

## Separation of aliphatic carboxylic acids and benzenecarboxylic acids by ion-exclusion chromatography with various cation-exchange resin columns and sulfuric acid as eluent

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### Abstract

The application of various hydrophilic cation-exchange resins for high-performance liquid chromatography (sulfonated silica gel: TSKgel SP-2SW, carboxylated silica gel: TSKgel CM-2SW, sulfonated polymethacrylate resin: TSKgel SP-5PW, carboxylated polymethacrylate resins: TSKgel CM-5PW and TSKgel OA-Pak A) as stationary phases in ion-exclusion chromatography for C<sub>1</sub>–C<sub>7</sub> aliphatic carboxylic acids (formic, acetic, propionic, butyric, isovaleric, valeric, isocaproic, caproic, 2-methylhexanoic and heptanoic acids) and benzenecarboxylic acids (pyromellitic, trimellitic, hemimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic, salicylic acids and phenol) was carried out using diluted sulfuric acid as the eluent. Silica-based cation-exchange resins (TSKgel SP-2SW and TSKgel CM-2SW) were very suitable for the ion-exclusion chromatographic separation of these benzenecarboxylic acids. Excellent simultaneous separation of these benzenecarboxylic acids was achieved on a TSKgel SP-2SW column (150×6 mm I.D.) in 17 min using a 2.5 mM sulfuric acid at pH 2.4 as the eluent. Polymethacrylate-based cation-exchange resins (TSKgel SP-5PW, TSKgel CM-5PW and TSKgel OA-Pak A) acted as advanced stationary phases for the ion-exclusion chromatographic separation of these C<sub>1</sub>–C<sub>7</sub> aliphatic carboxylic acids. Excellent simultaneous separation of these C<sub>1</sub>–C<sub>7</sub> acids was achieved on a TSKgel CM-5PW column (150×6 mm I.D.) in 32 min using a 0.05 mM sulfuric acid at pH 4.0 as the eluent.

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**Keywords:** Stationary phases, LC; Carboxylic acids; Benzenecarboxylic acids; Sulfuric acid

### 1. Introduction

Ion-exclusion chromatography developed by Wheaton and Baumann [1] is widely recognized as a simple and powerful analytical technique for the

determination of various carboxylic acids. Highly sulfonated styrene–divinylbenzene co-polymer resin (PS–DVB) in the H<sup>+</sup>-form is exclusively utilized as a stationary phase in the ion-exclusion chromatography of carboxylic acids [2]. Sulfonated PS–DVB column is successfully applied to the ion-exclusion chromatography of hydrophilic carboxylic acids and lower aliphatic carboxylic acids. However, due to the large hydrophobicity, ion-exclusion chromatographic

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separation of hydrophobic carboxylic acids, such as higher aliphatic carboxylic acids and benzenecarboxylic acids, on the sulfonated PS–DVB column is very difficult. This is because strongly tailed peaks and too long retention times for hydrophobic carboxylic acids are commonly obtained on the sulfonated PS–DVB column.

The addition of organic solvent to eluent was carried out, in order to improve peak shapes and to reduce the retention times for hydrophobic carboxylic acids on the sulfonated PS–DVB column. Various organics, such as alcohols [3,4], acetonitrile [5], sugar alcohols and sugars [6] and polyvinyl alcohols [7], were utilized as elution modifiers for hydrophobic carboxylic acids. Unfortunately, the concentration of organics added to eluent is strongly limited, because shrinkage of the PS–DVB occurs. Furthermore, since organic solvent itself is also retained on the sulfonated PS–DVB [8], vacant peak corresponding to organic solvent in the eluent appears and often interferes seriously with the determination of carboxylic acids.

Another approach for successful achievement of the ion-exclusion chromatographic separation of hydrophobic carboxylic acids was to utilize hydrophilic cation-exchange resins as the stationary phases. Ng et al. reported that hydrophobicity of the cation-exchange resin increased following sequence: sulfonated silica gel < sulfonated polymethacrylate resin < sulfonated PS–DVB [9]. However, the detailed study of the ion-exclusion chromatographic behavior of hydrophobic carboxylic acids on various commercially available hydrophilic cation-exchange resins columns has not been carried out yet. This study is very important for the expansion of the utility of ion-exclusion chromatography for carboxylic acids.

The aim of this study was to demonstrate the effectiveness of ion-exclusion chromatography for hydrophobic carboxylic acids. Then, the application of various commercially available hydrophilic cation-exchange resins for high-performance liquid chromatography (HPLC) (sulfonated silica gel: TSKgel SP-2SW, carboxylated silica gel: TSKgel CM-2SW, sulfonated polymethacrylate resin: TSKgel SP-5PW, carboxylated polymethacrylate resins: TSKgel CM-5SW and TSKgel OA-Pak A) as the stationary phases in the ion-exclusion chromatog-

raphy using diluted sulfuric acid as the eluent was carried out for the separation of both (a) C<sub>1</sub>–C<sub>7</sub> aliphatic carboxylic acids (formic, acetic, propionic, butyric, isovaleric, valeric, isocaproic, caproic, 2-methylhexanoic and heptanoic acids), and (b) benzenecarboxylic acids (pyromellitic, trimellitic, hemimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic, salicylic acids and phenol). Silica-based cation-exchange resins (TSKgel SP-2SW and TSKgel CM-2SW) were suitable for the ion-exclusion chromatographic separation of these benzenecarboxylic acids. Excellent simultaneous separation of these benzenecarboxylic acids was achieved on a TSKgel SP-2SW column (150×6 mm I.D.) in 17 min using a 2.5 mM sulfuric acid at pH 2.4 as the eluent. Polymethacrylate-based cation-exchange resins (TSKgel SP-5PW, TSKgel CM-5PW and TSKgel OA-Pak A) behaved as an advanced stationary phases for the ion-exclusion chromatography of these C<sub>1</sub>–C<sub>7</sub> aliphatic carboxylic acids. Excellent simultaneous separation of these aliphatic carboxylic acids was achieved on a TSKgel CM-5PW column (150×6 mm I.D.) in 32 min using a 0.05 mM sulfuric acid at pH 4.0 as the eluent.

## 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<sup>-1</sup>, 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 the measurement of pH of eluents.

### 2.2. Separation columns

Table 1 shows physical and chemical properties of various cation-exchanger resins used in the study.

Table 1  
Properties of various cation-exchange resins used in this study

Cation-exchange resin	Matrix	Particle size ( $\mu\text{m}$ )	Cation-exchanger form	Capacity (mequiv. $\text{ml}^{-1}$ )
TSKgel SCX	PS–DVB	5	– $\text{SO}_3\text{H}$	1.5
TSKgel SP-2SW	Silica	5	SP	0.1
TSKgel CM-2SW	Silica	5	CM	0.1
TSKgel SP-5PW	PMA	10	SP	0.1
TSKgel CM-5PW	PMA	10	CM	0.1
TSKgel OA-Pak A	PMA	7	CM	0.1

PS–DVB, styrene–divinylbenzene co-polymer resin; PMA, polymethacrylate resin; SP, sulfopropyl group; and CM, carboxymethyl group.

Separation columns (150×6 mm I.D.) were packed with these resins by using surrey-packing method.

### 2.3. Chemicals

All chemicals were of analytical-reagent grade and were purchased from Aldrich (Milwaukee, WI, USA), Wako (Osaka, Japan) or Tokyo Kasei (Tokyo, Japan). Distilled, deionized water was used for the preparation of the eluents and standard solutions.

## 3. Results and discussion

### 3.1. Ion-exclusion chromatographic behavior of $\text{C}_1$ – $\text{C}_7$ aliphatic carboxylic acids on various cation-exchange resins columns

Highly sulfonated and low cross-linked PS–DVB is exclusively utilized as a stationary phase in ion-exclusion chromatography for carboxylic acids [2]. Firstly, the ion-exclusion chromatographic behavior of  $\text{C}_1$ – $\text{C}_7$  aliphatic carboxylic acids (formic, acetic, propionic, butyric, isovaleric, valeric, isocaproic, caproic, 2-methylhexanoic and heptanoic acids) on this type of resin column (TSKgel SCX, 150×6 mm I.D.) was investigated using a 0.05 mM sulfuric acid at pH 4.0 as the eluent. Fig. 1A shows chromatogram of these  $\text{C}_1$ – $\text{C}_7$  acids on the TSKgel SCX column. Although symmetrical peaks of the  $\text{C}_1$ – $\text{C}_4$  acids (formic, acetic, propionic, isovaleric, and butyric acids) were obtained, peak shapes of hydrophobic carboxylic acids ( $\text{C}_5$ – $\text{C}_7$  acids) were tailed strongly and their retention times were extremely long. The retention time of heptanoic acid reached over 60

min. The ion-exclusion chromatographic behavior of these  $\text{C}_1$ – $\text{C}_7$  acids was due mainly to the large hydrophobicity of the TSKgel SCX resin. These above results strongly suggested that both excellent ion-exclusion chromatographic separation and symmetrical peaks for these  $\text{C}_1$ – $\text{C}_7$  acids could not be achieved on the TSKgel SCX column in a reasonable period of time (ca. 30 min) when using diluted sulfuric acid as the eluent.

To use hydrophilic cation-exchange resins was expected to be one of the best ways for the elimination of these above drawbacks. Ng et al. reported that hydrophobicity of the cation-exchange resin increased following sequence: sulfonated silica gel < sulfonated polymethacrylate resin < sulfonated PS–DVB [9]. Next, the application of silica-based and polymethacrylate-based cation-exchange resins as the stationary phases in ion-exclusion chromatography for these  $\text{C}_1$ – $\text{C}_7$  acids was carried out using a 0.05 mM sulfuric acid as the eluent.

Fig. 1B and C show chromatograms of these  $\text{C}_1$ – $\text{C}_7$  acids on a sulfonated silica gel (TSKgel SP-2SW) column and a carboxylated silica gel (TSKgel CM-2SW) column, respectively. It was very easy to elute these  $\text{C}_1$ – $\text{C}_7$  acids from these silica gels columns. This is because these silica-based cation-exchange resins have large hydrophilicity and, as a consequence, hydrophobic interaction between these resins and these  $\text{C}_1$ – $\text{C}_7$  acids was very weak. The retention behavior of these  $\text{C}_1$ – $\text{C}_7$  acids on the TSKgel SP-2SW column was very similar to that on the TSKgel CM-2SW column. This result suggested that these  $\text{C}_1$ – $\text{C}_7$  acids were mainly retained on the surface of silica gel. The retention times of these  $\text{C}_1$ – $\text{C}_7$  acids on the TSKgel SP-2SW

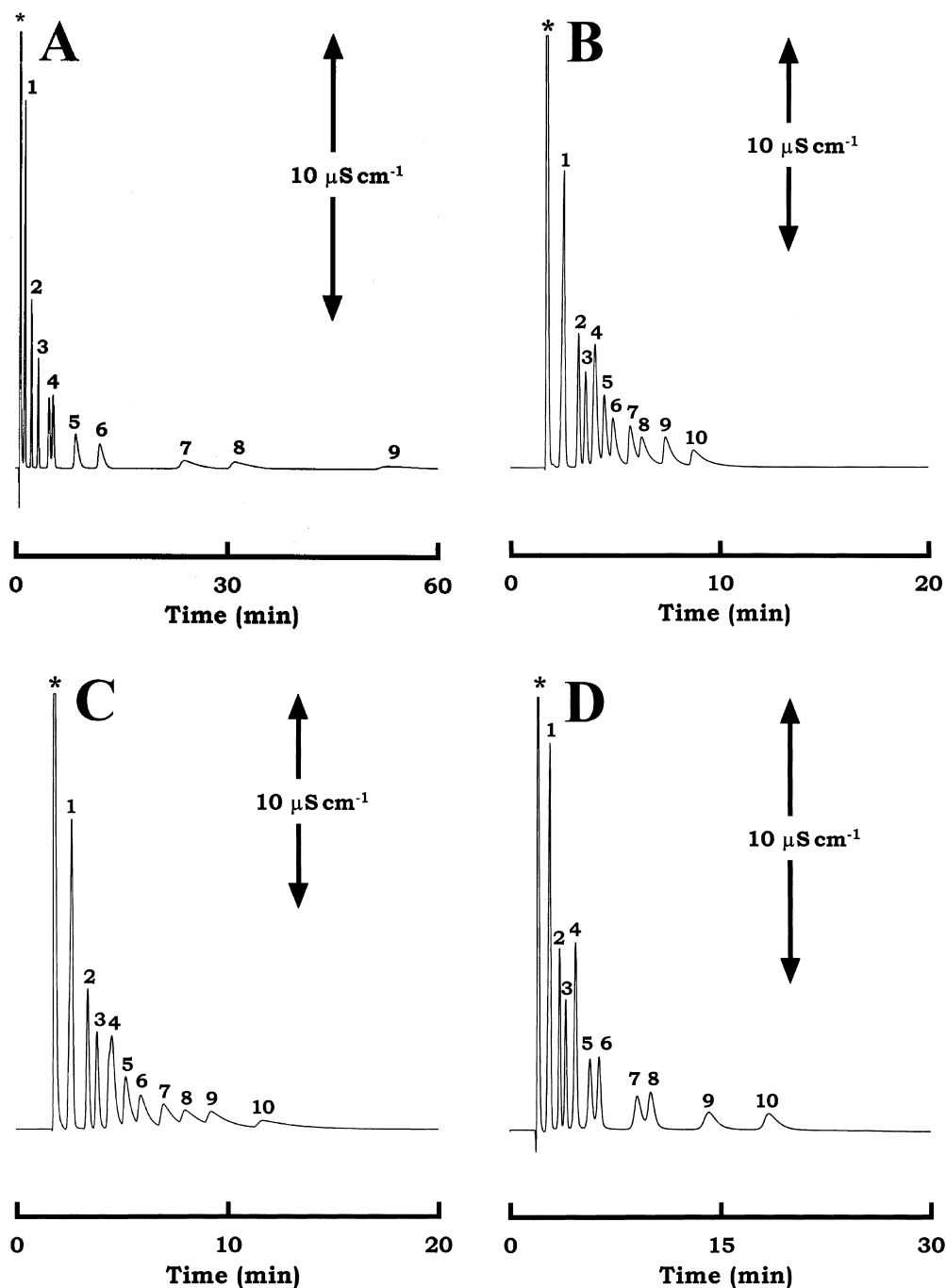


Fig. 1. Chromatograms of  $C_1$ – $C_7$  aliphatic carboxylic acids on various cation-exchange resins columns. Conditions: columns, (A) TSKgel SCX, (B) TSKgel SP-2SW, (C) TSKgel CM-2SW, (D) TSKgel SP-5PW, (E) TSKgel CM-5PW and (F) TSKgel OA-Pak A; column size,  $150 \times 6$  mm I.D.; column temperature,  $35^\circ\text{C}$ ; eluent,  $0.05\text{ mM}$  sulfuric acid at pH 4.0; flow-rate,  $1\text{ ml min}^{-1}$ ; detection, conductivity; injection volume,  $100\ \mu\text{l}$ ; sample concentration,  $0.1\text{ mM}$  for formic acid and  $0.2\text{ mM}$  for other aliphatic carboxylic acids. Peaks: \*, sulfuric acid; 1, formic acid; 2, acetic acid; 3, propionic acid; 4, isobutyric+butyric acids; 5, isovaleric acid; 6, valeric acid; 7, isocaproic acid; 8, caproic acid; 9, 2-methylhexanoic acid; 10, heptanoic acid.

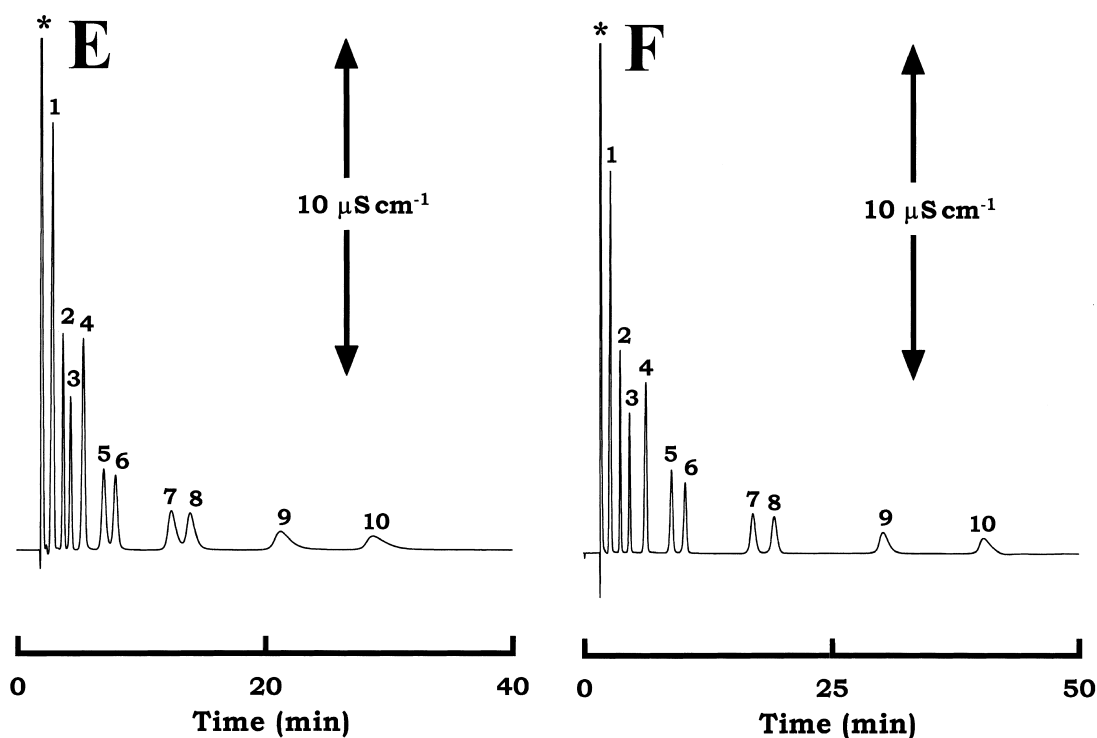


Fig. 1. (continued)

column were shorter than those on the TSKgel CM-2SW column. This is because the TSKgel SP-2SW is strongly acidic cation-exchanger and the TSKgel CM-2SW is weakly acidic cation-exchanger, and, then, these  $C_1$ – $C_7$  acids, which were partly dissociated under the ion-exclusion chromatographic conditions, were strongly excluded from the TSKgel SP-2SW by the electrostatic repulsion, in comparison to TSKgel CM-2SW. Unfortunately, peaks of higher aliphatic carboxylic acids were tailed strongly. Although no conclusive reasons for the explanation on the peak tailing, it was evident that these silica-based cation-exchange resins were not applicable for the ion-exclusion chromatography of these  $C_1$ – $C_7$  aliphatic carboxylic acids when using diluted sulfuric acid as the eluent. The detailed study on the peak tailing will be the subject of future work.

Fig. 1D–F show chromatograms of these  $C_1$ – $C_7$  acids on a sulfonated polymethacrylate resin (TSKgel SP-5PW) column, a carboxylated polymethacrylate resin (TSKgel CM-5PW) column and a carboxylated polymethacrylate resin (TSKgel OA-

Pak A) column, respectively. Both excellent ion-exclusion chromatographic separation and good peak shapes for these  $C_1$ – $C_7$  acids were achieved on these columns. The retention behavior of these  $C_1$ – $C_7$  acids on these columns was very similar. These chromatograms strongly indicated that polymethacrylate-based cation-exchange resins were very suitable for the ion-exclusion chromatography of these  $C_1$ – $C_7$  acids. The retention times of these  $C_1$ – $C_7$  acids on these polymethacrylate-based cation-exchange resins columns were considerably longer than those on these silica-based cation-exchange resins columns. This is due mainly to large hydrophobicity of polymethacrylate resin in comparison to silica gel. The retention times of these  $C_1$ – $C_7$  acids on the TSKgel SP-5PW column were shorter than those on the TSKgel CM-5PW column. This is because the TSKgel SP-5PW is strongly acidic cation-exchanger and the TSKgel CM-5PW is weakly acidic cation-exchanger. Although the TSKgel CM-5PW and TSKgel OA-Pak A are carboxylated polymethacrylate resins having almost the

same cation-exchange capacity (ca. 0.1 mequiv  $\text{ml}^{-1}$ ), the retention times of these  $\text{C}_1$ – $\text{C}_7$  acids on the TSKgel OA-Pak A column were considerably longer than those on the TSKgel CM-5PW column. This might be due to differences in physical and chemical properties between these resins.

From these above results, it was concluded that polymethacrylate-based cation-exchange resins were the most suitable for the ion-exclusion chromatographic separation of these  $\text{C}_1$ – $\text{C}_7$  aliphatic carboxylic acids. As shown in Fig. 1E, excellent simultaneous separation of these  $\text{C}_1$ – $\text{C}_7$  aliphatic carboxylic acids could be achieved in 32 min on the TSKgel CM-5PW column using a 0.05 mM sulfuric acid as the eluent.

### 3.2. Ion-exclusion chromatographic behavior of benzenecarboxylic acids on various cation-exchange resin columns

Fig. 2A show chromatogram of several benzenecarboxylic acids (pyromellitic, trimellitic, hemimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic, salicylic acids and phenol) on the TSKgel SCX column using a 2.5 mM sulfuric acid at pH 2.4 as the eluent. Although good separation and symmetrical peaks for pyromellitic, trimellitic, hemimellitic and *o*-phthalic acids were achieved, peak shapes of other benzenecarboxylic acids were tailed strongly. Furthermore, the retention times of other benzenecarboxylic acids were extremely long. The retention time of salicylic acid reached 100 min. The ion-exclusion chromatographic behavior of these benzenecarboxylic acids was due mainly to strongly hydrophobic interaction between these benzenecarboxylic acids and the TSKgel SCX resin. This chromatogram also suggested that both excellent ion-exclusion chromatographic separation and symmetrical peaks for these benzenecarboxylic acids could not be achieved on the TSKgel SCX column in a reasonable period of time (ca. 30 min) when using sulfuric acid as the eluent.

Fig. 2B and C show chromatograms of these benzenecarboxylic acids on the TSKgel SP-2SW and TSKgel CM-2SW columns, respectively. Both good separation and good peak shapes for these benzenecarboxylic acids were achieved in 20 min. These chromatograms strongly suggested that silica-based

cation-exchange resins were very suitable for the ion-exclusion chromatographic separation of these benzenecarboxylic acids. Chromatogram on the TSKgel SP-2SW column was considerably different from that on the TSKgel CM-2SW column. Perhaps, this might be because these benzenecarboxylic acids were adsorbed on both the surface of silica gel and functional group bonded to the silica gel. A detailed study on the ion-exclusion chromatographic behavior of these benzenecarboxylic acids on the TSKgel SP-2SW and CM-2SW columns will be the subject of future work.

Fig. 2D–F show chromatograms of these benzenecarboxylic acids on the TSKgel SP-5PW, TSKgel CM-5PW and TSKgel OA-Pak A columns, respectively. Similar chromatograms of these benzenecarboxylic acids were obtained on these columns. This might be because the affinity of these benzenecarboxylic acids for polymethacrylate resin was very strong, and, as a consequence, these benzenecarboxylic acids were mainly retained on the surface of polymethacrylate resin. Due to the strong affinity, very long chromatographic time was required for the elution of these benzenecarboxylic acids. It took ca. 90 min for the elution of salicylic acids from the TSKgel OA-Pak A column. The retention times of these benzenecarboxylic acids on the TSKgel SP-5PW column was shorter than those on the TSKgel CM-5PW column. This is because the TSKgel SP-5PW is strongly acidic cation-exchanger and the TSK gel CM-5PW is the weakly acidic cation-exchanger. The difference of the retention volumes of these benzenecarboxylic acids between the TSKgel CM-5PW and TSKgel OA-Pak A columns was attributed to be differences in physical and chemical properties between these resins. Unfortunately, due to very long chromatographic time, polymethacrylate-based cation-exchange resins were not applicable for the ion-exclusion chromatographic separation of these benzenecarboxylic acids.

From these above results, it was concluded that silica-based cation-exchange resins were the most suitable for the ion-exclusion chromatographic separation of these benzenecarboxylic acids. As shown in Fig. 2B, excellent simultaneous separation and symmetrical peaks for these benzenecarboxylic acids were achieved in 17 min on the TSKgel SP-2SW column using a 2.5 mM sulfuric acid as the eluent.

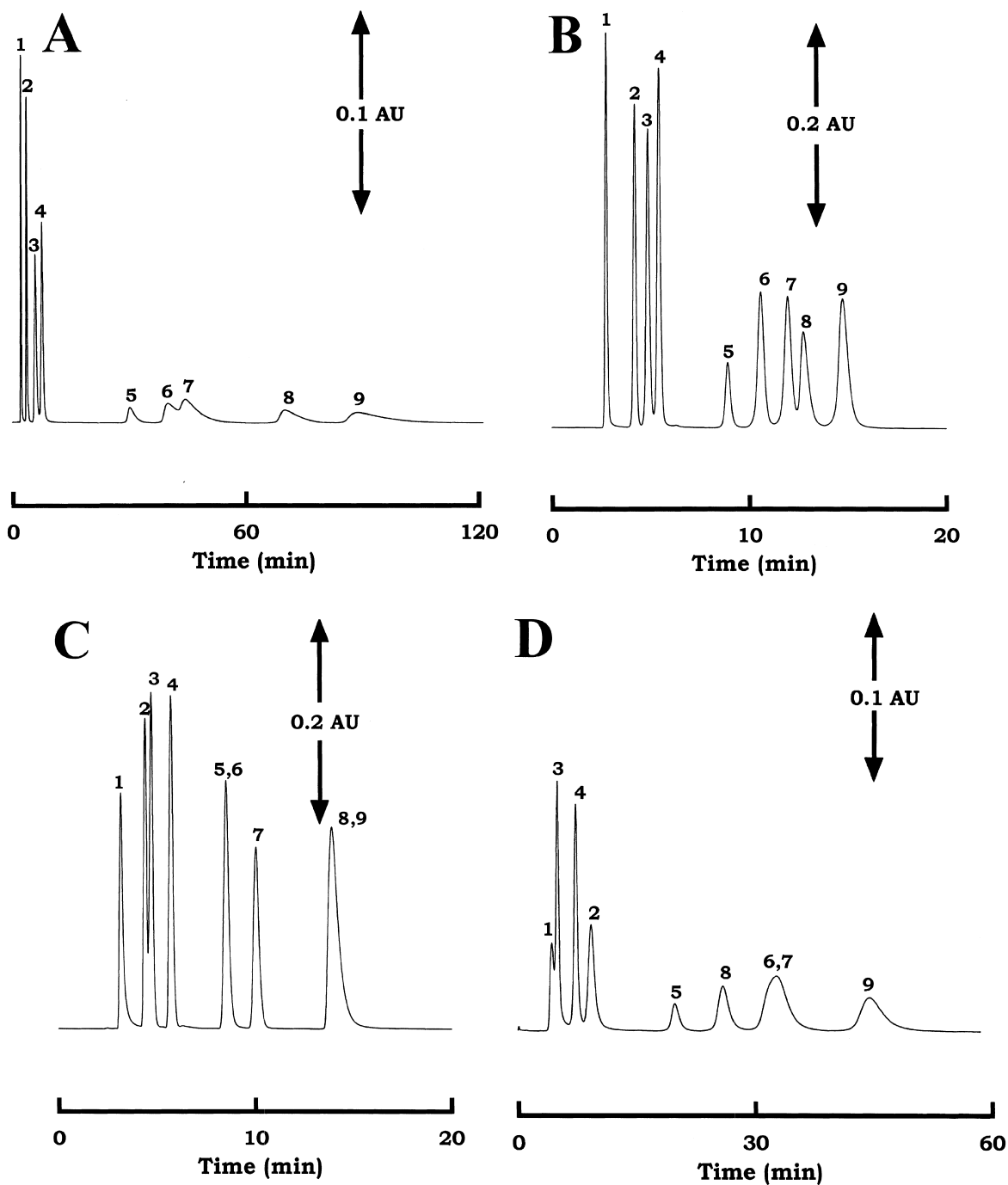


Fig. 2. Chromatograms of benzenecarboxylic acids on various cation-exchange resins columns. Conditions: columns, (A) TSKgel SCX, (B) TSKgel SP-2SW, (C) TSKgel CM-2SW, (D) TSKgel SP-5PW, (E) TSKgel CM-5PW, and (F) TSKgel OA-Pak A; eluent, 2.5 mM sulfuric acid at pH 2.4; detection, UV at 200 nm; sample concentration, 0.02 mM. 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. 1.

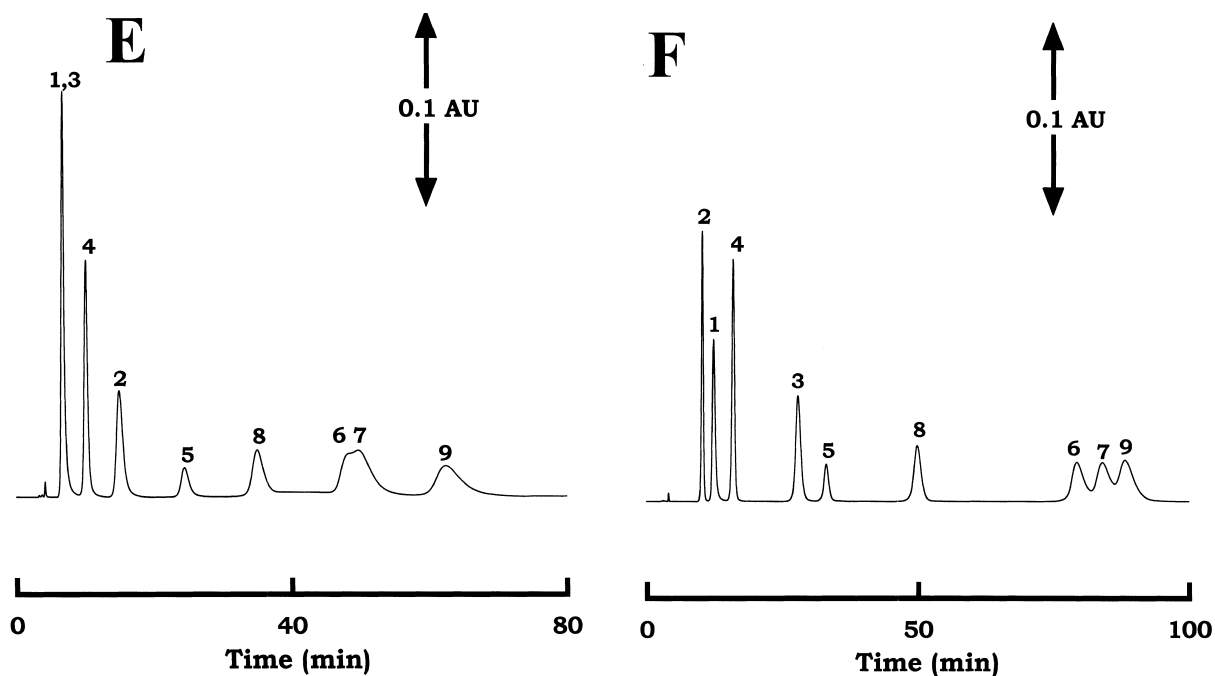


Fig. 2. (continued)

#### 4. Conclusions

In order to demonstrate the effectiveness of ion-exclusion chromatography for the determination of hydrophobic carboxylic acids, the application of various commercially available hydrophilic cation-exchange resins (sulfonated silica gel: TSKgel SP-2SW, carboxylated silica gel: TSKgel CM-2SW, sulfonated polymethacrylate resin: TSKgel SP-5PW, carboxylated polymethacrylate resin TSKgel CM-5PW and TSKgel OA-Pak A) for high performance liquid chromatography as the stationary phases in ion-exclusion chromatographic separations of both  $C_1$ – $C_7$  aliphatic carboxylic acids (formic, acetic, propionic, butyric, isovaleric, valeric, isocaproic, caproic, 2-methylhexanoic and heptanoic acids) and benzenecarboxylic acids (pyromellitic, trimellitic, hemimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic, salicylic acids and phenol) was carried out using diluted sulfuric acid as the eluent.

As a result, it was found: (a) silica-based cation-exchange resins (TSKgel SP-2SW and TSKgel CM-2SW) were one of the most suitable stationary

phases in ion-exclusion chromatography for the separation of these benzenecarboxylic acids; and (b) polymethacrylate-based cation-exchange resins (TSKgel SP-5PW, TSKgel CM-5PW and TSKgel OA-Pak A) were one of the most suitable stationary phases in ion-exclusion chromatography for the separation of these for  $C_1$ – $C_7$  carboxylic acids. These results contribute largely the development of advanced ion-exclusion chromatography for carboxylic acids.

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