



ELSEVIER

Journal of Chromatography A, 956 (2002) 209–214

JOURNAL OF  
CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

## Vacancy ion-exclusion chromatography of carboxylic acids on a weakly acidic cation-exchange resin

Kazuhiko Tanaka<sup>a,\*</sup>, Ming-Yu Ding<sup>b</sup>, Murad I.H. Helaleh<sup>a</sup>, Hiroshi Taoda<sup>a</sup>, Hayato Takahashi<sup>c</sup>, Wenzhi Hu<sup>c</sup>, Kiyoshi Hasebe<sup>c</sup>, Paul R. Haddad<sup>d</sup>, James S. Fritz<sup>e</sup>, C. Sarzanini<sup>f</sup>

<sup>a</sup>National Institute of Advanced Industrial Science and Technology (Former name: National Industrial Research Institute of Nagoya) and Graduate School of Engineering, Chubu University, Seto 489-0884, Japan

<sup>b</sup>Tsinghua University, Beijing 100084, China

<sup>c</sup>Hokkaido University, Sapporo 060-0810, Japan

<sup>d</sup>Australian Centre for Research on Separation Science, School of Chemistry, University of Tasmania, GPO Box 252-75, Hobart 7001, Australia

<sup>e</sup>Iowa State University, Ames, IA 50011, USA

<sup>f</sup>University of Turin, Turin I-10125, Italy

### Abstract

In this preliminary study, a new approach to ion-exclusion chromatography is proposed to overcome the relatively poor conductivity detection response which occurs in ion-exclusion chromatography when acids are added to the eluent in order to improve peak shape. This approach, termed vacancy ion-exclusion chromatography, requires the sample to be used as eluent and a sample of water to be injected onto a weakly acidic cation-exchange column (TSKgel OApak-A). Vacancy peaks for each of the analytes appear at the retention times of these analytes. Highly sensitive conductivity detection is possible and sharp, well-shaped peaks are produced, leading to efficient separations. Retention times were found to be affected by the concentration of the analytes in the eluent, and also by the presence of an organic modifier such as methanol in the eluent. Detection limits for oxalic, formic, acetic, propionic, butyric and valeric acids were 0.1, 0.2, 0.3, 0.3, 0.4 and 0.5  $\mu\text{M}$ , respectively, and linear ranges for some acids extended over two orders of magnitude. Precision values for retention times were 0.21% and for peak areas were <1.90%. The vacancy ion-exclusion chromatography method was found to give detection responses four to 10 times higher than conventional ion-exclusion chromatography using sulfuric acid eluent and two to five times higher than conventional ion-exclusion chromatography using benzoic acid eluent. © 2002 Elsevier Science B.V. All rights reserved.

**Keywords:** Vacancy chromatography; Ion-exclusion chromatography; Carboxylic acids

### 1. Introduction

Ion-exclusion chromatography is used frequently

for the chromatographic separation of weak acids, especially hydrophilic aliphatic carboxylic acids. The stationary phase is typically a polystyrene–divinylbenzene (PS–DVB)-based strongly acidic cation-exchange resin in the  $\text{H}^+$  form, having a high ion-exchange capacity (e.g. 1.5 mequiv./ml resin) [1–5]. However, ion-exclusion chromatography can

\*Corresponding author. Tel.: +81-561-82-2141; fax: +81-561-82-2141.

E-mail address: [kazuhiko-tanaka@aist.go.jp](mailto:kazuhiko-tanaka@aist.go.jp) (K. Tanaka).

also be performed on weakly acidic cation-exchange resins with low capacity (e.g. 0.1 mequiv./ml resin) and can be applied to the separation of hydrophilic and hydrophobic carboxylic acids. We have reported the ion-exclusion chromatography separation of aliphatic carboxylic acids on a polymethacrylate-based weakly acidic cation-exchange resin (Tosoh TSKgel OApak-A) [6–8]. The eluents used in these separations were water, mixtures of water with organic solvents, or dilute solutions of strong or weak acids [1–8]. When ion-exclusion chromatography was performed on both the strongly or weakly acidic cation-exchange resins with water eluents, the resolution of aliphatic carboxylic acids was generally low and the peaks obtained were fronted owing primarily to the lack of buffer capacity of the eluent. 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) must be employed to ensure that the degree of ionisation remains constant across the entire sample band. However, the use of such eluents decreases the sensitivity of conductivity detection as a result of the increased background conductivity of the eluent.

As an alternative approach, we have reported the use of carboxylic acids such as tartaric acid and sulfosalicylic acid (SSA) as very effective eluents for combined ion-exclusion chromatography–cation-exchange chromatography on a weakly acidic cation-exchange resin column (Tosoh TSKgel OApak-A). This approach has been used for the simultaneous separation of common inorganic strong acid anions and mono- and divalent cations [9–16]. In this ion-exclusion chromatography–cation-exchange chromatography system, a system peak caused by the adsorption of the eluent on the stationary phase was noted, with the retention time of the system peak being strongly dependent on the properties (ionization and hydrophilicity) of the carboxylic acid used as the eluent. This system peak could be induced by the injection of water and was therefore attributed to a vacancy of the eluent acid.

In the present study, we made a preliminary investigation of this effect as a means of determining weak acids. According to this principle, a new approach to separate some carboxylic acids on a weakly acidic ion-exclusion separation column (Tosoh TSKgel OApak-A) was developed in which a mixture of aliphatic carboxylic acids was used as the

eluent and water was injected into the separation column as the sample. Greater resolution, increased conductivity detection sensitivity and better peak shape were obtained in comparison to conventional ion-exclusion chromatography on the same column using water as eluent.

## 2. Experimental

### 2.1. Reagents and procedures

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 prepared using Wako (Osaka, Japan) 1.0 M sulfuric acid. Aliphatic carboxylic acids were prepared as 20–40 mM stock solutions and these were used to prepare standard mixtures of these species.

### 2.2. Instrumentation

A Tosoh (Tokyo, Japan) 8020 ion chromatograph was employed and consisted of a DP-8020 pump, a CM-8020 conductivity detector, a CO-8020 column oven a Rheodyne 7125 sample loop injector, and a SD-8022 on-line vacuum degasser. A Tosoh chromatography work station (LC-8020) was utilized to control the instrument and for data processing. The separation of the aliphatic carboxylic acids was carried out using a Tosoh TSKgel OApak-A column (150×7.8 mm I.D.) packed with polymethacrylate-based weakly acidic cation-exchange resin in the H<sup>+</sup>-form (particle size: 5 μm, cation-exchange capacity: 0.1 mequiv./ml). A mixture of six common aliphatic carboxylic acids was used as eluent at 1.5 ml/min and the column temperature was maintained at 35 °C. Sample injection volumes were 100 μl.

## 3. Results and discussion

### 3.1. Vacancy ion-exclusion chromatographic separation of carboxylic acids

In conventional ion-exclusion chromatography, fronted and broadened peaks are obtained for ali-

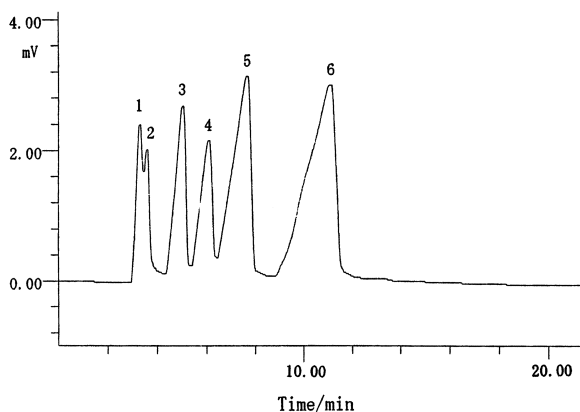


Fig. 1. Conventional ion-exclusion chromatogram of some aliphatic carboxylic acids obtained using pure water as eluent. Column: TSKgel OApak-A (150×7.8 mm I.D.); column temperature: 35 °C; flow-rate: 1.5 ml/min; injection volume: 100  $\mu$ l. Peaks ( $\mu$ M): 1=oxalic acid (1.6), 2=formic acid (1.6), 3=acetic acid (8.0), 4=propionic acid (8.0), 5=butyric acid (18), 6= valeric acid (24).

phatic carboxylic acids when water is used as an eluent. As shown in Fig. 1, the chromatogram obtained for ion-exclusion chromatography of six common aliphatic carboxylic acids using water as eluent poor resolution, especially for hydrophobic carboxylic acids such as butyric and valeric acids. When an eluent containing an aromatic carboxylic acid such as benzoic acid was used with a weakly acidic cation-exchange resin, an improved separation is obtained (Fig. 2) and the background conductance of the eluent is low due to the low limiting ionic conductance of the eluent anion (benzoate). The system peak caused by the adsorption of the eluent acid is clearly evident at a retention time of 42 min. When the chromatographic system was altered so that a sample comprising a mixture of six common aliphatic carboxylic acids was used as eluent and a sample of water was injected, the chromatogram shown in Fig. 3 resulted. Negative “vacancy” peaks corresponding to each of the acids present in the sample appear, with these peaks being well-shaped and well-resolved from each other. The identities of these peaks were confirmed by using eluents containing each species separately.

### 3.2. Effects on retention

The retention times of the aliphatic carboxylic

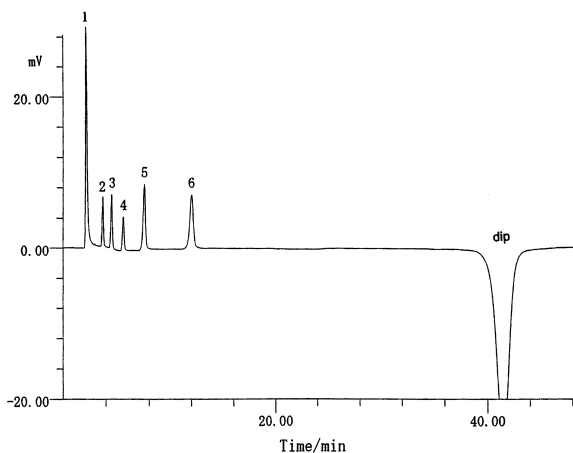


Fig. 2. Conventional ion-exclusion chromatogram of some aliphatic carboxylic acids obtained using 2 mM benzoic acid as eluent. Conditions as in Fig. 1. Peaks ( $\mu$ M): 1=oxalic acid (40), 2=formic acid (40), 3=acetic acid (200), 4=propionic acid (200), 5=butyric acid (460), 6=valeric acid (600). Other conditions as in Fig. 1.

acids were increased by increasing the concentrations of these analytes in the eluent. Fig. 4 shows plots of the concentration of aliphatic carboxylic acids in the eluent vs. the observed retention time. The shift of retention time with concentration can be explained by changes in the degree of ionization of the aliphatic carboxylic acids. Decreased ionization of the aliphatic carboxylic acids would weaken their interaction with the stationary phase, leading to increased retention without decreased peak resolu-

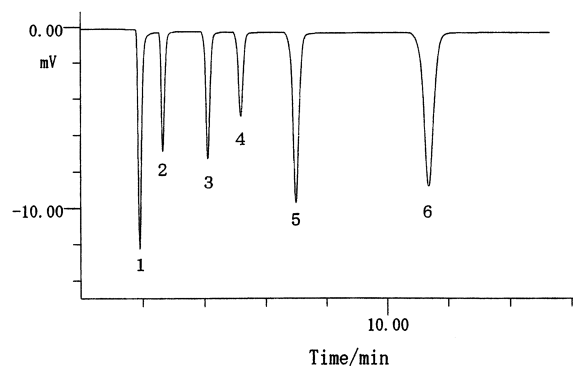


Fig. 3. Vacancy ion-exclusion chromatogram of some aliphatic carboxylic acids. Concentration ( $\mu$ M) of aliphatic carboxylic acids in the mobile phase: 1=oxalic acid (8), 2=formic acid (8), 3=acetic acid (40), 4=propionic acid (40), 5=butyric acid (92), 6=valeric acid (120). A 100  $\mu$ l sample of water was injected onto the column. Other conditions as in Fig. 1.

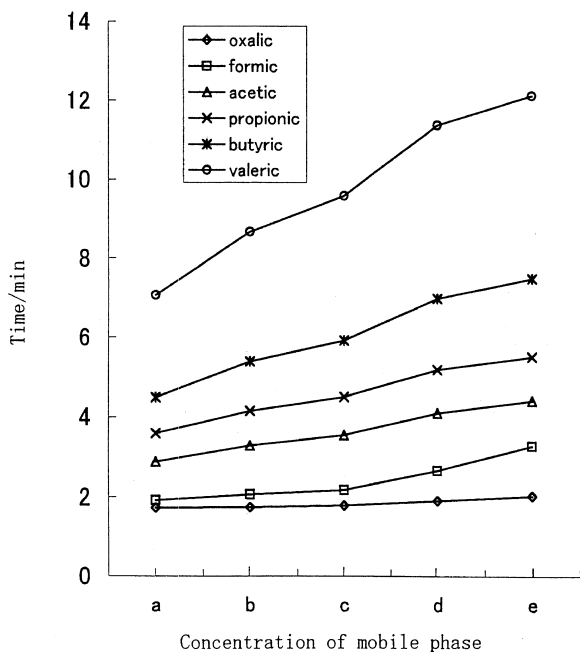


Fig. 4. Effect of the concentration of aliphatic carboxylic acids in the mobile phase on retention time. Concentration of mobile phase ( $\mu\text{M}$ ): (a)=mixture of oxalic acid(0.32), formic acid(1.6), acetic acid(1.6), propionic acid(1.6), butyric acid(3.7) and valeric acid(4.8); (b)=(a) $\times$ 2.5; (c)=(a) $\times$ 5; (d)=(a) $\times$ 25; (e)=(a) $\times$ 125.

tion. This feature would be most problematic when the separation was applied to real samples and it would therefore be necessary for the pH of the eluent to be maintained at a constant value.

Previous studies [2,4–8] have shown that the retention times of aliphatic carboxylic acids in ion-exclusion chromatography can be decreased by adding organic modifiers, such as methanol, to the eluent. However, the organic modifier itself can also be retained on the stationary phase and can be detected conductimetrically [17], which implies that a further vacancy peak for methanol would be produced in the system under study. Fig. 5 shows that such a peak does appear and under the conditions used in this particular separation, the methanol peak is co-eluted with propionic acid. Therefore, the use of higher alcohols, such as propanol and butanol, having stronger retention on the resin phase would be necessary to avoid this interference.

The effect of column temperature on the retention of the aliphatic carboxylic acids was examined over

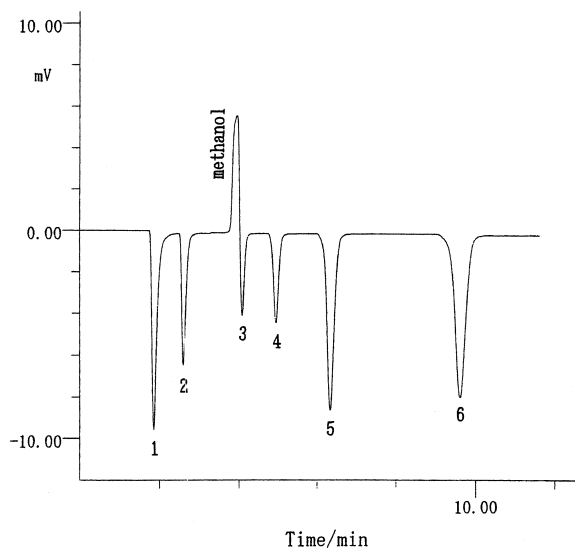


Fig. 5. Vacancy ion-exclusion chromatogram obtained using 5% (v/v) methanol in the mobile phase. Other conditions as in Fig. 3.

the range 25–45 °C. No significant change in retention time was observed for oxalic acid and formic acid, whilst the other acids showed a minor decrease in retention time as the column temperature was increased.

### 3.3. Analytical performance parameters

In vacancy ion-exclusion chromatography, the sensitivity of conductivity detection is based on the difference in conductivity between the analytes in the eluent and the injected water sample. High detection sensitivity can therefore be expected because water of the very low conductivity of the water sample. Table 1 shows the conductivity detection responses (based on peak heights) for a number of ion-exclusion chromatography systems, together with that for the vacancy ion-exclusion chromatography approach (to which the responses in the other systems were normalised). Similar results were obtained when peak areas were used instead of peak heights. As shown in Table 1, the relative conductivity response values for vacancy ion-exclusion chromatography were very similar to those for conventional ion-exclusion chromatography using a water eluent, but were substantially higher than ion-exclusion chroma-

Table 1

Comparison of the conductivity detection response values for aliphatic carboxylic acids in different ion-exclusion chromatography systems

Acid	Relative detector response values			
	A <sup>a</sup>	B <sup>b</sup>	C <sup>c</sup>	D <sup>d</sup>
Oxalic acid	1.00	0.82	ND	0.48
Formic acid	1.00	0.85	0.25	0.34
Acetic acid	1.00	1.10	0.17	0.25
Propionic acid	1.00	1.30	0.16	0.20
Butyric acid	1.00	0.91	0.12	0.21
Valeric acid	1.00	0.86	0.10	0.19

The values are normalised using the vacancy ion-exclusion chromatography system as unity.

<sup>a</sup> Vacancy ion-exclusion chromatography system (eluent: aliphatic carboxylic acids).

<sup>b</sup> Conventional ion-exclusion chromatography (eluent: water).

<sup>c</sup> Conventional ion-exclusion chromatography (eluent: 0.5 mM sulfuric acid).

<sup>d</sup> Conventional ion-exclusion chromatography (eluent: 2.0 mM benzoic acid).

tography systems using sulfuric acid or benzoic acid eluents. This difference can be attributed both to the difference in conductance between the eluent and the sample, and also to the level of baseline noise which is present.

Detection limits for the vacancy ion-exclusion chromatography system were determined at a signal-to-noise ratio of three and the results were shown in Table 2. The values shown indicate the highly sensitive nature of the method. The calibration curves of all aliphatic carboxylic acids were obtained using both peak areas and peak heights and linear calibration was maintained for up to two orders of magnitude (for oxalic acid and formic acid) but narrower linear ranges were obtained for the other aliphatic carboxylic acids. The linear ranges for peak

Table 2

Linear calibration ranges and detection limits for the ion-exclusion chromatography system

Acid	Linear range for peak area calibration ( $\mu\text{M}$ )	Detection limit ( $\mu\text{M}$ )
Oxalic acid	0.8–40	0.2
Formic acid	0.8–40	0.1
Acetic acid	0.8–8	0.3
Propionic acid	0.8–8	0.3
Butyric acid	1.8–18	0.4
Valeric acid	2.4–24	0.5

Table 3

Reproducibility data for retention time and peak area of carboxylic acids

Acid (Conc. $\mu\text{M}$ )	Reproducibility ( $n=7$ ), RSD (%)	
	For retention times	For peak areas
Oxalic acid (8)	0.25	1.78
Formic acid (8)	0.26	1.28
Acetic acid (40)	0.21	1.47
Propionic acid (40)	0.19	1.68
Butyric acid (92)	0.21	1.90
Valeric acid (120)	0.17	0.82

area calibrations are listed in Table 2. Reproducibility data were obtained for retention times and peak areas using seven replicate injections and the results are shown in Table 3.

#### 4. Conclusions

Vacancy ion-exclusion chromatography, in which the mixture of the carboxylic acid analytes is used as the mobile phase and water is injected as the sample, provides higher peak resolution, better peak shapes, and higher conductimetric detection sensitivity than conventional ion-exclusion chromatography with conductimetric detection using water or dilute sulfuric acid or benzoic acid as the eluent. Elimination of the effect of the concentration of the carboxylic acids in the eluent on retention time will be necessary for practical implementation of this approach and will be the subject of future investigations using different eluents and cation-exchange resins.

#### Acknowledgements

This work was supported in part by the Ministry of the Environment and the Ministry of Economy, Trade and Industry in Japan through a Cooperative Agreement of Science and Technology for the study on development of environmental monitoring by advanced ion chromatography between National Institute of Advanced Industrial Science and Technology, University of Tasmania, Tsinghua University, Iowa State University, and University of Turin.

## References

- [1] V.T. Turkelson, M. Richards, *Anal. Chem.* 50 (1978) 1420.
- [2] K. Tanaka, J.S. Fritz, *J. Chromatogr.* 409 (1987) 271.
- [3] J.S. Fritz, *J. Chromatogr.* 546 (1991) 111.
- [4] K. Tanaka, K. Ohta, J.S. Fritz, Y.-S. Lee, S.-B. Shim, *J. Chromatogr. A* 706 (1995) 385.
- [5] K. Tanaka, K. Ohta, J.S. Fritz, *J. Chromatogr. A* 770 (1997) 211.
- [6] K. Tanaka, H. Chikara, W. Hu, K. Hasebe, *J. Chromatogr. A* 850 (1999) 187.
- [7] T. Abe, H. Baba, I. Soloshonok, K. Tanaka, *J. Chromatogr. A* 884 (2000) 93.
- [8] T. Abe, H. Baba, E. Itoh, K. Tanaka, *J. Chromatogr. A* 920 (2001) 173.
- [9] K. Tanaka, K. Ohta, J.S. Fritz, S. Matsushita, A. Miyanaga, *J. Chromatogr. A* 671 (1994) 239.
- [10] K. Tanaka, J.S. Fritz, *Am. Environ. Lab.* 7 (1995) 30.
- [11] K. Tanaka, P.R. Haddad, *Trends Anal. Chem.* 15 (1996) 266.
- [12] K. Tanaka, K. Ohta, P.R. Haddad, J.S. Fritz, K.-P. Lee, K. Hasebe, A. Ieuji, A. Miyanaga, *J. Chromatogr. A* 850 (1999) 311.
- [13] S.M. Kwon, K.P. Lee, K. Tanaka, K. Ohta, *J. Chromatogr. A* 884 (1999) 79.
- [14] K. Tanaka, K. Ohta, P.R. Haddad, J.S. Fritz, A. Miyanaga, W. Hu, K. Hasebe, *J. Chromatogr. A* 884 (2000) 167.
- [15] K. Tanaka, K. Ohta, P.R. Haddad, J.S. Fritz, A. Miyanaga, W. Hu, K. Hasebe, C. Sarzanini, *J. Chromatogr. A* 920 (2001) 239.
- [16] M.-Y. Ding, K. Tanaka, W. Hu, K. Hasebe, P.R. Haddad, *Analyst* 126 (2001) 567.
- [17] K. Tanaka, P.R. Haddad, *Encyclopedia of Separation Science, Liquid Chromatography/Ion Exclusion Chromatography*, Academic Press, London, UK, 2000.