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Application of zirconium-modified silica gel as a stationary phase in the ion-exclusion chromatography of carboxylic acids

II. Separation of aliphatic carboxylic acids with pyromellitic acid as eluent and with suppressed conductimetric detection

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Abstract

The application of zirconium-modified silica gels (Zr-Silica) as stationary phases for ion-exclusion chromatography with conductimetric detection (IEC-CD) for C₁–C₈ aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, heptanoic and caprylic acids) was carried out using pyromellitic acid as the eluent. Zr-Silicas were prepared by the reaction of the silanol group on the surface of silica gel with zirconium tetrabutoxide [Zr(OCH₂CH₂CH₂CH₃)₄] in ethanol solution. An ASRS-Ultra anion self-regenerating suppressor in the K⁺ form was used for the enhancement of conductimetric detector response of these aliphatic carboxylic acids. A Zr-Silica adsorbed on 10 mg zirconium g⁻¹ silica gel was the most suitable stationary phase in IEC-CD for the separation of these aliphatic carboxylic acids. Excellent simultaneous separation and highly sensitive detection for these aliphatic carboxylic acids were achieved in 25 min by IEC-CD with the Zr-Silica column (250×4.6 mm I.D.) and a 0.2 mM pyromellitic acid containing 0.15% heptanol as the eluent. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Zirconized silica; Stationary phases, LC; Mobile phase composition; Silica, zirconized; Carboxylic acids; Pyromellitic acid

1. Introduction

Ion-exclusion chromatography (IEC) developed by Wheaton and Bauman [1] is commonly used for the separation of various kinds of carboxylic acids. The unique feature in IEC is that the separation of carboxylic acids proceeds on cation-exchange stationary phases. High-capacity sulfonated styrene-divinylbenzene co-polymer resins are exclusively employed as cation-exchange stationary phases in ion-exclusion chromatography for carboxylic acids

[2]. In the beginning of the development of IEC, water was used as eluent. However, when using water as the eluent, peak shapes of carboxylic acids were strongly fronted, because of an electrostatic repulsion between dissociated carboxylic acids and the sulfonic acid group on the stationary phase. Turkelson and Richard demonstrated the effectiveness of strongly acidic eluents for the improvement of peak shapes of carboxylic acids in IEC [3]. Now, dilute strong acids, such as sulfuric, hydrochloric acid and perchloric acids, are commonly used as eluents.

Since a silanol group on the surface of silica gel behaves as a weak acid at pK_a of ca. 7.1 [4], it is

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possible to apply unmodified silica gel as a cation-exchange stationary phase for the ion-exclusion chromatographic separation of carboxylic acids. Zenki et al. have applied an unmodified silica gel in IEC for the determination of hydrogen carbonate (HCO_3^-) in river water samples using borate buffer at pH 7.1 as the eluent [5]. However, since strongly acidic eluents cause the suppression of the dissociation of the silanol group, the application of unmodified silica gel for ion-exclusion chromatography was strongly limited.

It is well known that commercially available silica gels contain various kinds of metals as impurities in the silica matrix and several polyvalent metals cause the enhancement of the acidity of the silanol group on the surface of silica gel [6–8]. This suggested that there were some commercially available silica gels, which acted as cation exchangers under strongly acidic conditions. Fortunately, the author has found a commercially available silica gel (Develosil 30-5), which acted as a cation exchanger under strongly acidic conditions [9]. The Develosil 30-5 silica gel was successfully applied for the ion-exclusion chromatographic separation of various carboxylic acids [10,11]. The advantage of the use of silica gel in IEC was that the separation of hydrophobic carboxylic acids, such as higher aliphatic carboxylic acids and aromatic carboxylic acids, could be easily achieved. This is due to large hydrophilic nature of silica gel. The main cause of the cation-exchange characteristics was attributed to aluminium in the silica matrix [12]. Laboratory-made aluminium-modified silica gel (Al-Silica) was also successfully applied as a cation-exchange stationary phase in IEC for the separation of various carboxylic acids [13].

In a previous study [14], the author has prepared zirconium-modified silica gel (Zr-Silica) by the reaction of the silanol group on the surface of silica gel with zirconium tetrabutoxide [$\text{Zr}(\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_4$] in ethanol. The Zr-Silica acted as a cation exchanger under strongly acidic conditions and was successfully applied for the simultaneous separation of monovalent cations (Li^+ , Na^+ , NH_4^+ , K^+ , Rb^+ and Cs^+) using 10 mM tartaric acid at pH 2.5 as eluent. However, the application of the Zr-Silica as a cation-exchange stationary phase for the ion-exclusion chromatographic separation of carboxylic acids has not been carried out yet.

The aim of this study was to expand the utility of Zr-Silicas as cation-exchange stationary phases in IEC for carboxylic acids. Zr-Silicas adsorbed on various amount of zirconium were prepared and were applied as cation-exchange stationary phases in IEC with conductimetric detection (IEC-CD) for the simultaneous separation of C_1 – C_8 aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, heptanoic and caprylic acids) using pyromellitic acid as the eluent. An ASRS-Ultra anion self-regenerating suppressor in the K^+ form was used for the enhancement of conductimetric detector response of these aliphatic carboxylic acids. Excellently simultaneous separation and highly sensitive detection for these aliphatic carboxylic acids were achieved in 25 min by IEC-CD using a Zr-Silica (10 mg zirconium g^{-1} silica gel) column (250×4.6 mm I.D.) and 0.2 mM pyromellitic acid containing 0.15% heptanol as the eluent.

2. Experimental

2.1. Preparation of zirconium-modified silica gel

A Pia Tec (Suzuka, Japan) Pia Seed 5S-100-SIL high-purity porous spherical silica gel for high-performance liquid chromatography (HPLC) was employed as a matrix. The silica gel was dried overnight at 150°C. A 10-g amount of the silica gel was immersed in 100 ml ethanol. Stirring the solution, 100 ml ethanol solution containing given amount of zirconium tetrabutoxide [$\text{Zr}(\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_4$] solution [ca. 85% (w/w) zirconium tetrabutoxide in butanol] was gradually added to the solution. After adding, the solution was stirring for 1 h and then was filtered. The resultant gel was washed thoroughly with ethanol and then dried at 150°C, followed by calcining at 1000°C for 5 h.

Table 1 shows the physical and chemical properties of Pia Seed 5S-100-SIL and prepared zirconium-modified silica gels (Zr-Silicas). The determination of zirconium on Zr-Silicas was carried out using a Nippon Jarrel-Ash ICAP-1000 inductively coupled plasma atomic emission spectrometry (ICP-AES) system. The determination of the surface area, pore size and pore volume of Pia Seed 5S-100-SIL and Zr-Silicas by using nitrogen adsorption

Table 1
Physical and chemical properties of Pia Seed 5S-100-SIL and zirconium-modified silica gels (Zr-Silicas)

| Gel | Amount of zirconium (mg g ⁻¹ silica) | Drying or calcining (°C) | Particle size (μm) | Surface area (m ² g ⁻¹) | Pore size (Å) | Pore volume (ml g ⁻¹) | Packing density (g ml ⁻¹) | Surface area per column ^a (m ² column ⁻¹) |
|---------------------|---|--------------------------|--------------------|--|---------------|-----------------------------------|---------------------------------------|---|
| Pia Seed 5S-100-SIL | 0 | 150 | 6.9 | 499 | 102 | 1.14 | 0.37 | 7.7×10 ² |
| | 0 | 1000 | 6.0 | 250 | 59 | 0.54 | 0.60 | 6.3×10 ² |
| Zr-Silica | 5.2 | 1000 | 5.9 | 323 | 82 | 0.70 | 0.52 | 7.0×10 ² |
| | 10 | 1000 | 6.0 | 313 | 80 | 0.69 | 0.53 | 6.9×10 ² |
| | 15 | 1000 | 5.8 | 285 | 79 | 0.61 | 0.58 | 6.9×10 ² |
| | 20 | 1000 | 5.6 | 267 | 78 | 0.60 | 0.58 | 6.4×10 ² |
| | 39 | 1000 | 5.5 | 230 | 80 | 0.50 | 0.63 | 6.0×10 ² |
| | 57 | 1000 | 5.3 | 223 | 78 | 0.48 | 0.66 | 6.1×10 ² |
| | 87 | 1000 | 5.3 | 216 | 73 | 0.47 | 0.71 | 6.3×10 ² |
| | 100 | 1000 | 5.2 | 204 | 67 | 0.47 | 0.76 | 6.4×10 ² |

^a Column size: 250×4.6 mm I.D.

isotherms on the gels at 77 K was carried out using a Beckman-Coulter (Fullerton, CA, USA) Ominisorp 360 gas sorption analyzer. The surface area was calculated from the BET equation.

The separation columns (250×4.6 mm I.D., stainless steel) were packed with Pia Seed 5S-100-SIL and Zr-Silicas using the slurry-packing method.

2.2. Instrument

The ion chromatograph consisted of a Tosoh (Tokyo, Japan) LC-8020 chromatographic data processor, a Tosoh DP-8020 eluent delivery pump operated at a flow-rate of 0.35 ml min⁻¹, a Tosoh CCPD regenerant delivery pump operated at a flow-rate of 0.7 ml min⁻¹, a Tosoh CO-8020 column oven operated at 35°C, a Tosoh CM-8020 conductimetric detector, a Tosoh DS-8023 on-line degasser, a Dionex (Sunnyvale, CA, USA) ASRS-Ultra (2-mm) anion self-regenerating suppressor and a Reodyne (Cotati, CA, USA) Model 9125 injector equipped with a 20-μl of sample loop.

2.3. Chemicals

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

The pH of eluents was measured with a Toa

Denpa (Tokyo, Japan) IM-40S ion meter with a glass electrode.

3. Results and discussion

3.1. Eluent and detection

For the improvement of peak shapes of carboxylic acid in IEC, dilute strong acids, such as sulfuric, hydrochloric and perchloric acids, are commonly used as eluents. As reported before [14], laboratory-made Zr-Silica was unstable when using a dilute strong acid (3 mM nitric acid at pH 2.5) as an eluent, while the Zr-Silica was stable when using a dilute weak acid (10 mM tartaric acid at pH 2.5) as an eluent. These results suggested that dilute weak acids, such as dilute carboxylic acids, were suitable eluents in IEC with the Zr-Silica column for the separation of carboxylic acids.

First, the effect of various dilute carboxylic acids (0.5 mM tartaric acid at pH 3.4, 0.5 mM malonic acid at pH 3.4, 0.5 mM citric acid at pH 3.5, 2 mM succinic acid at pH 3.5, 2.0 mM benzoic acid at pH 3.5, 0.4 mM *o*-phthalic acid at pH 3.5, 0.3 mM trimellitic acid at pH 3.5 and 0.2 mM pyromellitic acid at pH 3.5) as eluents on chromatographic behavior of C₁–C₅ aliphatic carboxylic acids (formic, acetic, propionic, butyric and valeric acids) on the Zr-Silica column was investigated in IEC–CD.

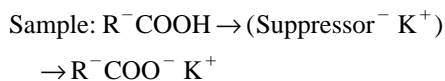
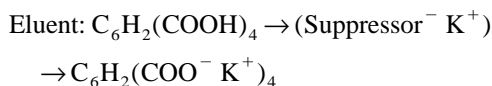
Zr-Silica adsorbed on 10 mg zirconium g^{-1} silica gel was used as a stationary phase.

Good separation of these aliphatic carboxylic acids was achieved on the Zr-Silica column using these carboxylic acids as eluents. The elution order was formic acid < acetic acid < propionic acid < butyric acid < valeric acid. These results indicated that these aliphatic carboxylic acids were mainly separated by an ion-exclusion chromatographic process. However, when using 0.5 mM tartaric acid, 0.5 mM malonic acid, 0.5 mM citric acid or 0.4 mM *o*-phthalic acid as the eluents, an eluent dip peak appeared close to a peak of formic acid, which interfered with the detection of formic acid. When using 2 mM succinic acid as the eluent, an eluent dip peak appeared close to a peak of acetic acid, which interfered with the detection of acetic acid. When using 2.0 mM benzoic acid as the eluent, an eluent dip peak appeared between peaks of butyric and valeric acids, which interfered with the detection of valeric acid. In contrast, when using 0.3 mM trimellitic acid or 0.2 mM pyromellitic acid as eluents, an eluent dip peak was eluted rapidly and no interferences were observed for the detection of these aliphatic carboxylic acids. The difference in retention times of eluent dip peaks was due mainly to both the difference in acidic strength between these carboxylic acids (tartaric, malonic, citric, succinic, benzoic, *o*-phthalic, trimellitic and pyromellitic acids) and the difference in affinity of these carboxylic acids to the Zr-Silica stationary phase. From the above results, it was concluded that pyromellitic acid was the suitable eluent under the ion-exclusion chromatography conditions.

Fig. 1A shows chromatogram of these aliphatic carboxylic acids using 0.2 mM pyromellitic acid as the eluent. Although complete separation of these aliphatic carboxylic acids was achieved in 20 min, the conductimetric detector responses of acetic, propionic, butyric and valeric acids were much lower than that of formic acid. This is because formic acid is a relatively strong acid in comparison with the other aliphatic carboxylic acids and then is well dissociated under the ion-exclusion chromatographic conditions. For the enhancement of the detector response of aliphatic carboxylic acids in IEC-CD, a cation-exchange membrane suppressor in the K^+ form is often employed [15]. Next, the effect of an

ASRS-Ultra anion self-regenerating suppressor in the K^+ form was investigated.

Fig. 1B shows chromatogram of these aliphatic carboxylic acid using an ASRS-Ultra anion self-regenerating suppressor in the K^+ form. The detector response of acetic, propionic, butyric and valeric acids increased greatly and the peak areas of these aliphatic carboxylic acids were closed to that of formic acid. The background eluent conductivity also decreased. These results indicated that following reactions proceeded successfully in the suppressor:



From the above results, it was demonstrated that the use of the suppressor in the K^+ form was very effective for the enhancement of conductimetric detector responses of aliphatic carboxylic acids under the IEC-CD conditions.

3.2. Effect of amount of zirconium-adsorbed on silica gel on chromatographic behavior of aliphatic carboxylic acids

The effect of the amount of zirconium adsorbed on silica gel (0–100 mg zirconium g^{-1} silica gel) on chromatographic behavior of C_1 – C_8 aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, heptanoic and caprylic acids) was investigated using 0.2 mM pyromellitic acid as the eluent.

Fig. 2A–D show chromatograms of these aliphatic carboxylic acids on Zr-Silicas adsorbed on 10, 20, 100 and 0 mg zirconium g^{-1} silica gel as stationary phases, respectively. As shown in Fig. 2A, when using Zr-Silica adsorbed on 10 mg zirconium g^{-1} silica gel as the stationary phase, complete separation of these aliphatic carboxylic acids was achieved, but it took a very long time for the elution of higher aliphatic carboxylic acids (caproic, heptanoic and caprylic acids). Peak shapes of these higher aliphatic carboxylic acids indicated strong tailing. This is due mainly to strongly hydrophobic interaction between

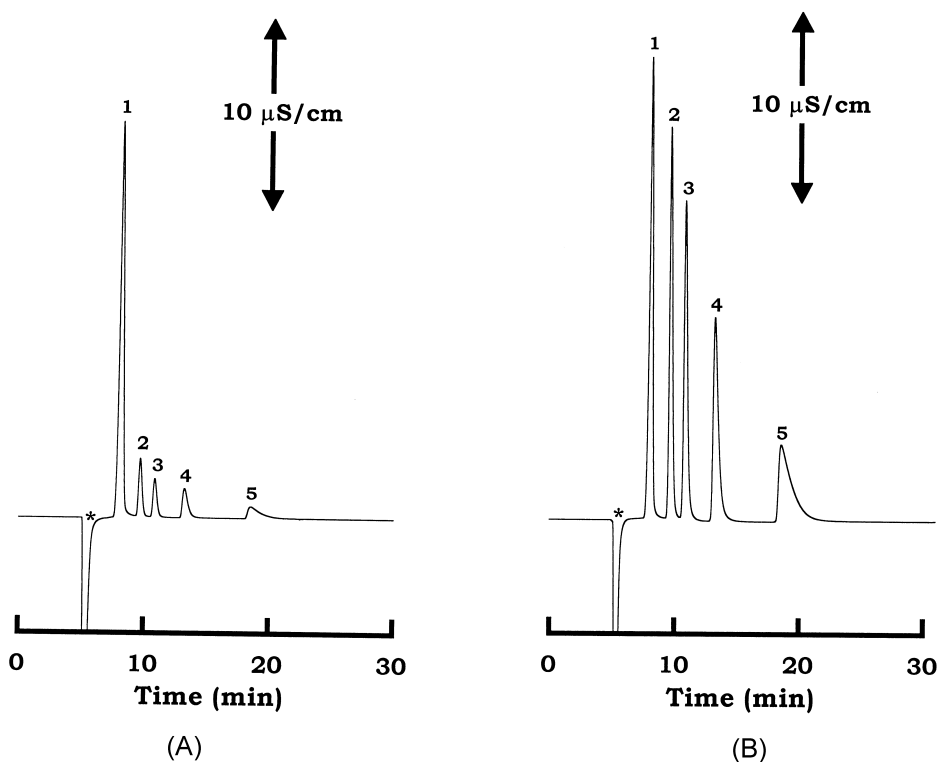


Fig. 1. Chromatogram of C₁-C₅ aliphatic carboxylic acids on zirconium-modified silica gel (Zr-Silica) column using 0.2 mM pyromellitic acid at pH 3.5 as eluent. (A) Without ASRS-Ultra anion self-regenerating suppressor in K⁺ form; (B) with ASRS-Ultra anion self-regenerating suppressor in K⁺ form. Column: Zr-Silica adsorbed on 10 mg zirconium g⁻¹ silica gel; column size: 250×4.6 mm I.D.; column temperature: 35°C; eluent: 0.2 mM pyromellitic acid at pH 3.5; eluent flow-rate: 0.35 ml min⁻¹; regenerant: 20 mM K₂SO₄; regenerant flow-rate: 0.7 ml min⁻¹; detection: conductivity; injection volume: 20 μl; sample concentration: 1.0 mM. Peaks: *=eluent dip, 1=formic acid, 2=acetic acid, 3=propionic acid, 4=butyric acid, 5=valeric acid.

these carboxylic acids and the Zr-Silica. As shown in Fig. 2B and C, with increasing the amount of zirconium adsorbed on silica gel, the peak shapes of these aliphatic carboxylic acids were largely destroyed. Although no conclusive reasons for the peak destruction were found, it was evident that strong interactions between these aliphatic carboxylic acids and zirconium on the surface of the Zr-Silica occurred. A detailed study on retention behavior of these aliphatic carboxylic acids on Zr-Silica stationary phases will be the subject of future work.

On the other hand, as shown in Fig. 2D, when using Zr-Silica adsorbed on 0 mg zirconium g⁻¹ silica gel (Pia Seed 5S-100-SIL calcined at 1000°C) as the stationary phase, the separation of these aliphatic carboxylic acids was also achieved. Unfortunately, peaks of cations co-existing in the

sample solution appeared. As a result, the detection of these aliphatic carboxylic acids was interfered. This is due to very low cation-exchange capacity of the Pia Seed 5S-100-SIL and perhaps due to incomplete conversion of cations including H⁺ in the eluent into K⁺ in the suppressor.

Considering peak shapes and interference, it was concluded that Zr-Silica adsorbed on 10 mg zirconium g⁻¹ silica gel was the most suitable stationary phase in the IEC-CD for the simultaneous separation of these aliphatic carboxylic acids.

3.3. Effect of concentration of pyromellitic acids on retention behavior of aliphatic carboxylic acids on Zr-Silica column

The effect of the concentration of pyromellitic

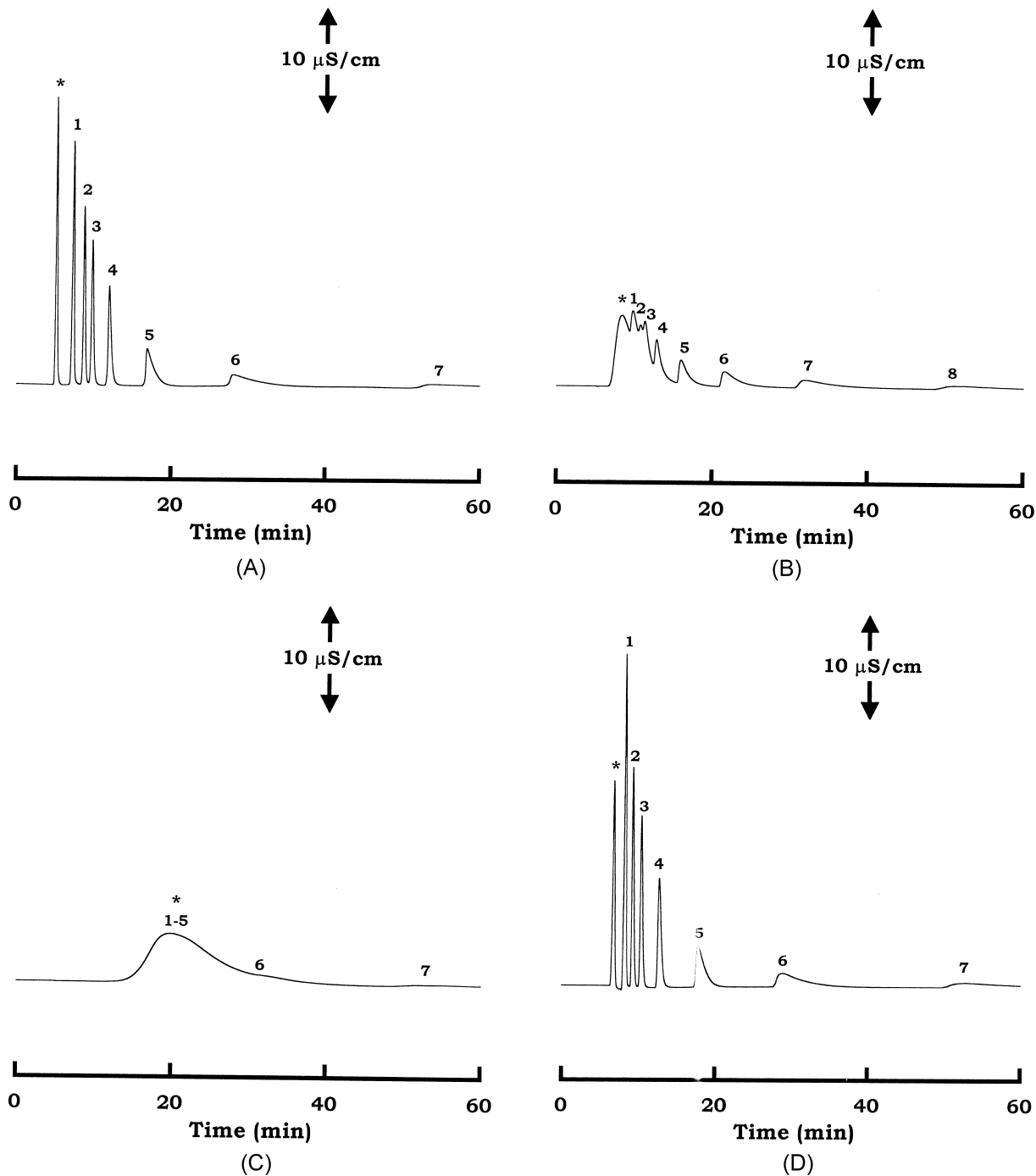


Fig. 2. Chromatograms of C_1 - C_8 aliphatic carboxylic acids on various Zr-Silica columns using 0.2 mM pyromellitic acid as eluent. (A) Zr-Silica adsorbed on 10 mg zirconium g^{-1} silica gel, (B) Zr-Silica adsorbed on 20 mg zirconium g^{-1} silica gel, (C) Zr-Silica adsorbed on 100 mg zirconium g^{-1} silica gel, (D) Zr-Silica adsorbed on 0 mg zirconium g^{-1} silica gel (Pia Seed 5S-100-SIL calcined at 1000°C). Regenerant: 20 mM K_2SO_4 ; regenerant flow-rate: 0.7 ml min^{-1} ; sample concentration: 1.0 mM for aliphatic carboxylic acids. Peaks: * = pyromellitic acid, 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid, 5 = valeric acid, 6 = caproic acid, 7 = heptanoic acid, 8 = caprylic acids. Other conditions as in Fig. 1.

acid in the eluent on chromatographic behavior of C_1 – C_8 aliphatic carboxylic acids on the Zr-Silica (10 mg zirconium g^{-1} silica gel) as the stationary phase was investigated for the complete separation of these aliphatic carboxylic acids.

Fig. 3 shows relationship between the concentration of pyromellitic acid in the eluent and the retention times of these aliphatic carboxylic acids. With increasing the concentration of pyromellitic acid in the eluent, the retention times of these aliphatic carboxylic acids increased at first and then remained almost the same. The retention times of higher carboxylic acids (caproic, heptanoic and caprylic acids) increased drastically. This is due to both a decrease in electrostatic repulsion and an increase in hydrophobic interaction between these aliphatic carboxylic acids and the Zr-Silica by sup-

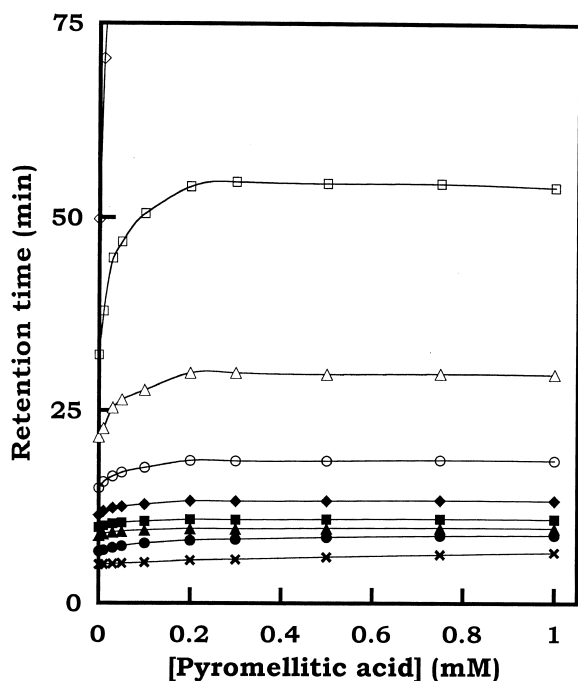


Fig. 3. Effect of concentration of pyromellitic acids in eluent on retention times of C_1 – C_8 aliphatic carboxylic acids on the Zr-Silica column. Column: Zr-Silica adsorbed on 10 mg zirconium g^{-1} silica gel; eluent: 0–1.0 mM pyromellitic acid. Symbols: \times = pyromellitic acid, \bullet = formic acid, \blacktriangle = acetic acid, \blacksquare = propionic acid, \blacklozenge = butyric acid, \circ = valeric acid, \triangle = caproic acid, \square = heptanoic acid, \diamond = caprylic acid. Other conditions as in Fig. 2.

pressing of the dissociation of these aliphatic carboxylic acids and the silanol group on the Zr-Silica.

Fig. 4A–D show chromatograms of these aliphatic carboxylic acids using 0 mM pyromellitic acid (water) at pH 5.5, 0.01 mM pyromellitic acid at pH 4.6, 0.1 mM pyromellitic acid at pH 3.8 and 1 mM pyromellitic acid at pH 2.9 as the eluents, respectively. (The chromatogram using 0.2 mM pyromellitic acid as the eluent has been already shown in Fig. 2A). As shown in Fig. 4A, when using water as eluent, peak shapes of these aliphatic carboxylic acid were fronted. This is because these aliphatic carboxylic acids and the silanol group on the Zr-Silica were well dissociated, and these carboxylic acids were exclusively separated by the ion-exclusion process. As shown in Fig. 4B–D and Fig. 2A, with increasing the concentration of pyromellitic acid in the eluent, peak shapes of lower aliphatic carboxylic acids (formic, acetic, propionic and butyric acids) were improved. In contrast, peak shapes of higher carboxylic acids (caproic, heptanoic, caprylic acids) were not improved. This is due mainly to strongly hydrophobic interaction between these higher aliphatic carboxylic acids and the Zr-Silica stationary phase caused by suppressing the dissociation of these acids and the silanol group on the Zr-Silica.

Considering peak shapes, peak resolution, retention time and background eluent conductivity, it was concluded that a reasonable concentration of pyromellitic acid in the eluent was 0.2 mM.

3.4. Effect of alcohols added to eluent on chromatographic behavior of aliphatic carboxylic acids on Zr-Silica column

The addition of organic solvent to the eluent is very effective for both reduction of retention times and improvement of peak shapes of hydrophobic carboxylic acids in IEC [2]. Morris and Fritz have demonstrated the effectiveness of higher alcohol added to eluent for the improvement of peak shapes of carboxylic acids in IEC–CD [16]. The authors have also demonstrated the effectiveness of higher alcohol added to acidic eluents for the reduction of retention times and improvement of peak shapes of hydrophobic aliphatic carboxylic acids in IEC using an unmodified silica gel column [10] and a laboratory-made aluminium-modified silica gel column

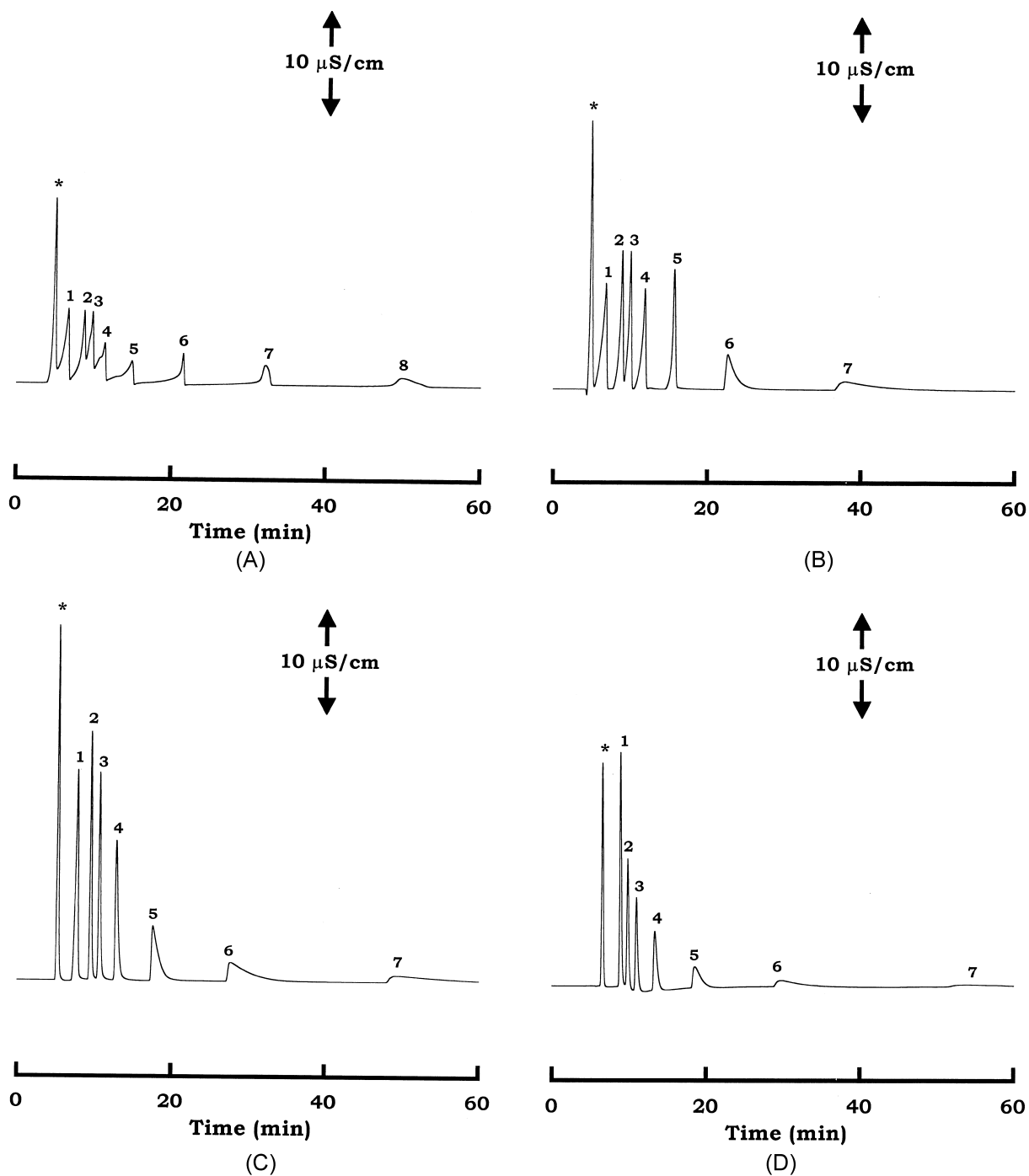


Fig. 4. Chromatograms of C₁-C₈ aliphatic carboxylic acids on the Zr-Silica column using various concentration of pyromellitic acid as eluent. (A) 0 mM pyromellitic acid (water) at pH 5.5, (B) 0.01 mM pyromellitic acid at pH 4.6, (C) 0.1 mM pyromellitic acid at pH 3.8, (D) 1 mM pyromellitic acid at pH 2.9. Peaks: * = pyromellitic acid, 1 = formic acid, 2 = acetic acid, 3 = propionic acid, 4 = butyric acid, 5 = valeric acid, 6 = caproic acid, 7 = heptanoic acid, 8 = caprylic acid. Other conditions as in Fig. 3.

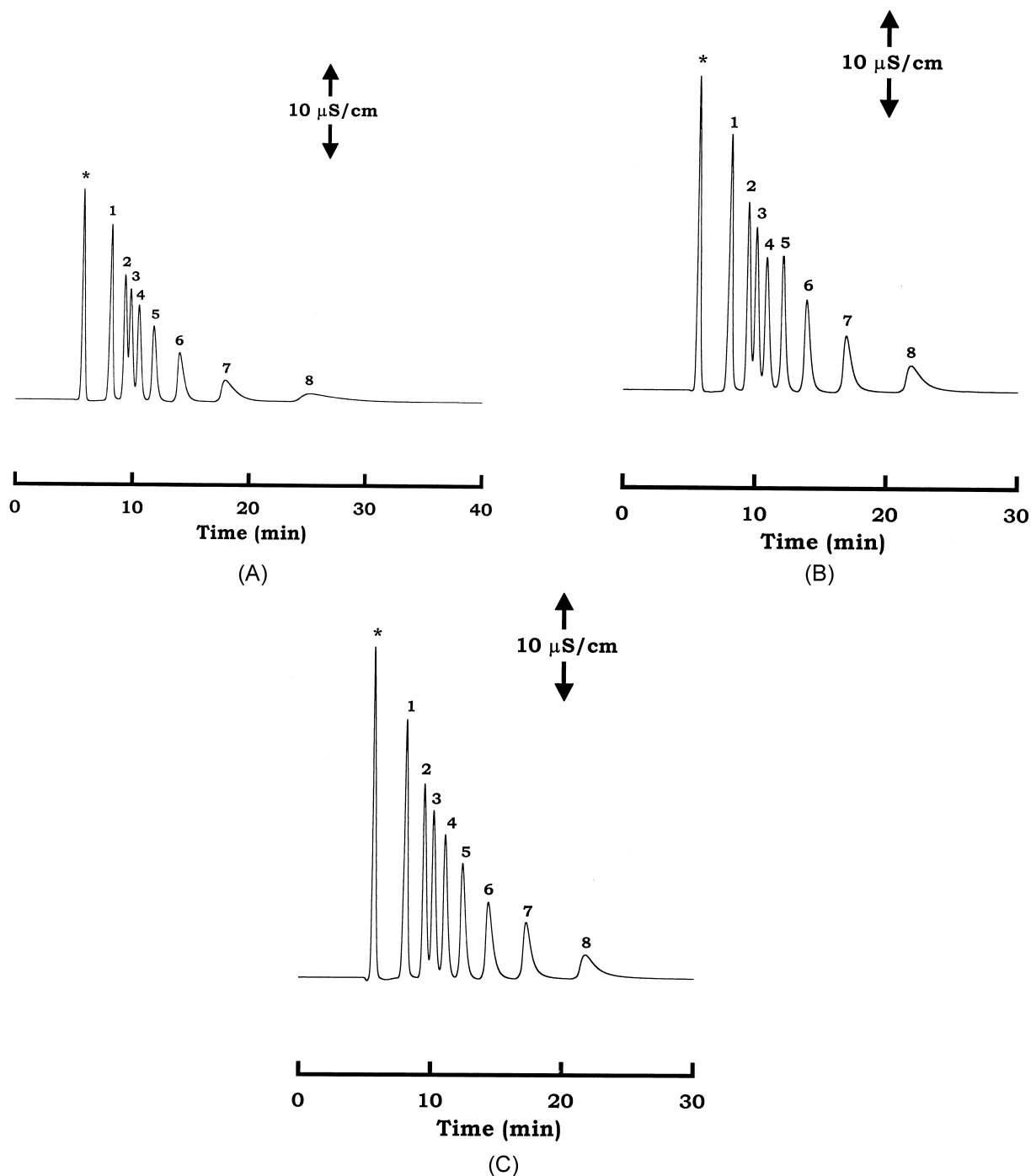


Fig. 5. Chromatograms of C_1 – C_8 aliphatic carboxylic acids on the Zr-Silica column using pyromellitic acid containing various alcohols. (A) 0.2 mM pyromellitic acid containing 10% methanol, (B) 0.2 mM pyromellitic acid containing 0.75% butanol, (C) 0.2 mM pyromellitic acid containing 0.15% heptanol. Other chromatographic conditions as in Fig. 4.

[13]. Therefore, the addition of C₁–C₇ alcohols [10% (v/v) methanol, 5% ethanol, 2% propanol, 0.75% butanol, 0.3% pentanol, 0.2% hexanol and 0.15% heptanol] to the eluent was carried out for the simultaneous separation of C₁–C₈ aliphatic carboxylic acid in a reasonable time (≤ 30 min). Alcohols >C₇ (heptanol) were not applicable due to the limitation of solubility.

Fig. 5A–C show chromatograms of these aliphatic carboxylic acid using 0.2 mM pyromellitic acids containing 10% methanol, 0.75% butanol and 0.15% heptanol as eluents, respectively. These chromatograms indicated that higher alcohols (heptanol) were more effective modifier than lower alcohols (methanol and butanol) for a decrease in the retention times and improvement of peak shapes of higher aliphatic carboxylic acids under the IEC–CD conditions. This is because higher alcohol was strongly adsorbed on the Zr-Silica and then reduced the hydrophobic interaction between the higher aliphatic carboxylic acids and the Zr-Silica.

From the above results, it was concluded that the most effective modifier was heptanol. As shown in Fig. 5C, excellently simultaneous separation of C₁–C₈ aliphatic carboxylic was achieved acids on the Zr-Silica column in 25 min using a 0.2 mM pyromellitic acid containing 0.15% heptanol as the eluent.

3.5. Analytical performance parameters

The optimum column and eluent conditions in IEC–CD for the simultaneous separation of C₁–C₈ aliphatic carboxylic acids were concluded to be the Zr-Silica (10 mg zirconium g⁻¹ silica gel) column and 0.2 mM pyromellitic acid containing 0.15% heptanol as the eluent. Various analytical performance parameters were investigated.

Calibration graphs were obtained by plotting peak area versus the concentration of these carboxylic acids. Linear calibration graphs ($r^2 \geq 0.99$) were obtained in the concentration range between 0.005 and 2.0 mM for these carboxylic acids.

The relative standard deviations of the chromatographic peak areas of these carboxylic acids, whose concentrations were 1.0 mM, were less than 0.8% ($n = 10$). Reproducible chromatograms were obtained during repeated chromatographic runs.

Table 2

Detection limits of C₁–C₈ aliphatic carboxylic acids with (A) or without (B) ASRS-Ultra anion self-regenerating suppressor in the K⁺ form

| Carboxylic acid | A | | B | |
|-----------------|---------------|---------------------|---------------|---------------------|
| | μM | ng ml^{-1} | μM | ng ml^{-1} |
| Formic acid | 0.18 | 8.3 | 0.17 | 7.8 |
| Acetic acid | 0.24 | 14 | 2.1 | 72 |
| Propionic acid | 0.28 | 21 | 1.7 | 1.3×10^2 |
| Butyric acid | 0.33 | 29 | 1.7 | 1.5×10^2 |
| Valeric acid | 0.42 | 43 | 2.1 | 2.2×10^2 |
| Caproic acid | 0.56 | 65 | 2.6 | 3.0×10^2 |
| Heptanoic acid | 1.2 | 1.5×10^2 | 4.5 | 5.9×10^2 |
| Caprylic acid | 2.1 | 3.0×10^2 | 5.6 | 8.1×10^2 |

Signal-to-noise ratio = 3. Injection volume: 20 μl .

Table 2 shows the detection limits of these aliphatic carboxylic acids with and without the suppressor in K⁺ form. Highly sensitive conductimetric detection for these aliphatic carboxylic acids was achieved using the suppressor in the K⁺ form.

4. Conclusion

In order to demonstrate the effectiveness of laboratory-made zirconium-modified silica gels (Zr-Silicas) as cation-exchange stationary phases in IEC, the application of Zr-Silicas to IEC–CD for the simultaneous separation of C₁–C₈ aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, heptanoic and caprylic acids) was carried out. A Zr-Silica adsorbed on 10 mg zirconium g⁻¹ silica gel was the most suitable stationary phase in the IEC–CD for the separation of these aliphatic carboxylic acids. An ASRS-Ultra anion self-regenerating suppressor in K⁺ form was used for the enhancement of conductimetric detector response of these aliphatic carboxylic acids. Excellent simultaneous separation and highly sensitive detection for these aliphatic carboxylic acids were achieved in 25 min by IEC–CD using the Zr-Silica column (250 \times 4.6 mm I.D.) and 0.2 mM pyromellitic acid containing 0.15% heptanol as the eluent. This result expanded the utility of Zr-Silica stationary phases in IEC for carboxylic acids.

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