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Separation and conductimetric detection of C_1–C_7 aliphatic monocarboxylic acids and C_1–C_7 aliphatic monoamines on unfunctionized polymethacrylate resin columns

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Abstract

The application of unfunctionized polymethacrylate resin (TSKgel G3000PW$_{XL}$) as a stationary phase in liquid chromatography with conductimetric detection for C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) and C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) was attempted with C_8 aliphatic monocarboxylic acids (2-propylvaleric acid, 2-ethylhexanoic acid, 2-methylheptanoic acid and octanoic acid) and C_8 aliphatic monoamines (1,5-dimethylhexylamine, 2-ethylhexylamine, 1-methylheptylamine and octylamine) as eluents, respectively. Using 1 mM 2-methylheptanoic acid at pH 4.0 as the eluent, excellent separation and relatively high sensitive detection for these C_1–C_7 carboxylic acids were achieved on a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.) in 60 min. Using 2 mM octylamine at pH 11.0 as the eluent, excellent separation and relatively high sensitive detection for these C_1–C_7 amines were also achieved on the TSKgel G3000PW$_{XL}$ column in 60 min.

Keywords: Stationary phases, LC; Carboxylic acids; Amines; Polymethacrylate; Methylheptanoic acid; Octylamine

1. Introduction

Ion-exclusion chromatography developed by Wheaton and Baumann [1] is commonly employed as a simple and convenient analytical method for the determination of low-molecular-mass organic acids (carboxylic acids) [2,3]. Low cross-linked styrene–divinylbenzene co-polymer (PS–DVB)-based strongly acidic cation-exchange resin in the H^+-form is mainly utilized as the stationary phase in for carboxylic acids. Ion-exclusion chromatographic separation of low-molecular-mass organic bases (amines) is also possible. The separation mainly proceeds on low cross-linked PS–DVB-based strongly basic anion-exchange resin in the OH^--form as the stationary phase [2,4].

In early works, water was used as the eluent in ion-exclusion chromatography for carboxylic acids and amines [2,4,5]. Largely fronted peaks of carboxylic acids and amines were obtained. This is because carboxylic acids and amines were well dissociated, consequently, carboxylic acids and amines were strongly excluded from stationary phases by electrostatic repulsion. Acidic and basic solutions were utilized as the eluents for improving peak shapes of carboxylic acids and amines, respectively [6,7]. Peak shapes of hydrophilic carboxylic acids and amines were improved drastically. In contrast, largely tailed peaks and extremely large retention volumes for hydrophobic carboxylic acids and amines were obtained, because of suppressing their dissociation. The addition of organic solvent to the eluent was very effective way for improving peak shapes and reducing the retention volumes for hydrophobic carboxylic acids [8–10] and amines [4,7]. However, the concentration of organic solvent added to eluent is strongly limited, because shrinkage of these low cross-linked PS–DVB resins occurs.

Recently, Li and Fritz applied an unfunctionized high cross-linked PS–DVB resin as the stationary phase in liquid chromatography (LC) for carboxylic acids and amines

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[11]. Good separations of several carboxylic acids and amines were achieved on the resin by only hydrophobic interaction process. This result suggested that hydrophobic interaction would be main separation mechanism in ion-exclusion chromatography for carboxylic acids and amines. Unfortunately, due to large hydrophobicity, aquatic solution containing large amount of organic solvent was required as the eluent for utilizing this type resin. Hydrophilic unfunctionized polymer resin (polymethacrylate resin) was expected to be one of the most suitable stationary phases in LC with aquatic solution as the eluent for carboxylic acids and amines. However, the application has not been carried out yet.

The aim of this study was to demonstrate the effectiveness of an unfunctionized polymethacrylate resin as the stationary phase in LC for carboxylic acids and amines. Then, the application of this type resin (TSKgel G3000PW$_{XL}$) column (150 mm × 6 mm i.d.) in LC with conductimetric detection for C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) and C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) was attempted. Excellent separation, relatively high sensitive detection and symmetrical peak shapes for these C_1–C_7 carboxylic acids and those for these C_1–C_7 amines were achieved in 60 min with 1 mM C_8 carboxylic acid (2-methylheptanoic acid) at pH 4.0 and 2 mM C_8 amine (octylamine) at pH 11.0 as the eluents, respectively.

2. Experimental

2.1. Instruments

The liquid 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 CO-8020 column oven operated at 35 °C, a Tosoh CM-8020 conductimetric detector, a Tosoh SD-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 equipped with a glass electrode was used for the measurement of pH of eluents. A Toa Denpa CM-30S conductimetric detector was also employed for the measurement of conductivities of the eluents.

2.2. Separation column

A separation column (150 mm × 6 mm i.d., stainless steel) packed with a Tosoh TSKgel G3000PW$_{XL}$ unfunctionized polymethacrylate resin (particle size of ca. 7 μm) by using slurry-packing method was used in this work.

The TSKgel G3000PW$_{XL}$ resin shows small cation-exchange behavior under basic condition, due to small amount of carboxylic group on the resin, originated from starting materials [12]. The determination of the cation-exchange capacity of the resin was carried out by use of the following cation-exchange reaction;

$$\text{Resin–COO}^-\text{Na}^+ + \text{KOH} \rightarrow \text{Resin–COO}^-\text{K}^+ + \text{NaOH}$$

The cation-exchange capacity (A, meq. ml^{-1}) of the resin was calculated from the equations:

$$A = (V_R - V_o)\frac{C}{1000\,V}$$

where V_R is the breakthrough volume of the column (ml), V_o the total dead volume (column void volume+connected tube volume, ml), C the concentration of potassium hydroxide solution (mM) and V is column volume (4.24 ml).

Fig. 1 shows a titration curve of the TSKgel G3000PW$_{XL}$ column. First, the column was equilibrated with 1 mM sodium hydroxide solution. Sample of 1 mM sodium hydroxide solution containing 1% methanol was injected. Elution volume of peak corresponding to methanol was considered as V_o (3.34 ml). Next, 1 mM potassium hydroxide solution was passed through the column and the conductimetric detector response (breakthrough curve) was monitored. Volume corresponding to breakthrough point in the detector response curve was considered as V_R (49.0 ml).

The amount of cation-exchange capacity of the TSKgel G3000PW$_{XL}$ resin was ca. 0.011 meq. ml^{-1}.

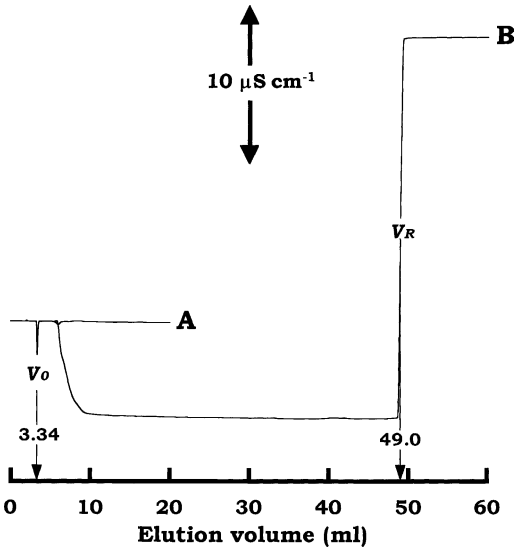

Fig. 1. Titration curve of TSKgel G3000PW$_{XL}$ column. Conditions: column: TSKgel G3000PW$_{XL}$, column size: 150 mm × 6 mm i.d., column temperature: 35 °C, eluents: (A) 1 mM NaOH, (B) 1 mM KOH, flow rate: 1 ml min^{-1}, detection: conductivity, injection volume: 20 μl, sample: 1% methanol in 1 mM NaOH, V_o: total dead volume (column void volume + connected tube volume, 3.34 ml), V_R: breakthrough volume (49.0 ml).

2.3. Chemicals

All chemicals were of analytical regent grade and were purchased form 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. Chromatographic behavior of C_1–C_7 carboxylic acids on TSKgel G3000PW$_{XL}$ column

3.1.1. Effect of C_8 carboxylic acids as eluent on chromatographic behavior of C_1–C_7 carboxylic acids

Firstly, the application of an unfunctionized polymethacrylate resin (TSKgel G3000PW$_{XL}$) as a stationary phase in liquid chromatography with conductimetric detection (LC–CD) for C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) was attempted with dilute sulfuric acid as an eluent. Fig. 2 shows a chromatogram of these C_1–C_7 carboxylic acids on a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.) with 0.05 mM sulfuric acid at pH 4.0 (eluent conductivity: 39 μS cm^{-1}) as the eluent.

As shown in Fig. 2, excellent separation of these C_1–C_7 carboxylic acids was achieved in 50 min. Elution order was C_1 acid (formic acid) < C_2 acid (acetic acid) < C_3 acid (propionic acid) < C_4 acid (butyric acid) < C_5 acids (isovaleric acid < valeric acid) < C_6 acids (3,3-dimethylbutyric acid < 4-methylvaleric acid < hexanoic acid) < C_7 acids (2-methylhexanoic acid < 5-methylhexanoic acid < heptanoic acid). These results indicated that (a) the resin was a very suitable stationary phase for the separation of these C_1–C_7 carboxylic acids and (b) these C_1–C_7 carboxylic acids were mainly separated by a hydrophobic interaction process. Unfortunately, (a) a system peak corresponding to sulfuric acid in the eluent appeared and interfered seriously for the determination of formic acid and (b) peaks of higher carboxylic acids (C_7 acids) were tailed largely. Therefore, a further investigation was required for eliminating these above drawbacks.

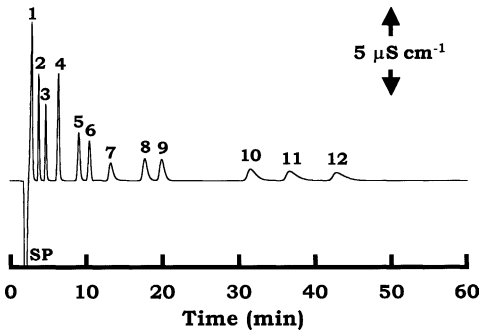

Fig. 2. Chromatograms of C_1–C_7 carboxylic acids with sulfuric acid as eluent. Conditions: eluent: 0.05 mM sulfuric acid at pH 4.0 (eluent conductivity: 39 μS cm^{-1}), injection volume: 100 μl, sample: 0.1 mM for formic acid and 0.2 mM for other carboxylic acids in water. Other chromatographic conditions as in Fig. 1. Peaks: (1) formic acid, (2) acetic acid, (3) propionic acid, (4) isobutyric and butyric acids, (5) isovaleric acid, (6) valeric acid, (7) 3,3-dimethylbutyric acid, (8) 4-methylvaleric acid, (9) hexanoic acid, (10) 2-methylhexanoic acid, (11) 5-methylhexanoic acid, (12) heptanoic acid, SP, system peak corresponding to sulfuric acid in eluent.

In previous study, we demonstrated the effectiveness of C_7 carboxylic acid (5-methylhexanoic acid) as the eluent in ion-exclusion chromatography with conductimetric detection for C_1–C_6 carboxylic acids (formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, 4-methylvaleric acid and hexanoic acid) on a sulfonated PS–DVB resin (TSKgel SCX) column [13]. 5-Methylhexanoic acid in the eluent was strongly adsorbed on the resin at first and then reduced hydrophobic interaction between the resin and these carboxylic acids. As a result, although a system peak corresponding to 5-methylhexanoic acid in the eluent appeared, excellent separation, relatively high sensitive detection and symmetrical peaks for these C_1–C_6 carboxylic acids were achieved on a TSKgel SCX column (150 mm × 6 mm i.d.) in a reasonable chromatographic time (≤25 min). Hence, the application of C_8 carboxylic acids (2-propylvaleric acid, 2-ethylhexanoic acid, 2-methylheptanoic acid and octanoic acid) as the eluents in the LC–CD was carried out. Fig. 3A–D show chromatograms of these C_1–C_7 carboxylic acids on the TSKgel G3000PW$_{XL}$ column with (A) 1 mM 2-propylvaleric acid at pH 4.0 (50 μS cm^{-1}), (B) 1 mM 2-ethylhexanoic acid at pH 4.0 (49 μS cm^{-1}), (C) 1 mM 2-methylheptanoic acid at pH 4.0 (46 μS cm^{-1}) and (D) 1 mM octanoic acid at pH 4.0 (43 μS cm^{-1}) as the eluents.

As shown in Figs. 2 and 3A–D, the retention times of higher carboxylic acids (C_6–C_7 acids) with the C_8 carboxylic acids as the eluents were considerably shorter than those with 0.0 mM sulfuric acid as the eluent. System peaks corresponding to the C_8 carboxylic acids in the eluent appeared and their retention times were longer than that of the C_7 carboxylic acid (heptanoic acid). These results indicated clearly that the C_8 carboxylic acids were strongly adsorbed on the TSKgel G3000PW$_{XL}$ resin at first and then reduced hydrophobic interaction between the resin and these carboxylic acids, effectively. As shown in Fig. 3A and B, when using 1 mM 2-propylvaleric acid or 1 mM 2-ethylhexanoic acid as the eluents, system peaks corresponding to 2-propylvaleric acid or 2-ethylhexanoic acid interfered for the determination of heptanoic acid. As show in Fig. 3C and D, when using 1 mM 2-methylheptanoic acid or 1 mM octanoic acid as the eluents, no interferences of system peaks corresponding to 2-methylheptanoic acid or octanoic acid were observed. However, as shown in Fig. 3D, since the retention time of the system peak corresponding to octanoic acid was ca. 66 min, it took

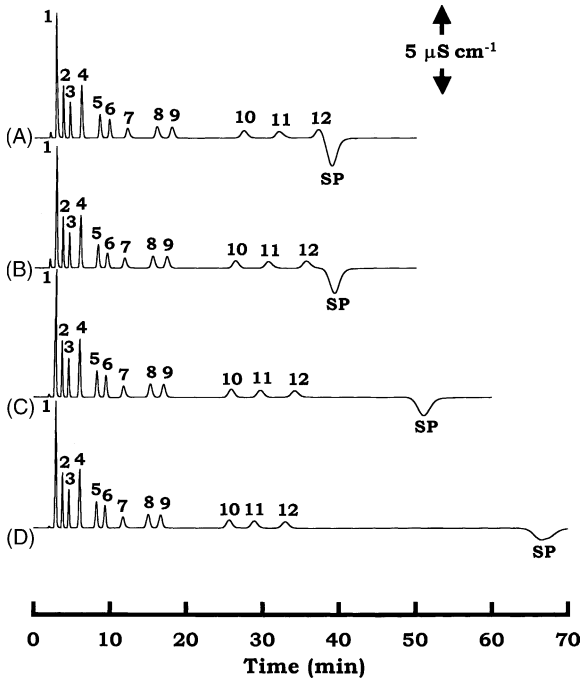

Fig. 3. Chromatograms of C_1–C_7 carboxylic acids with various C_8 carboxylic acids as eluents. Conditions: eluents: (A) 1 mM 2-propylvaleric acid at pH 4.0 (50 μS cm^{-1}), (B) 1 mM 2-ethylhexanoic acid at pH 4.0 (49 μS cm^{-1}), (C) 1 mM 2-methylheptanoic acid at pH 4.0 (46 μS cm^{-1}), (D) 1 mM octanoic acid at pH 4.0 (43 μS cm^{-1}). Other chromatographic conditions as in Fig. 2. Peaks: (SP), system peak corresponding to C_8 acid in eluent. Other peak identifications as in Fig. 2.

a very long time (ca. 70 min) for each chromatographic run.

Considering peak shape, interference of system peak and chromatographic time, the most suitable eluent was 2-methylheptanoic acid in LC–CD for these C_1–C_7 carboxylic acids (Fig. 3C).

3.1.2. Effect of concentration of 2-methylheptanoic acid in eluent on chromatographic behavior of C_1–C_7 carboxylic acids

The effect of the concentration of 2-methylheptanoic acid in the eluent on the chromatographic behavior of these C_1–C_7 carboxylic acids was investigated. Fig. 4 shows the relationship between the concentration of 2-methylheptanoic acid and the retention volumes of these C_1–C_7 carboxylic acids. Fig. 5A–D show chromatograms of these C_1–C_7 carboxylic acids with (A) 0.125 mM 2-methylheptanoic acid at pH 4.5 (15 μS cm^{-1}), (B) 0.25 mM 2-methylheptanoic acid at pH 4.3 (22 μS cm^{-1}), (C) 0.5 mM 2-methylheptanoic acid at pH 4.1 (31 μS cm^{-1}) and (D) 2 mM 2-methylheptanoic acid at pH 3.8 (62 μS cm^{-1}) as the eluents. A chromatogram with 1 mM 2-methylheptanoic acid as the eluent has been already shown in Fig. 3C.

As shown in Fig. 4, the retention volumes of these C_1–C_7 carboxylic acids were almost no variations at the concentration range of 2-methylheptanoic acid between 0.125 and

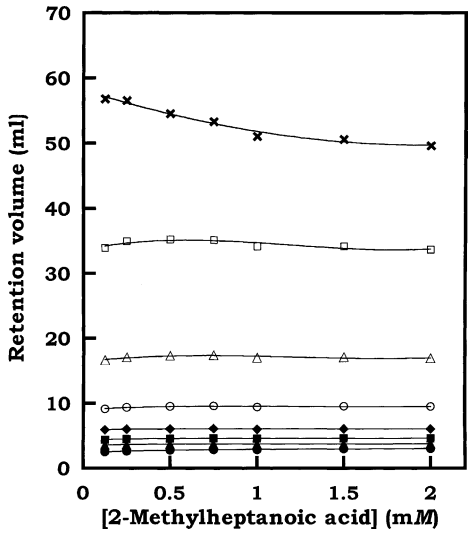

Fig. 4. Effect of concentration of 2-methylheptanoic acid in eluent on retention volumes of C_1–C_7 carboxylic acids. Conditions: eluent: 0.125–2 mM 2-methylheptanoic acid. Other chromatographic conditions as in Fig. 3. Symbols: (●) formic acid, (▲) acetic acid, (■) propionic acid, (◆) butyric acid, (○) valeric acid, (△) caproic acid, (□) heptanoic acid, (✖) system peak corresponding to 2-methylheptanoic acid in eluent.

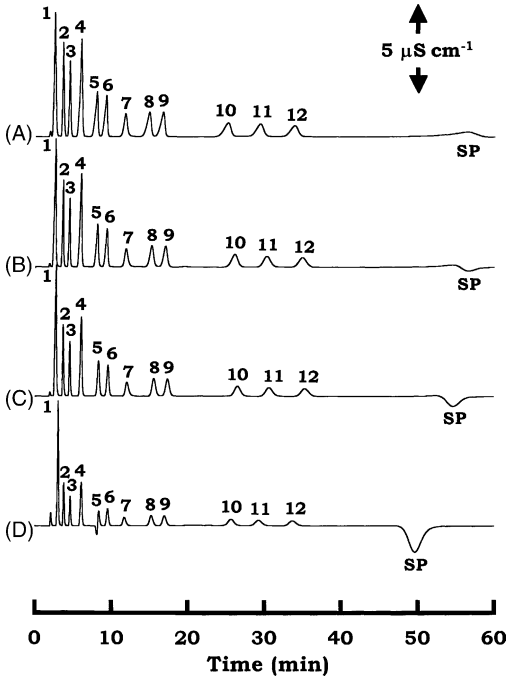

Fig. 5. Chromatograms of C_1–C_7 carboxylic acids with various concentrations of 2-methylheptanoic acid as eluents. Conditions: eluent: (A) 0.125 mM 2-methylheptanoic acid at pH 4.5 (15 μS cm^{-1}), (B) 0.25 mM 2-methylheptanoic acid at pH 4.3 (22 μS cm^{-1}), (C) 0.5 mM 2-methylheptanoic acid at pH 4.1 (31 μS cm^{-1}), (D) 2 mM 2-methylheptanoic acid at pH 3.8 (62 μS cm^{-1}). Other chromatographic conditions as in Fig. 4. Peaks: (SP), system peak corresponding to 2-methylheptanoic acid in eluent. Other peak identification as in Fig. 2.

2 mM. This might be due to (a) narrow pH range of eluent (pH 4.5–3.8) and (b) relatively weak hydrophobic interaction between the resin and these C_1–C_7 carboxylic acids. In contrast, with increasing the concentration of 2-methylheptanoic acid, the retention volume of the system peak corresponding to 2-methylheptanoic acid decreased remarkably. As shown in Figs. 3C and 5A–D, with increasing the concentration of 2-methylheptanoic acid, peak shapes of these C_1–C_7 carboxylic acids were improved. As shown in Fig. 5A, when using 0.125 mM 2-methylheptanoic acid as the eluent, largely fronted peaks of these C_1–C_7 carboxylic acids were observed. This is because these C_1–C_7 carboxylic acids were well dissociated in the conditions. Symmetrical peaks of these C_1–C_7 carboxylic acids were obtained at the concentration of 2-methylheptanoic acid $\geq$ 1 mM. In contrast, with increasing the concentration of 2-methylheptanoic acid, the detection sensitivities of these C_1–C_7 carboxylic acids decreased. This is due mainly to (a) an increase in the eluent conductivity and (b) a decrease in the conductimetric detector responses of these C_1–C_7 carboxylic acids caused by suppressing their dissociation.

Considering peak shape, peak resolution detection sensitivity and chromatographic time, the optimum concentration of 2-methylheptanoic acid was 1 mM in the LC–CD for these C_1–C_7 carboxylic acids (Fig. 3C).

3.1.3. Analytical performance parameters

Table 1 shows the detection limits (signal-to-noise ratio of 3, injection volume of 100 μl) of these C_1–C_7 carboxylic acids. Relatively high sensitive conductimetric detection was achieved by the proposed LC–CD method. The main reasons were that (a) the eluent conductivity was considerably low (46 $\mu S\,cm^{-1}$, noise 1.3×10^{-3} $\mu S\,cm^{-1}$) and (b) these carboxylic acids were partly dissociated under the optimum LC conditions.

Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of these C_1–C_7 carboxylic acids. Linear calibration graphs ($r^2 \geq$ 0.99) were obtained in the concentration range between 0.005 and 1.5 mM for these carboxylic acids.

The relative standard deviations of the chromatographic peak area of these C_1–C_7 carboxylic acids, whose concentrations were 0.1 mM for formic acids and 0.2 mM for the other carboxylic acids, were less than 0.6% ($n = 10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

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

Carboxylic acid	Detection limit	
	μM	ng ml^{-1}
Formic acid	0.057	2.6
Acetic acid	0.26	15
Propionic acid	0.37	27
Butyric acid	0.50	44
Isovaleric acid	0.55	56
Valeric acid	0.66	67
3,3-Dimethylbutyric acid	1.3	1.5×10^2
4-Methylvaleric acid	1.1	1.2×10^2
Hexanoic acid	1.1	1.3×10^2
2-Methylhexanoic acid	1.8	2.3×10^2
5-Methylhexanoic acid	2.0	2.6×10^2
Heptanoic acid	2.2	2.8×10^2

3.2. Chromatographic behavior of C_1–C_7 amines on TSKgel G3000PW$_{XL}$ column

3.2.1. Effect of C_8 amines in eluent on chromatographic behavior of C_1–C_7 amines

The application of the TSKgel G3000PW$_{XL}$ resin as the stationary phase in LC–CD for C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) was attempted with dilute C_8 aliphatic monoamines (1,5-dimethylhexylamine, 2-ethylhexylamine, 1-methylheptylamine and octylamine) as the eluents. Fig. 6A–D show chromatograms of these C_1–C_7 amines on the TSKgel G3000PW$_{XL}$ column with (A) 2 mM 1,5-dimethylhexylamine at pH 10.9 (148 $\mu S\,cm^{-1}$), (B) 2 mM 2-ethylhexylamine

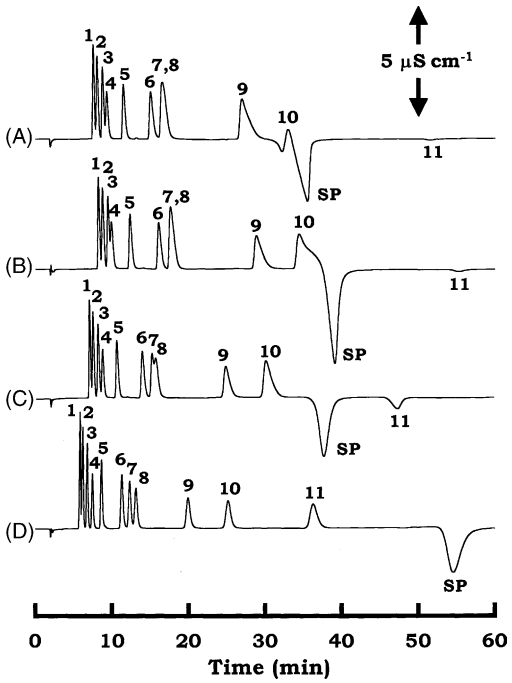

Fig. 6. Chromatograms of C_1–C_7 amines with various C_8 amines acids as eluents. Conditions: eluents: (A) 2 mM 1,5-dimethylhexylamine at pH 10.9 (148 $\mu S\,cm^{-1}$), (B) 2 mM 2-ethylhexylamine at pH 10.9 (152 $\mu S\,cm^{-1}$), (C) 2 mM 1-methylheptylamine at pH 11.0 (162 $\mu S\,cm^{-1}$), (D) 2 mM octylamine at pH 11.0 (170 $\mu S\,cm^{-1}$); sample: 0.2 mM amines in water. Other chromatographic conditions as in Fig. 5. Peaks: (1) methylamine, (2) ethylamine, (3) propylamine, (4) isobutylamine, (5) butylamine, (6) isoamylamine, (7) amylamine, (8) 1,3-dimethylbutylamine, (9) hexylamine, (10) 2-heptylamine, (11) heptylamine, (SP) system peak corresponding to C_8 amine in eluent.

at pH 10.9 (152 μS cm^{-1}), (C) 2 mM 1-methylheptylamine at pH 11.0 (162 μS cm^{-1}) and 2 mM octylamine at pH 11.0 (170 μS cm^{-1}) as the eluents.

As shown in Fig. 6A and B, when using 2 mM 1,5-dimethylhexylamine or 2 mM 2-ethylhexylamine as the eluents, the retention times of system peaks corresponding to 1,5-dimethylhexylamine or 2-ethylhexylamine in the eluent were closed to that of 2-heptylamine and so the system peaks interfered seriously for the determination of 2-heptylamine. Both extremely low detector response of heptylamine and completely overlapped peaks of amylamine and 1,3-dimethylbutylamine were observed. As shown in Fig. 6C, when using 2 mM 1-methylheptylamine as the eluent, although no interferences of a system peak corresponding to 1-methylheptylamine for the determination of these C_1–C_7 amines were observed, peak resolution between amylamine and 1,3-dimethylbutylamine was still very poor. Heptylamine was detected indirect-conductimetrically. In contrast, as shown in Fig. 6D, when using 2 mM octylamine as the eluent, excellent separation and symmetrical peaks for these C_1–C_7 amines were achieved in 60 min with no interferences of a system peak corresponding to octylamine.

Considering peak shape, interference of system peak and chromatographic time, the most suitable eluent was octylamine in the LC–CD for these C_1–C_7 amines (Fig. 6D).

3.2.2. *Effect of concentration of octylamine in the eluent on chromatographic behavior of C_1–C_7 amines*

The effect of the concentration of octylamine in the eluent on the chromatographic behavior of these C_1–C_7 amines was investigated. Fig. 7 shows the relationship between the concentration of octylamine and the retention volumes of these C_1–C_7 amines. Fig. 8A–D show chromatograms of these C_1–C_7 amines with (A) 0.25 mM octylamine at pH 10.2 (37 μS cm^{-1}), (B) 0.5 mM octylamine at pH 10.5 (65 μS cm^{-1}), (C) 1 mM octylamine at pH 10.8 (107 μS cm^{-1}) and (D) 4 mM octylamine at pH 11.2 (250 μS cm^{-1}) as the eluents. A chromatogram with 2 mM octylamine as the eluent has been already shown in Fig. 6D. As shown in Fig. 7, with increasing the concentration of octylamine, the retention volumes of these C_1–C_7 amines decreased drastically, whereas the retention volume of system peak corresponding to octylamine increased. The retention behavior of these C_1–C_7 amines and the system peak on the TSKgel G3000PW$_{XL}$ column with octylamine as the eluent was quite different from that of these C_1–C_7 carboxylic acids and the system peak corresponding to 2-methylheptanoic acid on the column with 2-methylheptanoic acid as the eluent (Fig. 4). The drastic decrease in the retention volumes of these C_1–C_7 amines might be attributed to (a) an increase in concentration of octylammonium ion as competing ion in the eluent and (b) the low cation-exchange capacity of the TSKgel G3000PW$_{XL}$ resin (cation-exchange capacity: ca. 0.011 meq. ml^{-1} at pH 11.0) under basic eluent condition [12]. These results suggested that these C_1–C_7 amines were separated on the TSKgel G3000PW$_{XL}$ column by not only hydrophobic interaction process but also cation-exchange process. A detailed study on the clarification of the retention behavior of these C_1–C_7 amines will be the subject

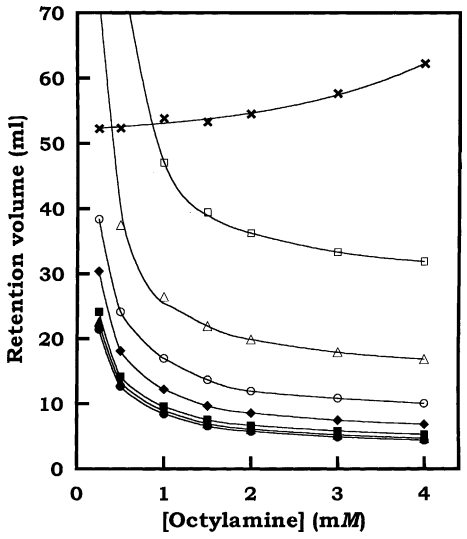

Fig. 7. Effect of concentration of octylamine in eluent on retention volumes of C_1–C_7 amines. Conditions: eluent: 0.25–4 mM octylamine. Other chromatographic conditions as in Fig. 6. Symbols: (●) methylamine, (▲) ethylamine, (■) propylamine, (◆) butylamine, (○) amylamine, (△) hexylamine, (□) heptylamine, (✖) system peak corresponding to octylamine in eluent.

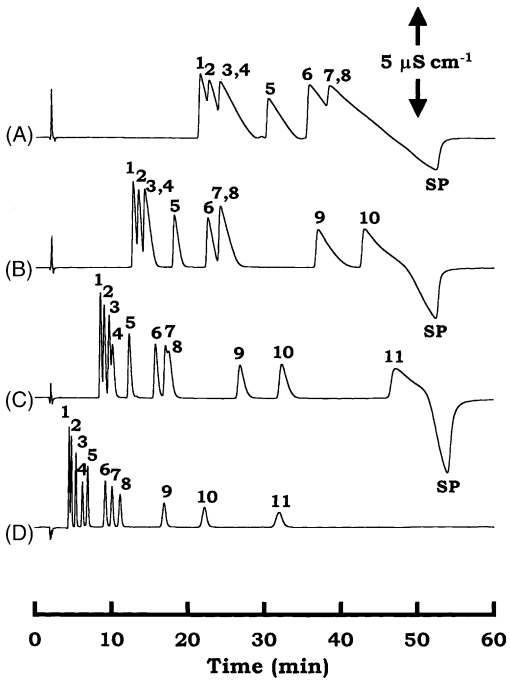

Fig. 8. Chromatograms of C_1–C_7 amines with various concentrations of octylamine as eluents. Conditions: eluents: (A) 0.25 mM octylamine at pH 10.2 (37 μS cm^{-1}), (B) 0.5 mM octylamine at pH 10.5 (65 μS cm^{-1}), (C) 1 mM octylamine at pH 10.8 (107 μS cm^{-1}), (D) 4 mM octylamine at pH 11.2 (250 μS cm^{-1}). Other chromatographic conditions as in Fig. 7. Peaks: (SP), system peak corresponding to octylamine in eluent. Other peaks identification is as in Fig. 6.

Table 2
Detection limits (signal-to-noise ratio of 3, injection volume of 100 μl) of C_1–C_7 amines

Amine	Detection limit	
	μM	ng ml^{-1}
Methylamine	0.23	7.3
Ethylamine	0.27	12
Propylamine	0.32	19
Isobutylamine	0.50	36
Butylamine	0.40	29
Isoamylamine	0.51	44
Amylamine	0.58	51
1,3-Dimethylbutylamine	0.68	69
Hexylamine	0.90	91
2-Heptylamine	0.99	1.2×10^2
Heptylamine	1.2	1.3×10^2

of future work. As shown in Fig. 6D and Fig. 8A–D, with changing the concentration of octylamine, the chromatographic behavior of these C_1–C_7 amines changed dramatically. With increasing the concentration of octylamine, peak shapes of these C_1–C_7 amines were improved largely. Symmetrical peaks of these C_1–C_7 amines were obtained at the concentration of octylamine $\geq$ 2 mM. Peak resolution between these C_1–C_7 amines and the system peak was also improved. No interferences of the system peak for the determination of these C_1–C_7 amines were observed at the concentration of octylamine $\geq$ 1.5 mM. In contract, with increasing the concentration of octylamine, the detection sensitivities of these C_1–C_7 amines decreased. This is due mainly to (a) an increase in the eluent conductivity and (b) a decrease in the conductimetric detector responses of these C_1–C_7 amines, caused by suppressing their dissociation.

Considering peak shape, peak resolution, interference of system peak, detection sensitivity and chromatographic time, the optimum concentration of octylamine was 2 mM in the LC–CD for these C_1–C_7 amines (Fig. 6D).

3.2.3. Analytical performance parameters

Table 2 shows the detection limits (signal-to-noise ratio of 3, injection volume of 100 μl) of these C_1–C_7 amines. Relatively high sensitive detection was achieved by the proposed LC–CD method. The main reasons were that (a) the eluent conductivity was relatively low (170 μS cm^{-1}, noise 2.1×10^{-3} μS cm^{-1}) and (b) these amines were partly dissociated under the optimum LC conditions.

Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of these C_1–C_7 amines. Linear calibration graphs ($r^2 > 0.99$) were obtained in the concentration range between 0.005 and 2 mM for these amines.

The relative standard deviations of the chromatographic peak area of these C_1–C_7 amines, whose concentrations were 0.2 mM, were less than 0.6% ($n = 10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

4. Conclusions

Chromatographic behavior of C_1–C_7 aliphatic monocarboxylic acids (formic acid, acetic acid, propionic acid, butyric acid, isovaleric acid, valeric acid, 3,3-dimethylbutyric acid, 4-methylvaleric acid, hexanoic acid, 2-methylhexanoic acid, 5-methylhexanoic acid and heptanoic acid) and C_1–C_7 aliphatic monoamines (methylamine, ethylamine, propylamine, isobutylamine, butylamine, isoamylamine, amylamine, 1,3-dimethylbutylamine, hexylamine, 2-heptylamine and heptylamine) on an unfunctionized polymethacrylate resin (TSKgel G3000PW$_{XL}$) column (150 mm × 6 mm i.d.) was investigated, for demonstrating the effectiveness of this type resin as the stationary phase in LC of organic compounds. The resin behaved as an advanced stationary phase for these C_1–C_7 carboxylic acids and C_1–C_7 amines with C_8 carboxylic acid and C_8 amine as the eluents, respectively. Excellent separation, relatively high sensitive conductimetric detection and symmetrical peaks for these C_1–C_7 carboxylic acids were achieved in 60 min with 1 mM 2-methylheptanoic acid as the eluent. Excellent separation, relatively high sensitive conductimetric detection and symmetrical peaks for these C_1–C_7 amines were also achieved in 60 min with 2 mM octylamine as the eluent. These results expand largely the utility of this type resin as the stationary phase in LC of various organic compounds.

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