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Use of potassium-form cation-exchange resin as a conductimetric enhancer in ion-exclusion chromatography of aliphatic carboxylic acids

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ABSTRACT

In this study, a cation-exchange resin (CEX) of the K^+-form, i.e., an enhancer resin, is used as a postcolumn conductimetric enhancer in the ion-exclusion chromatography of aliphatic carboxylic acids. The enhancer resin is filled in the switching valve of an ion chromatograph; this valve is usually used as a suppressor valve in ion-exchange chromatography. An aliphatic carboxylic acid (e.g., CH_3COOH) separated by a weakly acidic CEX column of the H^+-form converts into that of the K^+-form (e.g., CH_3COOK) by passing through the enhancer resin. In contrast, the background conductivity decreases because a strong acid (e.g., HNO_3) with a higher conductimetric response in an eluent converts into a salt (e.g., KNO_3) with a lower conductimetric response. Since the pH of the eluent containing the resin enhancer increases from 3.27 to 5.85, the enhancer accelerates the dissociations of analyte acids. Consequently, peak heights and peak areas of aliphatic carboxylic acids (e.g., acetic acid, propionic acid, butyric acid, and valeric acid) with the enhancer resin are 6.3–8.0 times higher and 7.2–9.2 times larger, respectively, than those without the enhancer resin. Calibrations of peak areas for injected analytes are linear in the concentration range of 0.01–1.0 mM. The detection limits (signal-to-noise ratio = 3) range from 0.10 μM to 0.39 μM in this system, as opposed to those in the range of 0.24–7.1 μM in the separation column alone. The developed system is successfully applied to the determination of aliphatic carboxylic acids in a chicken droppings sample.

1. Introduction

Ion-exclusion chromatography (IEC) has been a useful analytical method mainly for the separation and determination of weak acids, e.g., carboxylic acids, bicarbonates, and silicates, and weak bases, e.g., ammonium ion, and amines [1–7]. Acids analyzed by IEC (i.e., analytes) can be classified into fully ionized species and partially ionized species, depending on ion-exclusion/penetration effect on pseudo Donnan's membrane effect between stationary phase and mobile phase, and hydrophobic adsorption to the resin phase [1].

Conductivity detection has been commonly used for IEC, because it is possible to simultaneously detect many kinds of ionized species by this detection technique. However, responses of partially ionized species are low due to their low dissociation in an acidic eluent [8]. Therefore, many researchers have attempted and reported [9–15]. In particular, postcolumn ion-exchange reactions are useful (1) for achieving linear calibrations in wide concentration ranges and (2) for carrying out sensitive detection by converting partially ionized species into fully ionized species. Tanaka and Fritz [9] have reported that the response of a bicarbonate by a combination of two different ion-exchange resin columns connected after a separation column is approximately ten times that by a single separation column; this result is attributed to the fact that the columns caused the conversion of the bicarbonate from a weak acid to a strong base. Further, Hayashi carried out sensitive conductimetric detection by accelerating the ionization of weak acids by using a (bis-[2-hydroxyethyl]-iminotris-[hydroxy methyl]-methane: Bis-Tris) buffer with a pH of 6.5 connected after a separation column [11]. Guillén et al. [12] have applied this method to the determination of organic acids in brandy samples.

The purpose of this study is to develop a postcolumn conductimetric enhancement system by using a cation-exchange resin (CEX) in the alkali metal form, packed in a switching valve of the ion chromatograph Tosoh IC-2001 [16]. This CEX functions as a conductimetric enhancer for a weak acid used as a sample in IEC, because the CEX converts from a species with low conductivity (e.g., CH_3COOH) into that with high conductivity (e.g., CH_3COOK). Conversely, the resin functions as a conductimetric suppressor for strong acids used as eluents, because it converts from a species with high conductivity (e.g., HNO_3) into that with low conductivity (e.g., KNO_3). Consequently, the conductimetric responses of weak acid analytes improve with the use of the CEX.

This paper reports that a CEX of the K^+-form is an effective conductimetric enhancer for the IEC of monocarboxylic acids in terms

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of sensitive detection, calibration linearity, and its applicability to the determination of aliphatic carboxylic acids in a chicken droppings sample.

2. Experimental conditions

2.1. Reagents

Standard solutions of aliphatic carboxylic acids were purchased from Wako Pure Chemicals (Osaka, Japan), and they were dissolved in deionized water obtained from a Milli-Q reagent grade water system (Millipore).

2.2. Separation column and enhancer resin

The separation column was a weakly acidic CEX (Tosoh TSKgel Super IC-A/C, 150 mm × 6 mm ID) of the H^+-form.

The enhancer resin was Tosoh TSKsuppress IC-A (200 μm particles). The resin was converted from the H^+-form into the alkali metal form by an SR-2W Recipro shaker (TAITEC, Koshigaya, Japan) in a 0.5 M salt solution (e.g., LiCl, Na_2SO_4, and K_2SO_4) for 1 h. The enhancer resin was filled into a bottle connected just before the switching valve.

2.3. Enhancement process

All IEC measurements were carried out using the Tosoh IC-2001 ion chromatograph, which consists of an eluent pump, auto-sample injector, conductimetric detector, column oven, and suppression system. In this study, the suppression system was used as the enhancer system for the conductivity detection of aliphatic carboxylic acids in IEC. Therefore, we refer to the valve as the “enhancer valve.”

A summarized description of the enhancement procedure is schematically shown in Fig. 1. The enhancer valve consists of a six-port electronic rotary valve equipped with three grooves for packing the resin. Since the enhancer valve is switched before each injection, a new resin is always used for enhancement in analyzing each sample. While the first groove (a) is used for measuring the sample, the resin used is discharged to the drain from the second groove (b). Simultaneously, a new resin is filled into the third groove (c) in order to measure the next sample [16].

The filling and discharging of the resin and the washing of the groove are carried out by means of water pressure achieved using a syringe pump. The resin that is used in the groove is a strongly acidic cation exchanger of the alkali metal (M)-form; therefore, the chemical reaction occurring in the enhancer valve is as follows:

$$\text{Resin–SO}_3\text{M} + \text{R–COOH} \rightarrow \text{Resin–SO}_3\text{H} + \text{R–COOM}$$

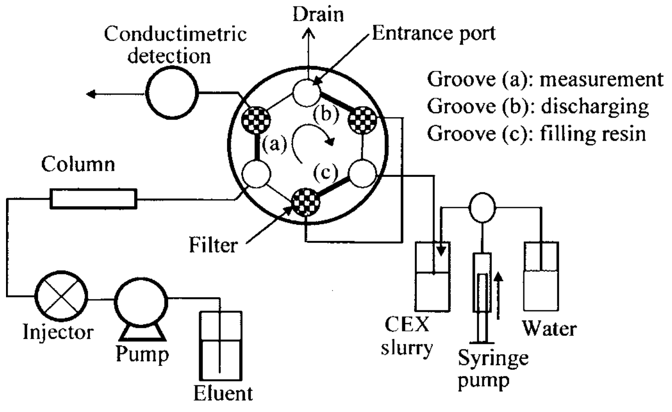

Fig. 1. Configuration of enhancer valve in Tosoh IC-2001. The details are described in the text.

Since the resin used for enhancement in the groove is disposable, no regeneration of the enhancer resin is required, and by-products and high molecular weight of matrix in a real sample do not damage the enhancer valve. The small volume of the grooves (200 μL) in the enhancer valve helps to eliminate band broadening; at the same time, the volume of the groves is sufficient for measuring one sample.

2.4. Analytical conditions

Acids added to the eluent were 0.5 mM nitric acid (HNO_3, pH 3.24), 0.5 mM perchloric acid ($HClO_4$, pH 3.27), 0.25 mM sulfuric acid (H_2SO_4, pH 3.27), and 0.55 mM phosphoric acid (H_3PO_4, pH 3.27). The flow rate of the eluent was 0.6 mL min^{-1}. The temperature of the column oven was 40 °C. Further, the injection volume was 30 μL.

2.5. Preparation of sample

A chicken droppings sample was obtained from a chicken farm in Gunma Prefecture, Japan. After the decomposition of 0.1 g of the sample in 50 mL of distilled-deionized water using a microwave, the sample was filtered using a syringe filter (0.45 μm). At the time of determining the aliphatic carboxylic acids in the sample, a strongly acidic CEX column of the H^+-form, TSKgel OApak-P (60 mm × 6 mm ID), was connected before the separation column in order to unify counter-cations to H^+ in the sample.

3. Results and discussion

3.1. Selection of enhancer resin

Three different strongly acidic CEXs of the alkali metal form were compared in terms of their conductimetric enhancement effects on the aliphatic carboxylic acids after ion-exclusion chromatographic separation. CEXs of the Li^+-form, Na^+-form, and K^+-form were tested as enhancer resins. Fig. 2 shows typical ion-exclusion chromatograms of five aliphatic carboxylic acids; the K^+-form CEX was used as the enhancer resin and the eluent was 0.55 mM phosphoric acid. From the chromatograms, it could be observed that the signals of analyte acids with the enhancer were considerably stronger than those without it.

Table 1 summarizes enhancement ratios of analytical signals obtained with and without the enhancer in the 0.55 mM phosphoric acid eluent. The enhancement ratios of five aliphatic carboxylic acids with the K^+-form CEX were the highest in this study, though the background conductivity obtained with the K^+-form CEX (61.0 μS cm^{-1}) was higher than those obtained with the Li^+-form (40.6 μS cm^{-1}) and Na^+-form (48.7 μS cm^{-1}) CEXs. Analytical signals of all acids, except for formic acid, with K^+-form CEX were 6.3–8.0 times higher and 7.2–9.2 times larger, respectively, than those in the case of acids without K^+-form CEX. This would be attributed to the fact that the limiting equivalent conductivity of K^+ is higher than those of Li^+ and Na^+ [17]. From these results, it was concluded that the K^+-form CEX was the most suitable enhancer resin among all resins considered in this study.

3.2. Selection of acid added to eluent

The role of acid in the eluent used for carrying out the IEC of aliphatic carboxylic acids with the K^+-form enhancer resin was investigated. As mentioned earlier, the acids added to the eluent

Table 1
Ratio of analytical signals with and without enhancer resin[a].

Analyte	Peak height			Peak area		
	Li^+-form	Na^+-form	K^+-form	Li^+-form	Na^+-form	K^+-form
Formic acid	0.9	0.9	1.1	0.9	0.9	1.1
Acetic acid	3.2	5.5	6.3	3.7	5.8	7.2
Propionic acid	3.7	7.0	8.0	4.3	7.3	9.2
n-Butyric acid	3.5	6.3	7.4	3.5	6.2	8.3
n-Valeric acid	3.7	6.5	7.6	4.1	7.0	8.6

[a] (Enhancement ratio) = (analytical signal with enhancer resin)/(analytical signal without enhancer resin). The experimental conditions are described in Fig. 2.

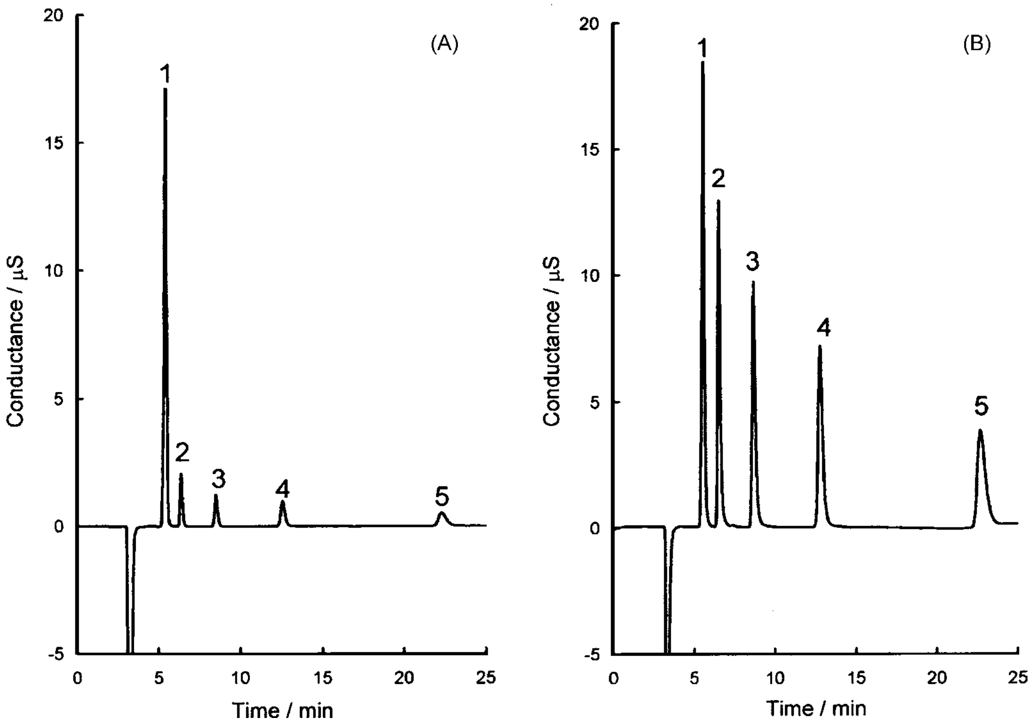

Fig. 2. Ion-exclusion chromatograms of aliphatic carboxylic acids with and without enhancer resin. Chromatogram: (A) without enhancer resin and (B) with enhancer resin. Separation column: weakly acidic cation-exchange resin column TSKgel Super IC-A/C (150 mm × 6 mm ID). Eluent: 0.55 mM H_3PO_4. Flow rate: 0.6 mL min^{-1}. Column temperature: 40 °C. Detection: conductivity. Injection volume: 30 µL. Sample concentration: 0.5 mM. Peak: 1 = formic acid; 2 = acetic acid; 3 = propionic acid; 4 = *n*-butyric acid; and 5 = *n*-valeric acid.

were 0.5 mM nitric acid, 0.5 mM perchloric acid, 0.25 mM sulfuric acid, and 0.55 mM phosphoric acid. The conductimetric responses of the analyte acids could be increased by the enhancer irrespective of the kind of acid in the eluent.

Conversely, the background conductivity was decreased by the enhancer resin (Table 2), because the acid with a higher conductimetric response in the eluent was converted into a salt with a lower conductimetric response.

Additionally, pH values of the eluent discharged from separation column and from the enhancer valve with the K^+-form enhancer resin were measured. The pH values of 0.5 mM nitric acid, 0.25 mM sulfuric acid, 0.5 mM perchloric acid, and 0.55 mM phosphoric acid from the enhancer valve were 5.65, 5.82, 5.61, and 5.85, as opposed to corresponding pH values of 3.27, 3.24, 3.26, and 3.27, respectively, from the separation. These values imply that the ionization of the aliphatic carboxylic acids was accelerated by an increase in the pH of the eluent as well as by the conversion of the analyte from an acid to a salt.

Table 2
Background conductivity of acidic eluent with and without enhancer resin.

Eluent	Background conductivity (µS cm^{-1})	
	Without enhancer resin	With enhancer resin[a]
0.5 mM HNO_3	186	68.4
0.25 mM H_2SO_4	182	71.3
0.5 mM $HClO_4$	184	66.6
0.55 mM H_3PO_4	173	61.0

[a] Enhancer resin: K^+-form CEX. The other conditions are the same as those described in Fig. 2.

Table 3 summarizes the limit of detection (LOD) of the analyte acids at a signal-to-noise ratio (S/N) of 3. The LODs in the phosphoric acid eluent were lower than those in other acid eluents. Moreover, in the phosphoric acid eluent, the LODs with the enhancer resin were 1/10 those without the enhancer resin. This is attributed to the decrease in the noise level by the enhancer resin with an increase in the peak response.

3.3. Relationship between conductimetric enhancement and pK_{a1} of carboxylic acids

As mentioned above, the conductimetric enhancement effect of the developed system in the case of formic acid was small. Fig. 3 shows a plot of enhancement ratios of analytical signals against the pK_{a1} values of analyte carboxylic acids. The system was found to be effective for carboxylic acids with pK_{a1} greater than 4; that is, the pK_{a1} value of formic acid was found

Table 3
Limits of detection of monocarboxylic acids at S/N = 3 with and without enhancer resin.

Analyte	Limit of detection (μM)				
	With enhancer resin				Without enhancer resin
	0.5 mM HNO_3	0.25 mM H_2SO_4	0.5 mM $HClO_4$	0.55 mM H_3PO_4	0.55 mM H_3PO_4
Formic acid	0.23	0.12	0.42	0.10	0.24
Acetic acid	0.30	0.24	0.39	0.11	1.89
Propionic acid	0.39	0.30	0.53	0.16	3.10
n-Butyric acid	0.54	0.42	0.72	0.21	4.19
n-Valeric acid	1.01	0.79	1.40	0.39	7.10

Enhancer resin: K^+-form CEX. Sample concentration: 0.01 μM. The other conditions are the same as those described in Fig. 2.

to be closely related to the small conductimetric enhancement effect.

3.4. Comparison between conductimetric enhancement effects of developed system and postcolumn enhancement system with two ion-exchange resin columns

The conductimetric enhancement effect of the developed system was compared with that of a postcolumn enhancement system containing two different ion-exchange resin columns connected after a separation column. The specifications of the postcolumn system with two columns were the same as those in the study by Tanaka and Fritz [9], and other conditions such as the type of separation column or eluent were the same as those in the present study. In this case, the analyte acids were finally converted into KOH by means of the K^+-form strongly acidic CEX column TSKgel SCX (50 mm × 4.6 mm ID) and the OH^--form strongly basic anion-exchange resin column TSKgel SAX (50 mm × 4.6 mm ID). The peak areas of acids in the case of the system with two ion-exchange resin columns were 1.24–1.40 times larger than those in the developed system. However, since the system with two columns cannot regenerate the resins packed in the columns after the completion of one measurement, the enhancement effect began to weaken after 12 measurements, as shown in Fig. 4. Additionally, the pressure applied to the separation column by the system with two columns (115 kgf cm^{-2} at a rate of 0.6 mL min^{-1}) was considerably higher than that applied by the developed enhancer system (62 kgf cm^{-2} at a rate of 0.6 mL min^{-1}).

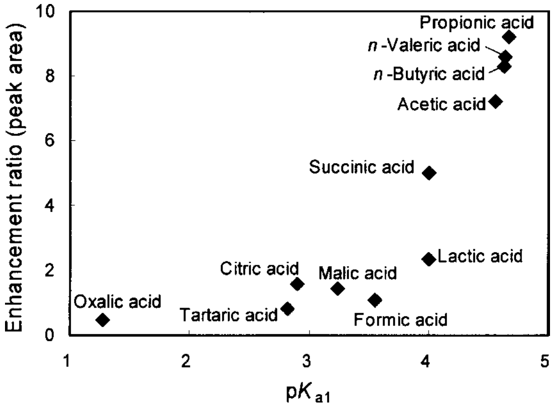

Fig. 3. Plot of conductimetric enhancement ratio against pK_{a1} of aliphatic carboxylic acids. These ratios were calculated from peak areas. Enhancer resin: K^+-form CEX. Eluent: 0.55 mM H_3PO_4. The other experimental conditions are the same as those described in Fig. 2.

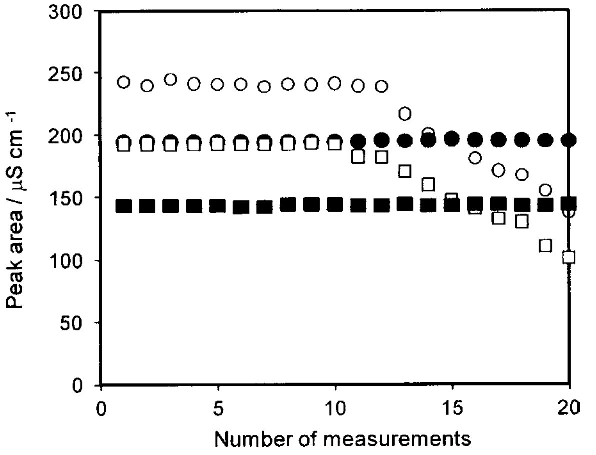

Fig. 4. Transitions of peak areas of acetic acid and *n*-valeric acid against repeated measurements in ion-exclusion chromatography between two different conductimetric enhancement systems. Separation column: weakly acidic cation-exchange resin column TSKgel Super IC-A/C (150 mm × 6 mm ID). Eluent: 5.5 mM phosphoric acid. Postcolumn enhancer: (developed system) enhancer resin, K^+-form CEX and (postcolumn system with two columns) K^+-form strongly acidic cation-exchange resin column Tosoh TSKgel SCX (50 mm × 4.6 mm ID) and OH^--form strongly basic anion-exchange resin column Tosoh TSKgel SAX (50 mm × 4.6 mm ID). Plot identities: (developed system) ● = acetic acid and ■ = *n*-valeric acid and (postcolumn system with two columns) ○ = acetic acid and □ = *n*-valeric acid. Sample concentration: 0.5 mM. The other experimental conditions are the same as those described in Fig. 2.

3.5. Analytical performances

Table 4 shows calibration data in the case of the IEC of aliphatic carboxylic acids with the K^+-form CEX used as the enhancer. With the use of the enhancer system, it was possible to achieve linear calibration in a concentration range of 0.01–1.0 mM for all analyte acids. The correlation coefficients (r^2) of the linear calibrations were 0.9985–0.9998 for the analytes.

Table 5 summarizes the relative standard deviations (RSD) of the retention time, peak area, and peak height for continuous measurements (n = 20). The RSD values of all these parameters were fairly good.

3.6. Application of developed system to prepared sample

Recently, chicken droppings, which are one of the waste products generated at a chicken farm, have been subjected to waste

Table 4
Calibration data for ion-exclusion chromatography with enhancer resin.

Analyte	Linear range for peak area (mM)	Regression equation	Correlation coefficient (r^2) (n = 5)
Formic acid	0.01–1.0	$y = 389.4x - 0.457$	0.9997
Acetic acid	0.01–1.0	$y = 306.2x - 0.165$	0.9994
Propionic acid	0.01–1.0	$y = 278.9x - 1.087$	0.9998
n-Butyric acid	0.01–1.0	$y = 289.5x - 2.284$	0.9985
n-Valeric acid	0.01–1.0	$y = 282.6x - 1.794$	0.9994

Enhancer resin: K^+-form CEX resin. Eluent: 0.55 mM H_3PO_4. The other experimental conditions are the same as those described in Fig. 2.

Table 5
RSD values of aliphatic acids in ion-exclusion chromatography with enhancer resin.

Analyte	RSD (%) ($n = 20$)		
	Retention time	Peak area	Peak height
Formic acid	0.09	0.26	1.20
Acetic acid	0.07	0.26	1.20
Propionic acid	0.07	0.47	0.96
n-Butyric acid	0.07	0.56	0.66
n-Valeric acid	0.09	0.44	0.48

Enhancer resin: K^+-form CEX resin. Eluent: 0.55 mM H_3PO_4. The other experimental conditions are the same as those described in Fig. 2.

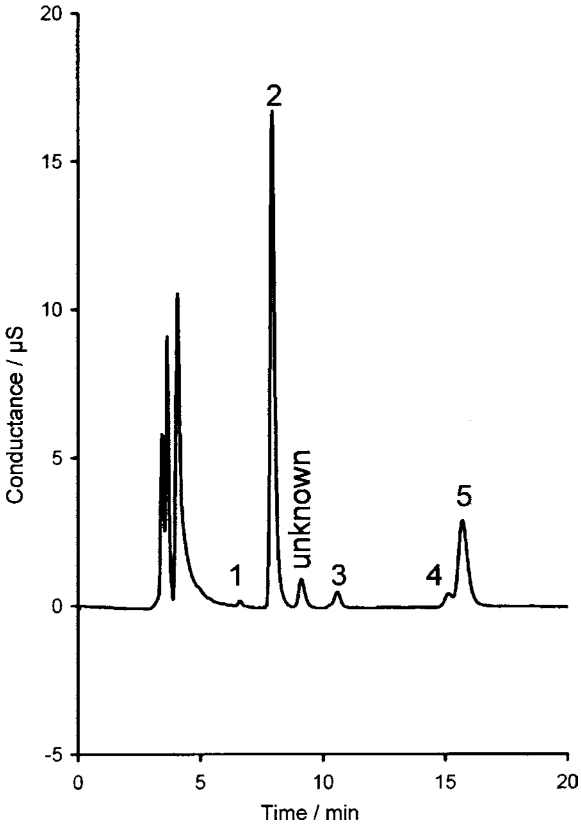

Fig. 5. Ion-exclusion chromatogram of aliphatic carboxylic acids in chicken droppings sample. Enhancer resin: K^+-form CEX. Eluent: 0.55 mM H_3PO_4. The other experimental conditions are the same as those described in Fig. 2. Peak identities: 1 = formic acid; 2 = acetic acid; 3 = propionic acid; 4 = isobutyric acid; and 5 = *n*-butyric acid.

treatment and used for biogas production by an anaerobic digestion process [18]. Further, aliphatic carboxylic acids are the by-products of this process. Then, for monitoring the metabolic state of the process, it is important to analyze formic, acetic, propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acids [19,20]. As a fundamental study, concentrations of aliphatic acids in a chicken droppings sample were determined using the developed system. The sample diluted 10-fold with distilled-deionized water prior to injection. As shown in Fig. 5, formic acid, acetic acid, propionic acid, isobutyric acid, and *n*-butyric acid were effectively separated. By the calibration method, their concentrations were found to be 0.004mM, 0.780mM, 0.038mM, 0.038mM, and 0.282mM, respectively. The reproducibility ($n = 5$) of peak areas of carboxylic acids was lower than 1.8% RSD. Recoveries when standard samples were spiked into the original sample were 96% for formic acid, 90% for acetic acid, 99% for propionic acid, 76% for isobutyric acid, and 106% for *n*-butyric acid. The poor recovery of isobutyric acid could be attributed to the low resolution between isobutyric and *n*-butyric acids.

It was found that the developed system could yield a well-reproduced peak without weakening of the conductimetric enhancement effect due to the presence of various matrices in the droppings.

4. Conclusions

In this study, a CEX of the alkali metal form was shown to be effective as a postcolumn conductimetric enhancer in the IEC of aliphatic carboxylic acids, particularly for acids with $pK_{a1} > 4$. Other advantages of the enhancer resin were linear calibration in a wide concentration range, decrease in detection limits, and well-reproduced conductimetric enhancement effect. When the developed system was applied to the determination of aliphatic carboxylic acids in a chicken droppings sample, satisfactory results were obtained without any interference. For practically applying this system to other types of samples, a further improvement in the resolution of this system is required, which will be the subject of a future work.

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