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# Retention behavior of $C_1$–$C_6$ aliphatic monoamines on anion-exchange and polymethacrylate resins with heptylamine as eluent

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**Abstract**

Retention behavior of $C_1$–$C_6$ aliphatic monoamines (methylamine, ethylamine, propylamine, butylamine, amylamine and hexylamine) on columns (150 mm × 6 mm i.d.) packed with various anion-exchange resins (styrene–divinylbenzene (PS–DVB) copolymer-based strongly basic anion-exchange resin: TSKgel SAX, polymethacrylate-based strongly basic anion-exchange resin: TSKgel SuperQ-5PW and polymethacrylate-based weakly basic anion-exchange resin: TSKgel DEAE-5PW) and unfunctionized polymethacrylate resins (TSKgel G5000PW and TSKgel G3000$PW_{XL}$) was investigated with basic solutions (sodium hydroxide and heptylamine) as the eluents. Due to strongly electrostatic repulsion (ion-exclusion effect) between these anion-exchange resins and these amines, peak resolution between these amines on these anion-exchange resin columns was unsatisfactory with both sodium hydroxide and heptylamine as the eluents. In contrast, these polymethacrylate resins were successfully applied as the stationary phases for the separation of these $C_1$–$C_6$ amines with heptylamine as eluent, because of both small hydrophobicity and small cation-exchange ability of these resins. Excellent simultaneous separation, highly sensitive conductimetric detection and symmetrical peaks for these $C_1$–$C_6$ amines were achieved on the TSKgel G3000$PW_{XL}$ column in 35 min with 5 mM heptylamine at pH 11.1 as the eluent.


*Keywords:* Retention behavior; Stationary phases, LC; Amines; Heptylamine

## 1. Introduction

Ion-exclusion chromatography developed by Wheaton and Baumann [1] is recognized as a simply and convenient analytical technique for the determination of low molecular weak acids (carboxylic acids) [2]. Carboxylic acids are mainly separated on cation-exchange resins (low cross-linked styrene–divinylbenzene (PS–DVB) copolymer-based strongly acidic cation-exchange resin and polymethacrylate-based weakly and strongly acidic cation-exchange resins) in the $H^+$ form as the stationary phase and acidic solution as the eluent [2,3]. The application of ion-exclusion chromatographic separation of low molecular weak bases (aliphatic and aromatic amines) was also attempted with both PS–DVB-based strongly basic anion-exchange resin in the $OH^-$ form as the stationary phase and basic solution as the eluent [4,5]. However, due to strongly hydrophobic interaction and electrostatic repulsion between this type resin and solute amines, separation of $C_1$–$C_6$ aliphatic monoamines (methylamine, ethylamine, propylamine, butylamine, amylamine and hexylamine) was not satisfactory. Furthermore, the application of various commercially available anion-exchange resins as the stationary phases in ion-exclusion chromatography for these $C_1$–$C_6$ aliphatic amines has not been carried out yet, for improving their peak resolution and peak shapes.

Recently, Li and Fritz applied unfunctionized high cross-linked PS–DVB resin as the stationary phase in liquid chromatography for aromatic amines [6]. Several amines were successful separated on the resin column by only hy-

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drophobic interaction process. This result implied that this type resin would be successfully applied for the separation of these aliphatic monoamines. Unfortunately, due to large hydrophobicity, aqueous 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 liquid chromatography for various aliphatic monoamines with aqueous solution as the eluent. However, the application has not been carried out yet.

The aim of this study was to develop a simple and effective chromatographic method for simultaneous separation and highly sensitive conductimetric detection for $C_1$–$C_6$ aliphatic monoamines (methylamine, ethylamine, propylamine, butylamine, amylamine and hexylamine). Then, chromatographic behavior of these $C_1$–$C_6$ amines on columns (150 mm × 6 mm i.d.) packed with various commercially available anion-exchange resins (PS–DVB-based strongly basic anion-exchange resin: TSKgel SAX, polymethacrylate-based strongly basic anion-exchange resin: TSKgel SuperQ-5PW and polymethacrylate-based weakly basic anion-exchanger resin: TSKgel DEAE-5PW) in the $OH^-$ form and unfunctionized polymethacrylate resins (TSKgel G5000PW and TSKgel G3000PW$_{XL}$) was investigated with basic solutions (sodium hydroxide and heptylamine) as the eluents. Unfortunately, these anion-exchange resins were not suitable for the separation of these $C_1$–$C_6$ amines with both sodium hydroxide and heptylamine as the eluent. In contrast, unfunctionized polymethacrylate resins acted as an advanced stationary phase for these $C_1$–$C_6$ amines with both sodium hydroxide and heptylamine as the eluent. Excellent simultaneous separation, relatively high sensitive conductimetric detection and symmetrical peaks for these $C_1$–$C_6$ amines were achieved on the TSKgel G3000PW$_{XL}$ column in 35 min with 5 mM heptylamine at pH 11.1 as the eluent.

## 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 Tosho 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 columns

Table 1 shows physical and chemical properties of various anion-exchange resins (styrene–divinylbenzene copolymer-based strongly basic anion-exchange resin: a Tosoh TSKgel SAX, polymethacrylate-based strongly basic anion-exchange resin: TSKgel SuperQ-5PW and polymethacrylate-based weakly basic anion-exchange resin: TSKgel DEAE-5PW) and unfunctionized polymethacrylate resins (TSKgel G5000PW and TSKgel G3000PW$_{XL}$). Separation columns (150 mm × 6 mm i.d.) were prepared by packing these resins with slurry-packing method.

The TSKgel G5000PW and TSKgel G3000PW$_{XL}$ resins show small cation-exchange behavior under basic condition, due to small amount of carboxylic group on these resins, originated from starting materials [7]. The determination of cation-exchange capacities of these resins was carried out according to following cation-exchange reaction;

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

Cation-exchange capacity ($A$, meq. ml$^{-1}$) was calculated from the equations:

$$A = \frac{(V_R - V_0)\,C}{1000\,V}$$

where $V_R$ is the breakthrough volume of the column (ml), $V_0$ the total dead volume (column void volume+connected tube volume, ml), $C$ is the concentration of potassium hydroxide solution (mM) and $V$ is column volume (4.24 ml).

First, the column was equilibrated with 1 mM sodium hydroxide solution. Sample of 1 mM sodium hydroxide containing 1% methanol was injected. Elution volume of

Table 1
Properties of various anion-exchange resins and unfunctionized polymethacrylate resins used in this study

| Resin | Matrix | Particle size (μm) | Ion-exchange form | Capacity (meq. ml$^{-1}$) |
|---|---|---|---|---|
| TSKgel SAX | PS–DVB[a] | 5 | –N$^+$(CH$_3$)$_3$ | 1.5 |
| TSKgel SuperQ-5PW | PMA[b] | 10 | –N$^+$(CH$_3$)$_3$ | 0.15 |
| TSKgel DEAE-5PW | PMA[b] | 10 | –C$_2$H$_4$–N(C$_2$H$_5$)$_2$ | 0.10 |
| TSKgel G5000PW | PMA[b] | 10 | –COOH | 0.007 |
| TSKgel G3000PW$_{XL}$ | PMA[b] | 7 | –COOH | 0.011 |

[a] Styrene–divinylbenzene co-polymer resin.
[b] Polymethacrylate resin.

peak corresponding to methanol was considered as $V_0$ (TSKgel G5000PW column: 3.57 ml, TSKgel G3000PW$_{XL}$ column: 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 $V_R$ (TSKgel G5000PW column: 34.8 ml, TSKgel G3000PW$_{XL}$ column: 49.0 ml). The amount of cation-exchange capacities of the TSKgel G5000PW and TSKgel G3000PW$_{XL}$ resins were ca. 0.007 and 0.011 meq. ml$^{-1}$, respectively.

### 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 $C_1$–$C_6$ aliphatic monoamines on various anion-exchange resin columns with sodium hydroxide and heptylamine as eluent

#### 3.1.1. Behavior of $C_1$–$C_6$ aliphatic monoamines on TSKgel SAX column

Styrene–divinylbenzene copolymer-based strongly basic anion-exchange resin is employed as a stationary phase in ion-exclusion chromatography for inorganic and organic bases [2,4,5]. However, a detailed study on the retention behavior of aliphatic monoamines on this type resin column has not been well investigated yet. Hence, the application of this type resin (TSKgel SAX) as the stationary phase in ion-exclusion chromatography with conductimetric detection for $C_1$–$C_6$ aliphatic monoamines (methylamine, ethylamine, propylamine, butylamine, amylamine and hexylamine) was attempted with basic solutions (sodium hydroxide and heptylamine) as eluents. First, the effect of concentration of sodium hydroxide in the eluent on the chromatographic behavior of these $C_1$–$C_6$ amines on a TSKgel SCX column (150 mm × 6 mm i.d.) was investigated. Fig. 1 shows the relationship between the concentration of sodium hydroxide in the eluent and the retention volumes of these $C_1$–$C_6$ amines on the TSKgel SAX column. Fig. 2A–C show chromatogram of these $C_1$–$C_6$ amines on the TSKgel SAX column with (A) 0.1 mM sodium hydroxide at pH 9.9 (eluent conductivity: 23 μS cm$^{-1}$), (B) 1 mM sodium hydroxide at pH 11.0 (eluent conductivity: 270 μS cm$^{-1}$), and (C) 10 mM sodium hydroxide at pH 11.9 (eluent conductivity: 2.4 mS cm$^{-1}$) as the eluents.

As shown in Fig. 1, the retention volumes of these $C_1$–$C_6$ amines increased drastically at the concentration range of sodium hydroxide in the eluent between 0.01 and 0.3 mM. The degree of the increase in the retention volumes of these amines gradually decreased at the concentration of sodium hydroxide in the eluent ≥0.3 mM. The increase in the retention volumes was due mainly to an increase in hydrophobic

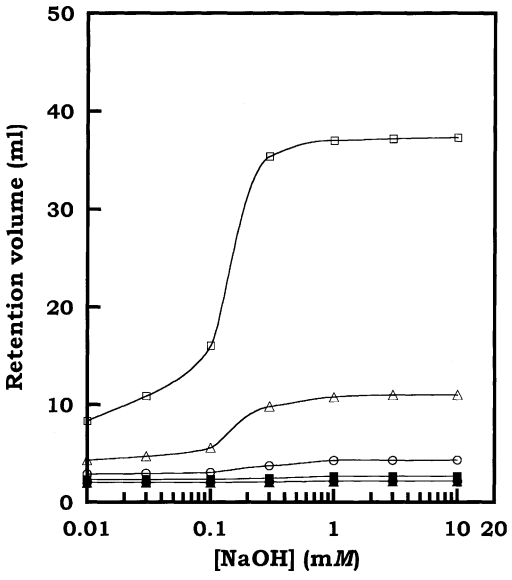


Fig. 1. Effect of concentration of sodium hydroxide in eluent on retention volumes of $C_1$–$C_6$ aliphatic monoamines on TSKgel SAX column. Conditions: column: TSKgel SAX, column size: 150 mm × 6 mm i.d., column temperature: 35 °C, eluent: 0.01–10 mM sodium hydroxide, flow rate: 1 ml min$^{-1}$, detection: direct conductivity, injection volume: 100 μl, sample concentration: 0.5 mM. Symbols: (●) methylamine, (▲) ethylamine, (■) propylamine, (○) butylamine, (△) amylamine, (□) hexylamine.

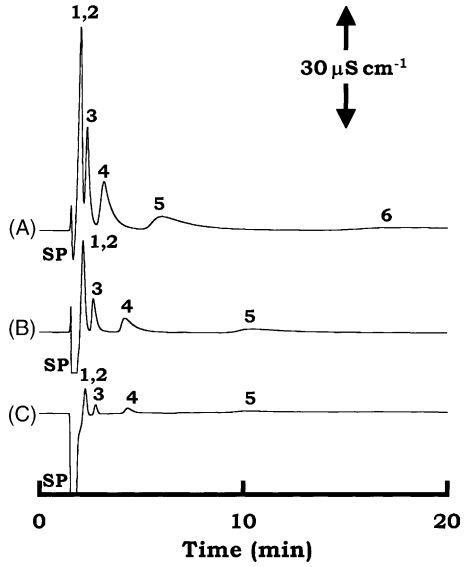


Fig. 2. Chromatograms of $C_1$–$C_6$ aliphatic monoamines on TSKgel SAX column with various concentrations of sodium hydroxide as eluents. Eluents: (A) 0.1 mM sodium hydroxide at pH 9.9 (eluent conductivity: 23 μS cm$^{-1}$), (B) 1 mM sodium hydroxide at pH 11.0 (eluent conductivity: 270 μS cm$^{-1}$), (C) 10 mM sodium hydroxide at pH 11.9 (eluent conductivity: 2.4 mS cm$^{-1}$). Peaks: (SP) system peak corresponding to sodium hydroxide in eluent (1) methylamine, (2) ethylamine, (3) propylamine, (4) butylamine, (5) amylamine, (6) hexylamine. Other conditions are as in Fig. 1.

interaction between the resin and these amines caused by suppressing the dissociation of these amines. As shown in Fig. 2A–C, tailed peaks of the $C_3$–$C_6$ amines were observed. This is due mainly to large hydrophobicity of the resin and the amines. These results indicated that these $C_1$–$C_6$ amines were mainly separated by hydrophobic interaction process. With increasing the concentration of sodium hydroxide in the eluent, conductimetric detection sensitivities of these $C_1$–$C_6$ amines decreased drastically. This is because (a) an increase in the eluent conductivity and (b) a decease in the conductimetric detector responses of these amines caused by suppressing their dissociation. Furthermore, peaks of methylamine and ethylamine were completely overlapped and a system peak corresponding to sodium hydroxide in the eluent interfered seriously with the determination of methylamine and ethylamine. These results strongly indicated that excellent simultaneous separation and symmetrical peaks for these $C_1$–$C_6$ amines could not achieved on the TSKgel SAX column with sodium hydroxide as the eluent.

In previous study [8], we demonstrated that $C_7$ carboxylic acid (5-methylhexanoic acid) acted as an advanced eluent in ion-exclusion chromatography with conductimetric detection for $C_1$–$C_6$ aliphatic mono carboxylic acids (formic acid, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, isocaproic acid and caproic acid) on a sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) column. 5-Methylhexanoic acid in the eluent was strongly adsorbed on the resin at first and then reduced hydrophobic interaction between the TSKgel SCX resin and these carboxylic acids. Although a system peak corresponding to 5-methylhexanoic acid in the eluent appeared, excellent simultaneous separation, highly sensitive detection and symmetrical peaks for these $C_1$–$C_6$ carboxylic acids were achieved on the TSKgel SCX column in a reasonable period of time. Hence, the application of $C_7$ amine (heptylamine) as the eluent was carried out, for improving peak shapes of these $C_1$–$C_6$ amines and peak resolution between these $C_1$–$C_6$ amines. Fig. 3 shows the relationship between the concentration of heptylamine in the eluent and the retention volumes of these $C_1$–$C_6$ amines on the TSKgel SAX column. Fig. 4A–C show chromatograms of these $C_1$–$C_6$ amines on the TSKgel SAX column with (A) 1 mM heptylamine at pH 10.7 (eluent conductivity: 104 μS cm$^{-1}$), (B) 5 mM heptylamine at pH 11.1 (eluent conductivity: 283 μS cm$^{-1}$) and (C) 10 mM heptylamine at pH 11.3 (eluent conductivity: 450 μS cm$^{-1}$) as the eluents.

As shown in Fig. 3, with increasing the concentration of heptylamine in the eluent, the retention volumes of these $C_1$–$C_6$ amines decreased. A system peak corresponding to heptylamine in the eluent appeared and the retention volume also decreased. The order of the decrease in the retention volumes was methylamine ($C_1$) < ethylamine ($C_2$) < propylamine ($C_3$) < butylamine ($C_4$) < amylamine ($C_5$) < hexylamine ($C_6$) < system peak corresponding to heptylamine ($C_7$). These results suggested that heptylamine was strongly adsorbed on the TSKgel SAX resin at first and

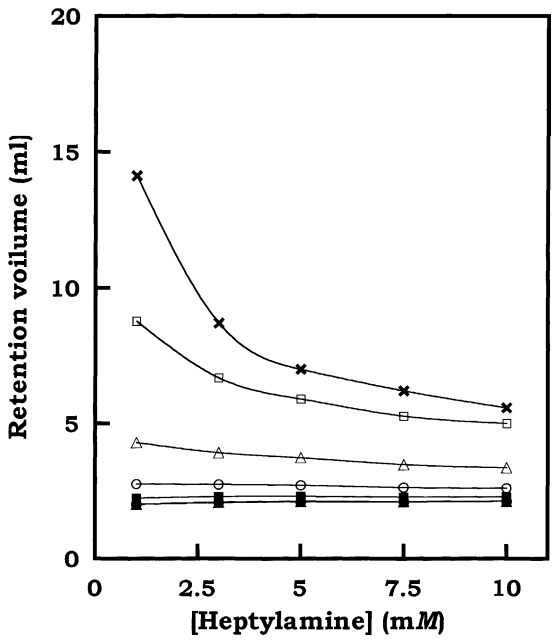


Fig. 3. Effect of concentration of heptylamine in eluent on retention volumes of $C_1$–$C_6$ aliphatic monoamines on TSKgel SAX column. Eluent: 1–10 mM heptylamine. Symbols: (●) methylamine, (▲) ethylamine, (■) propylamine, (○) butylamine, (△) amylamine, (□) hexylamine, (✖) system peak corresponding to heptylamine in eluent. Other conditions are as in Fig. 2.

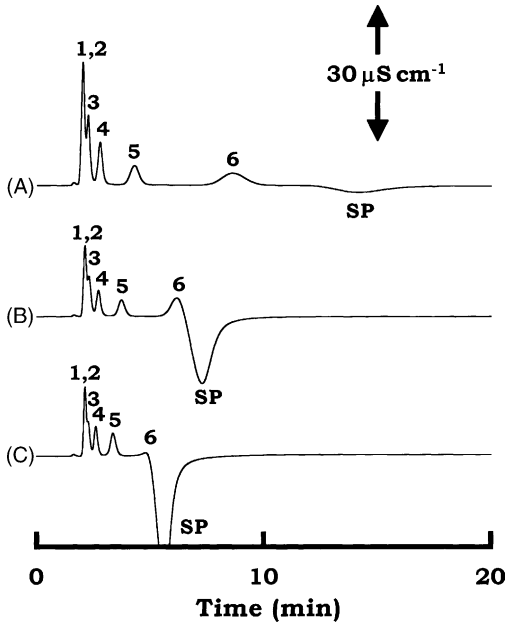


Fig. 4. Chromatograms of $C_1$–$C_6$ aliphatic monoamines on TSKgel SAX column with various concentrations of heptylamine as eluents. Eluents: (A) 1 mM heptylamine at pH 10.7 (eluent conductivity: 104 μS cm$^{-1}$), (B) 5 mM heptylamine at pH 11.2 (eluent conductivity: 283 μS cm$^{-1}$), (C) 10 mM heptylamine at pH 11.3 (eluent conductivity: 450 μS cm$^{-1}$). Peaks: (1) methylamine, (2) ethylamine, (3) propylamine, (4) butylamine, (5) amylamine, (6) hexylamine, (SP) system peak corresponding to heptylamine in eluent. Other conditions are as in Fig. 3.

then reduced hydrophobic interaction between the resin and these $C_1$–$C_6$ amines and heptylamine itself. As shown in Fig. 4A–C, with increasing the concentration of heptylamine in the eluent, peak shapes of higher amines were improved remarkably. This is because heptylamine acted as an effective elution modifier for higher amines. Unfortunately, peak resolution between these $C_1$–$C_6$ amines was not improved and the system peak interfered seriously for the determination of hexylamine. These chromatograms strongly suggested that excellent simultaneous separation of these $C_1$–$C_6$ amines could not be achieved on the TSKgel SAX column with heptylamine as the eluent.

Considering the above results, it was concluded that TSKgel SAX column was not always suitable for ion-exclusion chromatographic separation of these $C_1$–$C_6$ amines with both sodium hydroxide and heptylamine as the eluents.

### 3.1.2. Behavior of $C_1$–$C_6$ aliphatic monoamines on TSKgel SuperQ-5PW and TSKgel DEAE-5PW columns

The application of polymethacrylate-based strongly basic anion-exchange resin (TSKgel SuperQ-5PW) and polymethacrylate-based weakly basic anion-exchange resin (TSKgel DEAE-5PW) as the stationary phases in ion-exclusion chromatography with conductimetric detection for these $C_1$–$C_6$ amines was attempted with dilute sodium hydroxide and heptylamine as the eluents.

Fig. 5A and B show chromatograms of these $C_1$–$C_6$ amines on (A) TSKgel SuperQ-5PW and (B) TSKgel DEAE-5PW columns (150 mm × 6 mm i.d.) with 1 mM sodium hydroxide as the eluent. Both (a) completely overlapped peaks of methylamine and ethylamine and (b) largely tailed peaks of the $C_5$–$C_6$ amines were observed. Chromatogram of these amines on the TSKgel SuperQ-5PW column was similar to that on TSKgel DEAE-5PW column. The retention volumes of these $C_1$–$C_6$ amines on the TSKgel SuperQ-5PW column were somewhat different form those on the TSKgel DEAE-5PW column. The difference was due to both difference in the strength of electric repulsion and difference in the strength of hydrophobic interaction between anion-exchange groups and these amines. These chromatograms indicated that excellent simultaneous separation and symmetrical peaks for these $C_1$–$C_6$ amines could not be achieved on these columns with sodium hydroxide as the eluent.

Fig. 6A and B show chromatograms of these $C_1$–$C_6$ amines on (A) TSKgel SuperQ-5PW and (B) TSKgel DEAE-5PW columns with 5 mM heptylamine as the eluent. Although peak shapes of the $C_5$–$C_6$ amines were improved, peak resolution of methylamine and ethylamine was not improved. These chromatograms also indicated that excellent simultaneous separation of these $C_1$–$C_6$ amines could not be achieved on these columns with heptylamine as the eluent.

Considering the above results, it was concluded that TSKgel SuperQ-5PW and TSKgel DEAE-5PW columns were also unsuitable for ion-exclusion chromatographic separation of these $C_1$–$C_6$ amines with both sodium hydroxide and heptylamine as the eluents.

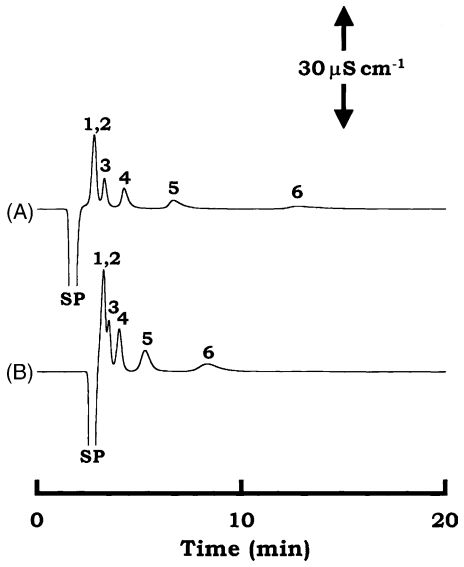


Fig. 5. Chromatograms of $C_1$–$C_6$ aliphatic monoamines on (A) TSKgel SuperQ-5PW and (B) TSKgel DEAE-5PW columns with 1 mM sodium hydroxide as eluent. Conditions: column: (A) TSKgel SuperQ-5PW, (B) TSKgel DEAE-5PW, column size: 150 mm × 6 mm i.d., eluent: 1 mM sodium hydroxide at pH 11.0. Peaks: (SP) system peak corresponding to sodium hydroxide in eluent (1) methylamine, (2) ethylamine, (3) propylamine, (4) butylamine, (5) amylamine, (6) hexylamine. Other conditions are as in Fig. 4.

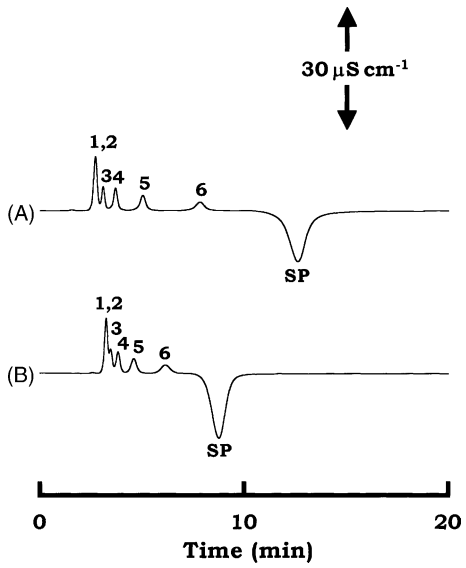


Fig. 6. Chromatograms of $C_1$–$C_6$ aliphatic monoamines on (A) TSKgel SuperQ-5PW and (B) TSKgel DEAE-5PW columns with 5 mM heptylamine as eluents. Conditions: column: (A) TSKgel SuperQ-5PW, (B) TSKgel DEAE-5PW, eluent: 5 mM heptylamine. Peaks: (1) methylamine, (2) ethylamine, (3) propylamine, (4) butylamine, (5) amylamine, (6) hexylamine, (SP) system peak corresponding to heptylamine in eluent. Other conditions are as in Fig. 5.

### 3.2. Chromatographic behavior $C_1$–$C_6$ aliphatic monoamines on unfunctionized polymethacrylate resin columns with sodium hydroxide and heptylamine as eluents

#### 3.2.1. Behavior of $C_1$–$C_6$ aliphatic monoamines on TSKgel G5000PW column

Recently, Li and Fritz applied successfully unfunctionalized high cross-linked styrene–divinylbenzene co-polymer resin as the stationary phase in liquid chromatography for the separation of several aromatic amines [6]. However, due to the large hydrophobicity, aqueous solution containing large amount of organic solvent was required, for employing this type resin. Hence, the application of unfunctionalized hydrophilic polymer resin (polymethacrylate resin: TSKgel G5000PW) as the stationary phases in liquid chromatography with conductimetric detection was attempted with basic solutions (sodium hydroxide and heptylamine) as the eluents, for excellent simultaneous separation and symmetrical peak shapes for these $C_1$–$C_6$ amines. The TSKgel G5000PW resin is base substrate for the preparation of the TSKgel SuperQ-5PW and TSK gel DEAE-5PW resins. Fig. 7 shows the relationship between the concentration of sodium hydroxide in the eluent and the retention volumes of these $C_1$–$C_6$ amines on a TSKgel G5000PW column (150 mm × 6 mm i.d.). Fig. 8A–C show chromatograms of these $C_1$–$C_6$ amines on the TSKgel G5000PW column with (A) 1 mM sodium hydroxide, (B) 5 mM sodium hydroxide at pH 11.6 (eluent conductivity: 1.2 mS cm$^{-1}$) and (C) 10 mM sodium hydroxide as the eluents.

As shown in Fig. 7, with increasing the concentration of sodium hydroxide in the eluent, the retention volumes of these $C_1$–$C_6$ amines decreased drastically. The retention behavior of these $C_1$–$C_6$ amines on the TSKgel G5000PW column with sodium hydroxide as the eluent was completely different from that on these anion-exchange resin columns with sodium hydroxide as the eluent. Since the TSKgel G5000PW resin showed small cation-exchange behavior (ca. 0.007 meq. ml$^{-1}$ at pH 11) as listed in Table 1 [7], it was expected that these $C_1$–$C_6$ amines were separated by not only hydrophobic interaction process but also cation-exchange process. As shown in Fig. 8A–C, these $C_1$–$C_6$ amines were detected indirect-conductimetrically. The detector responses of these $C_1$–$C_6$ amines were almost the same as those with potassium hydroxide as the eluent. Since sodium hydroxide and potassium hydroxide were completely dissociated and these $C_1$–$C_6$ amines were partly dissociated under the eluent conditions, it was expected that these $C_1$–$C_6$ amines were mainly detected as the difference in the concentration hydroxide ion ($OH^-$), which has very high limiting equivalent ionic conductance (198.3 S cm$^2$ eq.$^{-1}$ at 25 °C). As shown in Fig, 8B, using 5 mM sodium hydroxide as the eluent, although good simultaneous separation of these $C_1$–$C_6$ amines was achieved in 30 min, peaks of the $C_5$–$C_6$ amines were tailed largely. Addition of $C_7$ alcohol (heptanol) to 5 mM sodium hydroxide as the eluent was carried out, for improving peak shapes of the $C_5$–$C_6$ amines [9,10]. Unfortunately, heptanol was not effective for improving peak shapes of the $C_5$–$C_6$ amines in the chromatographic conditions.

Fig. 9 shows the relationship between the concentration of heptylamine in the eluent and the retention volumes of these $C_1$–$C_6$ amines on the TSKgel G5000PW column.

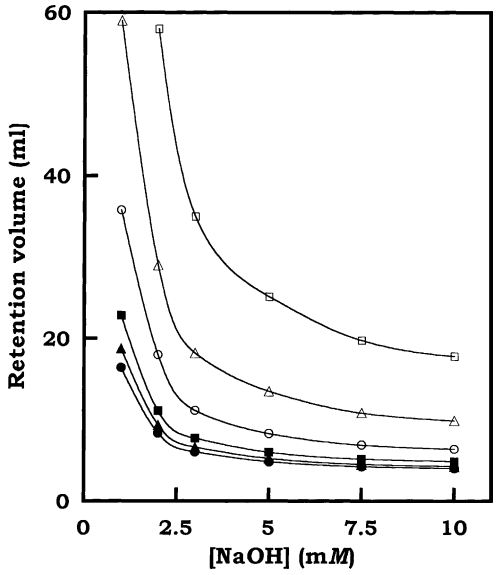


Fig. 7. Effect of concentration of sodium hydroxide in eluent on retention volumes of $C_1$–$C_6$ aliphatic monoamines on TSKgel G5000PW column. Conditions: column: TSKgel G5000PW, column size: 150 mm × 6 mm i.d., eluent: 1–10 mM sodium hydroxide. Symbols: (●) methylamine, (▲) ethylamine, (■) propylamine, (○) butylamine, (△) amylamine, (□) hexylamine. Other conditions are as in Fig. 6.

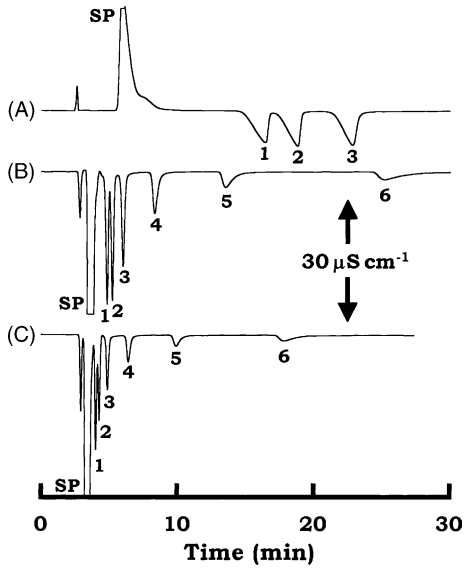


Fig. 8. Chromatograms of $C_1$–$C_6$ aliphatic monoamines on TSKgel G5000PW column with various concentrations of sodium hydroxide as eluents. Eluents: (A) 1 mM sodium hydroxide, (B) 5 mM sodium hydroxide at pH 11.6 (eluent conductivity: 1.2 mS cm$^{-1}$), (C) 10 mM sodium hydroxide. Peaks: (SP) system peak corresponding to sodium hydroxide in eluent (1) methylamine, (2) ethylamine, (3) propylamine, (4) butylamine, (5) amylamine, (6) hexylamine. Other conditions are as in Fig. 7.

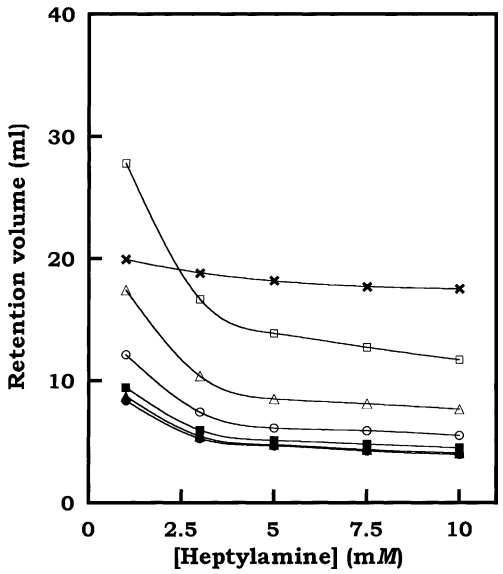


Fig. 9. Effect of concentration of heptylamine in eluent on retention volumes of $C_1$–$C_6$ aliphatic monoamines on TSKgel G5000PW column. Eluent: 1–10 mM heptylamine. Symbols: (●) methylamine, (▲) ethylamine, (■) propylamine, (○) butylamine, (△) amylamine, (□) hexylamine, (✖) system peak corresponding to heptylamine in eluent. Other conditions are as in Fig. 8.

Fig. 10A–C show chromatograms of these $C_1$–$C_6$ amines on the TSKgel G5000PW column with (A) 1 mM heptylamine, (B) 5 mM heptylamine and (C) 10 mM heptylamine as the eluents.

As shown in Fig. 9 with increasing the concentration of heptylamine in the eluent, the retention volumes of $C_1$–$C_6$ amines decreased largely. The degree of the decrease in the retention volume of a system peak corresponding to heptylamine in the eluent was smaller than those of these $C_1$–$C_6$ amines. This might be because these $C_1$–$C_6$ amines were separated by cation-exchange and hydrophobic interaction process. As shown in Fig. 10A–C, with increasing the concentration of heptylamine in the eluent, peak shapes of the $C_5$–$C_6$ amines were improved. Symmetrical peaks of these $C_1$–$C_6$ amines were obtained at the concentration of heptylamine in the eluent ≥5 mM. Unfortunately, peak resolution between methylamine and ethylamine was quite unsatisfactory.

Considering the above results, it was concluded that more highly efficient unfunctionized polymethacrylate resin was required for achieving the excellent simultaneous separation and symmetrical peaks for these $C_1$–$C_6$ amines with heptylamine as the eluent.

### 3.2.2. Behavior of $C_1$–$C_6$ aliphatic monoamines on TSKgel G3000PW$_{XL}$ resin columns

As listed in Table 1, due to small particle size (ca. 7 μm) and large cation-exchange capacity (ca. 0.011 meq. ml$^{-1}$ at pH 11), it was expected that the TSKgel G3000PW$_{XL}$ resin behaved more highly efficient stationary phase for these $C_1$–$C_6$ amines with basic solutions as the eluents in comparison to the TSKgel G5000PW resin. Then, application of a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.) in liquid chromatography with conductimetric detection for these $C_1$–$C_6$ amines was attempted with basic solutions (sodium hydroxide and heptylamine) as the eluents, for completely simultaneous separation and symmetrical peaks for these

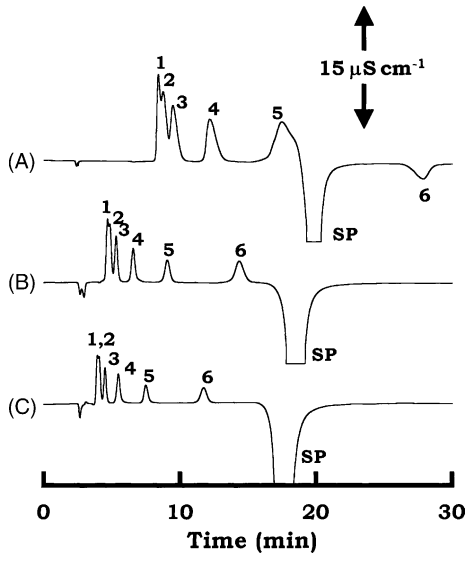


Fig. 10. Chromatograms of $C_1$–$C_6$ aliphatic monoamines on TSKgel G5000PW column with various concentrations of heptylamine as eluents. Eluents: (A) 1 mM heptylamine, (B) 5 mM heptylamine, (C) 10 mM heptylamine. Peaks: (1) methylamine, (2) ethylamine, (3) propylamine, (4) butylamine, (5) amylamine, (6) hexylamine, (SP) system peak corresponding to heptylamine in eluent. Other conditions are as in Fig. 9.

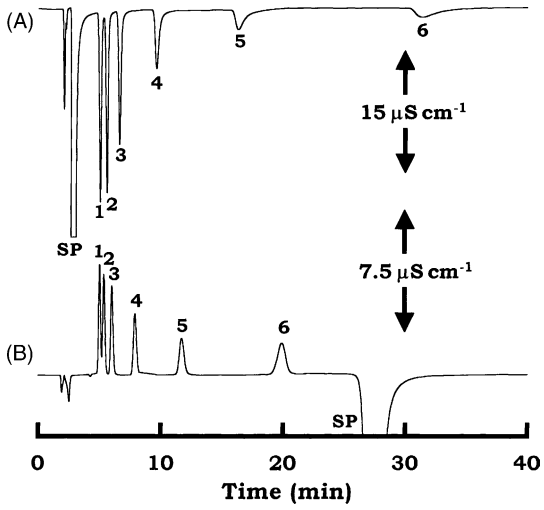


Fig. 11. Chromatograms of $C_1$–$C_6$ aliphatic monoamines on TSKgel G3000PW$_{XL}$ column with (A) 5 mM sodium hydroxide and (B) 5 mM heptylamine as eluents. Conditions: column: TSKgel G3000PW$_{XL}$, column size: 150 mm × 6 mm i.d., eluents: (A) 5 mM sodium hydroxide, (B) 5 mM heptylamine. Peaks: (1) methylamine, (2) ethylamine, (3) propylamine, (4) butylamine, (5) amylamine, (6) hexylamine, (SP) system peak corresponding to sodium hydroxide or heptylamine in eluent. Other conditions are as in Fig. 10.

Table 2
Detection limits (signal-to-noise ration of 3, injection volume of 100 μl) of $C_1$–$C_6$ aliphatic monoamines with 5 mM heptylamine at pH 11.1 as eluent

| Amines | Detection limit | |
|---|---|---|
| | μM | ng ml$^{-1}$ |
| Methylamine | 0.34 | 11 |
| Ethylamine | 0.39 | 17 |
| Propylamine | 0.47 | 28 |
| Butylamine | 0.60 | 44 |
| Amylamine | 0.88 | 77 |
| Hexylamine | 1.4 | $1.4 \times 10^2$ |

Eluent conductivity: 283 μS cm$^{-1}$. Noise: $2.4 \times 10^{-3}$ μS cm$^{-1}$.

amines. Fig. 11A and B chromatograms of these $C_1$–$C_6$ amines on the TSKgel G3000PW$_{XL}$ column with (A) 5 mM sodium hydroxide and (B) 5 mM heptylamine as the eluents.

As shown in Fig. 11A and B, it was evident that the column performance of the TSKgel G3000PW$_{XL}$ column was superior to that of the TSKgel G5000PW column for these $C_1$–$C_6$ amines. Excellent simultaneous separation of these $C_1$–$C_6$ amines was achieved in 35 min with both 5 mM sodium hydroxide and 5 mM heptylamine as the eluents. However, using 5 mM NaOH as the eluent, peaks of the $C_5$–$C_6$ amines were tailed largely and the eluent conductivity was very high (1.2 mS cm$^{-1}$). In contrast, using 5 mM heptylamine as the eluent, although system peak corresponding to heptylamine in the eluent appeared in ca. 28 min, excellent simultaneous separation and symmetrical peaks for these $C_1$–$C_6$ amines were achieved in 35 min and the eluent conductivity was reasonable (283 μS cm$^{-1}$).

Various analytical performance parameters were investigated with 5 mM heptylamine as the eluent. Table 2 shows the detection limits (signal-to-noise ratio of 3, injection volume of 100 μl) of these $C_1$–$C_6$ aliphatic monoamines. Relatively high sensitive conductimetric detection was achieved. This is due mainly to (a) partly dissociation of these amines (p$K_b$ = ca. 3.4) and (b) reasonable eluent conductivity (283 μS cm$^{-1}$, noise: $2.4 \times 10^{-3}$ μS cm$^{-1}$). Calibration graphs were obtained by plotting the chromatographic peak area against the concentration of these $C_1$–$C_6$ 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 $C_1$–$C_6$ amines, whose concentrations were 0.5 mM, were less than 0.7% ($n = 10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

Considering the above results, it was concluded that The TSKgel G3000PW$_{XL}$ resin was very suitable stationary phase in liquid chromatography with conductimetric detection for the simultaneous separation, highly sensitive detection and symmetrical peak shapes for these $C_1$–$C_6$ amines with heptylamine as the eluent.

## 4. Conclusion

The application of commercially available various anion-exchange resins (styrene–divinylbenzene copolymer-based strongly basic anion-exchange resin: TSKgel SAX, polymethacrylate-based strongly basic anion-exchange resin: TSKgel SuperQ-5PW and polymethacrylate-based weakly basic anion-exchange resin: TSKgel DEAE-5PW) and unfunctionized polymethacrylate resins (TSKgel G5000PW and TSKgel G3000PW$_{XL}$) as stationary phases in liquid chromatography with conductimetric detection for $C_1$–$C_6$ aliphatic monoamines (methylamine, ethylamine, propylamine, butylamine, amylamine and hexylamine) was performed with basic solutions (sodium hydroxide and heptylamine) as eluents. Unfortunately, these anion-exchanger resins were not successful applied as the stationary phases for these $C_1$–$C_6$ amines with both sodium hydroxide and heptylamine as the eluents. Due to small hydrophobicity and small cation-exchange ability, these polymethacrylate resins acted as advanced stationary phases for these $C_1$–$C_6$ amines with sodium hydroxide and heptylamine as the eluents. Excellent simultaneous separation, relatively high sensitive detection and symmetrical peaks for these $C_1$–$C_6$ amines were achieved on a TSKgel G3000PW$_{XL}$ column (150 mm × 6 mm i.d.) in 35 min with 5 mM heptylamine at pH 11.1 as the eluent. These above results extend largely the utility of unfunctionized polymethacrylate resin as the stationary phase in liquid chromatography for various organic and inorganic compounds.

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