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Vacancy ion-exclusion/adsorption chromatography of aliphatic amines on a polymethacrylate-based weakly basic anion-exchange column

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Abstract

Vacancy ion-exclusion/adsorption chromatography has been applied to investigate the separation behavior of five aliphatic amines (ethylamine, propylamine, butylamine, pentylamine and hexylamine) on a polymethacrylate-based weakly basic anion-exchange column (Tosoh TSKgel DEAE-5PW). This system is consisted of analytes as a mobile phase and water as an injected sample. In the vacancy ion-exclusion/adsorption chromatography, the elution order was as follows: ethylamine < propylamine < butylamine < pentylamine < hexylamine, depending on their hydrophobicity. The retention times of the amines were decreased with decreasing their concentrations in the mobile phase. The retention times and resolutions of the amines were increased by adding a basic compound (e.g., lithium hydroxide or heptylamine) and by increasing the pH of mobile phase (pH > 11). This was because the dissociations of amine samples in the mobile phase were suppressed and thus the hydrophobic adsorption effects were enhanced. The linearity of calibration graphs could be obtained from the peak areas of the amine samples injected to the 0.05, 0.5 and 5 mM of amine mobile phase at pH 11 by heptylamine. The detection limits of aliphatic amines as injected samples were around 1 μM for five aliphatic amines at three different amine mobile phases. From these results, the retention behaviors of aliphatic amines on vacancy ion-exclusion/adsorption chromatography were concluded to be governed by the hydrophobic adsorption effect.

Keywords: Vacancy chromatography; Ion-exclusion/adsorption chromatography; Adsorption; Amines; Alkylamines

1. Introduction

Vacancy ion-exclusion/adsorption chromatography has been recently developed in terms of the ion-exclusion and the hydrophobic adsorption of analyte samples in the mobile phase with the resin phase [1–4]. Vacancy ion chromatography is based of analyte samples as a mobile phase and water as an injected sample. The peaks resulted with the conductivity detection were detected as negative "vacant" peaks (elution-dip peaks) corresponding to each of the analytes present in the sample appear. The peak areas and heights are dependent on the volume of sample water injected to the separation column and the degree of ion-exclusion/hydrophobic adsorption of analytes retained on the resin phase. This system can be provided a sensitive detection and the asymmetric peaks, compared with the conventional ion-exclusion chromatography on the same separation column using water as a mobile phase. This method has been adopted for the separation of several acids, i.e., aliphatic/aromatic carboxylic acids on a polymethacrylate-based weakly acidic cation-exchange resin [1,3,4] or a polystyrene-divinylbenzene-based strongly acidic cation-exchange resin [2]. Consequently, the obtained conductivity detection responses of analytes have been ca. 5–10 times higher than conventional ion-exclusion chromatography using strong or weak acid eluents.

The purpose in this study was to clarify the retention behavior of basic compounds on vacancy ion chromatography in order to spread its applicability to various samples. Here, aliphatic amines with different alkyl chain were chosen as a test sample, because of industrial interest widely distributed in the biological system as a useful indicator of spoilage of fish or meat [5–7].

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As has been reported previously, well-resolved separations by ion-exclusion chromatography of aliphatic amines using a basic anion-exchange resin column were obtained by the pH increased adding a basic compound (e.g., sodium hydroxide) to the mobile phase [8]. At the same time, their retention times were prolonged with increasing the concentration of base in the mobile phase. This was due mainly to enhancement of hydrophobic adsorption with resin phase, as a side-effect of ion-exclusion chromatography. That is to say, the pH of mobile phase has been recognized to be important parameters to manipulate both retention times and resolution on ion-exclusion chromatography.

In this paper, we report the feature of vacancy ion-exclusion/adsorption chromatography of amine in terms: (1) the change of the retention time of amine depending on amine concentrations in the mobile phase; (2) the control of the retention behavior by adding a basic compound; and (3) the analytical performance for the calibration graph and reproducibility of retention.

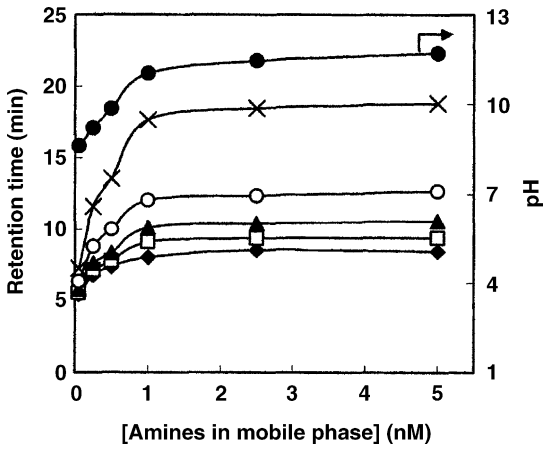


Fig. 1. Relationship between the retention times on the different concentrations of aliphatic amines in the mobile phase on vacancy ion-exclusion/adsorption chromatography and the pH values. Column: TSKgel DEAE-5PW (15 cm × 7.5 mm i.d.). Composition of amine mobile phase: ethylamine, propylamine, butylamine, pentylamine and hexylamine. Injected sample: water; injection volume: 100 μl; column oven: 40 °C; flow rate: 0.6 ml/min. Plot identities: (◆) ethylamine, (□) propylamine, (▲) butylamine, (○) pentylamine, (×) hexylamine and (●) pH value of each amine mobile phase.

2. Experimental

2.1. Instrumentation

The ion chromatograph consisted of a Tosoh (Tokyo, Japan) LC-8020-Model II chromatographic data processor, a DP-8020 dual pump operated at flow rate of 0.6 ml/min, an SD-8022 on-line degasser, a CO-8020 column oven operated at 40 °C and a CM 8020 conductimetric detector. The injection volume of injected sample was 100 μl.

A separation column used in this study was a polymethacrylate-based weakly basic anion-exchange resin in the OH^--form, TSKgel DEAE-5PW, packed with 10 μm particle size (150 mm × 7.5 mm i.d.). The column was equilibrated with eluting a mixture of aliphatic amines for 30 min before the chromatographic runs.

2.2. Reagents

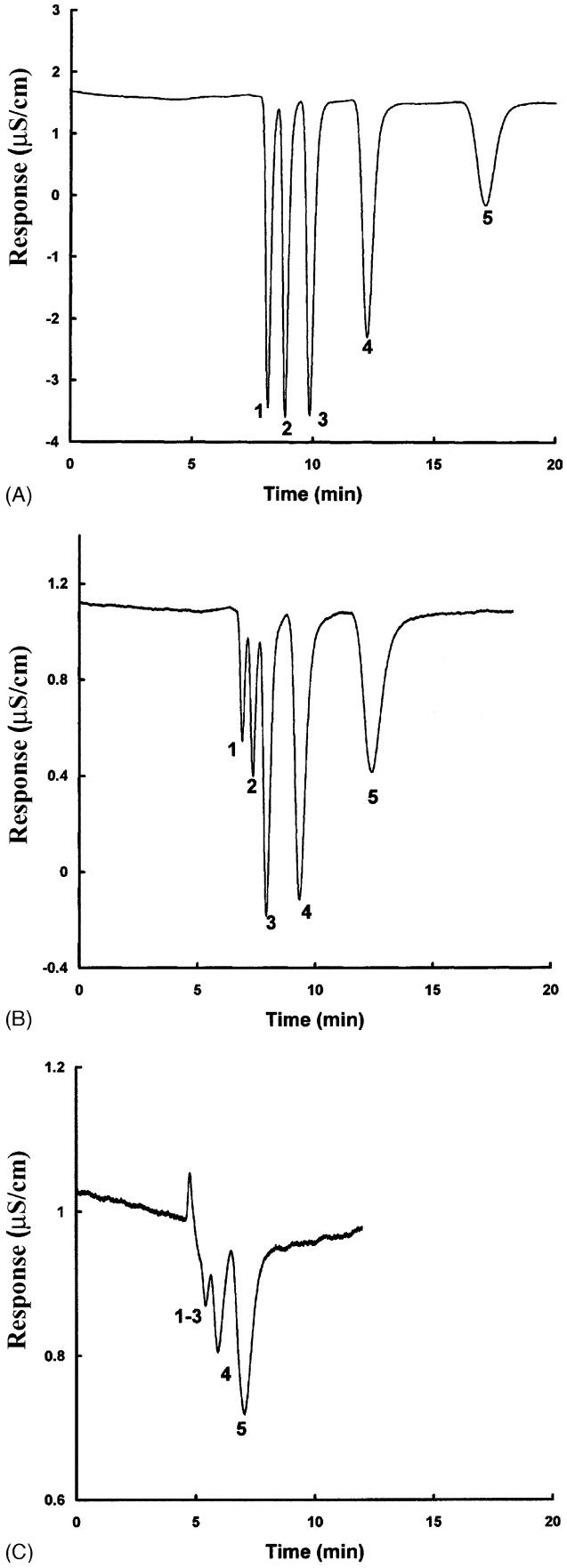
All reagents were of analytical reagent-grade, purchased from Wako (Osaka, Japan) and the preparation of the standard solutions and eluents were dissolved with distilled and deionized water. Appropriate amounts of analyte samples at the concentration of 0.1 M were diluted with water as necessary. In this report, the amine mobile phase was consisted of the following mixture: ethylamine, propylamine, butylamine, pentylamine and hexylamine.

3. Results and discussion

3.1. Vacancy ion-exclusion/adsorption chromatography of aliphatic amines

Fig. 1 shows the retention times of five aliphatic amines (i.e. ethylamine, propylamine, butylamine, pentylamine and hexylamine) obtained with their different concentrations in the mobile phase as well as the changes of pH values. The retention times of aliphatic amines were decreased when the total concentrations of amines in the mobile phase was less than 0.5 mM. The shift of retention times with varying the concentration was closely related on the degree of dissociation of aliphatic amines in the mobile phase. When the total concentration of amines in the mobile phase was higher than 0.5 mM, the pH values were higher than pK_a value of analyte amines (pK_a = 10.3–10.4) and the dissociations were consequently suppressed. The retention times were increased since the hydrophobic adsorption effect between amines and resin phase in separation column were enhanced. In contrast, when the total concentration of amines in the mobile phase was lower than 0.5 mM, the pH value was lower than pK_a value of amines and the dissociation of amines were consequently accelerated. The retention times were decreased since the increases of ion-exclusion of amines in mobile phase with the resin phase.

Fig. 2 shows the vacancy ion-exclusion/adsorption chromatographic separations of the aliphatic amines obtained when total concentration of the amines mixed in the mobile phase was 5 mM (Fig. 2A), 0.5 mM (Fig. 2B) and 0.05 mM (Fig. 2C), respectively. The 5 mM of five aliphatic amines was detected with the negative peak, which were well resolved and symmetrical as shown in Fig. 2A. However, in less than 0.5 mM of the mobile phase, the peak resolutions of the amines were poor and the retention times decreased largely, as shown in Fig. 2B and C.



3.2. Effect of adding basic compound

The separations of the aliphatic amines in conventional ion-exclusion chromatography have been controlled by using elution with basic compound, such as sodium hydroxide [8]. The retention volumes of weak bases were increased by the increase of hydrophobic adsorption because of the increase of molecular-form analyte amines with increasing the concentration of hydroxide ion.

Several basic compounds were added to the 0.5 mM amine mobile phase in order to improve the retention and resolution of the amines. The basic compound was added until the pH value of amine mobile phase was 11, referring to the results shown in Fig. 1. When 1 mM lithium hydroxide as a strong base was mixed to the 0.5 mM amines mobile phase, the vacant peak of lithium ion was appeared faster than amines and the retention times of amines were prolonged with increasing pH value of the mobile phase. Therefore, the addition of a basic compound to the mobile phase was effectively for the manipulation of retention of analyte amines in the vacancy ion-exclusion/adsorption chromatography. However, the separation was insufficient because the peak of lithium ion was overlapped with those of ethylamine and propylamine. Similar results were obtained using other strong bases (e.g., sodium hydroxide and potassium hydroxide).

On the other hand, when 2 mM heptylamine as a weak base with hydrophobic nature was added to 0.5 mM of amines mobile phase, a well-resolved separation of amines was obtained because the vacant peak of heptylamine was appeared later than all amines analytes as shown in Fig. 3. This effect of heptylamine was due mainly to a large hydrophobic adsorption effect to the resin phase than analyte amines. The addition of octylamine was also resulted in a good resolved separation. The use of indifferent bases to targeted amines, especially higher hydrophobic bases, was recognized to be extremely important for the separation of the amines by vacancy ion-exclusion/adsorption chromatography. Moreover, as shown in Fig. 4, the peak resolutions in the lower concentrations (less than 0.5 mM) of the amine mobile phases were simply improved by appropriate amounts of heptylamine added to maintain the pH 11. This feature was suggested to be the major factor when this separation system was applied to the real samples.

3.3. Analytical performance

The amine peaks in vacancy ion-exclusion/adsorption chromatography with conductimetric detection were

Fig. 2. Vacancy ion-exclusion/adsorption chromatogram obtained using different concentrations of amines in the mobile phase. Concentrations of amine mobile phases: (A) 5 mM (1 mM for each amine; pH 11.3), (B) 0.5 mM (0.1 mM for each; pH 9.85), and (C) 0.05 mM (0.01 mM for each; pH 8.60). Peak identities: (1) ethylamine, (2) propylamine, (3) butylamine, (4) pentylamine and (5) hexylamine. Other conditions as in Fig. 1.

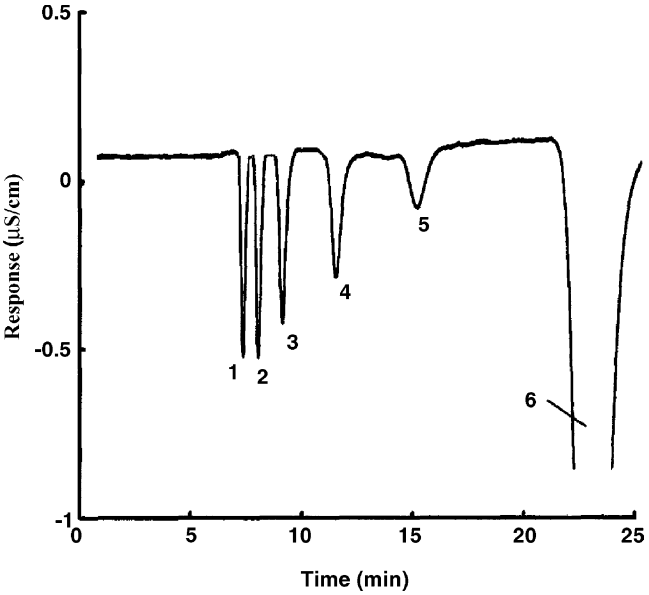


Fig. 3. Vacancy ion-exclusion/adsorption chromatogram of amines in the mobile phase obtained by adding heptylamine. Elution conditions: 0.5 mM amine mobile phase + 2 mM heptylamine (pH 11.02). Injection sample: water. Peaks: (1) ethylamine, (2) propylamine, (3) butylamine, (4) pentylamine, (5) hexylamine and (6) heptylamine. Other conditions as in Fig. 1.

identified as the decreases of the negative vacant peaks by injecting the same sample as the mobile phase. Fig. 5 shows the chromatograms of different concentrations of sample amines injected to 0.5 mM amine mobile phase at pH 11. When the concentrations of amine sample lower than 0.5 mM were injected to this system (Fig. 5A), the negative vacant peaks of amines was decreased with increasing the concentrations

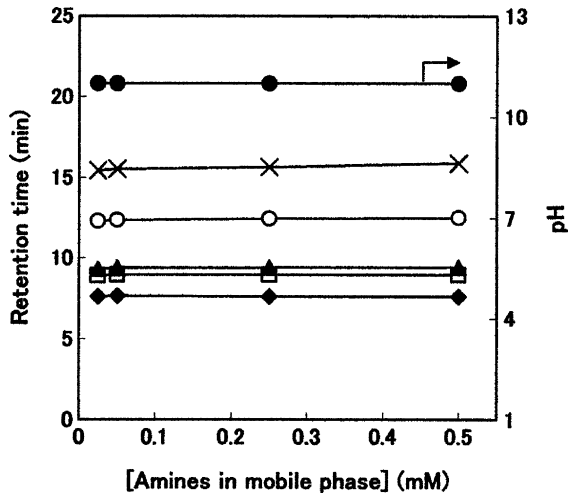


Fig. 4. Relationship between the retention times and the different concentration of amine mobile phases at pH 11 by adding heptylamine. Injection sample: water. The pH values of amine mobile phases of 0.025, 0.05, 0.25 and 0.5 mM were prepared to be 11, by adding 3.4, 3.0, 2.5 and 2 mM heptylamine, respectively. Plot identities: (◆) ethylamine, (□) propylamine, (▲) butylamine, (○) pentylamine, (×) hexylamine, (●) pH of each amine mobile phase. Other conditions as in Fig. 1.

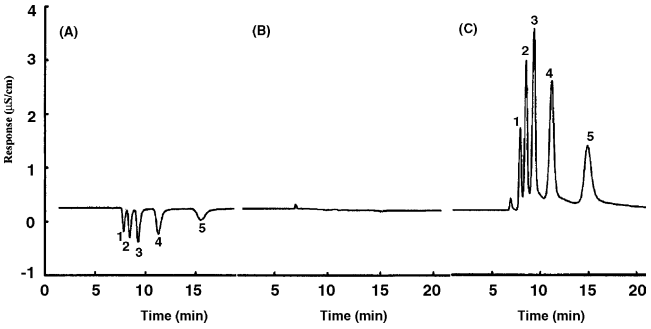


Fig. 5. Peak profile of different concentration of aliphatic amines at pH 11. Injection sample: mixture of ethylamine, propylamine, butylamine, pentylamine and hexylamine. Total concentrations of amines injected: (A) 0.05 mM, (B) 0.5 mM, and (C) 5 mM. Peaks: (1) ethylamine, (2) propylamine, (3) butylamine, (4) pentylamine and (5) hexylamine (heptylamine was detected at about 25 min). Other conditions as in Fig. 1.

of injected samples. When the concentration of the injected amines was the same as that of the amines mobile phase at pH 11, the conductivity response was almost unity (Fig. 5B). When the concentration of the injected amines was higher than 0.5 mM, positive peaks were observed, as shown in Fig. 5C. Therefore, it is found that there is no concentration dependency for retention times of analyte amines injected.

Fig. 6 shows the calibration graph obtained using the peak area of butylamine injected to the 0.5 mM amine mobile phase at pH 11. The calibration graph was linear in the range 0.001–5.00 mM. The linearity of calibration graphs for four other amines were ranged 0.001–5.00 mM for ethylamine and propylamine, and 0.001–1.00 mM for pentylamine and hexylamine. These correlation coefficients (r^2) of five amine samples were calculated for three different concentrations of amine mobile phase at pH 11, as summarized in Table 1. The detection limits of the sample amines in the vacancy ion chromatography system were calculated at a signal-to-noise

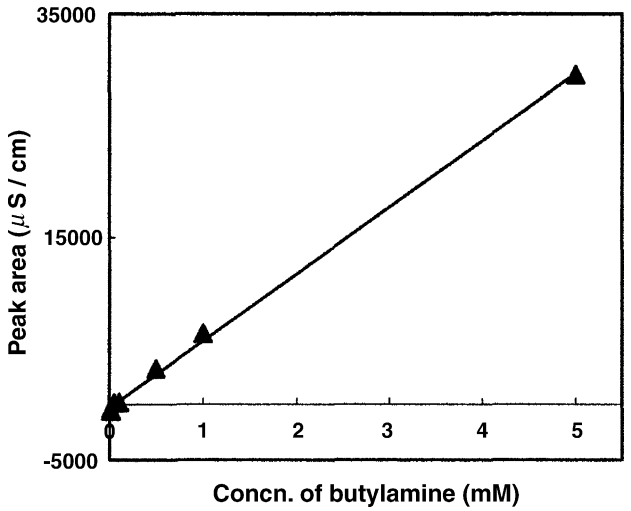


Fig. 6. Calibration graph of peak area on each concentration of butylamine. Injection volume: 100 μl. Other conditions as in Fig. 1.

Table 1
Correlation coefficients (r^2) of the peak areas of the amines samples injected in the region of 0.001–5 mM for each amine mobile

Concentrations of amine mobile phases[a] (mM)	Analytes				
	Ethylamine	Propylamine	Butylamine	Pentylamine	Hexylamine
0.05	0.9997	0.9990	0.9991	0.9994	0.9964
0.50	0.9978	0.9989	0.9993	0.9996	0.9996
5.00	0.9998	0.9980	0.9997	0.9989	0.9994

[a] The pH values of amine mobile phases of 0.05 and 0.5 mM were prepared to be 11, by adding 3 and 2 mM heptylamine, respectively. Column: TSKgel DEAE-5PW (15 cm × 7.5 mm i.d.). Composition of amine mobile phase: ethylamine, propylamine, butylamine, pentylamine and hexylamine. Injected sample: water; injection volume: 100 μl; column oven: 40 °C; flow rate: 0.6 ml/min.

Table 2
Detection limits for each analytes on different amine mobile phases for the vacant IC (S/N = 3)

Concentrations of amine mobile phases[a] (mM)	Analytes (μM)				
	Ethylamine	Propylamine	Butylamine	Pentylamine	Hexylamine
0.05	0.31	0.27	0.40	0.29	0.62
0.50	0.59	0.51	0.26	0.52	0.54
5.00	1.21	1.19	1.30	1.34	1.40

[a] The pH values of amine mobile phases of 0.05 and 0.5 mM were prepared to be 11, by adding 3 and 2 mM heptylamine, respectively. Column: TSKgel DEAE-5PW (15 cm × 7.5 mm i.d.). Composition of amine mobile phase: ethylamine, propylamine, butylamine, pentylamine and hexylamine. Injected sample: water; injection volume: 100 μl; column oven: 40 °C; flow rate: 0.6 ml/min.

ratio of 3 and the results were listed in Table 2. The obtained values indicated that the method was highly sensitive.

The reproducibility of the retention times of the amines in vacancy ion chromatography system using water as an injected samples were respectively calculated by running three different concentrations of the amine mobile phases. These values were found to be ranged 0.18–0.55% R.S.D. in the repeated chromatographic runs ($n = 11$). The reproducibility of the chromatographic peak was found to be ranged 2.89–5.29% R.S.D. for the peak areas and 2.92–5.00% for the peak heights ($n = 11$). Although the detail for this is not clear, insufficient column conditioning might be caused by poor reproducibility.

4. Conclusions

Vacancy ion-exclusion/adsorption chromatography of aliphatic amines on a polymethacrylate-based weakly basic anion-exchange column was newly developed. The reproducibility of retention times and peak resolutions were improved by keeping the mobile phase amine concentrations to pH 11 by adding heptylamine because the retention mechanism of vacancy ion-exclusion/adsorption chromatography is based mainly on hydrophobic adsorption effect to separate the amines as molecular-form. The linearity of calibration graphs in the wide range of amine concentrations could be obtained by injecting the same composition of samples as the amine mobile phase. The detection limits were around 1 μM for all of amine mobile phases at pH 11. Further investigation will be required towards solving the problems of vacancy ion-exclusion/adsorption chromatography in order to apply it to real samples.

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