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

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Abstract

Determination of aromatic carboxylic acids by conventional ion-exclusion chromatography is relatively difficult and methods generally rely on hydrophobic interaction between the solute and the resin. To overcome the difficulties in determining aromatic carboxylic acids a new approach is presented, termed vacancy ion-exclusion chromatography, which is based on use of the sample as mobile phase and an injection of aqueous 10% methanol onto a weakly acidic cation-exchange column (TSKgel OApak-A). Highly sensitive conductivity detection occurred with sharp and well-shaped peaks, leading to very efficient separations. The effects of sulfuric acid concentration added to the mobile phase, flow-rate, and column temperature on the retention volume of tested aromatic carboxylic acids was investigated. Retention times were found to be affected by the concentration of the analytes in the mobile phase and to some extent also by the addition of an organic modifier such as methanol to the injected water sample. Separation of sulfuric acid (SA), naphthalenetetracarboxylic acid (NTCA), phthalic acid (PA) and benzoic acid (BA) was satisfactory using this new approach. Detection limits were 0.66, 0.67, 0.42 and 0.86 μM and detector responses were linear in the range 1–100, 1–80, 2.5–100 and 10–40 μM , for SA, NTCA, PA and BA, respectively. Precision for retention times was 0.36% and for peak areas was 1.5%.

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1. Introduction

Ion-exclusion chromatography has been commonly used for the separation of inorganic and weak

organic acids of low-molecular mass. In contrast, only relatively few methods for the ion-exclusion chromatography separation of aromatic carboxylic acids have been reported [1]. Aliphatic carboxylic acids are usually separated on sulfonated polystyrene–divinylbenzene (PS–DVB) resins functionalised with sulfonic acid groups to give strongly acidic cation-exchange resins (generally used in the H^+ form) having high ion-exchange capacity (e.g., 1.5 mequiv/ml) and with sulfuric acid used as

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mobile phase [2–4]. Hydrophobic weak acids, such as aromatic carboxylic acids of higher molecular mass, are retained strongly under these conditions due to hydrophobic interactions with the resin and thus the peaks obtained are usually tailed [5,6] and the separation obtained is often poor [7]. However, addition of organic modifier has been found to improve the peak shapes of the hydrophobic weak acids and various modifiers have been used for this purpose, such as aliphatic alcohols, acetonitrile and sugars. Alternatively, the separation can be performed using hydrophilic cation-exchange stationary phases. Ohta et al. reported the ion-exclusion chromatography separation of benzenecarboxylic acids and aliphatic acids (C_1 – C_{10}) using this approach with sulfuric acid eluent [6,8] and they proposed that the separation mechanism was based mainly on the ion-exclusion effect for low-molecular mass hydrophobic acids and on some contribution of adsorption effects for the more hydrophobic analytes.

The technique of vacancy ion-exclusion chromatography was reported by Tanaka et al. for the separation of aliphatic carboxylic acids [9]. In this approach, the sample is used as mobile phase and when an injection of water is made, vacancy peaks for each of the analytes in the sample are observed. In the present study, we have evaluated the applicability of vacancy ion-exclusion chromatography for the separation of aromatic carboxylic acids and a new approach using a weakly acidic cation-exchange column (Tosoh TSKgel OApak-A) has been developed. Here, a mixture of aromatic carboxylic acids is used as the mobile phase and aqueous 10% methanol is injected onto the separation column, with conductivity detection being used. The effects on the separation of a range of experimental factors have been investigated in detail in order to optimize the separation.

2. Experimental

2.1. Reagents and procedures

All reagents were of analytical grade and were dissolved in distilled water. Standard solutions were prepared using analytical-grade chemicals without further purification, as follows: 1.0 M sulfuric acid

(SA), 0.4 mM naphthalenetetracarboxylic acid (NTCA), 25 mM phthalic acid (PA) and 10 mM benzoic acid (BA). Standard mixtures were prepared by combining these solutions.

2.2. Instrumentation

A Dionex (Sunnyvale, CA, USA) IS20 ion chromatograph was used, comprising a pump and degasser, a column oven, injector (25 μ l), conductivity detector, and PeakNet workstation (Version 5.11). The separation of aromatic carboxylic acids was carried out using a Tosoh TSKgel OApak-A column (150 mm \times 7.8 mm I.D.) packed with a polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form (particle size 5 μ m, cation-exchange capacity 0.1 mequiv./ml). A mixture of sulfuric acid and the three aromatic carboxylic acids was used as mobile phase (flow-rate 1.25 ml/min, column temperature 35 $^\circ$ C). The column was equilibrated for 60 min prior to use.

3. Results and discussion

3.1. Vacancy ion-exclusion chromatography of aromatic carboxylic acids

Hydrophobic interactions between analytes and the ion-exclusion chromatography stationary phase offer a potentially useful separation mechanism for aromatic acids if the separation conditions can be modified to control retention behavior. However, there have been relatively few studies of retention of these compounds, their conductivity detection response is generally poor, and peak shapes are usually tailed (see Fig. 1 for a typical chromatogram obtained using water at pH 5.6 as mobile phase). Fig. 1 shows that poor resolution between NTCA and PA was obtained by conventional ion-exclusion chromatography. Addition of acids in order to improve peak shape in ion-exclusion chromatography has been used and analyte retention depends on the degree of ionization of the analyte, pK_{a1} , the mobile phase pH and on the amount of organic modifier present in the mobile phase.

The technique of vacancy ion-exclusion chromatography [9,10] offers an alternative method for

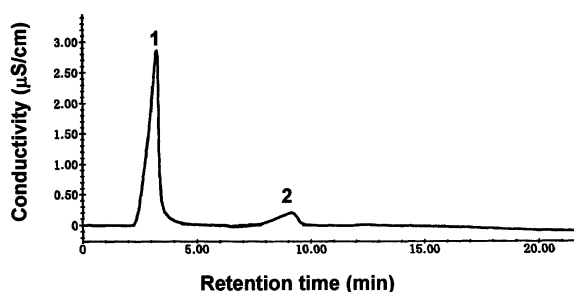


Fig. 1. Conventional ion-exclusion chromatogram of some aromatic carboxylic acids obtained by using pure water as mobile phase (pH 5.6). Peak identification (μM): (1) NTCA (50) and PA (50); (2) BA (35). Injected sample, mixture of aromatic carboxylic acids. Chromatographic conditions: column, Tosoh TSKgel OA-Pak-A polymethacrylate-based weakly acidic cation-exchange resin in the H^+ form (150 mm \times 7.8 mm I.D.); eluent flow-rate, 1.25 ml/min; column temperature, 35 °C; detector sensitivity, 100 mV=100 $\mu\text{s/cm}$; unit of retention time, min; injection volume, 0.025 ml.

separation of aromatic carboxylic acids. After equilibration of the a weakly acidic stationary phase with an eluent comprising a solution of the three aromatic acids under study, followed by injection of water, the chromatogram shown in Fig. 2 resulted. Good separation of the three species was obtained, with symmetrical peaks. The identities of these peaks were confirmed using mobile phases containing each species separately.

The relationship between the concentration of aromatic carboxylic acids in the mobile phase and the observed retention volumes is shown in Fig. 3. This figure shows that the retention increased with

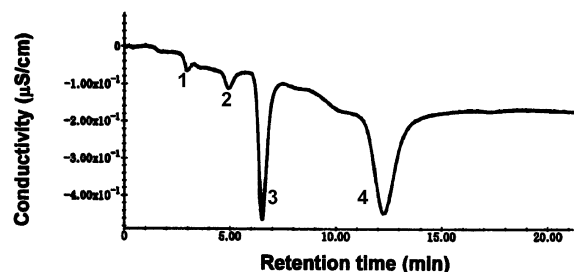


Fig. 2. Vacancy ion-exclusion chromatogram of three aromatic carboxylic acids. Column, Tosoh TSKgel OA-Pak-A (150 mm \times 7.8 mm I.D.); eluent flow-rate, 1.25 ml/min; column temperature, 35 °C; and mobile phase, pH 4.1. Concentration (μM) of aromatic carboxylic acids in the mobile phase: (1) unknown; (2) NTCA (50); (3) PA (50); and (4) BA (35). Injected sample, pure water.

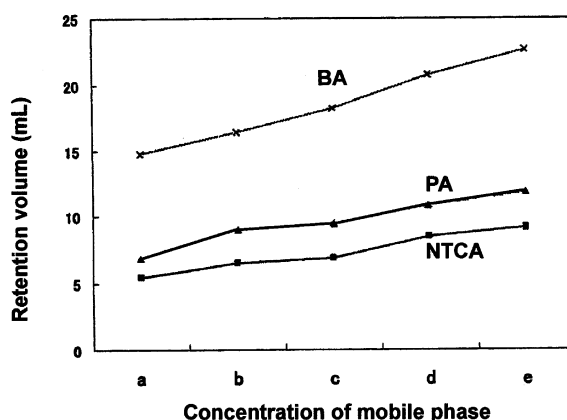


Fig. 3. Effect of the concentration of aromatic carboxylic acids in the mobile phase on retention volumes. Concentration of mobile phase (μM): (a) mixture of H_2SO_4 (0.5); NTCA (1.0); PA (2.5) and BA (10); (b)=(a) \times 5.0; (c)=(a) \times 50; (d)=(a) \times 75 and (e)=(a) \times 80. Other chromatographic conditions as in Fig. 1.

increased concentration of the analytes in the mobile phase, which can be explained by changes in the degree of ionization of the aromatic carboxylic acids. As analyte concentration increased, the degree of dissociation would be reduced, leading to both increased adsorption effects and decreased ion-exclusion effects, which both lead to increased retention. This observation suggests that application of the separation to real samples would be problematic unless the mobile phase was buffered to maintain the degree of ionisation of the analytes at a constant value.

3.2. Effect of concentration of sulfuric acid in the mobile phase

Strong acids (such as sulfuric acid) are often used as the mobile phase in ion-exclusion chromatography owing to their effective control of the degree of ionization of weak acids, resulting in improved peak shape and resolution (e.g., Ref. [11]). The same approach was examined in vacancy ion-exclusion chromatography, again using a mixture of NTCA, PA and BA as the mobile phase. Fig. 4 shows the effect of 0–0.4 mM sulfuric acid in the mobile phase and indicates that V_R values of the aromatic carboxylic acids were not strongly dependent on sulfuric acid concentration. A further factor was the effects of

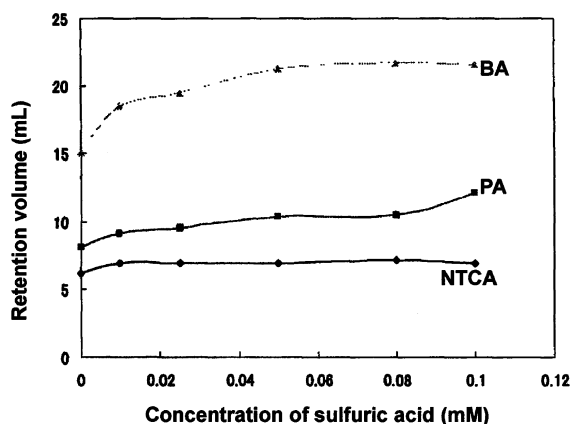


Fig. 4. Effect of H_2SO_4 concentration in the mobile phase on retention volumes (V_R) of aromatic carboxylic acids. Mobile phase (μM): mixture of H_2SO_4 (50); NTCA (50); PA (50); and BA (40). Sample: water containing 10% methanol. Other chromatographic conditions as in Fig. 1.

sulfuric acid in the mobile phase on the conductivity detection signal. Whilst at lower sulfuric acid concentration (0.01–0.1 mM), the detection signal was satisfactory (as also were the chromatographic resolution and peak shapes) at higher sulfuric acid concentration (≥ 0.4 mM) the detection signal and the chromatographic resolution both deteriorated. Therefore, 0.05 mM sulfuric acid (pH 4.1) was selected for further experiments with vacancy ion-exclusion chromatography.

3.3. Effects of organic modifier and column temperature

In order to improve the peak shape and to accelerate the elution of aromatic carboxylic acids, the effect of organic modifiers such as methanol was investigated. Addition of methanol to the injected water sample produced only small effects on retention, but baseline stability, resolution and peak shape were all improved and for these reasons, 10% methanol was included in the injected volume in future studies.

The influence of column temperature on the separation of aromatic carboxylic acids was investigated over the range 30–45 °C. Temperature effects on retention were relatively minor for sulfuric acid, NTCA and PA but in the case of benzoic acid,

retention volumes decreased markedly above 40 °C, probably due to the higher solubility of this hydrophobic acid at higher temperature. Different temperature effects for different analytes therefore resulted from the chemical properties of the analytes, leading to slight changes on retention behavior [12,13]. An optimal column temperature of 35 °C was used in subsequent studies.

Using the optimal conditions, the separation of aromatic carboxylic acids by vacancy ion-exclusion chromatography shown in Fig. 5 was obtained. Well-shaped and well-resolved peaks were obtained with a stable baseline.

3.4. Retention volumes and distribution coefficients

In order to characterize the TSKgel OApak-A column used in the vacancy ion-exclusion mode, the distribution coefficients (K_d) for aromatic carboxylic acids were determined according to the following equation [14]:

$$V_R = V_o + V_i K_d \quad (1)$$

where V_R is the retention volume, V_o is the column void volume and V_i is the volume of liquid inside the resin in the column. V_o was taken as the V_R for sulfuric acid, which was completely excluded from the resin phase ($V_o = 2.78$ ml, $V_o + V_i = 5.7$ ml). Table

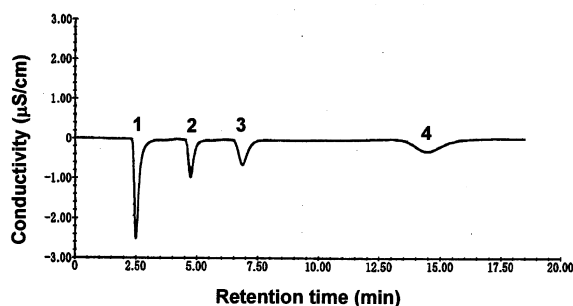


Fig. 5. Vacancy ion-exclusion chromatogram of aromatic carboxylic acids obtained under optimal conditions. Column, TSK gel OApak-A (150 mm \times 7.8 mm I.D.); column temperature, 35 °C; mobile phase, pH 4.1; flow-rate, 1.25 ml/min. Concentration (μM) of aromatic carboxylic acids in the mobile phase: (1) H_2SO_4 (50), (2) NTCA (50), (3) PA (50) and (4) BA (35). Sample: water containing 10% methanol was injected onto the column.

Table 1
Retention volumes (V_R) and distribution coefficients (K_d) of aromatic carboxylic acids in vacancy ion-exclusion chromatography

Aromatic carboxylic acid	K_d	pK_{a1}	V_R (ml)
Naphthalenetetracarboxylic acid (NTCA)	1.07	1.98	5.9
Phthalic acid (PA)	1.99	2.95	8.6
Benzoic acid (BA)	5.25	4.2	18.1
H ₂ SO ₄	0.0	-4.0	2.78
Methanol	1.0	15.5	5.7

$$V_o = 2.78 \text{ ml.}$$

$$(V_o + V_i) + 5.7 \text{ ml; } V_i + (V_o + V_i) - V_o = 2.92 \text{ ml.}$$

$$V_R = V_o + K_d V_i.$$

1 summarizes the distribution coefficients for these acids together, with their pK_{a1} values. Table 1 shows that strong acids, such as sulfuric acid, are eluted first and the weak acids were eluted later, indicating that an ion-exclusion mechanism was take place on the weakly acidic cation-exchange resin. The K_d values of the aromatic carboxylic acids tended to increase with increasing pK_{a1} and hydrophobicity. K_d values greater than unity (benzoic acid and phthalic acid) indicated that adsorption effects on the surface of the resin matrix contributed to the retention process.

3.5. Analytical performance parameters

Under the optimum chromatographic conditions described above, a mixture of aromatic carboxylic acids was chromatographed in the concentration range 1–100 μM . Correlation coefficients for the resultant calibration plots were in the range 0.9955–0.9983 and Table 2 summarizes the linearity range for peak area calibrations.

Table 2
Calibration data and detection limits for vacancy ion-exclusion chromatography of aromatic carboxylic acids

Aromatic carboxylic acid	Linear range for peak area calibration (μM)	Regression equation	Correlation coefficient (r^2) ($n=15$)	Detection limit (μM), ($S/N=3$)
H ₂ SO ₄	1–100	$y = 7515.5x - 16631$	0.9987	0.66
NTCA	1–80	$y = 2046.3x + 1590.9$	0.9991	0.67
PA	2.5–100	$y = 2071.3x + 48434$	0.9955	0.42
BA	10–40	$y = 2572.7x + 165476$	0.9999	0.86

The reproducibility of the retention times obtained using nine replicate injection samples was 0.35, 0.46, 0.39 and 0.23% (RSD) for sulfuric acid (20 μM), NTCA (50 μM), PA (50 μM) and BA (20 μM), respectively. The reproducibility for peak area was 0.69, 1.2, 1.8 and 2.2% (RSD) for sulfuric acid (20 μM), NTCA (50 μM), PA (50 μM) and BA (20 μM), respectively.

The detection limits of aromatic carboxylic acids using vacancy ion-exclusion chromatography system were determined at a signal-to-noise ratio (S/N) of 3. These detection limits were two to five times lower than those obtained by conventional ion-exclusion chromatography using conductivity detection.

4. Conclusions

A vacancy ion-exclusion chromatography method in which a mixture of aromatic carboxylic acids is used as mobile phase and water containing 10% methanol is used as injected sample has been developed. The chromatographic resolution, peak shapes and conductivity detection sensitivity were all better than those observed with conventional ion-exclusion chromatography for the same analytes. When a weakly acidic cation-exchange resin was used as stationary phase, retention occurred by a mixture of ion-exclusion and adsorption effects. Because of the presence of adsorption effects, addition of 10% methanol to the water injected sample gave decreased retention and also resulted in improved separation. Other aromatic carboxylic acids such as naphthoic acid, trimellitic, naphthalenedisulfonic, trimesic, salicylic, and pyromellitic acids were difficult to separate because of similar pK_{a1} values.

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