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Effect of Eluants and Regenerants on the Separation of Organic Acids in Ion-Exclusion Chromatography

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Abstract

Effect of eluants and regenerants on the separation of organic acids in ion-exclusion chromatography are described. For the determination of organic acids, heptafluorobutylic acid and tetraalkylammonium salts such as tetrabutylammonium hydroxide play an important role in separation and determination of carboxylic acids.

Introduction

Many analytical methods have been reported in the literature for the separation of organic acids, and a number of these have been devoted to the determination of the acids in fluids of biological interest such as blood, urine and so on, and food additives. In many of these methods, complete separation of all acids of interest is not always achieved, some of which have found very limited application in analysis of authentic samples; False identification, especially in environmental samples containing unexpected analytes, is possible and coelution problems also exist¹. Ion exchange resins have a well-known ability to provide excellent separations of ionic species and there are many instances where ion exchange chromatography has been successfully applied. In recent years, ion-exclusion chromatography has been applied to the separation and determination of weak organic acids²⁻⁴. This method also has an advantage that inorganic anions such as chloride and nitrate which are perfectly dissociated, are excluded from the column. Another advantage is the high selectivity for weak carboxylic acids on the basis of Donnan exclusion effect, partition and van der Waals forces. Above all, it is useful to separate and determine incompletely dissociated electrolytes such as carboxylic acids and amino acids. In regard to conductivity detector, a disadvantage on the conductivity detection is the low sensitivity towards weak carboxylic acids because of their elution as unionized forms³. In order to improve the sensitivity, several techniques have been developed as to decreasing of background conductivity prior to detection⁵⁻⁸. Amperometric detector also could not be determined non-reducible or non-oxidizable species in the potential domain of interest. Due to the development of the elegant suppresser, conductivity detection is superior to UV detection from the viewpoint of selectivity and sensitivity⁴.

In this paper, we describe the effect of eluant and regenerant on the separation

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of carboxylic acids by ion-exclusion chromatography. This work gives some of basic informations of separation of carboxylic acids.

Experimental

Reagents

All carboxylic acids and other chemicals were of analytical reagents grade and used without further purification. Water used was purified by using deionized and double-distilled quartz distillor. Heptafluoro-n-butyric acid (for sequential analysis of peptides) and tetra-n-butylammonium hydroxide were obtained from Nakarai Chemicals Co. Ltd. (Kyoto, Japan).

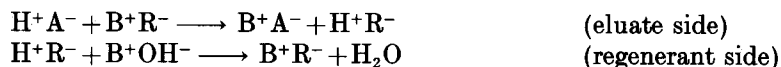
Instruments

The QIC analyzer with the suppresser, AMMS-ICE manufactured by Dionex Co., USA was used for the chromatographic separation and detection of carboxylic acids. It consisted of a pump, a 50 μ l sample loop, a separation column, a cation exchange micromembrane suppresser and a conductivity detector. A separation column packed with a high capacity, fully sulphonated styrene-divinyl-benzene cation-exchange resin, HPICE-AS-5 (250 mm \times 9 mm I.D., Dionex Co.) was used. The eluants and the regenerants were flowed through at a flow-rate of 0.3 ml/min and of 2 ml/min, respectively. The chromatograms were recorded and stored with a chromato-integrator D-2500 (Hitachi Co., Ltd., Japan). All the separations and detections were performed at room-temperature.

Results and Discussion

Selection of eluant

In the analysis of organic acids by using ion-exclusion chromatography, H⁺ typed cation exchange resin column and diluted acid solution were often used as the eluant. It is necessary to give full consideration to the concentration of hydrogen ion on the dissociation constant of weak acids. This technique is based on the Donnan exclusion phenomenon. Therefore, charged species such as chloride ion and nitrate ion are eliminated in the ion-exclusion column. In addition, the following reactions occur in both sides through cation exchange micromembrane in the suppresser.



where H⁺A⁻ represents acids in the eluant or organic acid as an analyte, B⁺OH⁻ and R⁻ are base in the regenerant and a cation exchange resin, respectively. In order to enhance the sensitivity in conductivity detection, it is necessary to suppress or decrease the conductivity of B⁺A⁻ produced in the above reaction as much as possible. The relationship between the background conductivity and the retention time (t_R) of weak carboxylic acids of interest has been studied by using a dilute solution of acids. One mM L-lactic acid (pK_a 3.8), acetic acid (pK_a 4.8) and citric acid (pK_a 3.1, 4.8 and 6.4) were used as the analytes for the separation of carboxylic acids in standard samples, respectively. The carboxylic acids of interest here are

well separated from each other in the cation exchange resin (H^+) with high capacity using a dilute solution of strong acids such as 1.6 mM hydrochloric acid ($155 \mu S/cm$), 1.6 mM perchloric acid ($150 \mu S/cm$) and 0.8 mM sulfuric acid ($169 \mu S/cm$) as the eluant. There are almost no differences in their peak areas and the column efficiency among the eluants studied. As can be seen from the values of the ionic conductivities at infinite dilution of corresponding anions⁹, their background conductivities became large. When 0.53 mM phosphoric acid (pK_a 2.15, 7.20 and 12.55) was used as the eluant, the dissociation of citric acid accelerated owing to increasing pH and its elution time was too fast. As a necessary consequence, it is difficult to determine perfectly these carboxylic acids each other. When monochloroacetic acid (MCA, pK_a 2.68), dichloroacetic acid (DCA, pK_a 1.30) and trichloroacetic acid (TCA, pK_a 0.66) (each acid concentration: 1.6 mM) were used as the eluant, the separation for these carboxylic acids obtained by using DCA or TCA were much better than those obtained by using MCA; the responses were observed at decreasing background conductivity with a sharp single peak individually. This might be caused by smaller equivalent conductance of DCA or TCA anions compared with that of corresponding MCA anion and by larger pH of MCA solution than the others. The peak areas obtained by using MCA were also somewhat larger than those obtained by using inorganic strong acids or TCA. This means possibly that nondissociated MCA was adsorbable on the resin in the separation column and

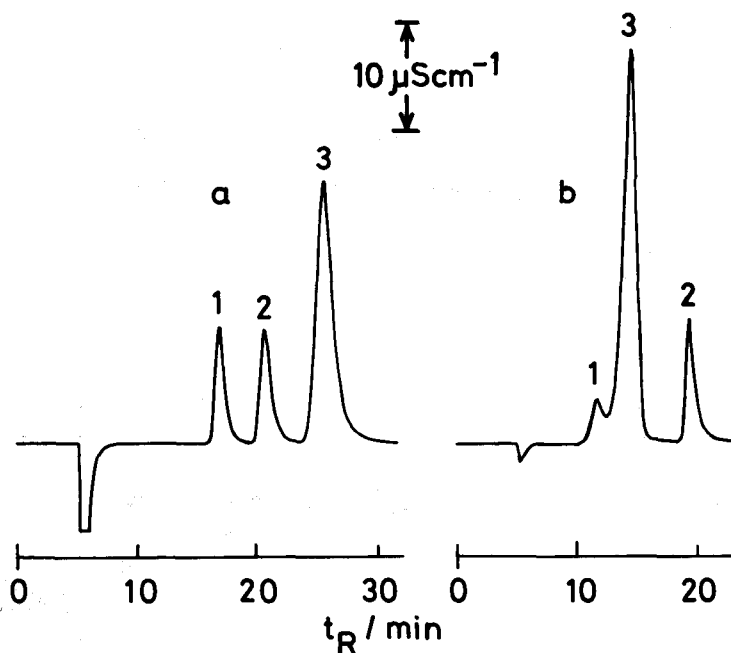


Fig. 1. Typical chromatograms of carboxylic acids using two different concentrations of heptafluorobutyric acid as the eluant.

Conditions: Eluant; (a) 1.6 mM HFB, (b) 0.2 mM HFB, flow rate; 0.3 ml/min.

Regenerant; 5 mM TBAOH, flow rate: 2 ml/min. Injection volume; 50 μ l. Peak No.

1: L-lactic acid. No. 2: acetic acid, No. 3: citric acid.

eluted into the sample zone. If 1.6 mM trifluoroacetic acid (TFA, pK_a 0.2) or 1.6 mM heptafluorobutyric acid (HFB, pK_a 0.17) was used as the eluant, similar results were observed in the case of TCA as the eluant. The typical chromatogram using 1.6 mM HFB is shown in Fig. 1a. From the aforementioned results, halogenized carboxylic acids or alkyl sulphonic acids ($pK_a < 1$) are suitable for the eluant because of the corresponding counter anion with larger molecular cross sections and lower conductances than chloride and sulfate ions and so on.

Effect of concentration of eluant

The separation of carboxylic acids in standard samples has been studied by using various concentrations of HFB as the eluant (Fig. 2). These three carboxylic acids could be separated satisfactorily in the order of lactic acid, acetic acid and citric acid by using more than 1.6 mM HFB (pH 2.9), but the chromatographic peak of citric acid showed a broadening profile with a large background conductivity using above 2.4 mM HFB (pH 2.7). When less than 0.8 mM HFB (pH 3.1) was used as the eluant, the shapes of the peak produced are sharp profiles, but the peak of citric acid replaced the peak of acetic acid reversely. Moreover, the peak area of L-lactic acid became very small with decreasing the concentration of HFB. The

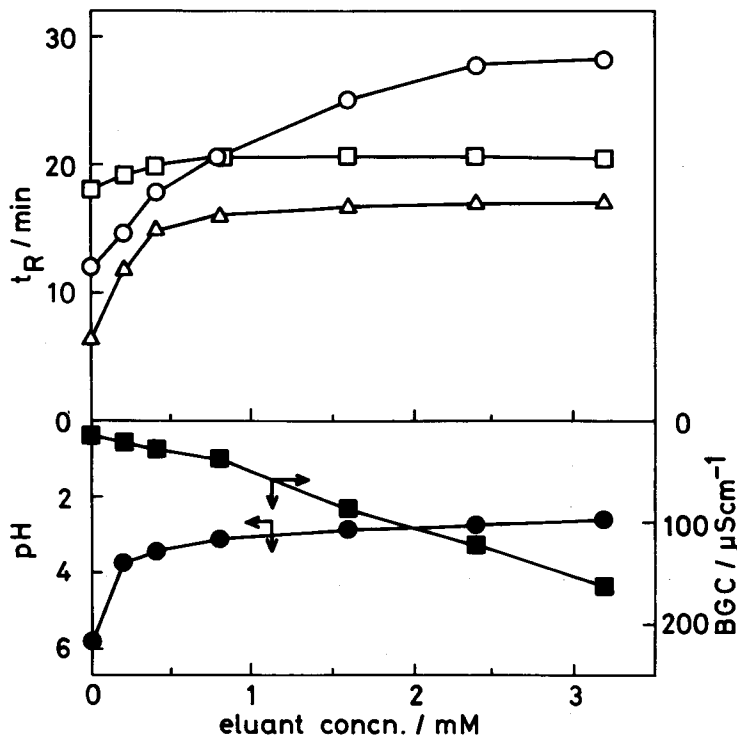


Fig. 2. Effect of eluant concentration on chromatographic performance.

Conditions: Eluant; HFB, others as same as in Fig. 1.

○: citric acid, □: acetic acid, △: L-lactic acid.

Table 1. Background conductivities using some bases as the regenerant

Regenerant	TBAOH	NaOH	KOH	NH ₃
BGC/(μ S/cm)	85	150	218	189

Concentration of each base: 5 mM, eluant: 1.6 mM HFB. Abbreviation: see in Text.

other two acids also showed similar tendency although the reasons are still unknown (Fig. 1b). In the elution of L-lactic acid and citric acid, the larger the pH aperted from pK_a of both the carboxylic acids, the shorter the retention time (t_R) significantly. This might be due to strong exclusion of the corresponding anions produced by the dissociation from the cation exchange resin. If deionized water (pH 5.8) was used as the eluant instead of HFB, acetic acid could not be eluted fast like the other carboxylic acids. This may be presumably adsorbed on the resin by the corresponding anions of acetic acid.

Selection of regenerant

Contrary to the selection of eluant, it is preferable to be a base composed of counter cation (B^+) with low conductance. When tetra-n-butylammonium hydroxide (TBAOH), sodium hydroxide, potassium hydroxide, and aqueous ammonia were used as the regenerant, respectively, the background conductivities are shown in Table 1. The good chromatograms with lower background conductivity that those with the other bases were observed only when TBAOH was used, as can be expected from the limiting equivalent conductivities. Moreover, there is almost no difference in the background conductivity over the concentration range of 2.5–10 mM TBAOH as the eluant. This result supports the view that ion exchange membrane is generated satisfactorily in these concentrations. As one would expect, this makes it much more advantageous to work at these concentrations to obtain better results.

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