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Ion-exclusion chromatography of carboxylic acids on silica gel modified with aluminium

Kazutoku Ohta*, Kazuhiko Tanaka

National Industrial Research Institute of Nagoya, 1-1 Hirate-cho, Kita-ku, Nagoya 462-8510, Japan

Abstract

The modification of silica gel with aluminium by a coating method was effective for the preparation of a silica-based stationary phase, which acted as a cation exchanger under strongly acidic conditions. In order to expand the utility of the laboratory-made aluminium-adsorbing silica gel it was applied as a stationary phase to the ion-exclusion chromatography of various carboxylic acids. Good separations for both aliphatic carboxylic acids and benzenecarboxylic acids with a hydrophobic nature under acidic eluent conditions were achieved in 25 min. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Silica, aluminium-modified; Stationary phases, LC; Carboxylic acids; Benzenecarboxylic acids

1. Introduction

Ion-exclusion chromatography, developed by Wheaton and Bauman [1] is commonly employed for the separation of inorganic and organic weak acids. Both a high-capacity sulfonated styrene–divinylbenzene co-polymer resin and a high-capacity carboxylated polyacrylate resin are exclusively used as a stationary phase for the ion-exclusion chromatographic separation of weak acids. Therefore, the separation of hydrophobic weak acids such as higher aliphatic carboxylic acids and aromatic carboxylic acids was very difficult due to strongly hydrophobic interaction between these carboxylic acids and the stationary phases [2]. The use of cation-exchange stationary phase with a large hydrophilic nature was expected to be one of the best ways to achieve the

ion-exclusion chromatographic separation of these hydrophobic carboxylic acids.

In previous studies [3–5], we found that one commercially available unmodified silica gel (Develosil 30-5) acted as a cation exchanger under strongly acidic conditions and the Develosil 30-5 was successfully applied to the ion-exclusion chromatographic separation of higher aliphatic carboxylic acids and benzenecarboxylic acids. We also found that the cation-exchange characteristics of the Develosil 30-5 were attributable to a trace amount of aluminium present in the silica gel [6]. However, since the Develosil 30-5 gel was developed as a stationary phase for normal-phase HPLC, the reproducibility of the cation-exchange characteristics is not guaranteed. We then developed a simple modification of silica gel with aluminium by a coating method for the preparation of silica-based stationary phase which acts as cation exchanger under strongly acidic conditions [7]. The laboratory-made aluminium-adsorbing silica gel (Al-silica) developed was

*Corresponding author. Tel.: +81-52-9112111 (ext. 2615); fax: +81-52-9112428.

E-mail address: kazuhta@nirin.go.jp (K. Ohta)

successfully applied to the simultaneous separation of common mono- and divalent cations in conductimetric detection ion chromatography with strongly acidic eluents. However, the characteristics of the Al-silica as a stationary phase in ion-exclusion chromatography for carboxylic acids has not been yet investigated.

The aim of this study was to evaluate the characteristics of the Al-silica stationary phase for the ion-exclusion chromatographic separation of various carboxylic acids, including aliphatic and aromatic carboxylic acids. As a result, when using a 0.5 *M* sulfuric acid containing 0.15% heptanol as eluent, a good separation of C₁–C₈ aliphatic carboxylic acids (formic, acetic, propionic, butyric, valeric, caproic, heptanoic and caprylic acids) was successfully achieved in 25 min. When using a 2.5 *mM* sulfuric acid as the eluent, a good separation of benzenecarboxylic acids (pyromellitic, hemimellitic, trimellitic, *o*-phthalic, *m*-phthalic, *p*-phthalic, salicylic and benzoic acids) was successfully achieved in 25 min.

2. Experimental

2.1. Preparation of aluminium-adsorbing silica gel column

A Fuji-Silysia (Kasugai, Japan) Super Micro Bead Silica Gel B-5 (SMBSG B-5) porous spherical silica gel was employed as a matrix. A 10-g amount of the silica gel was immersed in 200 ml of Al₂(SO₄)₃ solution [1 g Al₂(SO₄)₃·14–18H₂O/100 ml distilled deionized water (DIW)]. After stirring for 1 h, the solution was filtered. The resultant gel was washed thoroughly with DIW and then was dried overnight at 130°C followed by calcining at 1000°C for 5 h.

The determination of aluminium on the Al-silica preparation was carried out by using a Nippon

Jarrell-Ash (Kyoto, Japan) ICAP-1000 inductively coupled plasma-atomic emission spectrometry (ICP-AES) system [7]. The amount of aluminium adsorbed on the 1 g of silica gel was ≈500 µg.

Table 1 shows the physical properties of the Al-silica. The determination of the surface area and pore volume by the nitrogen adsorption isotherms on the gels at 77 K was carried out using a Carlo Erba (Milan, Italy) Sorptomatic 2400 surface analyzer. The surface area was calculated from the BET equation. The pore volume was obtained from the liquid nitrogen volume evaluated from the nitrogen adsorption volume at $P/P_0 = 0.95$. A decrease of surface area and pore volume and an increase of packing density were due mainly to shrinkage of silica matrix.

2.2. Ion chromatograph

The ion chromatograph consisted of a Tosoh (Tokyo, Japan) SC-8010 chromatographic data processor, a Tosoh CCPD delivery pump operated at a flow-rate 0.35 ml min⁻¹, a Tosoh CO-8000 column oven operated at 35°C, a Tosoh UV-8010 UV–vis spectrophotometric detector, a Tosoh SD-8013 on-line degasser and a Reodyne (Cotati, CA, USA) 7125 injector equipped with a 50-µl sample loop.

2.3. Reagents

All reagents were of analytical-reagent grade or HPLC grade and were purchased from Wako (Osaka, Japan). DIW was employed for the preparation of eluent and standard solutions. The eluents were prepared by diluting a 0.5 *M* sulfuric acid standard solution with DIW or organic solvent–DIW. The eluent pH was measured using a Toa Denpa (Tokyo, Japan) IM-40S ion-meter with a glass electrode.

Table 1
Physical properties of Super Micro Beads Silica Gel B-5 (SMBSG B-5) and aluminium-adsorbing silica gel (Al-silica)

Packing	Form	Particle size (µm)	Surface area (m ² /g)	Pore size (Å)	Pore volume (ml/g)	Packing density (g/ml)
SMBSG B-5	Spherical	6.1	541	68	0.83	0.44
Al-silica	Spherical	5.3	310	64	0.48	0.65

3. Results and discussion

3.1. Elution behavior of various acids on Al-silica column by elution with water

For the characterization of the laboratory-made Al-silica as a stationary phase in ion-exclusion chromatography, the retention behavior of various inorganic and organic acids was investigated by elution with water. Table 2 lists the retention times of various acids on the Al-silica column together

Table 2
Retention times of common inorganic and organic acids on Al-silica and SMBSG B-5 columns by elution with water^a

Solute	pK _{a1}	Retention time (min)	
		Al-silica	SMBSG B-5
HCl	-7.0	5.0	5.0
H ₂ SO ₄	-4.0	5.1	5.1
HNO ₃	-1.5	5.1	5.5
Formic acid	3.75	7.4	7.8
Acetic acid	4.76	9.1	9.8
Propionic acid	4.87	10.2	10.7
Butyric acid	4.82	12.5	11.9
Valeric acid	4.86	16.6	13.4
Caproic acid	4.86	23.4	14.9
Heptanoic acid	4.87	35.7	16.2
Caprylic acid	4.89	56.5	17.6
Lactic acid	3.89	7.8	8.2
Oxalic acid	1.27	6.4	7.3
Maleic acid	1.94	6.0	6.4
Malonic acid	2.84	6.4	6.8
Fumalic acid	3.02	6.6	6.9
Tartaric acid	3.04	6.5	6.8
Malic acid	3.46	7.0	7.3
Succinic acid	4.21	8.2	8.4
Citric acid	3.13	6.6	7.2
Pyromellitic acid	1.92	5.0	5.0
Trimellitic acid	2.52	5.0	5.0
Hemimellitic acid	2.62	5.0	5.2
<i>o</i> -Phthalic acid	2.95	5.0	5.0
<i>m</i> -Phthalic acid	3.72	5.0	5.0
<i>p</i> -Phthalic acid	3.54	5.0	5.0
Salicylic acid	2.75	5.0	5.0
Benzoic acid	4.20	7.3	5.9
Phenol	9.70	13.0	11.5

^a Conditions: column size, 250×4.6 mm I.D.; column temperatures, 35°C; flow-rate, 0.35 ml min⁻¹.

with those on the SMBSG B-5 column. The values of the first acid dissociation constant (pK_{a1}) of these acids are cited from Refs. [2,8,9]

As listed in Table 2, it can be seen that strong acids (HCl, HNO₃ and H₂SO₄) were eluted simultaneously and weak acids (except for benzenecarboxylic acids) were eluted later than these strong acids. This result indicated that these acids were excluded from the surface of the Al-silica stationary phase by electrostatic repulsion from dissociated silanol group and, in other words, these acids were separated by the ion-exclusion chromatographic process.

The retention times of higher C₅–C₈ aliphatic carboxylic acids (valeric, caproic, heptanoic and caprylic acids) on the Al-silica column were considerably longer than those on the SMBSG B-5 column. However, since the retention times of these carboxylic acids on the Al-silica column were much shorter than those on the conventional ion-exclusion chromatographic column packed with a sulfonated styrene–divinylbenzene co-polymer resin (TSKgel SCX) [4], it is considered that the Al-silica column is applicable to the ion-exclusion chromatographic separation of these hydrophobic carboxylic acids.

The retention times of benzenecarboxylic acids (except for benzoic acid) were the same as those of the strong acids. This is because these acids are relatively strong, and are therefore strongly excluded from the Al-silica stationary phase by electrostatic repulsion. As reported before [5], unmodified silica gel (Develosil 30-5), which acted as a cation exchanger under strongly acidic conditions, was successfully applied to the ion-exclusion chromatographic separation of benzenecarboxylic acids. It was expected that the Al-silica would also be applicable to the separation of these benzenecarboxylic acids.

In contrast, the retention times of hydrophilic carboxylic acids (oxalic, maleic, malonic, fumalic, tartaric and citric acids) on the Al-silica column were almost the same. A column packed with TSKgel SCX is commonly used for the ion-exclusion chromatographic separation of these carboxylic acids [2]. This is because these acids are separated by both ion-exclusion and adsorption processes. Since the hydrophobicity of the Al-silica was much lower than that of the TSKgel SCX, it was considered that the Al-silica column was not effective for the separation of these hydrophilic carboxylic acids.

It was concluded that the Al-silica column was suitable for the ion-exclusion chromatographic separation of aliphatic carboxylic acids and benzenecarboxylic acids of a hydrophobic nature.

3.2. Separation of aliphatic carboxylic acid on Al-silica column

As shown in Fig. 1a, when using water as an

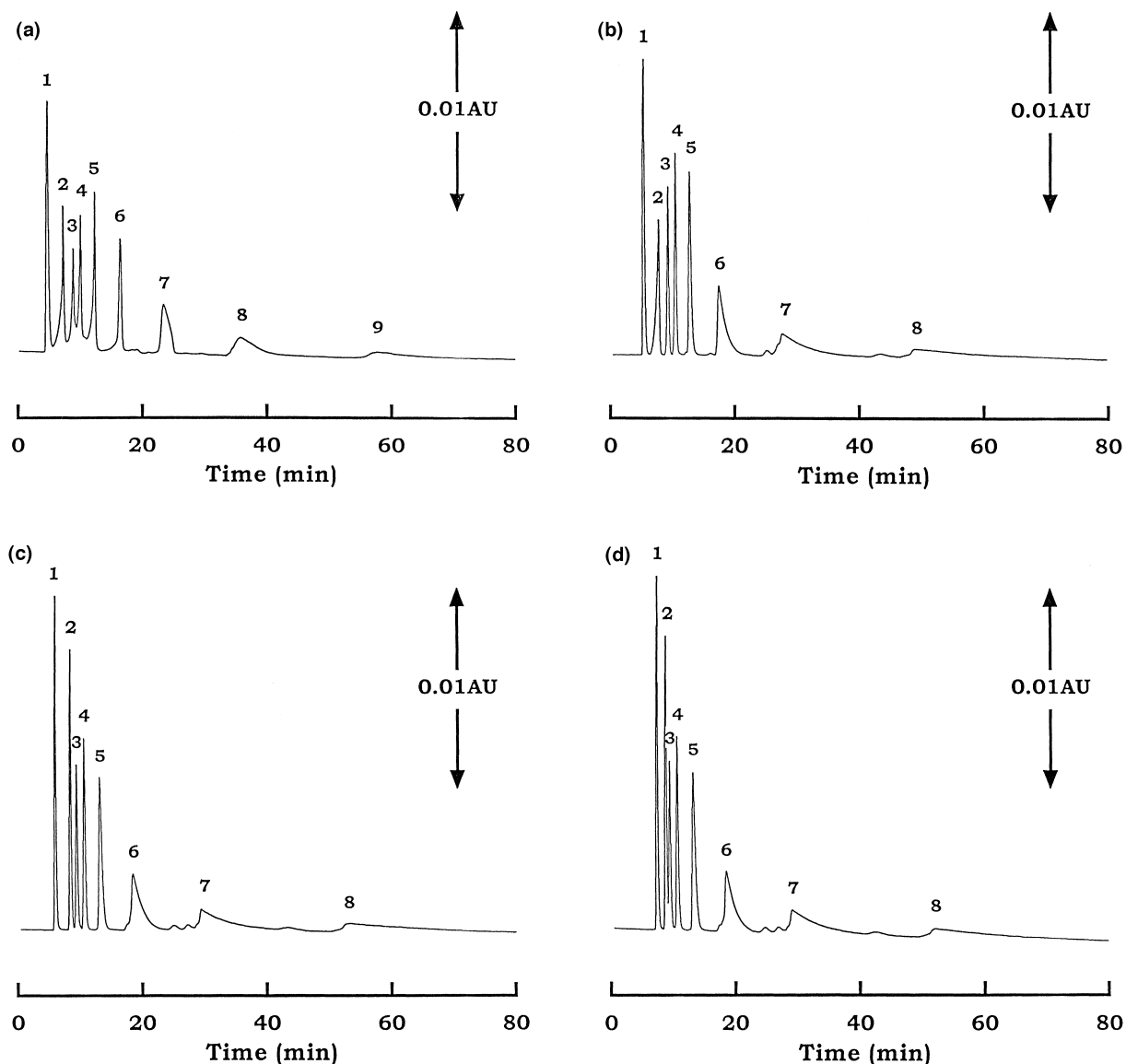


Fig. 1. Chromatograms of aliphatic carboxylic acids on aluminium-adsorbing silica gel column by elution with various concentration of sulfuric acid. Conditions: column, aluminium-adsorbing silica gel ($500 \mu\text{g}$ aluminium g^{-1} silica gel) calcined at 1000°C for 5 h; column size: 250×4.6 mm I.D.; column temperature: 35°C ; eluents: (a) water (pH 5.65), (b) 0.05 mM sulfuric acid (pH 4.01), (c) 0.5 mM sulfuric acid (pH 3.04), (d) 5 mM sulfuric acid (pH 2.08); flow-rate: 1 ml min^{-1} ; detection: UV at 220 nm ; injection volume: $50 \mu\text{l}$; sample concentration: 0.01 mM for nitric and 1 mM for aliphatic carboxylic acids. Peaks: 1=nitric acid, 2=formic acid, 3=acetic acid, 4=propionic acid, 5=butyric acid, 6=valeric acid, 7=caproic acid, 8=heptanoic acid, 9=caprylic acid.

eluent, although C_1 – C_8 aliphatic carboxylic acids (formic, acetic propionic, butyric, valeric, caproic, heptanoic and caprylic acids) were separated on the Al-silica column, the peak shapes of these acids were all destroyed. The peak shapes of C_1 – C_5 aliphatic carboxylic acids were fronted and those of C_6 – C_8 aliphatic carboxylic acids were tailed. These results indicated that the C_1 – C_5 aliphatic carboxylic acids were mainly separated by the ion-exclusion chromatography process and the C_6 – C_8 aliphatic carboxylic acids were mainly separated by hydrophobic adsorption process. Turkelson and Richards [10] have reported that the use of acidic eluents was very effective for the improvement of peak shapes in ion-exclusion chromatography. Therefore, in order to improve peak shape, the effect of the concentration of sulfuric acid in the eluent was investigated between 0 and 5 mM (pH 5.65–2.08).

As shown in Fig. 2, the retention times of these aliphatic carboxylic acids increased with decreasing

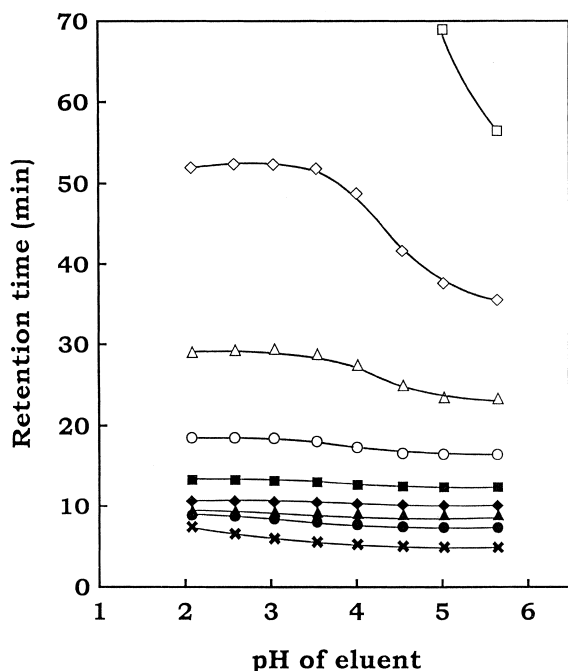


Fig. 2. Effect of pH of eluent on retention times of aliphatic carboxylic acids on aluminium-adsorbing silica gel column. Symbols: \times = nitric acid, \bullet = formic acid, \blacktriangle = acetic acid, \blacklozenge = propionic acid, \blacksquare = butyric acid, \circ = valeric acid, \triangle = caproic acid, \diamond = heptanoic acid, \square = caprylic acid. Chromatographic conditions as in Fig. 1.

pH of the eluent. This is due mainly to an increase of hydrophobic interaction between these acids and the surface of the Al-silica by suppressing the dissociation of both these acids and silanol group on the Al-silica. As a result, the retention times of higher aliphatic carboxylic acids drastically increased. The retention time of nitric acid also increased. This is due mainly to a decrease of electrostatic repulsion. Fig. 1b–d shows typical chromatograms of these acids obtained by elution with 0.05, 0.5 and 5 mM sulfuric acid, respectively. The peak shapes of C_1 – C_4 aliphatic carboxylic acids, which were separated mainly by the ion-exclusion chromatographic process, were improved. In contrast, the peak shapes of C_5 – C_8 aliphatic carboxylic acids, which were separated by hydrophobic adsorption, were extensively tailed. Considering the peak shapes and retention times of higher aliphatic carboxylic acids, a reasonable concentration of sulfuric acid in the eluent was chosen at 0.5 mM.

The addition of organic solvent to the eluent has often been carried out for the reduction of retention times and improvement of peak shapes of hydrophobic carboxylic acids in ion-exclusion chromatography [2]. Morris and Fritz [11] reported that a higher alcohol content was very effective for the improvement of peak shape of carboxylic acids. Therefore, various alcohols (methanol, ethanol, propanol, butanol, pentanol, hexanol and heptanol) in 0.5 mM sulfuric acid eluent were added. Alcohols $>C_7$ ($>$ heptanol) were not applicable due to the limitation of solubility. Fig. 3a–d show typical chromatograms of C_1 – C_8 aliphatic carboxylic acids on the Al-silica column obtained by elution with 10% methanol, 2% propanol, 0.5% pentanol and 0.15% heptanol in 0.5 mM sulfuric acid, respectively. These chromatograms indicated that higher alcohols (pentanol and heptanol) were more effective for the reduction of retention times and improvement of peak shapes of higher aliphatic carboxylic acid. This indicated that higher alcohols were strongly adsorbed on the Al-silica reducing the hydrophobic interaction between the higher aliphatic carboxylic acids and the Al-silica.

As shown in Fig. 3d, when using 0.5 mM sulfuric acid containing 0.15% heptanol as eluent, an excellent separation of C_1 – C_8 aliphatic carboxylic acids with good peak shape was achieved in 25 min.

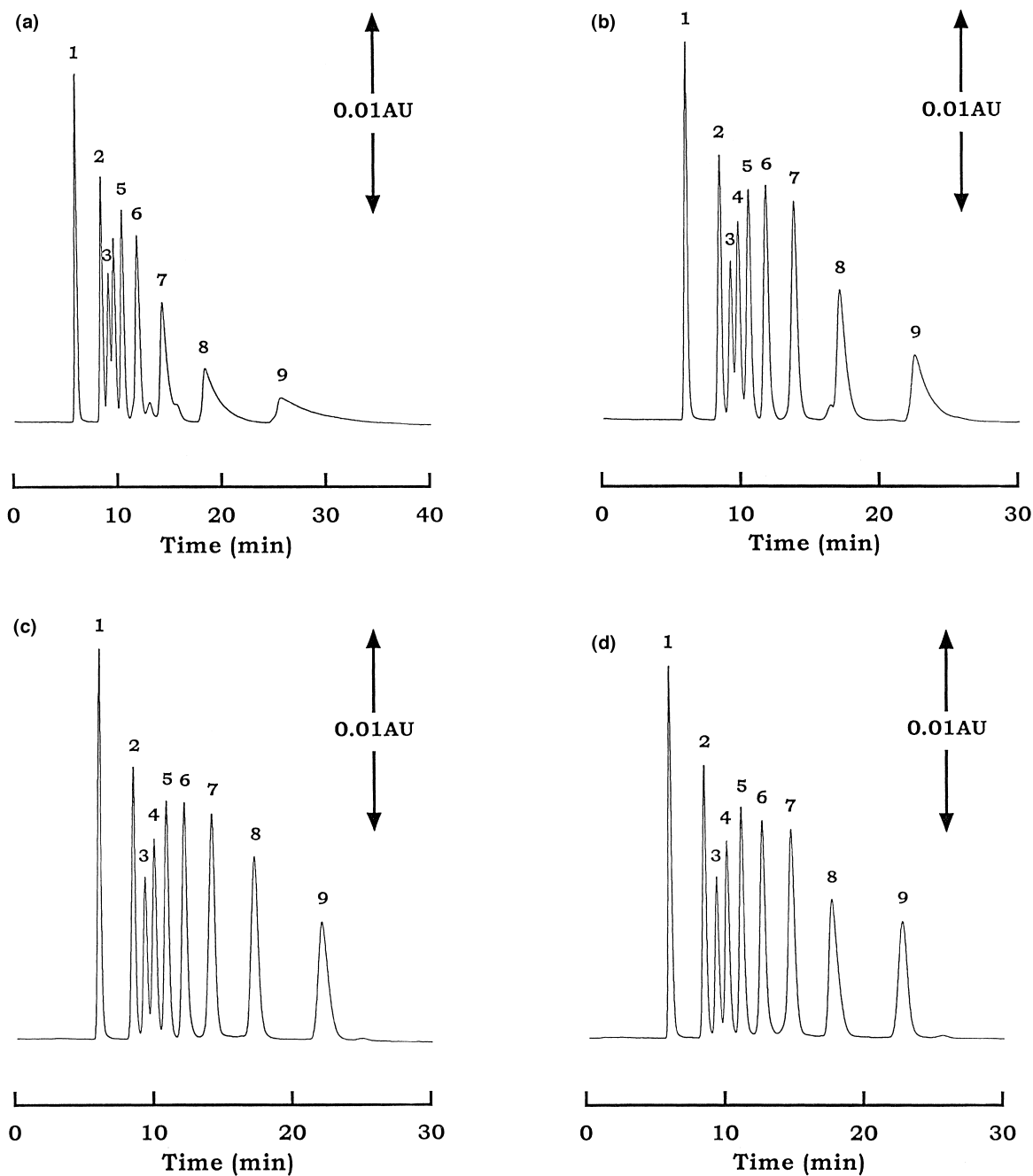


Fig. 3. Chromatograms of aliphatic carboxylic acids on aluminium-adsorbing silica gel column by elution with 0.5 mM sulfuric acid containing various alcohols. Conditions: eluent: (a) 10% methanol, (b) 2% propanol, (c) 0.5% pentanol, (d) 0.15% heptanol in 0.5 mM sulfuric acid; other chromatographic conditions as in Fig. 1.

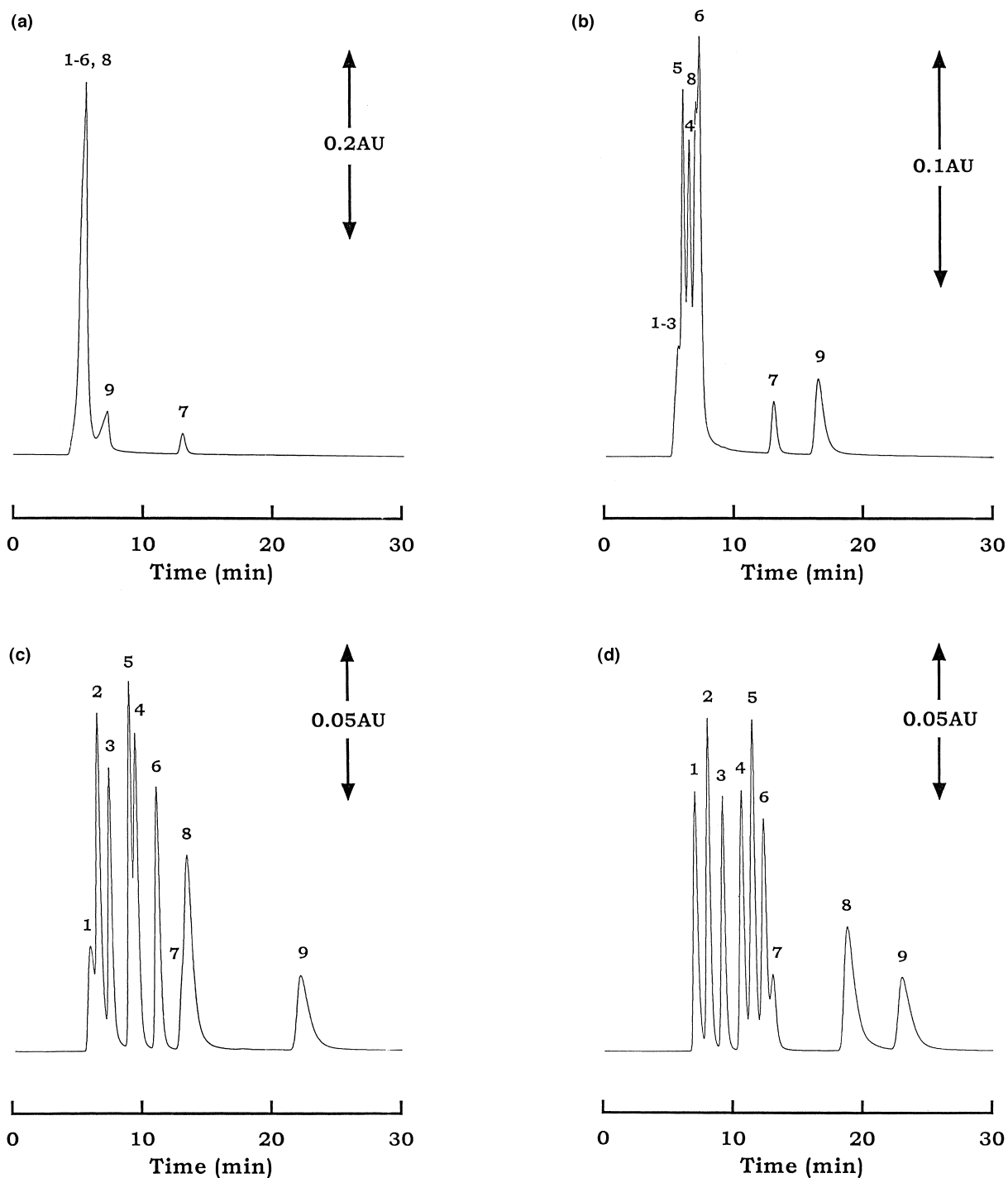


Fig. 4. Chromatograms of benzenecarboxylic acids on aluminium-adsorbing silica gel column by elution with various concentration of sulfuric acid. Conditions: eluent: (a) water, (b) 0.05 mM sulfuric acid, (c) 0.5 mM sulfuric acid, (d) 2.5 mM sulfuric acid (pH 2.36), (e) 5 mM sulfuric acid; detection: UV at 200 nm; sample concentration: 0.01 mM; other chromatographic conditions as in Fig. 1. Peaks: 1=pyromellitic acid, 2=trimellitic acid, 3=hemimellitic acid, 4=*p*-phthalic acid, 5=*o*-phthalic acid, 6=*m*-phthalic acid, 7=phenol, 8=salicylic acid, 9=benzoic acid. (Continued on next page)

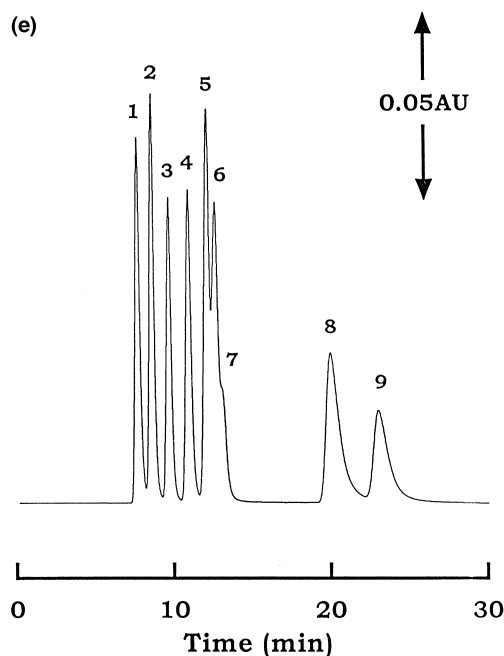


Fig. 4. (continued)

3.3. Separation of benzenecarboxylic acid on Al-silica column

Fig. 4a shows chromatogram of benzenecarboxylic acids (pyromellitic, hemimellitic, trimellitic acid, *o*-phthalic, *m*-phthalic, *p*-phthalic, benzoic and salicylic acids) and phenol on the Al-silica column by elution with water. As shown in Fig. 4a, almost all benzenecarboxylic acids (except for benzoic acid) co-eluted and their peak shapes were fronted. Therefore, in order to separate these carboxylic acids with a good peak shape, the effect of the concentration of sulfuric acid in the eluent was investigated between 0 and 5 mM.

As shown in Fig. 5, the retention times of the benzenecarboxylic acids increased with decreasing pH of the eluent. This is due to both a decrease of electrostatic repulsion and an increase of hydrophobic interaction between these benzenecarboxylic acids and the Al-silica by suppressing the dissociation of these acids and silanol group on the Al-silica. In contrast, the retention time of phenol was constant. This is because phenol is separated only by hydrophobic adsorption. The difference of the in-

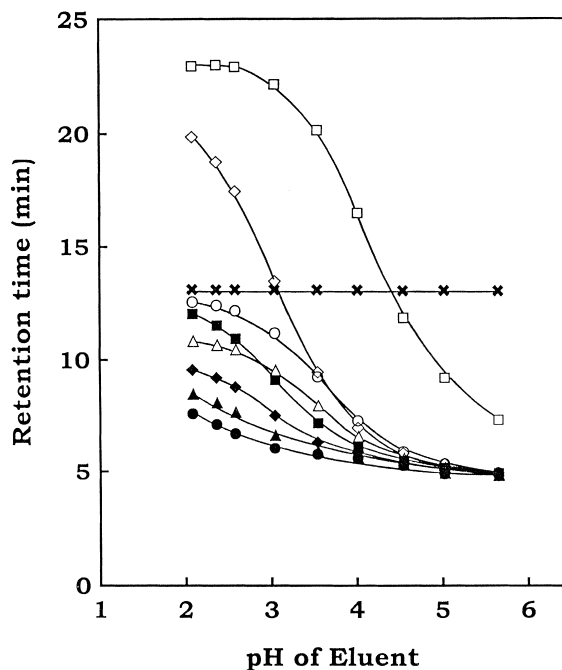


Fig. 5. Effect of pH of the eluent on retention times of benzenecarboxylic acids on aluminium-adsorbing silica gel column. Symbols: ●=pyromellitic acid, ▲=trimellitic acid, ◆=hemimellitic acid, ■=*p*-phthalic acid, ○=*o*-phthalic acid, △=*m*-phthalic acid, ◇=salicylic acid, □=benzoic acid, ×=phenol; chromatographic conditions as in Fig. 4.

creasing retention times of these benzenecarboxylic acids was due mainly to the difference of their pK_a values and hydrophobicity. Fig. 4b–e shows typical chromatograms of these benzenecarboxylic acids and phenol on the Al-silica column obtained by elution with 0.05, 0.5, 2.5 and 5 mM sulfuric acid, respectively. The peak shapes improved with increasing the concentration of sulfuric acid in the eluent. This is due mainly to a decrease of electrostatic repulsion. As shown in Fig. 4d, when using 2.5 mM sulfuric acid eluent, a good separation of these benzenecarboxylic acids and phenol was achieved in 25 min.

4. Conclusion

In this study, a laboratory-made aluminium-adsorbing silica gel (Al-silica), which acts as a cation exchanger under strongly acidic conditions, was applied to the ion-exclusion chromatographic separa-

ration of various carboxylic acids. The Al-silica stationary phase was found to be very effective in the ion-exclusion chromatographic separation for both aliphatic carboxylic acids and benzenecarboxylic acids with a hydrophobic nature. As has been reported previously [7], the Al-silica was successfully applied to the ion chromatographic separation of common mono- and divalent cations based on a cation-exchange mechanism. These results indicate that the Al-silica is one of the most useful cation exchangers in both ion chromatography and ion-exclusion chromatography.

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References

- [1] R.M. Wheaton, W.C. Bauman, *Ind. Eng. Chem.* 45 (1953) 228.
- [2] P.R. Haddad, P.E. Jackson, *Ion Chromatography: Principles and Applications*, Elsevier, Amsterdam, 1990.
- [3] K. Ohta, M. Sando, K. Tanaka, P.R. Haddad, *J. Chromatogr. A* 752 (1996) 167.
- [4] K. Ohta, K. Tanaka, P.R. Haddad, *J. Chromatogr. A* 739 (1996) 359.
- [5] K. Ohta, K. Tanaka, P.R. Haddad, *J. Chromatogr. A* 782 (1997) 33.
- [6] K. Ohta, H. Morikawa, K. Tanaka, Y. Uryu, B. Paull, P.R. Haddad, *Anal. Chim. Acta* 359 (1998) 255.
- [7] K. Ohta, H. Morikawa, K. Tanaka, P.R. Haddad, *J. Chromatogr. A* 804 (1998) 171.
- [8] S. Motomizu, I. Sawatani, T. Hironaka, M. Oshima, K. Toei, *Bunseki Kagaku* 36 (1987) 77.
- [9] K. Biinran (Ed.), *The Chemical Society of Japan*, Maruzen, Tokyo, 1966.
- [10] V.T. Turkelson, M. Richards, *Anal. Chem.* 50 (1978) 1420.
- [11] J. Morris, J.S. Fritz, *Anal. Chem.* 66 (1994) 2390.