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Journal of Chromatography A, 1039 (2004) 141–145
JOURNAL OF CHROMATOGRAPHY A
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Determination of some aliphatic carboxylic acids in anaerobic digestion process waters by ion-exclusion chromatography with conductimetric detection on a weakly acidic cation-exchange resin column

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Available online 18 March 2004

Abstract

The determination of seven aliphatic carboxylic acids, formic, acetic, propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acids in anaerobic digestion process waters was examined using ion-exclusion chromatography with conductimetric detection. The analysis of these biologically important carboxylic acids is necessary as a measure for evaluating and controlling the process. The ion-exclusion chromatography system employed consisted of polymethacrylate-based weakly acidic cation-exchange resin columns (TSKgel OApak-A or TSKgel Super IC-A/C), weakly acidic eluent (benzoic acid), and conductimetric detection. Particle size and cation-exchange capacity were 5 μm and 0.1 meq./ml for TSKgel OApak-A and 3 μm and 0.2 meq./ml for TSKgel Super IC-A/C, respectively. A dilute eluent (1.0–2.0 mM) of benzoic acid was effective for the high resolution and highly conductimetric detection of the carboxylic acids. The good separation of isobutyric and *n*-butyric acids was performed using the TSKgel Super IC-A/C column (150 mm × 6.0 mm i.d. × 2). The simple and good chromatograms were obtained by the optimized ion-exclusion chromatography conditions for real samples from mesophilic anaerobic digestors, thus the aliphatic carboxylic acids were successfully determined without any interferences.

Keywords: Water analysis; Mobile phase composition; Process monitoring; Carboxylic acids; Benzoic acids

1. Introduction

The anaerobic digestion process is useful for waste treatment and biogas production. In the process, total organic carbons of carbohydrates, fats, and protein were finally converted biologically to methane and carbon dioxide through aliphatic carboxylic acids, etc. by consortia of different bacteria with slow growth rate. However, the process is very sensitive to environmental changes such as a sudden increase in organic or hydraulic loading rate, change of carbon contents, temperature condition, operational failure, etc. For monitoring of the metabolic state of the process, analysis of aliphatic carboxylic acids, especially formic, acetic, propionic, isobutyric, *n*-butyric, isovaleric and *n*-valeric acids is one of most important items such as gas production, gas composition, and redox potential and pH of process waters (1–3). Although the analysis was usually performed by means of gas chromatography (GC) with flame ionization detection (FID) [1–3], ion-exclusion chromatography is suitable for the analysis, in terms of simplicity, sensitivity and simultaneity [4,5].

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doi:10.1016/j.chroma.2004.01.068

Ion-exclusion chromatography is a unique technique for the determination of aliphatic, aromatic carboxylic acids and haloacetic acids [4–13]. The acids can be separated on various types of ion-exchange resins with strongly or weakly acidic cation exchangers in the H^+ form. The stationary phases used are in general polymer gels of polymethacrylate and poly(styrene-divinylbenzene) (PS–DVB) copolymers. Ion-exclusion chromatography for the determination of carboxylic acids has the advantages that they are separated completely from inorganic anions because the inorganic anions are excluded from the resins via the electrostatic repulsion effects and have no retention on the resins. Thus, it is possible to get relatively simple chromatograms by weak organic and inorganic acids.

It has been already reported by Tanaka et al. that ion-exclusion chromatography on a polymethacrylate-based weakly acidic cation-exchange resin (Tosoh TSKgel OApak-A) is effective for the separation of some carboxylic acids [9–13]. The eluents used were water, its mixture with some organic solvents, and dilute solution of strong and weak acids (also containing β-cyclodextrin). Thus, both of high speed separation and sensitive conductimetric detection were performed with the column and eluents for aliphatic carboxylic acids, formic, acetic, propionic, *n*-butyric and *n*-valeric acids in standard solution [9–11]. The column is also useful for the simultaneous determination of common inorganic anions (Cl^-, NO_3^-, SO_4^{2-}) and cation (Na^+, NH_4^+, K^+, Mg^{2+}, and Ca^{2+}) by ion-exclusion chromatography–cation-exchange chromatography with conductimetric detection by elution with tartaric acid–crown ether [14].

In this study, the separation and detection of the carboxylic acids including isobutyric and isovaleric acids in addition to the above five carboxylic acids in anaerobic digestion process waters were examined using an ion-exclusion chromatography system consisting a polymethacrylate-based weakly acidic cation-exchange resin (Tosoh TSKgel OApak-A or TSKgel Super IC-A/C column), dilute weak acid (benzoic acid) eluent, and conductimetric detection. Especially, the separation behaviour of isobutyric and *n*-butyric acids was examined and discussed in detail because both acids have the similar retention times on the columns. Finally, the ion-exclusion chromatography method optimized was examined for sample waters in mesophilic anaerobic digestors.

2. Experimental

2.1. Instrumentation

The ion chromatographic system used consisted of a Shimadzu LC-6A or LC-10AD pump, a Shimadzu CTO-10AC column oven equipped with a 50 μl sample loop, a Tosoh CM-8000 conductivity detector, and a Shimadzu C-R8A data processing system.

2.2. Columns

The separation columns were Tosoh polyether ether ketone (PEEK) and stainless-steel columns (150 mm × 7.8 mm i.d. and 300 mm × 6.0 mm i.d., respectively) packed with a Tosoh TSKgel OApak-A, polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form with a particle size of 5 μm. The exchange capacity was 0.1 meq./ml resin. The columns were equilibrated thoroughly with eluents before the chromatographic run. A TSKgel Super IC-A/C column (150 mm × 6.0 mm i.d.) (polymethacrylate-based weakly acidic cation-exchange resin; particle size 3 μm; exchange capacity 0.2 meq./ml; Tosoh, Tokyo, Japan) was also used as the separation column with higher separation efficiency.

A Tosoh TSKgel OApak P (50 mm × 4.2 mm i.d.) (PS-DVB-based strongly acidic cation-exchange resin; particle size, 5 μm; exchange capacity, 1.5 meq./ml; Tosoh, Tokyo, Japan) was used as a precolumn for trapping of mono- and divalent inorganic cations.

Weak acid (benzoic acid) was used as the eluent at a flow rate of 1.0 ml/min. Column temperature was maintained at 40 °C. Sample injection volumes were 10 and 30 μl.

2.3. Reagents and samples

All solutions were prepared from analytical reagent grade chemicals in deionized water. Benzoic acid used as the eluents was purchased from Kanto Chemicals (Tokyo, Japan). Aliphatic carboxylic acids were purchased from Kanto Chemicals except for propionic acid (Nakarai Tesque, Kyoto, Japan). The standard solutions of carboxylic acids and their mixtures were prepared from each of 0.1 M stock solutions.

Anaerobic digestion process waters were obtained from a pilot-scale and a full scale of mesophilic anaerobic digestors for poultry manure and waste beverages, respectively. Both digestors consisted of two tanks, acid-forming and methanogenic tanks. The hydraulic retention times for the tanks were 5 and 45 days for the former (poultry manure) and 2 and 12 days for the latter (waster beverages), respectively.

3. Results and discussion

3.1. Effect of benzoic acid eluent on ion-exclusion chromatographic separation of carboxylic acids

In a preliminary experiment, the separation of seven carboxylic acids (each 1 mM) was examined on a Tosoh TSKgel OApak-A (PEEK) columns (150 mm × 7.8 mm i.d.) packed with a polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form with a particle size of 5 μm. When 2 mM benzoic acid was used as eluent, good chromatograms were obtained except for isobutyric and

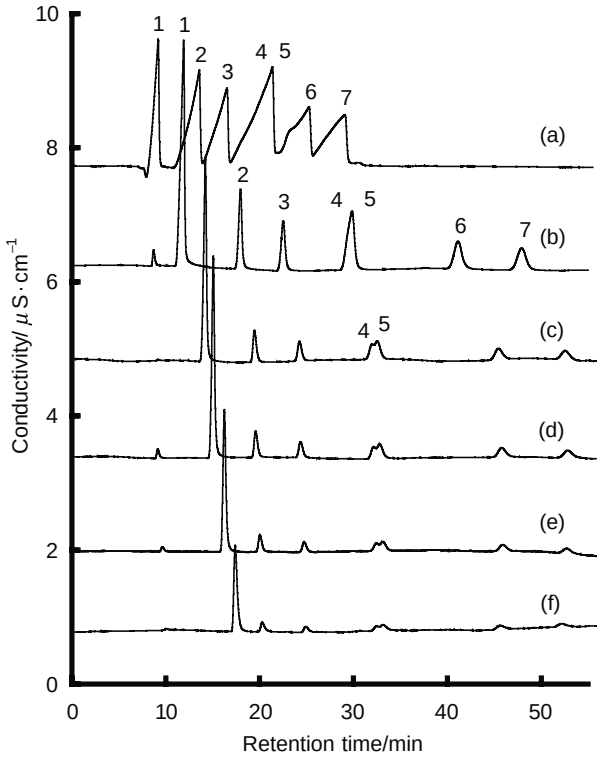

Fig. 1. Comparison of ion-exclusion chromatograms of aliphatic carboxylic acids by elution with (a) water; (b) 0.1 mM benzoic acid; (c) 0.5 mM; (d) 1.0 mM; (e) 2.0 mM; and (f) 4 mM. Column: TSKgel OApak-P (PS–DVB-based strongly acidic cation-exchange resin, 50 mm × 4.2 mm i.d.) + TSKgel OApak-A (polymethacrylate-based weakly acidic cation-exchange resin, 150 mm × 7.8 mm i.d. +300 mm × 6.0 mm i.d.); column temperature: 40 °C; flow-rate: 1.0 ml/min; detection: conductivity; injection volume: 10 μl; sample concentration: 1 mM for all aliphatic carboxylic acids. Peaks: (1) formic acid; (2) acetic acid; (3) propionic acid; (4) isobutyric acid; (5) *n*-butyric acid; (6) isovaleric acid; (7) *n*-valeric acid.

n-butyric acids. Isobutyric and *n*-butyric acids have almost the same retention volumes. Therefore, the optimization of separation and detection of the carboxylic acids was examined using the PEEK column (150 mm × 7.8 mm i.d.) and a stainless-steel column (300 mm × 6.0 mm i.d.) packed with the same resin.

Fig. 1 shows the separation of seven aliphatic carboxylic acids in the range of 0–4 mM benzoic acid as the eluents. As shown in Fig. 1a, the resolution of carboxylic acids was very low and the peaks were fronted when water was used as eluent. The peak shape is due to the presence of associated and dissociated forms for the acids, although the rate depends on the acid dissociation constant (K_a) of each acid. Isobutyric and *n*-butyric acids almost co-eluted. The use of a 0.1 mM benzoic acid (pH 4.31, background conductivity 28 μS/cm) as the eluent resulted in the sharpness in peak shape of carboxylic acid with higher sensitivity and also resulted in increase in the retention times due to increase in associated form. However, the separation of isobutyric and *n*-butyric acids was still bad. The further addition of benzoic acid to the eluent (0.5–4.0 mM) resulted in the improvement of separation of isobutyric and *n*-butyric acids with increase in the concentration of benzoic acid eluents. On the other hand, the sensitivity was decreased rapidly due to both of the decrease in dissociated form of the acids at lower pH and the increase in background conductivity of eluents. The pH and background conductivity of eluents were pH 4.31 and 28 μS/cm for 0.1 mM benzoic acid eluent, pH 3.85 and 61 μS/cm for 0.5 mM benzoic acid, pH 3.65 and 90 μS/cm for 1.0 mM benzoic acid, pH 3.48 and 127 μS/cm for 2.0 mM benzoic acid, and pH 3.31 and 190 μS/cm for 4.0 mM benzoic acid. The system peak was at ca. 127 min for 0.1 mM benzoic acid eluent and ca. 173 min for 0.5–4.0 mM benzoic acid.

As for the separation of isobutyric and *n*-butyric acids, the difference in retention times increased with benzoic acid concentration in the eluents from 0.38 min (0.1 mM benzoic acid) to 0.71 min (4 mM). In general, retention of carboxylic acids in ion-exclusion chromatography is controlled by the following factors: (1) mobile phase composition (concentration of strong and weak acids, organic solvent, etc. [6–13]); (2) acid dissociation constant of analyte (pK_a) [7,8], and (3) adsorption of the analyte to the stationary phase substrate through hydrophobic interaction [7,8]. As isobutyric and *n*-butyric acids have the same pK_a values of 4.63 and 4.63 [15], respectively, the difference of factor (2) between them is small. Similarly, the difference of factor (3) is small from the fact that they have similar retention times. On the other hand, although isovaleric and *n*-valeric acids have similar pK_a values of 4.58 and 4.64 [15], respectively, the difference of retention times is larger as shown in Fig. 1a-f, suggesting that the difference of factor (3) is predominant for them. Thus, these results indicate that hydrophobic properties of branched isomer of valeric acid are smaller that those of *n*-valeric acid which is a longer-chain acid, while the effect is smaller for butyric acid.

3.2. Optimization of separation of isobutyric acid and n-butyric acid

In a previous section, relatively good separation and detection of isobutyric and *n*-butyric acid was achieved on a longer column (15 cm + 30 cm, long) with 0.5–2.0 mM of benzoic acid as eluents. Fig. 2 shows the separation of 7 aliphatic carboxylic acids on two TSKgel Super IC-A/C columns (150 mm × 6.0 mm i.d. × 2). Tosoh TSKgel OApak-A and TSKgel Super IC-A/C columns have the similar properties basically as is described in Section 2 and the differences are that particle size is 5 and 3 μm, respectively, and cation-exchange capacity is 0.1 and 0.2 meq./ml, respectively. Thus, separation efficiency and speed is improved with maintaining both separation and detection patterns for carboxylic acids. For the separation of isobutyric and *n*-butyric acids, 2 mM benzoic acid as the eluent was effective compared to 0.5 and 1 mM benzoic acids although the reverse result was obtained for sensitivity of detection.

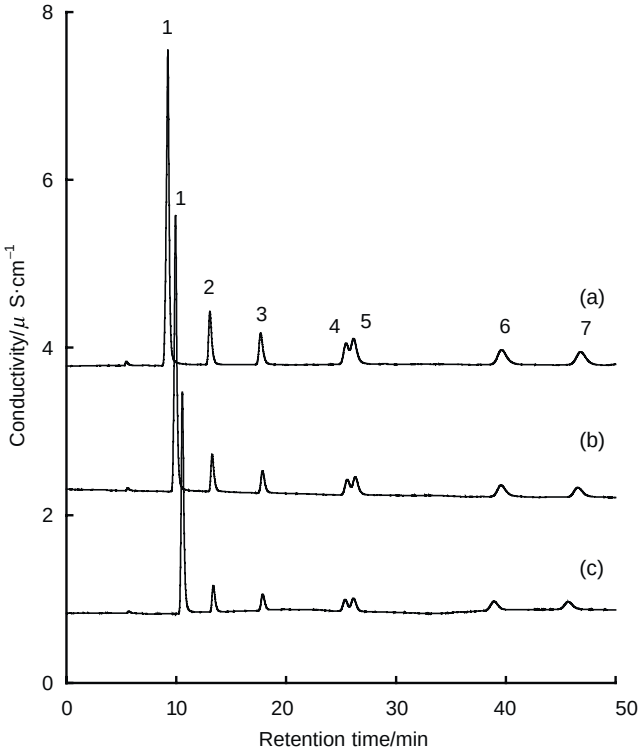

Fig. 2. Ion-exclusion chromatograms of aliphatic carboxylic acids by elution with (a) 0.5 mM benzoic acid; (b) 1.0 mM; and (c) 2.0 mM. Column: TSKgel OApak-P (PS–DVB-based strongly acidic cation-exchange resin, 50 mm × 4.2 mm i.d.) + TSKgel Super IC-A/C (polymethacrlate-based weakly acidic cation-exchange resin, particle size 3 μm; 150 mm × 6.0 mm i.d. × 2). Peak identification and other conditions as in Fig. 1.

3.3. Application to anaerobic digestion process waters

Fig. 3 shows good separation of anaerobic digestion process waters together with 1 mM standard solurion by elution with 2 mM benzoic acid. Sample waters were filtered through a 0.80 μm membrane filter (made of cellulose nitrate) and diluted 10-fold with benzoic acid (final concentration 2 mM). Samples (a) and (b) were acid-forming and methanogenic tank waters for poultry manure, respectively. The characteristics were as follows: (a) the concentration levels of acetic acid (acetate) were high for both samples and decreased from (a) to (b). (2) Isobutyric acid (isobutyrate) was lower to *n*-butyric acid (*n*-butyrate) for sample (a). (3) The concentration levels of formic acid (formate) were very low. These results indicate that anaerobic digestion process was in a good and steady state [1–3]. On the other hand, the concentration level of formic acid was higher for sample (c), suggesting the process might be in an incomplete state. The analytical results of the samples were shown in Table 1 together with detection limits under the optimal conditions.

Thus, the obtained results indicate that ion-exclusion chromatography established in this study is useful for monitoring of metabolic state of anaerobic digestion process. The detection limits of aliphatic carboxylic acids was enough for present purpose although the values were higher than those

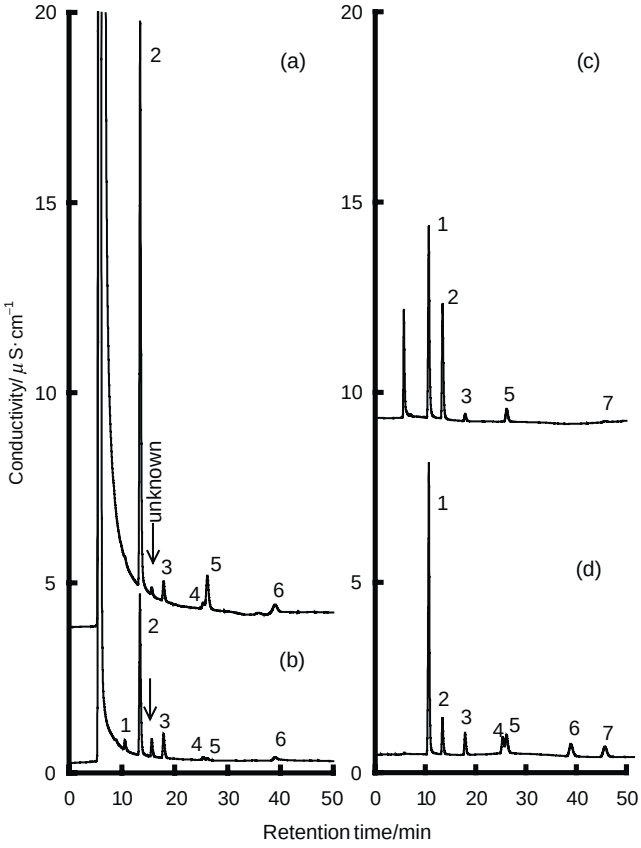

Fig. 3. Ion-exclusion chromatograms of anaerobic digestion process waters by elution with 2 mM benzoic acid. (a) Acid-forming tank water; (b) methanogenic tank water; (c) effluent of waste beverage (each, 10-fold dilution); (d) 1 mM of aliphatic carboxylic acids. Injection volume: 30 μl. Peak identification and other conditions as in Fig. 2.

Table 1
Analytical results of anaerobic digestion process waters (mM)

Samples	1	2	3	4	5	6	7
a	–	155	8.9	3.5	17.8	9.0	–
b	0.3	44	11.0	1.8	0.9	3.5	–
c	7.2	32	3.4	–	6.6	–	1.0
DL	0.001	0.006	0.01	0.01	0.01	0.02	0.02

(1) formic acid; (2) acetic acid; (3) propionic acid; (4) isobutyric acid; (5) *n*-butyric acid; (6) isovaleric acid; (7) *n*-valeric acid. DL: detection limit (S/N = 3); (–) not detectable.

by 0.05–0.2 mM sulfuric acid as eluents commonly employed in conventional ion-exclusion chromatography [9].

The reproducibility ($n = 7$) of peak areas of carboxylic acids (1 mM) except for isovaleric acid was good and less than 2.7% R.S.D.

4. Conclusion

In this study, good determination of seven aliphatic carboxylic acids in mesophilic anaerobic digestion process

waters was achieved on the combination of a weakly acidic cation-exchange resin column, benzoic acid eluent, and conductivity detection without any interferences. For the practical applications to other samples, further improvement of resolution and detection will be the subject of further work.

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