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Ion-exclusion chromatography with conductimetric detection of aliphatic carboxylic acids on a weakly acidic cation-exchange resin by elution with benzoic acid– β -cyclodextrin

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Abstract

In this study, an aqueous solution consisting of benzoic acid with low background conductivity and β -cyclodextrin (β -CD) of hydrophilic nature and the inclusion effect to benzoic acid were used as eluent for the ion-exclusion chromatographic separation of aliphatic carboxylic acids with different pK_a values and hydrophobicity on a polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form. With increasing concentration of β -cyclodextrin in the eluent, the retention times of the carboxylic acids decreased due to the increased hydrophilicity of the polymethacrylate-based cation-exchange resin surface from the adsorption of OH groups of β -cyclodextrin. Moreover, the eluent background conductivity decreased with increasing concentration of β -cyclodextrin in 1 mM benzoic acid, which could result in higher sensitivity for conductimetric detection. The ion-exclusion chromatographic separation of carboxylic acids with high resolution and sensitivity was accomplished successfully by elution with a 1 mM benzoic acid–10 mM cyclodextrin solution without chemical suppression.

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1. Introduction

Ion-exclusion chromatography has frequently been used for the chromatographic separation of weak acids, especially hydrophilic aliphatic carboxylic

acids [1–5]. Using an unbuffered mobile phase, it was found that the retention of mono-carboxylic acids by hydrophobic adsorption increases in the sequence: sulfonated silica gel < polymethacrylate resin < poly(styrene–divinylbenzene) stationary phase [1]. Therefore, typical stationary phases, polystyrene–divinylbenzene (PS–DVB)-based strongly acidic cation-exchange resins in the H^+ form with a high ion-exchange capacity (e.g. 1.5 mequiv./ml resin) and weakly acidic cation-exchange resins with

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low capacity (e.g. 0.1 mequiv./ml resin), are usually applied in the separation of hydrophilic and hydrophobic carboxylic acids in environmental samples [2].

Our group has already reported the ion-exclusion chromatographic separation of aliphatic carboxylic acids on a polymethacrylate-based weakly acidic cation-exchange resin (Tosoh TSKgel OApak-A) [6–11]. The eluents used in these separations were water, mixtures of water with organic solvents, or dilute solutions of strong or weak carboxylic acids. When ion-exclusion chromatography was performed on both weakly acidic cation-exchange resins with water as eluent, the resolution of aliphatic carboxylic acids was generally low and the peaks obtained were fronted, primarily due to hydrophobic adsorption at the resin surface. In order to improve the peak shapes for hydrophobic carboxylic acids, dilute solutions of strong acids (such as sulfuric acid) or weak acids (such as benzoic acid) should be employed to ensure that the degree of ionization remains constant across the entire sample band. However, the use of such eluents results in reduced sensitivity of conductivity detection as a result of the increased background conductivity of the eluent.

In our previous studies [12,13] we have shown that, in ion-exclusion chromatography on PS–DVB-based strongly acidic cation-exchange resins in the H^+ form, the addition of sugars, polyols, and poly(vinyl alcohol)s to the aqueous eluent is very effective for improvement of the peak shape and the peak resolution, based on the effect of the increased hydrophilicity of the cation-exchange resin surface due to adsorption of OH groups.

The purpose of this study was to demonstrate the effectiveness of a mixed aqueous eluent consisting of benzoic acid, with a reduced fronting effect, and cyclodextrin, with an increased hydrophilicity effect to the cation-exchange resin surface and inclusion effect to benzoic acid, in the ion-exclusion chromatographic separation of aliphatic carboxylic acids on a weakly acidic cation-exchange resin in the H^+ form. Using a 1 mM benzoic acid–10 mM β -cyclodextrin solution as eluent with a relatively low eluent background conductivity, the ion-exclusion chromatographic separation of carboxylic acids with high resolution, sharp and symmetric peak shapes and sensitive conductimetric detection was achieved without chemical suppression.

2. Experimental

2.1. Instrumentation

All experiments were carried out on a Shimadzu LC10-AT series (Osaka, Japan) with a data processing system (C-R6A) and including a non-metallic eluent delivery pump (LC-10AT), a conductimetric detector (CDD-6A), a column oven (CTO10A) and an injector equipped with a 100 μ l sample loop.

2.2. Column

The separation column was a Tosoh polyether ether ketone (PEEK) column (300 \times 7.8 mm I.D.) packed with a Tosoh TSKgel OApak-A polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form with a particle size of 5 μ m. The exchange capacity was 0.1 mequiv./ml resin. The column was equilibrated thoroughly with eluent before the chromatographic run.

A mixture of strong acid (sulfuric acid, 0.05 mM) and weak acid (benzoic acid, 1 and 10 mM) containing β -cyclodextrin (10 mM) was used as eluent at 1.0 ml/min and the column temperature was maintained at 35 °C. Sample injection volumes were 100 μ l.

2.3. Reagents

All solutions were prepared from analytical reagent-grade chemicals in distilled and deionized water. Benzoic acid used as the eluent was purchased from Katayama (Osaka, Japan) and sulfuric acid eluent was obtained from Wako (Osaka, Japan) as 1.0 M sulfuric acid. β -Cyclodextrin (β -CD) was purchased from Wako. Aliphatic carboxylic acids were prepared as 20–40 mM stock solutions and were used to prepare standard mixtures of these species.

3. Results and discussion

3.1. Ion-exclusion chromatographic separation of carboxylic acids by elution with water, a strong acid, and a weak acid

As shown in Fig. 1A, when using water as eluent

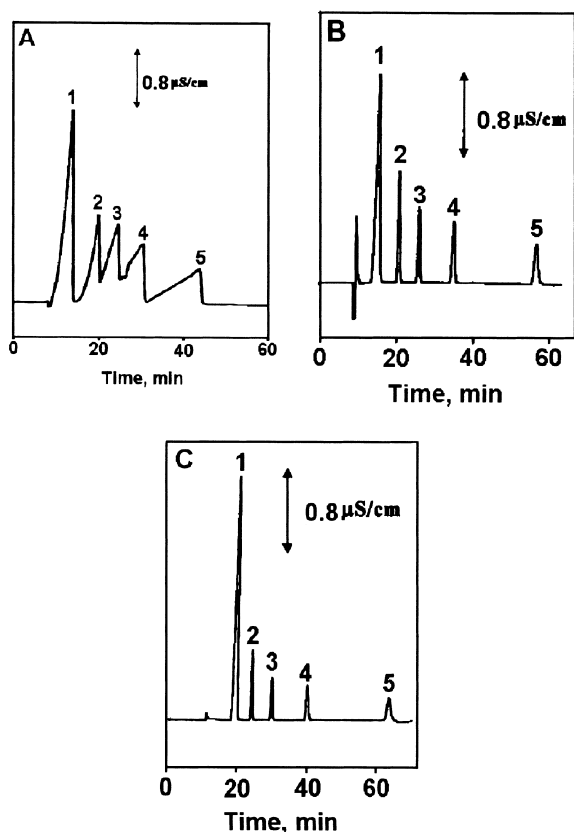


Fig. 1. Ion-exclusion chromatograms of aliphatic carboxylic acids eluted with (A) water, (B) 0.05 mM sulfuric acid, and (C) 1 mM benzoic acid. Column, TSKgel OA-PAK-A polymethacrylate-based weakly acidic cation-exchange resin, 300 mm \times 7.8 mm I.D.; column temperature, 35 $^{\circ}$ C; flow-rate, 1.0 ml/min; detection, conductivity detection; injection volume, 100 μ l; sample concentration, 0.5 mM for all aliphatic carboxylic acids. Peaks: 1=formic acid, 2=acetic acid, 3=propionic acid, 4=butyric acid, 5=valeric acid.

(pH 5.6), a low-resolution ion-exclusion chromatography separation and bad peak shape were obtained for the carboxylic acids. Using acid as eluent can result in an improvement of the peak resolution and peak shape [14,15]. From Figs. 1B and C, we can see that a high-resolution separation of the carboxylic acids was obtained when using a strong acid (sulfuric acid) as eluent at pH 4.0 and a weak acid (benzoic acid) as eluent at pH 3.6. The above result indicates that the H^{+} from these acid eluents represses ionization of the sample acids and sharpens their chromatographic peaks, indicating that the peak shapes are

dependent on the proton concentration (pH value) of the eluent.

The eluent background conductivity values obtained using 0.05 mM sulfuric acid and 1 and 10 mM benzoic acid as eluent were relatively high, as shown in Table 1. As the increased eluent background conductivity caused a decrease in the conductimetric detection sensitivity, eluent conductivity suppression is generally necessary.

3.2. Effect of β -cyclodextrin on the conductimetric detection sensitivity of carboxylic acids

In order to suppress eluent conductivity, the effect of β -CD with inclusion effect to eluent acid with hydrophobic nature was tested. As shown in Fig. 2, β -CD exhibits an inclusion effect to hydrophobic acids such as benzoic acid.

As can be seen from Table 1, the eluent background conductivity increases with increasing β -CD concentration when the benzoic acid concentration increases. However, when compared with sulfuric acid, we observed that (1) the conductivity in absolute value was much higher with benzoic acid than with sulfuric acid and, more importantly, (2) the so-called increase with sulfuric acid was from 64 μ S/cm (for 1 mM β -CD) to 72 μ S/cm (for 10 mM β -CD). For 10 mM β -CD, the conductivity was 72 μ S/cm for 0.05 mM sulfuric acid, 79 μ S/cm for 1 mM benzoic acid and 294 μ S/cm for 10 mM benzoic acid. The decrease of the eluent background conductivity could be attributed to the inclusion effect of benzoic acid by β -CD.

Table 1

Background conductivity of water, sulfuric acid and benzoic acid solutions used as eluent containing different concentrations of β -cyclodextrin

Eluent	Conductivity (μ S/cm)				
	β -Cyclodextrin concentration (mM)				
	0	1	2	5	10
Water ^a	0	2	3	8	17
0.05 mM Sulfuric acid ^a	55	64	68	69	72
1 mM Benzoic acid	122	113	105	87	79
10 mM Benzoic acid	439	414	385	319	294

^a The increased conductivity of water and sulfuric acid eluents is mainly due to the presence of ionic impurities in β -CD.

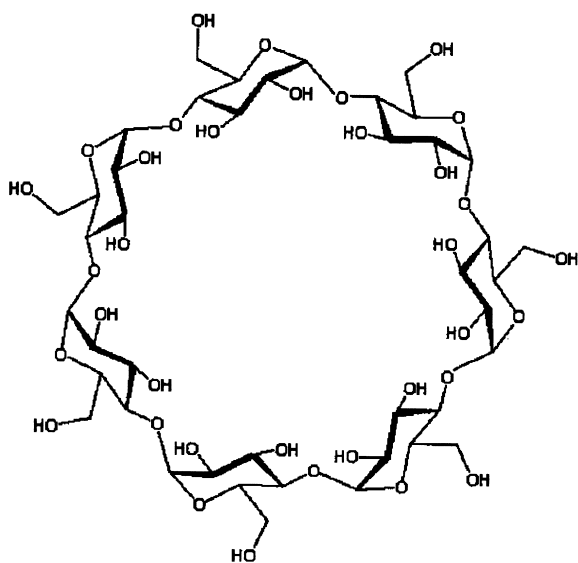


Fig. 2. Structure of β -cyclodextrin.

3.3. Effect of β -cyclodextrin on retention of aliphatic carboxylic acids

In order to determine the optimum concentration of β -CD in 1 mM benzoic acid eluent, the effect of the β -CD concentration on the ion-exclusion chromatographic separation of carboxylic acids was investigated between 0 and 10 mM. The relationship between the β -CD concentration and the retention volumes of carboxylic acids is shown in Fig. 3. We can see that the retention volumes of the carboxylic acids are not entirely dependent on the β -CD concentration for formic, acetic, and propionic acids. The reduced retention volume of butyric and valeric acids is due to the reduced hydrophobicity of the resin surface caused by the increased concentration of β -CD in the eluent.

As shown in Fig. 4, a good ion-exclusion chromatographic separation of carboxylic acids was achieved on elution with 1 mM benzoic acid–10 mM β -CD. Additionally, good ion-exclusion chromatographic separation of the hydrophilic carboxylic acids was also achieved under the same elution conditions, as shown in Fig. 5.

Additionally, there were no changes in the conductimetric detection sensitivity for all hydrophilic carboxylic acids because the inclusion effect occurs

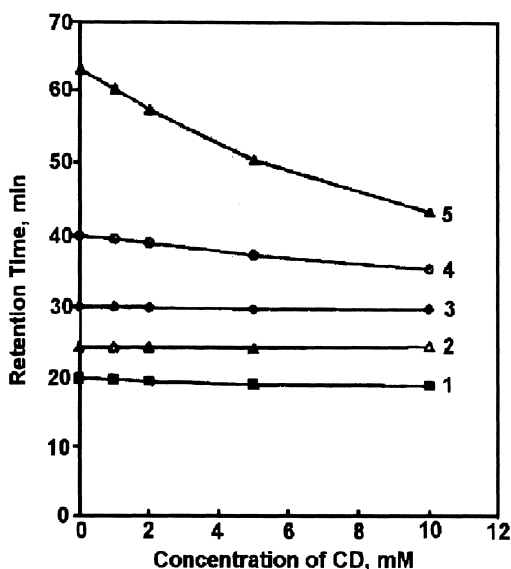


Fig. 3. Effect of β -cyclodextrin on the retention times of aliphatic carboxylic acids eluted with 1 mM benzoic acid. Other conditions as in Fig. 1. 1=formic acid, 2=acetic acid, 3=propionic acid, 4=butyric acid, 5=valeric acid.

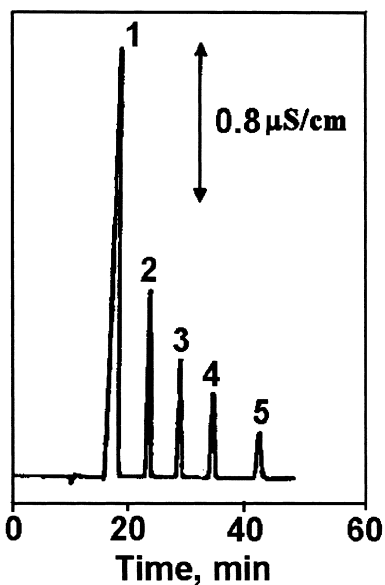


Fig. 4. Ion-exclusion chromatograms of carboxylic acids eluted with 1 mM benzoic acid–10 mM β -cyclodextrin. Other conditions as in Fig. 1.

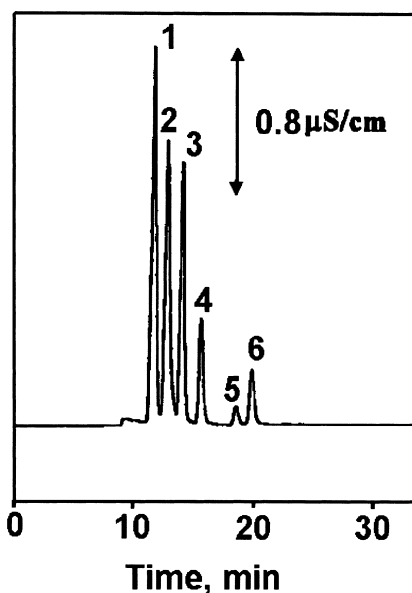


Fig. 5. Ion-exclusion chromatogram of hydrophilic carboxylic acids eluted with 10 mM benzoic acid–10 mM β -cyclodextrin; sample concentrations, 0.1 mM for all hydrophilic carboxylic acids. Peaks: 1=tartaric acid, 2=citric acid, 3=malic acid, 4=lactic acid, 5=acetic acid, 6=succinic acid. Other conditions as in Fig. 1.

only to hydrophobic carboxylic acids such as benzoic acid.

From the above results, an eluent consisting of benzoic acid and β -CD is concluded to be useful for the ion-exclusion chromatographic separation of hydrophilic carboxylic acids.

3.4. Effect of methanol concentration

In order to accelerate the elution of hydrophobic carboxylic acids, the effect of methanol on the retention volumes of aliphatic carboxylic acids was investigated. With increasing methanol concentration, the retention volumes of formic, acetic and propionic acids were virtually unaltered, whereas those of hydrophobic acids such as butyric and valeric acids decreased drastically. This decrease might be due to the effect of the lipophilic alkyl group in methanol rather than to the hydrophilic alcoholic OH group in methanol. This retention behavior was almost the same as for the ion-exclusion chromatographic separation

Table 2

Detection limits of carboxylic acids at $S/N = 3$

Carboxylic acid	Detection limit ($\mu\text{g}/\text{ml}$)
Tartaric	0.06
Citric	0.12
Malic	0.09
Lactic	0.15
Acetic	0.59
Succinic	0.38

of aliphatic carboxylic acids on the TSKgel OA-Pak A used in previous work [16].

From the above results, the addition of methanol to benzoic acid– β -CD is very effective in reducing the ion-exclusion chromatographic separation time of aliphatic carboxylic acids.

3.5. Calibration curves and detection limits of carboxylic acids

The calibration curves of all aliphatic carboxylic acids on elution with 1 mM benzoic acid–10 mM β -CD were obtained using peak heights, and linear calibration was maintained from 0 to 0.6 mM for tartaric, citric, malic, lactic, acetic and succinic acids.

The detection limits of carboxylic acids on elution with 1 mM benzoic acid–10 mM β -CD were determined at a signal-to-noise ratio of 3. As shown in Table 2, the detection limits of tartaric, citric, malic, lactic, acetic and succinic acids were in the range 0.06 $\mu\text{g}/\text{ml}$ (tartaric acid)–0.59 $\mu\text{g}/\text{ml}$ (acetic acid), depending on their acidity.

4. Conclusion

In this study, the ion-exclusion chromatography of carboxylic acids was investigated using a novel combination of a weakly acidic cation-exchange resin column and benzoic acid– β -CD. Good resolution and highly sensitive conductimetric detection of carboxylic acids was achieved by elution with diluted benzoic acid– β -CD, while the application of this method to practical samples will be the subject of future work.

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