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Ion-exclusion chromatography with conductimetric detection of aliphatic carboxylic acids on an H⁺-form cation-exchange resin column by elution with polyols and sugars

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Abstract

The Ion-exclusion chromatography (IEC) of normal aliphatic carboxylic acids of different acidity (pK_a) and hydrophobicity was investigated on a poly(styrene-divinylbenzene) (PS-DVB)-based strongly acidic cation-exchange resin column in the H⁺ form and conductimetric detection. When water was used as the eluent, although the C₁–C₅ carboxylic acids were separated from strong acid (HCl) depending on the pK_a and the hydrophobicity, the resolution was low and the peaks were accompanied by leading depending on their hydrophobicities. To improve the peak shape and the peak resolution, aqueous eluents of polyols and sugars containing 1–8 alcoholic OH groups (methanol, ethylene glycol, glycerol, erythritol, xylitol, fructose, sorbitol, and sucrose) were tested for the IEC separation of the carboxylic acids. When aqueous eluents of polyols and sugars were used, the tendency for leading peaks was decreased drastically with increasing number of OH groups in the polyols and sugars. This is due mainly to the increase in the hydrophilicity of the PS-DVB surface by the OH groups. An aqueous eluent of 10% methanol–0.15 M sucrose gave a reasonable resolution and highly sensitive detection for carboxylic acids. This eluent has a much lower background conductivity (ca. 4 $\mu\text{S cm}^{-1}$) and much higher detection sensitivity (ca. 170 times higher for valeric acid) than the 0.5 mM sulfuric acid (ca. 390 $\mu\text{S cm}^{-1}$) commonly used as an eluent in conventional IEC with conductimetric detection.

1. Introduction

Ion-exclusion chromatography (IEC) is a technique for the separation of organic and inorganic weak acids, especially those of a hydrophilic nature. Several reviews have been published on IEC [1–5]. Typically, a high-capacity cation-ex-

change resin in the H⁺ form is used in IEC. When water is used as an eluent, peaks with a leading front edge (fronted peaks) are obtained for carboxylic acids such as butyric acid. This is a result of adsorption as a side-effect in IEC. Accordingly, an aqueous solution of a strong acid such as sulfuric acid is generally used as the eluent for the separation of weak acids [1]. This is done to suppress the ionization of the sample

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acids and to ensure that they are entirely in their molecular forms. However, when conductivity detection is used, an acidic eluent causes a high background conductance and reduces the ability to detect sample acids.

In previous papers [6,7], we reported IEC using elution with a weak acid, such as benzoic or succinic acid, of low background conductivity. By using these weak acid eluents, highly sensitive conductimetric detection and high resolution for aliphatic carboxylic acids or F^- and PO_4^{3-} was accomplished.

A major aim of this research was to find an eluent that gives sharp chromatographic peaks for aliphatic carboxylic acids with some hydrophobicity, such as butyric and valeric acids, and highly sensitive detection for all aliphatic carboxylic acids without a high background conductance from the eluent itself. In this work, high-performance separation and highly sensitive conductimetric detection were achieved for aliphatic carboxylic acids by using a styrene-divinylbenzene copolymer (PS-DVB)-based strongly acidic cation-exchange resin in the H^+ form. The eluent was an aqueous solution containing a polyol or sugar with many OH groups. It is shown that excellent resolution and very sensitive conductimetric detection of aliphatic carboxylic acids are obtained using 0.15 M sucrose–10% methanol as the eluent.

2. Experimental

2.1. Apparatus

The ion chromatograph consisted of a Tosoh (Tokyo, Japan) CCPD metal-free eluent delivery pump with a flow-rate of 1 ml min^{-1} , equipped with a Rheodyne Model 7125 sample injector with a $100\text{-}\mu\text{l}$ PTFE loop.

Conductimetric detection was carried out with a Tosoh CM-8010 detector equipped with a constant-temperature controller maintained at 35°C .

The computing integrator was a System Instrument (Tokyo, Japan) Model 12 Chromatocorder.

2.2. Column

The separation column was a Tosoh glass column ($300 \text{ mm} \times 8 \text{ mm I.D.}$). The column was packed by the slurry packing technique and equilibrated thoroughly with the eluent before the chromatographic run.

2.3. Resin

A Tosoh TSKgel SCX styrene-divinylbenzene copolymer-based strongly acidic cation-exchange resin in the H^+ form with a particle size of $5 \mu\text{m}$ and an exchange capacity of $4.2 \text{ mequiv. g}^{-1}$ was used for all chromatographic runs.

2.4. Reagents and solutions

Standard solutions of aliphatic carboxylic acids and other inorganic acids were prepared as the acid or the salt from analytical-reagent grade chemicals without further purification.

Aqueous eluents containing polyols and sugars were prepared as 0.025–0.3 M solutions by dissolving the polyols (methanol, ethylene glycol, glycerol, erythritol, xylitol and sorbitol) and sugars (fructose and sucrose) in distilled, deionized water.

Methanol, ethylene glycol and glycerol were obtained from Wako (Osaka, Japan) and the other polyols, fructose and sucrose from Nikken Kasei (Tokyo, Japan).

3. Results and discussion

3.1. Effect of eluent composition on the separation and detection of carboxylic acids

As is well known, although carboxylic acids are separated from each other by elution with water, the resolution is very low and the peaks are fronted owing primarily to the hydrophobic adsorption effect as a side-effect in the IEC [6]. In an attempt to reduce peak fronting, polyols and sugars such as ethylene glycol, glycerol,

erythritol, xylitol, sorbitol, fructose and sucrose were tested and the elution performances were compared for the separation of various carboxylic acids. As shown in Table 1, these polyols and sugars have different numbers of alcoholic OH groups in the molecules.

Fig. 1a shows a complete IEC separation of strong acid (HCl) and C₁–C₅ aliphatic carboxylic acids with water as the eluent. As expected from the results in our previous papers on fundamental IEC research [4,6–9], the separation time was very long and the peaks were fronted, especially for the hydrophobic carboxylic acids such as propionic, butyric, and valeric acids. This is a consequence of an adsorption effect.

Fig. 1b shows a complete IEC separation of the strong acid (hydrochloric acid) and the carboxylic acids with 0.5 mM sulfuric acid as eluent. As expected from previous work [1,6], a high-resolution chromatogram was obtained by elution with 0.5 mM sulfuric acid, but the separation time was longer than with water as eluent and the conductimetric detection sensitivity was lower than with water as eluent. Additionally, the eluent background conductivity was extremely high and very noisy.

Fig. 1c–j show the IEC separation of the strong acid and the carboxylic acids by elution with polyols and sugars. As can be seen from the

peak shape of valeric acid, fronting was dramatically decreased with increasing number of OH groups in the polyols and sugars. This means that the hydrophobicity of the PS–DVB cation-exchange resin surface was decreased by the adsorption of the polyols and sugars. Hence it is possible to modify the polarity of the cation-exchange resin surface by adsorption of polyols and sugars, especially using sucrose with eight alcoholic OH groups.

The retention volumes (V_R) of all carboxylic acids gradually increased with increasing number of OH groups in the polyols except methanol and sugars. The V_R of valeric acid increased from 41.1 mL for water as eluent to 47.1 ml for 0.2 M sucrose–water.

On the other hand, the V_R s of carboxylic acids decreased with 0.2 M methanol–water as eluent compared with those with water and other polyol and sugar eluents. The V_R of valeric acid decreased from 41.1 ml with water to 34.5 ml with 0.2M methanol–water. This decrease might be due to the effect of the lipophilic property of the alkyl group in methanol rather than that of the hydrophilic property of the alcoholic OH group in methanol.

Although the mechanism of IEC is considered to involve partitioning of various analytes between the predominantly aqueous eluent and water inside the resin gel, several workers have proposed a mixed-mode mechanism in which there is also partitioning of the analytes due to the hydrophobic attraction of the analytes for the polymeric resin matrix [8,10,11].

The function of methanol in the eluent is to reduce this hydrophobic attraction by providing better solvation of the analytes in the mobile phase. However, sugar in the mobile phase is more effective in sharpening the sample peaks. The sugar additive coats the resin surface by a dynamic equilibrium between the liquid phase and the resin surface. The OH groups of the sugar make the resin surface more hydrophilic and decrease the attraction of the lipophilic parts of the analyte molecules.

Previous work has shown that sugars are indeed retained by ion-exchange resins [8]. Ali-

Table 1
Characteristics of polyols and sugars tested as eluents in ion-exclusion chromatography on a cation-exchange resin column for the separation of hydrochloric acid and some aliphatic acids

Polyol or sugar	Formula	No. of alcoholic OH groups
Methanol ^a	CH ₃ OH	1
Ethylene glycol ^a	C ₂ H ₆ O ₂	2
Glycerol ^a	C ₃ H ₈ O ₃	3
Erythritol ^a	C ₄ H ₁₀ O ₄	4
Xylitol ^a	C ₅ H ₁₂ O ₅	5
Fructose ^b	C ₆ H ₁₂ O ₆	5
Sorbitol ^a	C ₆ H ₁₄ O ₆	6
Sucrose ^b	C ₁₂ H ₂₂ O ₁₁	8

^a Alcohols (polyols).

^b Sugars.

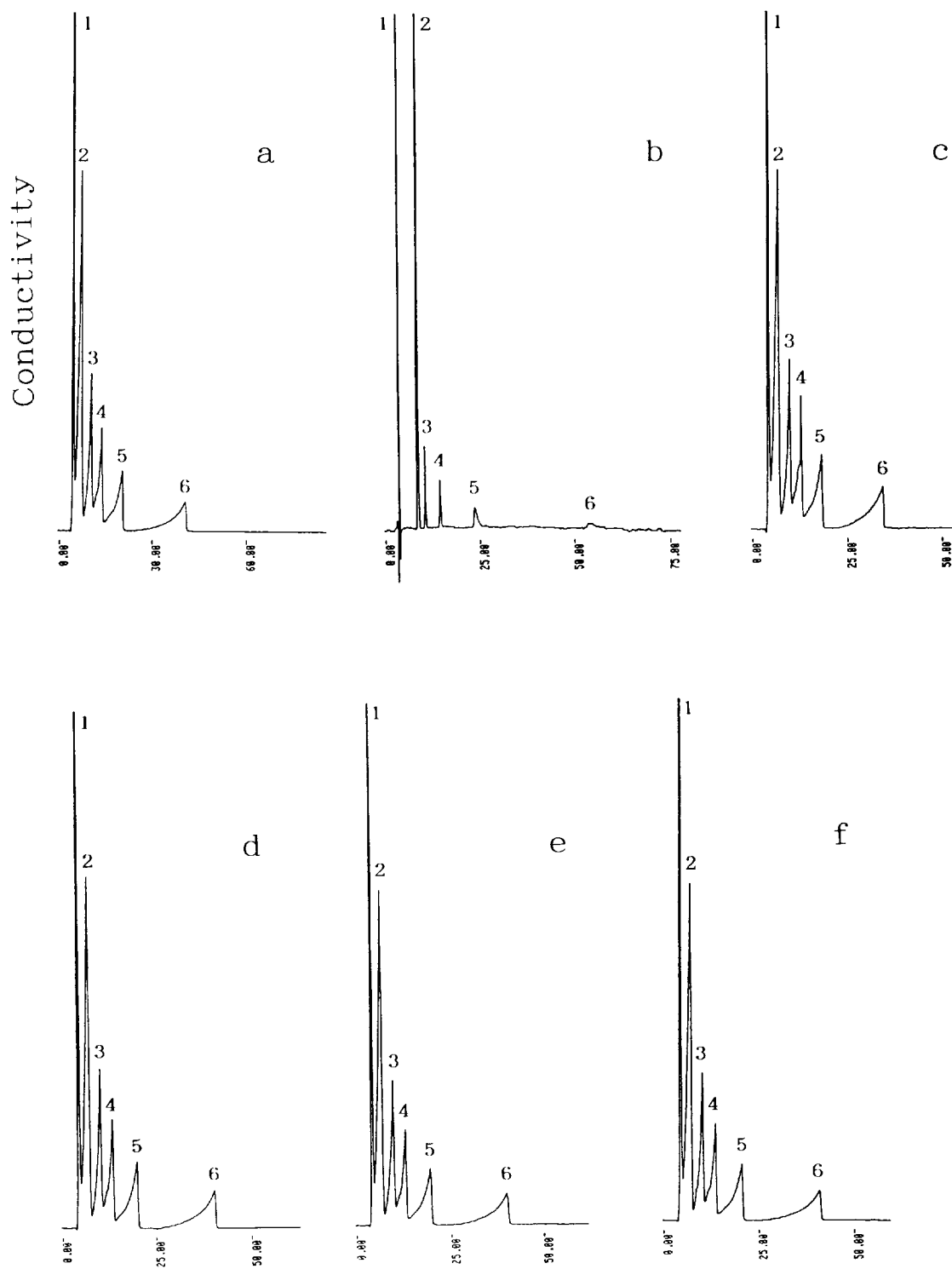


Fig. 1.

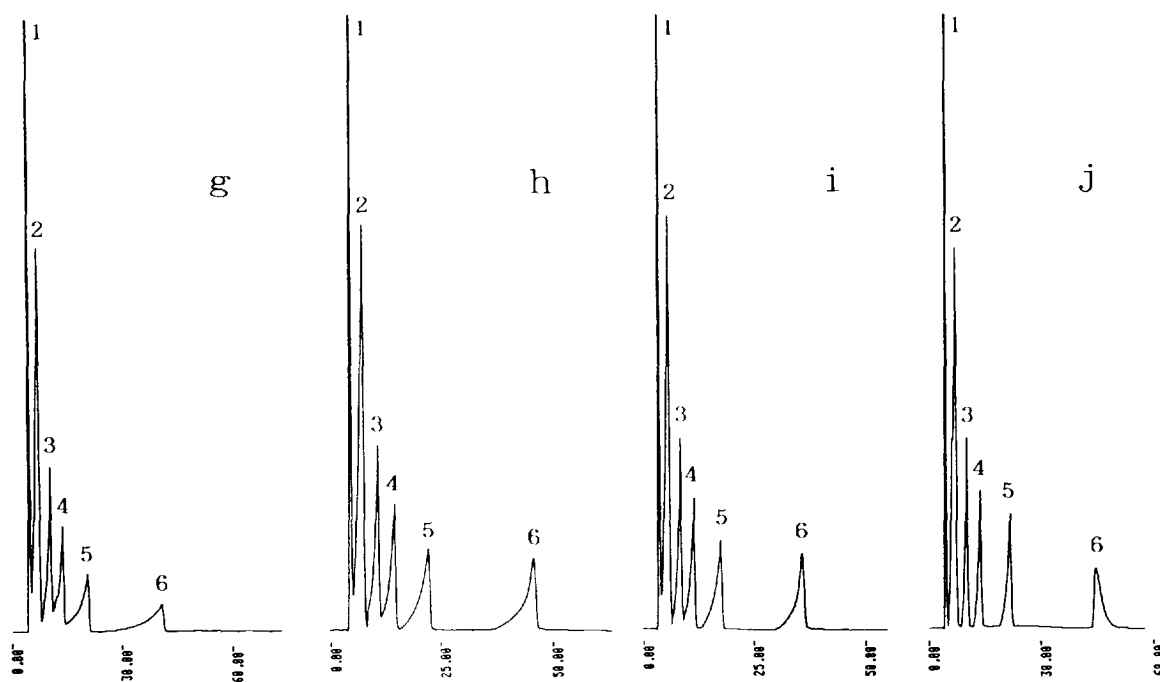


Fig. 1. Effect of eluent composition on IEC separation and conductimetric detection of a strong acid and some aliphatic carboxylic acids. Eluent: (a) water; (b) 0.5 mM sulfuric acid; (c) 0.2 M methanol–water; (d) 0.2 M ethylene glycol–water; (e) 0.2 M glycerol–water; (f) 0.2 M erythritol–water; (g) 0.2 M xylitol–water; (h) 0.2 M fructose–water; (i) 0.2 M sorbitol–water; (j) 0.2 M sucrose–water. Eluent flow-rate, 1 ml min⁻¹; column, Tosoh TSKgel SCX strongly acidic cation-exchange resin in the H⁺ form (300 mm × 8 mm I.D.); column temperature, room temperature (20°C); sample concentration, 1 mM each; sample volume, 100 μl; detection sensitivity, 40 μS cm⁻¹ full-scale (for sulfuric acid eluent, 16 μS cm⁻¹ full-scale); unit of retention time, min. Peaks: 1 = strong acid (hydrochloric acid); 2 = formic acid; 3 = acetic acid; 4 = propionic acid; 5 = butyric acid; 6 = valeric acid. All figures time scale in min.

phatic alcohols such as *n*-butanol have also been found to sharpen carboxylic acid peaks by altering the resin surface [9].

It is well known that in conventional IEC with conductimetric detection using sulfuric acid as eluent, the detector responses of carboxylic acids decrease owing to ionization suppression by the H⁺ ion of sulfuric acid [6,9]. To demonstrate the usefulness of polyols and sugars as eluents in IEC with conductimetric detection, the detector responses of carboxylic acids were compared.

As can be seen from the chromatograms of carboxylic acids with various polyols and sugars as eluents in Fig. 1, the detector responses of both formic and acetic acid were almost the same as those with water as eluent. The background conductivities of these polyol and sugar eluents

were very low, viz., just ca. 6 μS cm⁻¹ even with 0.3 M sucrose as eluent. Therefore, the noise level on the eluent background conductivity signal was extremely low.

From the above results, sucrose was judged to be the optimum eluent for the IEC separation and conductimetric detection of carboxylic acids.

3.2. Effect of sucrose concentration

In order to determine the optimum concentration of sucrose in the eluent, the effect of sucrose concentration on the IEC separation and conductimetric detection of some carboxylic acids was studied using 0.025–0.3 M sucrose. The V_{RS} of carboxylic acids increased slightly

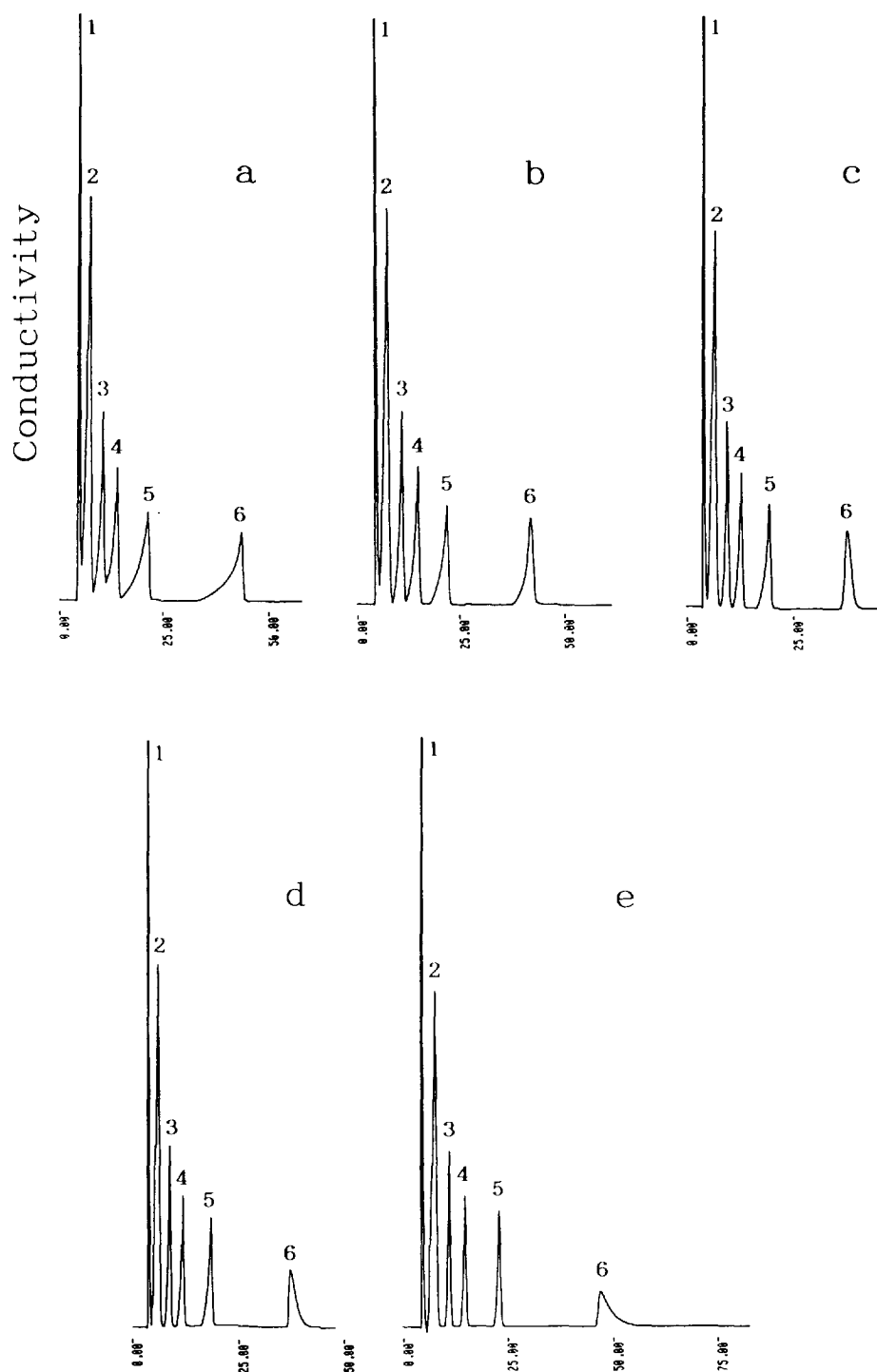


Fig. 2. Effect of sucrose concentration in eluent on IEC separation and conductimetric detection of hydrochloric acid and some carboxylic acids. Sucrose concentration: (a) 0.05; (b) 0.1; (c) 0.15; (d) 0.2; (e) 0.3 M. Other chromatographic conditions and peak identification as in Fig. 1.

with increasing concentration of sucrose in the eluent, as shown in Fig. 2. The background conductivity of the sucrose eluent ranged from ca. $0.5 \mu\text{S cm}^{-1}$ at $0.025 M$ to ca. $6 \mu\text{S cm}^{-1}$ at $0.3 M$.

Although the detector responses (peak height) of carboxylic acids decreased with increasing concentration of sucrose in the eluent, there was no effect on the peak-area method, as shown in Fig. 2. A good chromatogram of carboxylic acids without fronting was obtained by elution with $0.15 M$ sucrose, as shown in Fig. 2d.

3.3. Effect of methanol concentration

As described previously [2], addition of an organic solvent to the eluent might be expected to reduce the adsorption of propionic, butyric and valeric acid on the cation-exchange resin surface and thus decrease their V_{R} s. Additionally, the results described in the previous section (Fig. 1) indicated that the addition of methanol

to an aqueous eluent decreases the V_{R} s of hydrophobic aliphatic carboxylic acids such as propionic, butyric and valeric acid. Therefore, the effect of methanol concentration in the eluent on the V_{R} s of carboxylic acids was examined. The results are shown in Fig. 3 for the addition of methanol to the $0.15 M$ sucrose eluent.

The decrease in the V_{R} s of the hydrophobic carboxylic acids by addition of methanol to the sucrose eluent might be due to the effect of the lipophilic property of the alkyl group in methanol rather than that of the hydrophilic property of the alcoholic OH group in methanol.

The detector responses of carboxylic acids gradually decreased with increasing concentration of methanol in $0.15 M$ sucrose eluent, as shown in Fig. 3.

A reasonable separation and detection of carboxylic acids was accomplished by elution with 10% methanol in $0.15 M$ sucrose eluent, as shown in Fig. 3b. This eluent was chemically stable and there was no occurrence of fungi

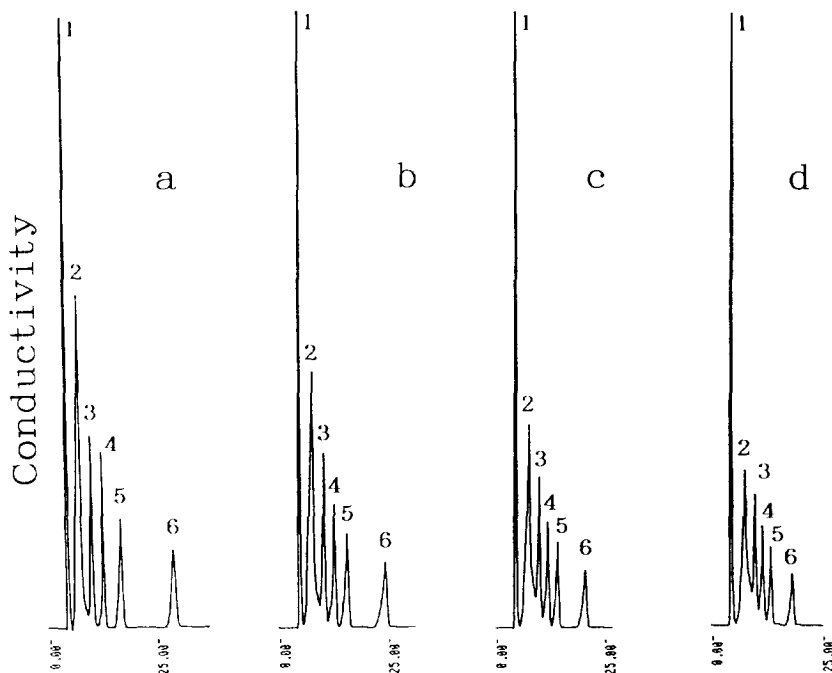


Fig. 3. Effect of methanol concentration in $0.15 M$ sucrose eluent on IEC separation and conductimetric detection of hydrochloric acid and some carboxylic acids. Methanol concentration: (a) 5; (b) 10; (c) 15; (d) 20%. Other chromatographic conditions and peak identification as in Fig. 1.

during chromatographic runs for several weeks, even at room temperature.

3.4. Retention volumes and distribution coefficients

The V_R s and the distribution coefficients (K_d) of various carboxylic acids with 10% methanol–0.15 M sucrose as eluent are given in Table 1. The K_d values were calculated according to the following equation, which was given in previous papers [4,6]:

$$V_R = V_0 + K_d V_i$$

where V_R is retention volume, V_0 the column void volume and V_i the volume of liquid inside the resin in the column.

As can be seen from Table 2, the K_d values of carboxylic acids tend to increase with increasing pK_1 and hydrophobicity. Most of the K_d values are between 0 and 1, but those of propionic, butyric and valeric acid are greater >1 . This means that adsorption by the resin matrix is a side-effect due to the greater hydrophobic nature of these acids.

Table 2

Retention volumes (V_R) and distribution coefficients (K_d) of a strong acid (hydrochloric acid), a very weak acid (carbonic acid) and weak acids (carboxylic acids) by elution with 10% methanol–0.15 M sucrose

Acid	pK_1	V_R (ml) ^a	K_d ^a
Hydrochloric acid	-7	4.98	0
Formic acid	3.8	8.02	0.34
Acetic acid	4.8	10.57	0.63
Propionic acid	4.9	12.58	0.86
Butyric acid	4.8	15.22	1.24
Valeric acid	4.8	25.22	2.28
Oxalic acid	1.3	5.00	0.00
Malic acid	1.9	6.45	0.17
Malonic acid	2.9	5.77	0.09
Tartaric acid	3.0	5.82	0.09
Citric acid	3.2	5.95	0.11
Succinic acid	4.2	8.54	0.40
Carbonic acid	6.4	13.85	1.00

Other chromatographic conditions as in Fig. 1.

^a $V_R = V_0 + K_d V_i$; $V_0 = 4.98$ ml, $V_i = 8.87$ ml.

3.5. Calibration graph

A calibration graph was obtained by plotting peak height against carboxylic acid concentration in the range 0–3 mM. The calibration graph obtained was non-linear at concentrations >2 mM. This might be due to mainly to the decrease in the degree of dissociation of carboxylic acids at higher concentrations.

3.6. Reproducibility

The reproducibility of peak heights obtained by repeated injections of 1 mM standards is ca. 0.8% (relative standard deviation) for all the carboxylic acids.

3.7. Detection limits

The detection limits of carboxylic acids at a signal-to-noise ratio (S/N) of 3 on elution with 10% methanol–0.15 M sucrose are given in Table 3. As this method uses a non-acidic eluent, the eluent background conductivity was much lower (ca. $4 \mu\text{S cm}^{-1}$) than that with 0.5 mM sulfuric acid as eluent (ca. $390 \mu\text{S cm}^{-1}$). There-

Table 3

Comparison of detection limits of carboxylic acids by elution with (A) 0.5 mM sulfuric acid, (B) water and (C) 10% methanol–0.15 M sucrose

Acid	Detection limit (S/N = 3) (μM)		
	A ^a	B ^b	C ^c
Formic acid	8.4	0.12	1.40
Acetic acid	84	0.27	2.50
Propionic acid	150	0.40	3.44
Butyric acid	330	0.70	4.34
Valeric acid	1200	1.61	6.88

Other chromatographic conditions as in Fig. 1.

^a Eluent background conductivity, $390 \mu\text{S cm}^{-1}$; noise level, $0.21 \mu\text{S cm}^{-1}$.

^b Eluent background conductivity, ca. $390 \mu\text{S cm}^{-1}$; noise level, $0.00321 \mu\text{S cm}^{-1}$.

^c Eluent background conductivity, ca. $4 \mu\text{S cm}^{-1}$; noise level, $0.0236 \mu\text{S cm}^{-1}$.

fore, the noise level on the eluent background conductivity was extremely low ($0.0236 \mu\text{S cm}^{-1}$). As there is no ionization suppression of carboxylic acids by H^+ ion in the acidic eluent, the detector response was as highly sensitive as the water eluent. The detection sensitivities of carboxylic acids (valeric acid) were ca. 6.5–174 times higher than those in conventional conductimetric IEC using sulfuric acid as the eluent, depending on their $\text{p}K_{\text{a}}$ and V_{R} values, as shown in Table 3.

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