_1–C_7 aliphatic carboxylic acids were achieved on the TSKgel SCX column in 30 min. When using 5 m*M* sulfuric acid containing 0.075% heptanol as the eluent, symmetric peaks and excellent simultaneous separation of these benzenecarboxylic acids were also achieved on the TSKgel SCX column in 30 min.

2. Experimental

2.1. Instruments

The ion-exclusion chromatograph consisted of a Tosoh (Tokyo, Japan) LC-8020 chromatographic data processor, a Tosoh CCPM-II solvent delivery pump operated at a flow-rate of 1 ml min^{-1}, a Tosoh CM-8020 conductimetric detector, a Tosoh UV-8020 UV–Vis spectrophotometric detector operated at 200 nm, a Tosoh CO-8020 column oven operated at 35 °C, a Tosoh DS-8023 on-line degasser and a Rheodyne (Cotati, CA, USA) Model 9125 injector equipped with a 100 μl sample loop.

A Toa Denpa (Tokyo, Japan) IM-40S ion meter with a glass electrode was used for measurement of the pH of the eluents. A Toa Denpa CM-20 conductimetric detector was also employed for measurement of the conductivities of the eluents.

2.2. Separation column

A separation column (150×6 mm I.D.) packed with a Tosoh TSKgel SCX highly sulfonated styrene–divinylbenzene co-polymer resin in the H^+

form (cation-exchange capacity of ca. 1.5 mequiv. ml^{-1} and particle size of ca. 5 μm) for HPLC was used.

2.3. Chemicals

All chemicals were of analytical reagent grade and were purchased from Aldrich (Milwaukee, WI, USA), Wako (Osaka, Japan) and Tokyo Kasei (Tokyo, Japan).

Distilled, deionized water was used for the preparation of the eluents and standard solutions.

3. Results and discussion

In order to expand the utility of the highly sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) in the H^+ form as the stationary phase in ion-exclusion chromatography for carboxylic acids, the application of the TSKgel SCX column (150×6 mm I.D.) for the ion-exclusion chromatographic separation of both (a) C_1–C_7 aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, 2-methylvaleric, isocaproic, caproic, 2,2-dimethyl-*n*-valeric, 2-methylhexanoic, 5-methylhexanoic and heptanoic acids) and (b) benzenecarboxylic acids (pyromellitic, hemimellitic, trimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic and salicylic acids and phenol) was carried out using sulfuric acid containing C_1–C_7 alcohols (methanol, ethanol, propanol, butanol, pentanol, hexanol and heptanol) as eluent.

3.1. Ion-exclusion chromatographic separation of C_1–C_7 aliphatic carboxylic acids on a TSKgel SCX column

3.1.1. Effect of the concentration of sulfuric acid in the eluent on the chromatographic behavior of C_1–C_7 aliphatic carboxylic acids

The effect of the concentration of sulfuric acid in the eluent on the chromatographic behavior of these C_1–C_7 aliphatic carboxylic acids on the TSKgel SCX column was investigated for the simultaneous separation of these carboxylic acids.

Fig. 1 shows the relationship between the concentration of sulfuric acid and the retention volumes of the C_1–C_7 aliphatic carboxylic acids. With increasing concentration of sulfuric acid, the retention volumes of the aliphatic carboxylic acids increased. The retention volumes of the higher aliphatic carboxylic acids increased greatly in comparison with those of the lower aliphatic carboxylic acids. This is due mainly to (a) a decrease in electrostatic repulsion and (b) an increase in the hydrophobic interaction between the aliphatic carboxylic acids and the surface of TSKgel SCX, caused by suppression of the dissociation of the carboxylic acids. This result also indicated that, with increasing concentration of sul-

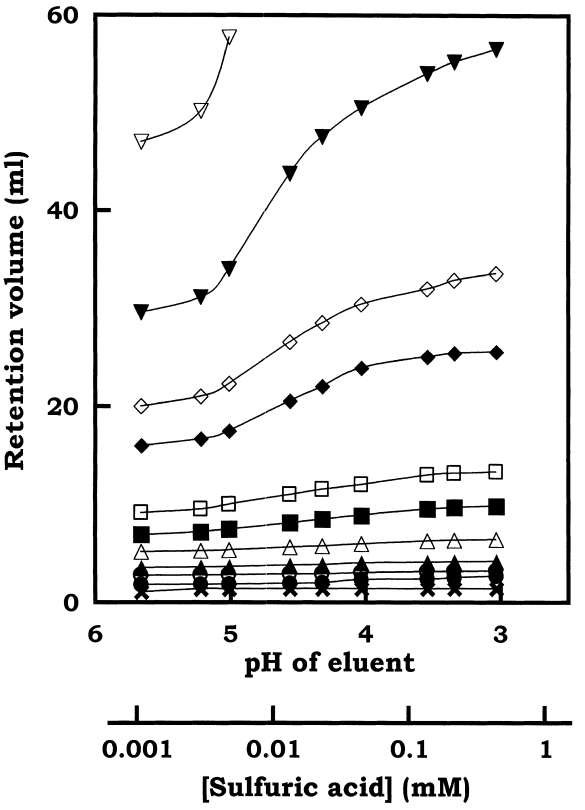

Fig. 1. Effect of the concentration of sulfuric acid in the eluent on the retention volumes of C_1–C_7 aliphatic carboxylic acids on a TSKgel SCX column. Conditions: column, TSKgel SCX, 150×6 mm I.D., 35 °C; eluent, 0.0015–0.5 m*M* sulfuric acid; flow-rate, 1 ml min^{-1}; detection, conductivity; injection volume, 100 μl; sample concentration, 0.1 m*M* for formic acid and 0.2 m*M* for the other aliphatic carboxylic acids. Symbols: (×) sulfuric acid, (●) formic acid, (○) acetic acid, (▲) propionic acid, (△) butyric acid, (■) isovaleric acid, (□) valeric acid, (♦) isocaproic acid, (◇) caproic acid, (▼) 2-methylhexanoic acid, (▽) heptanoic acid.

furic acid, the aliphatic carboxylic acids were separated mainly by the hydrophobic adsorption process.

Fig. 2A–C show chromatograms of the aliphatic carboxylic acids using 0.005 m*M* sulfuric acid at pH 5.0 (eluent conductivity 5.5 μS cm^{-1}), 0.05 m*M* sulfuric acid at pH 4.0 (41 μS cm^{-1}) and 0.5 m*M* sulfuric acid at pH 3.0 (391 μS cm^{-1}) as eluents. As shown in Fig. 2A, when using 0.05 m*M* sulfuric acid as eluent, fronted peaks of C_1–C_5 aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric and valeric acids) and tailed peaks of C_6–C_7 aliphatic carboxylic acids (2-methylvaleric, isocaproic, caproic, 2,2-dimethyl-*n*-valeric, 2-methylhexanoic, 5-methylhexanoic and heptanoic acids) were obtained. Since these C_1–C_7 aliphatic carboxylic acids were well dissociated, it was expected that the C_1–C_5 aliphatic carboxylic acids would mainly be separated by the ion-exclusion chromatographic process and the C_6–C_7 aliphatic carboxylic acids with large hydrophobicity would be separated by both the ion-exclusion chromatographic and hydrophobic adsorption processes. As shown in Fig. 2B, when using 0.05 m*M* sulfuric acid as eluent, largely tailed peaks of C_5–C_7 aliphatic carboxylic acids (isovaleric, valeric, 2-methylvaleric, isocaproic, caproic, 2,2-dimethyl-*n*-valeric, 2-methylhexanoic, 5-methylhexanoic and heptanoic acids) were obtained. This is because the dissociation of these C_1–C_7 aliphatic carboxylic acids was largely suppressed and, as a consequence, higher carboxylic acids (C_5–C_7 carboxylic acids) with large hydrophobicity were mainly separated by the hydrophobic adsorption process. Symmetric peaks of C_1–C_4 carboxylic acids (formic, acetic, propionic, isobutyric and butyric acids) were also obtained. This is due mainly to their small hydrophobicity. In contrast, as shown in Fig. 2A–C, with increasing concentration of sulfuric acid, the conductimetric detection sensitivities of these C_1–C_7 aliphatic carboxylic acids decreased drastically. This is due to (a) an increase in the eluent conductivity and (b) a decrease in the conductimetric detector responses of these carboxylic acids, caused by suppression of dissociation. Finally, as shown in Fig. 2C, when using 0.5 m*M* sulfuric acid as eluent, it was very difficult to determine the C_6–C_7 aliphatic carboxylic acids.

From the above results, it was found that the optimum concentration of sulfuric acid in the eluent was 0.05 m*M* (pH 4.0). However, a further investigation was required to improve the peak shapes and to reduce the retention volumes for hydrophobic aliphatic carboxylic acids.

3.1.2. Effect of C_1–C_7 alcohols added to sulfuric acid as eluent on the chromatographic behavior of C_1–C_7 aliphatic carboxylic acids

The addition of organic solvents to the eluent is very effective for both improving the peak shapes and reducing the retention volumes for higher carboxylic acids in ion-exclusion chromatography. However, the use of higher concentrations of organic solvents is limited, because shrinking of the styrene–divinylbenzene co-polymer resin occurs. Morris and Fritz have demonstrated the effectiveness of a higher alcohol (butanol) as the retention modifier for a C_5 aliphatic carboxylic acid (valeric acid) in ion-exclusion chromatography using a sulfonated styrene–divinylbenzene co-polymer resin column [12]. The authors have also reported that a higher alcohol (heptanol) is a very effective retention modifier for higher aliphatic carboxylic acids in ion-exclusion chromatography using an unmodified silica gel column [13]. The addition of C_1–C_7 alcohols (methanol, ethanol, propanol, butanol, pentanol, hexanol and heptanol) to 0.05 m*M* sulfuric acid as eluent was carried out for the simultaneous separation of these C_1–C_7 aliphatic carboxylic acids on a TSKgel SCX column in a reasonable period of time. Alcohols $>C_7$ (heptanol) were not applicable, due to their limited solubility. The concentrations of various alcohols added to the eluent was determined for the elution of the C_1–C_7 aliphatic carboxylic acids within 30 min.

Fig. 3A–D show chromatograms of the C_1–C_7 aliphatic carboxylic acids using 0.05 m*M* sulfuric acid containing 10% methanol, 2% propanol, 0.1% pentanol and 0.025% heptanol as eluent, respectively. When using the above eluents, the C_1–C_7 aliphatic carboxylic acids were readily eluted in 30 min. Furthermore, using a higher alcohol, the peak shapes of the higher aliphatic carboxylic acids were improved greatly and the peak resolution between the C_1–C_7 aliphatic carboxylic acids was also improved. This may be because the higher alcohol was strongly adsorbed on TSKgel SCX and reduced the hydrophobicity of the resin. The above results indi-

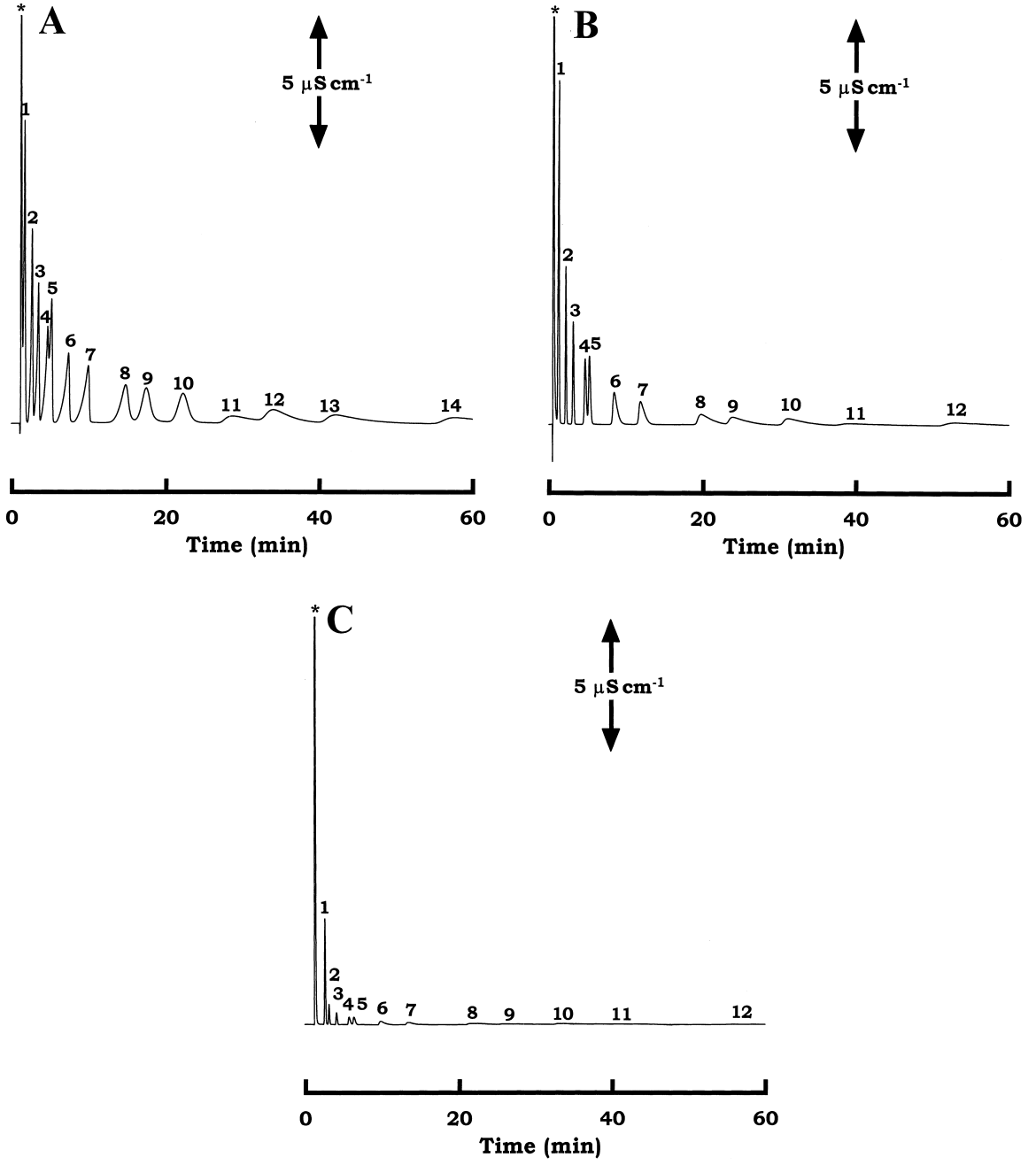

Fig. 2. Chromatograms of C_1–C_7 aliphatic carboxylic acids on a TSKgel SCX column using various concentrations of sulfuric acid as eluent. Conditions: eluents, (A) 0.005 m*M* sulfuric acid at pH 5.0 (eluent conductivity 5.5 μS cm^{-1}), (B) 0.05 m*M* sulfuric acid at pH 4.0 (eluent conductivity 41 μS cm^{-1}), (C) 0.5 m*M* sulfuric acid at pH 3.0 (eluent conductivity 391 μS cm^{-1}). Peaks: *=sulfuric acid, 1=formic acid, 2=acetic acid, 3=propionic acid, 4=isobutyric acid, 5=butyric acid, 6=isovaleric acid, 7=valeric acid, 8=2-methylvaleric acid, 9=isocaproic acid, 10=caproic acid, 11=2,2-dimethyl-*n*-valeric acid, 12=2-methylhexanoic acid, 13=5-methylhexanoic acid, 14=heptanoic acid. Other chromatographic conditions as in Fig. 1.

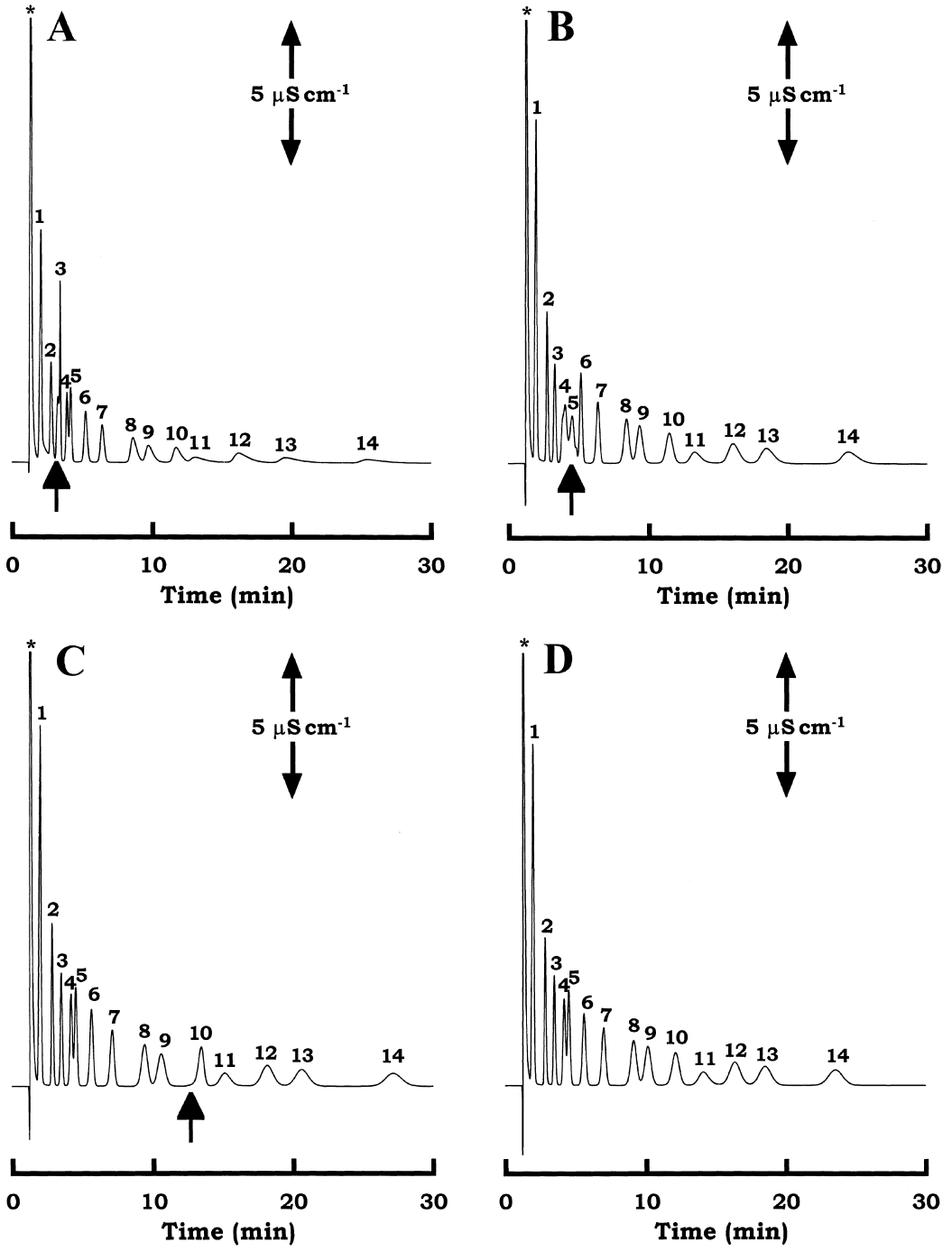

Fig. 3. Chromatograms of C_1–C_7 aliphatic carboxylic acids on a TSKgel SCX column using 0.05 m*M* sulfuric acid containing various alcohols as eluent. Conditions: eluents, (A) 0.05 m*M* sulfuric acid containing 10% methanol, (B) 0.05 m*M* sulfuric acid containing 2% propanol, (C) 0.05 m*M* sulfuric acid containing 0.1% pentanol, (D) 0.05 m*M* sulfuric acid containing 0.025% heptanol. Other chromatographic conditions as in Fig. 2.

cate that heptanol is the most effective modifier in ion-exclusion chromatography with conductimetric detection (ion-exclusion chromatography–CD) for improving the peak shapes and reducing the retention volumes for higher aliphatic carboxylic acids. Unfortunately, since alcohols themselves are retained on TSKgel SCX and can be detected conductimetrically [16], vacant peaks corresponding to alcohols in the eluent appeared and these peaks often interfered with the determination of the aliphatic carboxylic acids. For example, as shown in Fig. 3A–C, vacant peaks corresponding to methanol, propanol and pentanol interfere with the determination of propionic acid, isobutyric and butyric acids, and caproic acid, respectively. In contrast, as shown in Fig. 3D, when using 0.05 m*M* sulfuric acid containing 0.025% heptanol as eluent, since the concentration of heptanol was extremely low and heptanol was strongly retained on the TSKgel SCX column, a vacant peak corresponding to heptanol is not observed and, as a consequence, excellent simultaneous separation of the C_1–C_7 aliphatic carboxylic acids is achieved with no interferences from vacant peaks. The equilibrium time to obtain reproducible retention times for the C_1–C_7 aliphatic carboxylic acids was less than 1.5 h, after changing the eluent from 0.05 m*M* sulfuric acid to 0.05 m*M* sulfuric acid containing 0.025% heptanol.

From the above results, it was concluded that heptanol was the most effective organic modifier in ion-exclusion chromatography–CD for the simultaneous separation of the C_1–C_7 aliphatic carboxylic acids. As shown in Fig. 3D, symmetric peaks, excellent simultaneous separation and a relatively high sensitive conductimetric detection of the aliphatic carboxylic acids were achieved in 30 min.

3.1.3. Analytical performance parameters

Table 1 shows the detection limits (signal-to-noise ratio 3, injection volume 100 μl) of the C_1–C_7 aliphatic carboxylic acids. A relatively high sensitive conductimetric detection was achieved. The main reasons for this are that (a) the eluent conductivity was relatively low (42 μS cm^{-1}; noise, 1.4×10^{-3} μS cm^{-1}) and (b) the aliphatic carboxylic acids were partly dissociated under the ion-exclusion chromatography–CD conditions used.

Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of C_1–C_7 aliphatic carboxylic acids. Linear calibration graphs ($r^2 \geq 0.99$) were obtained in the concentration range between 0.005 and 2.0 m*M*.

Table 1
Detection limits (signal-to-noise ratio 3, injection volume 100 μl) of C_1–C_7 aliphatic carboxylic acids

Aliphatic carboxylic acid	pK_a at 25 °C	Detection limit	
		μ*M*	ng ml^{-1}
Formic	3.75	0.030	1.2
Acetic	4.76	0.14	8.1
Propionic	4.87	0.19	14
Isobutyric	4.86	0.24	21
Butyric	4.82	0.22	19
Isovaleric	4.77	0.27	28
Valeric	4.86	0.36	37
2-Methylvaleric	≈4.9	0.48	56
Isocaproic	4.82	0.56	65
Caproic	4.86	0.65	75
2,2-Dimethyl-*n*-valeric	≈4.9	1.6	2.0×10^2
2-Methylhexanoic	≈4.9	0.92	1.2×10^2
5-Methylhexanoic	≈4.9	1.1	1.4×10^2
Heptanoic	4.89	1.3	1.7×10^2

Eluent conductivity, 42 μS cm^{-1}; noise, 1.4×10^{-3} μS cm^{-1}.

The relative standard deviations (RSDs) of the chromatographic peak areas of the C_1–C_7 aliphatic carboxylic acids, the concentrations of which were 0.1 m*M* for formic acid and 0.2 m*M* for the other acids, were less than 0.7% ($n=10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

3.2. Ion-exclusion chromatographic separation of benzenecarboxylic acids on a TSKgel SCX column

3.2.1. Effect of the concentration of sulfuric acid in the eluent on the chromatographic behavior of benzenecarboxylic acids

The effect of the concentration of sulfuric acid in the eluent on the chromatographic behavior of these benzenecarboxylic acids on the TSKgel SCX column was investigated for their simultaneous separation.

Fig. 4 shows the relationship between the concentration of sulfuric acid and the retention volumes of these benzenecarboxylic acids. With increasing concentration of sulfuric acid, the retention volumes of the benzenecarboxylic acids increased. This is due mainly to (a) a decrease in the electrostatic repulsion

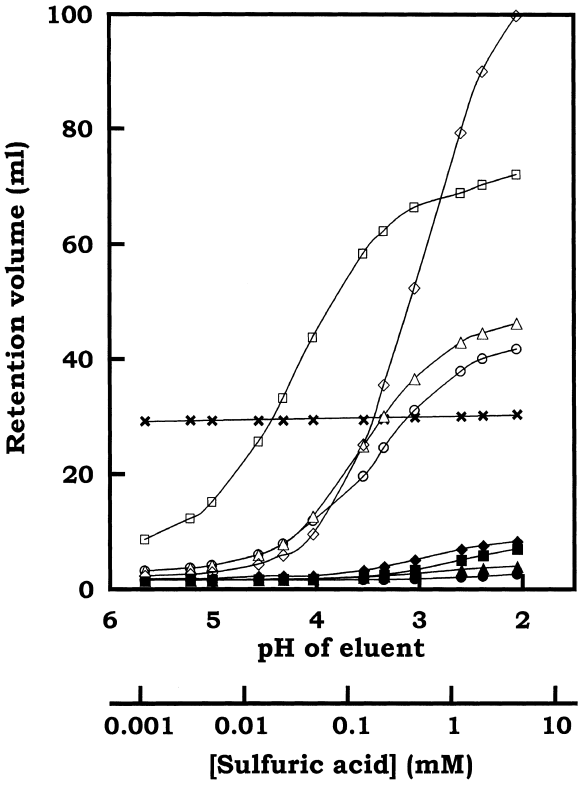

Fig. 4. Effect of the concentration of sulfuric acid in the eluent on the retention volumes of benzenecarboxylic acids on a TSKgel SCX column. Conditions: eluent, 0.0015–5 m*M* sulfuric acid; detection, UV at 200 nm; sample concentration, 0.02 m*M*. Symbols: (●) pyromellitic acid, (▲) trimellitic acid, (■) hemimellitic acid, (✦) *o*-phthalic acid, (×) phenol, (○) *p*-phthalic acid, (△) *m*-phthalic acid, (□) benzoic, (◇) salicylic acid. Other chromatographic conditions as in Fig. 3.

and (b) an increase in the hydrophobic interaction between the benzenecarboxylic acids and the surface of TSKgel SCX, caused by suppression of the dissociation of the benzenecarboxylic acids. The degree of increase in the retention volume for individual benzenecarboxylic acids was considerably different. The differences are mainly due to differences in both the acid dissociation constants and the hydrophobicity of individual benzenecarboxylic acids. In contrast, the retention volume of phenol remained almost constant. This is because phenol is a nonionic compound and is separated by only the hydrophobic adsorption process.

Fig. 5A–D show chromatograms of the benzenecarboxylic acids using 0.005 m*M* sulfuric acid at pH 5.0, 0.05 m*M* sulfuric acid at pH 4.0, 0.5 m*M* sulfuric acid at pH 3.0 and 5 m*M* sulfuric acid at pH 2.1 as eluent, respectively. With increasing concentration of sulfuric acid, the peak shapes of pyromellitic, trimellitic, hemimellitic and *o*-phthalic acids were improved. The peak resolution between the four benzenecarboxylic acids was also improved. As shown in Fig. 5D, when using 5 m*M* sulfuric acid as eluent, good peak shapes and good separation for the above four benzenecarboxylic acids were achieved. In contrast, with increasing concentration of sulfuric acid, the peaks of the remaining benzenecarboxylic acids were strongly tailed and the retention volumes increased drastically. The differences could also be attributed to differences in their chemical properties (dissociation constants, hydrophobicity, etc.).

From the above results, it was found that the optimum concentration of sulfuric acid in the eluent is 5 m*M* (pH 2.1). However, a further investigation was also required to improve the peak shapes and to reduce the retention volumes for the benzenecarboxylic acids.

3.2.2. Effect of C_1–C_7 alcohols added to sulfuric acid as eluent on the chromatographic behavior of benzenecarboxylic acids

The addition of C_1–C_7 alcohols to 5 m*M* sulfuric acid as eluent was carried out for the simultaneous separation of the benzenecarboxylic acids on the TSKgel SCX column in a reasonable period of time. The concentrations of various alcohols added to the eluent was determined for the elution of these benzenecarboxylic acids within 30 min.

Fig. 6A–D show chromatograms of the benzenecarboxylic acids using 5 m*M* sulfuric acid containing 20% methanol, 4% propanol, 0.5% pentanol and 0.075% heptanol as eluent, respectively. When using the above eluents, the benzenecarboxylic acids were readily eluted in 30 min. Furthermore, using a higher alcohol, the peak shapes of the strongly adsorbed benzenecarboxylic acids were improved drastically and the peak resolution between the acids was also improved. This might be because the higher alcohol was strongly adsorbed on the TSKgel SCX column, reducing its hydrophobicity. The above results also indicate that heptanol is the most effective modifier in ion-exclusion chromatography for improving the peak shapes and reducing

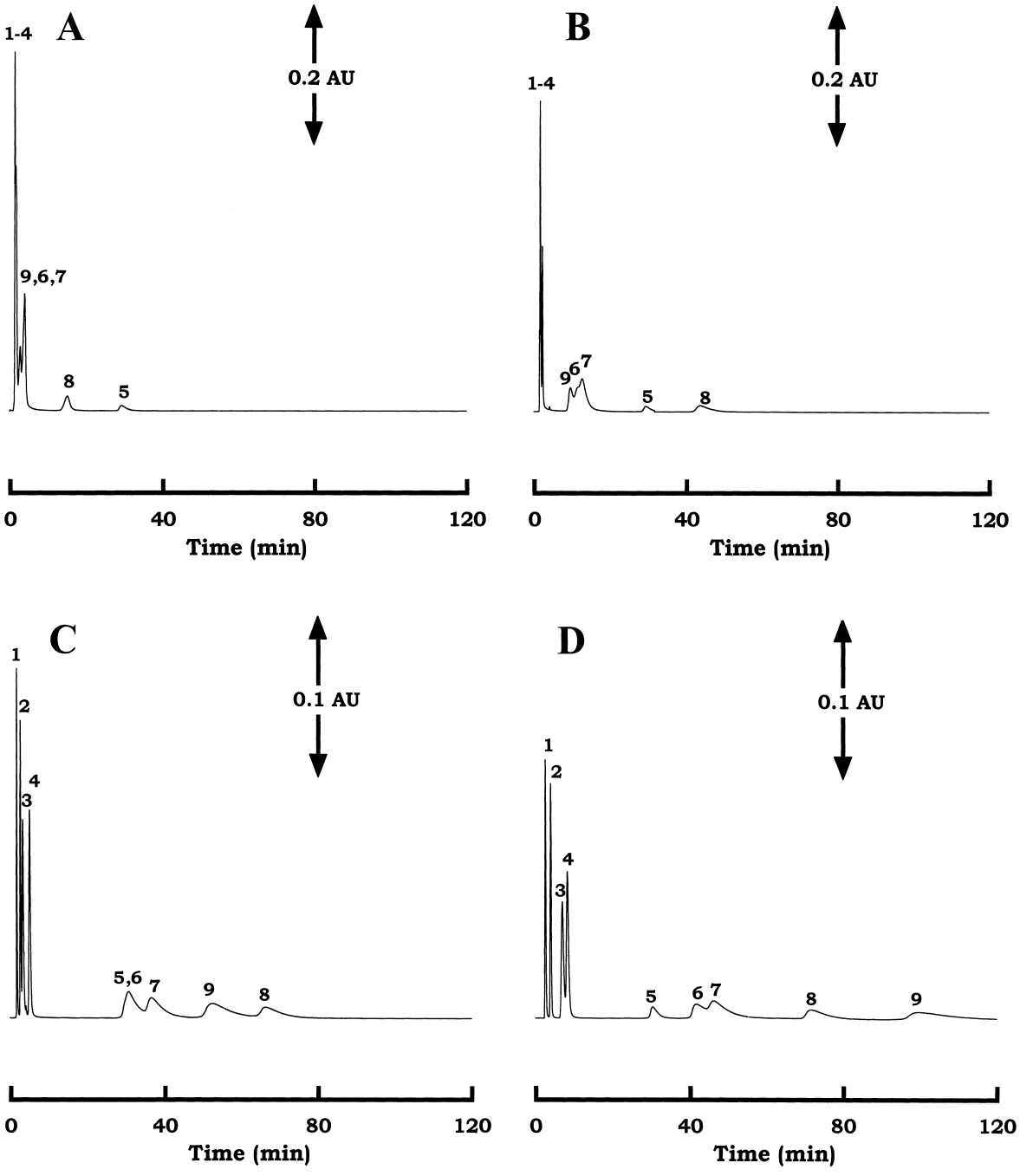

Fig. 5. Chromatograms of benzenecarboxylic acids on a TSKgel SCX column using various concentrations of sulfuric acid as eluent. Conditions: eluents, (A) 0.005 m*M* sulfuric acid at pH 5.0, (B) 0.05 m*M* sulfuric acid at pH 4.0, (C) 0.5 m*M* sulfuric acid at pH 3.0, (D) 5 m*M* sulfuric acid at pH 2.1. Peaks: 1=pyromellitic acid, 2=trimellitic acid, 3=hemimellitic acid, 4=*o*-phthalic acid, 5=phenol, 6=*p*-phthalic acid, 7=*m*-phthalic acid, 8=benzoic acid, 9=salicylic acid. Other chromatographic conditions as in Fig. 4.

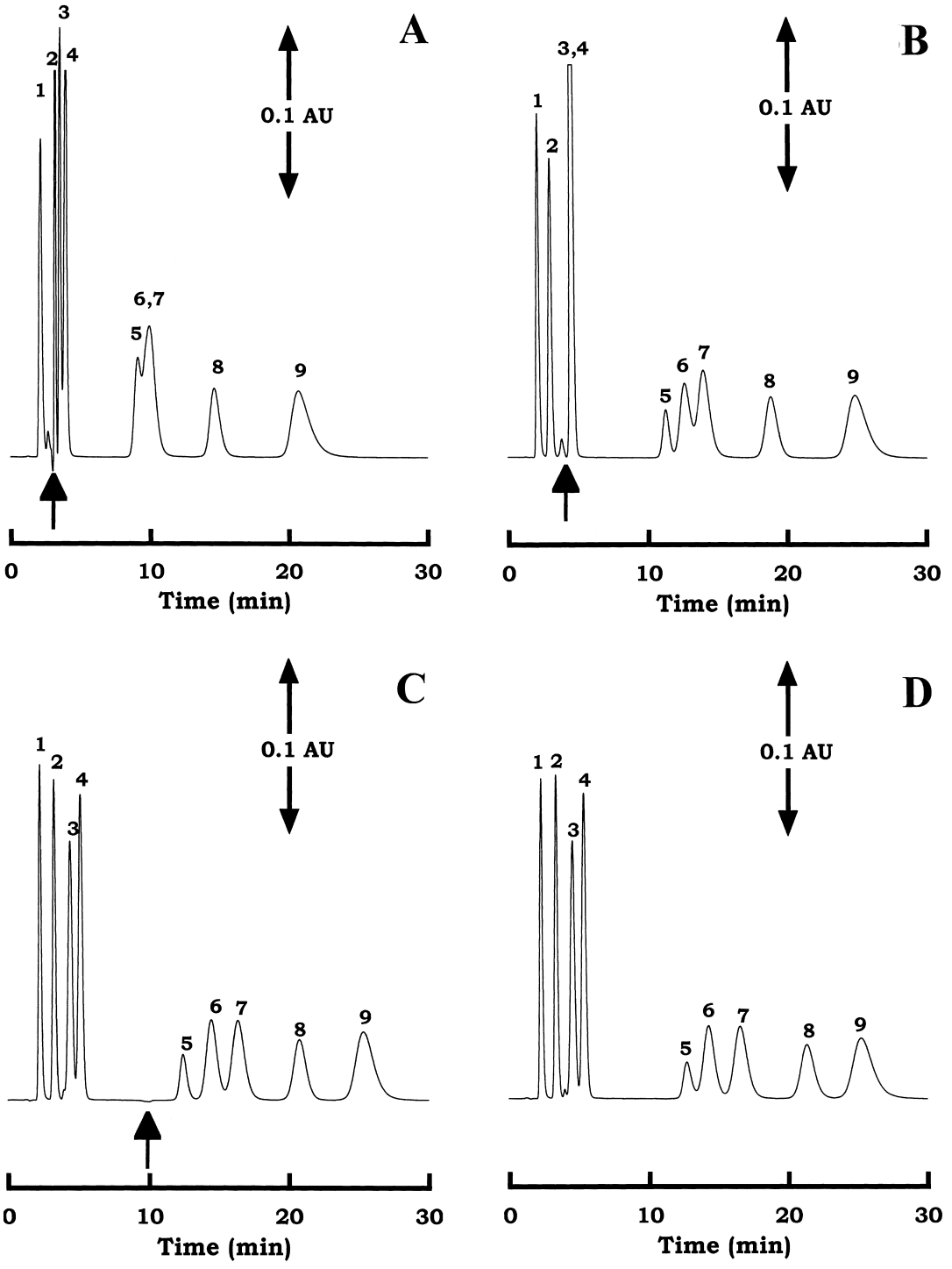

Fig. 6. Chromatograms of benzenecarboxylic acids on a TSKgel SCX column using 5 m*M* sulfuric acid containing various alcohols as eluent. Conditions: eluents, (A) 5 m*M* sulfuric acid containing 20% methanol, (B) 5 m*M* sulfuric acid containing 4% propanol, (C) 5 m*M* sulfuric acid containing 0.5% pentanol, (D) 5 m*M* sulfuric acid containing 0.075% heptanol. Other chromatographic conditions as in Fig. 5.

the retention volumes of benzenecarboxylic acids. As shown in Fig. 6A and B, when using 5 m*M* sulfuric acid containing 20% methanol or 4% propanol as eluent, vacant peaks corresponding to methanol and propanol in the eluents interfere with the determination of pyromellitic and trimellitic acids and hemimellitic and *o*-phthalic acids, respectively. As shown in Fig. 6C, when using 5 m*M* sulfuric acid containing 0.5% pentanol as eluent, a small vacant peak corresponding to pentanol in the eluent appears in ca. 10 min. In contrast, as shown in Fig. 6D, when using 5 m*M* sulfuric acid containing 0.075% heptanol as eluent, no interferences from a vacant peak corresponding to heptanol in the determination of the benzenecarboxylic acids was observed. This is mainly due to (a) the very low concentration of heptanol in the eluent and (b) the strong affinity of heptanol for TSKgel SCX. The equilibrium time to obtain reproducible retention times for these benzenecarboxylic acids was less than 1 h, after changing the eluent from 5 m*M* sulfuric acid to 5 m*M* sulfuric acid containing 0.075% heptanol.

From the above results, it was concluded that heptanol is the most effective organic modifier in ion-exclusion chromatography for the simultaneous separation of these benzenecarboxylic acids. As shown in Fig. 6D, symmetric peaks, excellent simultaneous separation and highly sensitive UV detection at 200 nm were achieved for these benzenecarboxylic acids in 30 min.

3.2.3. Analytical performance parameters

Table 2 shows the detection limits (signal-to-noise ratio 3, injection volume 100 μl) of the benzenecarboxylic acids. Highly sensitive UV detection at 200 nm was achieved. This is due to the large molar extinction coefficients of these benzenecarboxylic acids at 200 nm.

Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of the benzenecarboxylic acids. Linear calibration graphs ($r^2 \geq 0.99$) were obtained in the concentration range between 0.0005 and 0.2 m*M*.

The relative standard deviations (RSDs) of the chromatographic peak areas of the benzenecarboxylic acids, the concentrations of which were 0.02 m*M*, were less than 0.6% ($n=10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

Table 2
Detection limits (signal-to-noise ratio 3, injection volume 100 μl) of benzenecarboxylic acids

Benzenecarboxylic acid	pK_a at 25 °C	Detection limit	
		μ*M*	ng ml^{-1}
Pyromellitic	1.9, 2.8, 4.5, 5.6	0.0032	0.81
Trimellitic	2.5, 3.9, 5.2	0.0031	0.66
Hemimellitic	2.6, 3.8, 5.5	0.0045	0.95
o-Phthalic	3.1, 5.4	0.0038	0.63
m-Phthalic	3.7, 4.6	0.016	2.6
p-Phthalic	3.5, 4.5	0.016	2.6
Benzoic	4.2	0.020	2.4
Salicylic	2.8[a], 12.4[a]	0.018	2.5
Phenol	10.0	0.028	2.7

[a] At 30 °C.

4. Conclusion

In order to expand the utility of the highly sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) in the H^+ form as a stationary phase in ion-exclusion chromatography for carboxylic acids, the application of the TSKgel SCX column (150×6 mm I.D.) for the ion-exclusion chromatographic separation of both (a) C_1–C_7 aliphatic carboxylic acids (formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, 2-methylvaleric, isocaproic, caproic, 2,2-dimethyl-*n*-valeric, 2-methylhexanoic, 5-methylhexanoic and heptanoic acids) and (b) benzenecarboxylic acids (pyromellitic, hemimellitic, trimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic and salicylic acids and phenol) was carried out using dilute sulfuric acid containing C_1–C_7 alcohols (methanol, ethanol, propanol, butanol, pentanol, hexanol and heptanol) as eluent.

Heptanol (C_7 alcohol) was found to be the most effective modifier in ion-exclusion chromatography in order to improve the peak shapes and to reduce the retention volumes for hydrophobic aliphatic carboxylic acids and benzenecarboxylic acids. When using 0.5 m*M* sulfuric acid containing 0.025% heptanol as eluent, excellent simultaneous separation and relatively highly sensitive conductimetric detection of the aliphatic carboxylic acids were

achieved in 30 min. When using 5 m*M* sulfuric acid containing 0.075% heptanol as eluent, excellent simultaneous separation and highly sensitive UV detection at 200 nm was achieved for the benzenecarboxylic acids in 30 min.

The use of a higher alcohol, such as heptanol, as the retention modifier strongly expands the utility of the TSKgel SCX column in the ion-exclusion chromatography of various carboxylic acids.

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