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Preparation of monodispersed vinylpyridine–divinylbenzene porous copolymer resins and their application to high-performance liquid chromatographic separation of aromatic amines

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ABSTRACT

For the separation of aromatic amines, two types of monodispersed porous polymer resins were prepared by the copolymerization of 2-vinylpyridine and 4-vinylpyridine with divinylbenzene in the presence of template silica gel particles (particle size 5 μm), followed by dissolution of the template silica gel in an alkaline solution. The transmission electron micrographs and the scanning electron micrograph revealed that these templated polymer resins have a spherical morphology with a good monodispersity and porous structure. Using these monodispersed polymer resins, the high-performance liquid chromatographic separation of aromatic amines in the mobile phases of pHs 2.0, 2.9, 4.1, 7.2 and 11.7 were carried out. The 2-vinylpyridine–divinylbenzene copolymer resins showed slightly stronger retentions for aromatic amines than the 4-vinylpyridine–divinylbenzene copolymer resins. Under acidic conditions (around pH 2.0), aniline and the toluidines showed no retention on these copolymer resins due to the repulsion between the cationic forms of these amines and pyridinium cations in the stationary phase, whereas less basic aromatic amines or non-basic acetanilide showed slight retentions. Above pH 4.1, the separation of aromatic amines with these polymer resins showed a typical reversed-phase mode separation. Therefore, the separation patterns of aromatic amines are effectively tunable by changing the pH value of the mobile phases. A good separation of eight aromatic amines was achieved at pH 2.9 using the 2-vinylpyridine–divinylbenzene copolymer resins.

1. Introduction

There are various types of aromatic amines in the surrounding living environment as pollutants. Among the aromatic amines, aniline and its derivatives are known as human toxic agents causing cyanosis and methemoglobinemia [1], and *o*- and *p*-toluidines and *p*-chloroaniline are known as carcinogenic agents [2]. Hence, it is important to precisely assay these aromatic amines in environmental samples. High-performance liquid chromatography (HPLC) is an easy-to-perform analytical tool for the quantitative analysis of these aromatic amines [3–6].

Basic compounds are generally separated using reversed-phase HPLC [4,5], reversed-phase HPLC with an ion-paired reagent [7] and cation-exchange HPLC [8]. For weak basic compounds, such as aromatic amines, reversed-phase HPLC using basic mobile phases is thought to be effective because the protonation of amines are almost suppressed. However, the basic conditions of the assays make it difficult to use silica gel as a column packing support due to dissolution of the silica gel in an alkaline solution. Moreover, the separation of basic compounds with silica gel based packings is not suitable because of peak tailing due to the undesirable interactions between the polar basic compounds and residual acidic silanol groups of the silica gel [9]. Many polymer-coated silica-based phases have been studied in order to stabilize silica gels chemically. The columns packed with silica gels which are coated with homogenous silicon layers by polymerizing silicone monomers on their surfaces are commercially available. These columns possess higher pH stability than the conventional non-modified silica-based phases. However, even these columns can be used in the pH range of 1–10 [10,11]. On the other hand, polymer-based column packings usually can be used in the pH range of 1–12. Therefore, polymer resins would be a more efficient chromatographic support for the separation of basic compounds.

Polymer resins for stationary phases are extensively prepared by suspension, emulsion and precipitation polymerization techniques. Some of polymer resins have been still produced by a traditional suspension polymerization. In this technique, the particle size

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strongly depends on the stirring velocity and the shape of reaction instrument such as the vessel and the agitator blades [12]. These polymer resins have rather broad particle size distributions, and therefore cannot be used directly for HPLC column packings without time–consuming size classification. Emulsion polymerization affords the polymer lattices consisting of 0.05–0.5 μm sized polymer resins, therefore multi-step swelling techniques are usually necessary for larger polymer particles suitable for HPLC stationary phases. The preparation of 5–6 μm sized monodispersed polymer resins using these multi-step swelling techniques have been reported by Hosoya and Fréchet [13]. Ogino et al. reported on a single-step swelling and polymerization method that allowed the synthesis of mono–sized polymer beads in the range of 4.1–7.5 μm [14]. Moreover, 7 μm sized monodisperse polymer-based stationary phase for carbohydrate analysis was synthesized through a two-step swelling and polymerization method [15]. Recently, Haginaka et al. prepared uniform-sized molecular imprinted polymers of about 4 μm in diameter by a precipitation polymerization method [16].

The templating polymerization methods for the preparation of monodispersed porous polymer particles have been reported, i.e., the polymerization of a monomer in a porous matrix, such as silica gel, followed by dissolution of the matrix in an alkaline solution [17–19]. Therefore, the synthesized polymer products should reflect the characteristics of the template silica gel particles having a desired size, surface area and porosity. Accordingly, we can easily prepare not only 2–10 μm sized polymer resins for HPLC column packings, also ten to a few hundred μm sized polymer resins for column chromatography and solid phase extraction. We have reported the preparation of monodispersed porous polymer resins using this templating polymerization method and their application as stationary phases for the high-performance liquid chromatographic separation of carbohydrates [20].

In this paper, we will describe the preparation of monodispersed vinylpyridine–divinylbenzene porous copolymer resins using the templating polymerization technique and their application to the separation of aromatic amines. These polymer resins are weakly basic, and therefore, under acidic conditions could be converted to cationic copolymer resins which exclude the cationic aromatic amines. These polymer resins also work as the reversed-phase stationary phases in neutral and basic conditions. Therefore, on the vinylpyridine–divinylbenzene porous copolymer resins, the separation patterns of aromatic amines are tunable by changing the pH value of the mobile phases.

2. Experimental

2.1. Materials

As the template silica gels, Nucleosil Silica 100-5 (5 μm particle size, 10 nm pore size, 1.0 mL/g pore volume, 350 m^2/g surface (BET)) was purchased from Macherey-Nagel (Duren, Germany). 2-Vinylpyridine, 4-vinylpyridine and chlorotrimethylsilane were from Tokyo Kasei (Tokyo, Japan). Divinylbenzene (technical grade, 80%) and an inhibitor remover were from the Aldrich (Milwaukee, WI, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of monodispersed vinylpyridine–divinylbenzene porous copolymer resins

The schematic outline of the preparation method of the monodispersed porous copolymer resins is shown in Fig. 1. Initially, the hydrophilic surface of the silica gel was converted to a hydrophobic surface by silanization of the silica gel with chlorotrimethylsilane.

The template silica gel (Nucleosil Silica 100-5, 10 g) was dried in a flask under vacuum at 150 °C for 4 h. Dry toluene (80 mL), pyridine (7 mL) and chlorotrimethylsilane (4.29 g, 39.5 mmol) were added to the flask. The mixture was stirred at 60 °C for 5 h under an argon atmosphere. The silanized silica gel was filtered through a sintered-glass filter and washed with 200 mL of toluene followed by 200 mL of dichloromethane and 300 mL of methanol, then dried overnight under vacuum. The extent of the silanization calculated from an increase in weight was 0.85 mmol per gram of the silanized silica gel.

Silanized silica gel (2.96 g) and ultrapure water (30 mL) were added to a three-necked flask, and the mixture was aerated with argon gas for 30 min with gentle stirring. Three mL of the mixed monomer solutions consisting of 2-vinylpyridine (0.69 g), 80% divinylbenzene (1.7 g) and benzoyl peroxide (0.13 g) (molar ratio, 1:2:0.08), which were freed of the polymerization inhibitor by passing through the inhibitor remover column, was added to the flask, followed by 30 mL of 0.17 wt.% aqueous poly(vinyl alcohol) (average

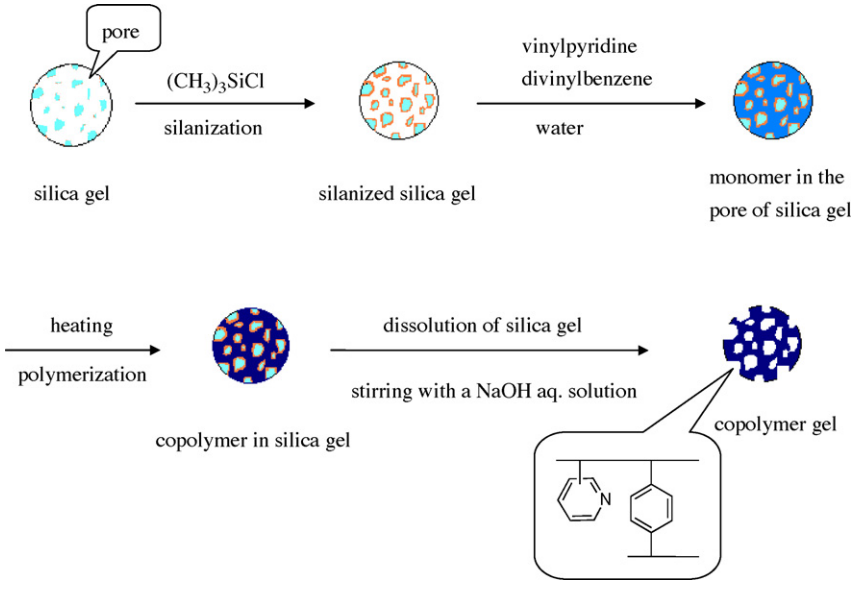

Fig. 1. Preparation of porous polymer resins by copolymerization of monomers in the presence of template silica gel.

degree of polymerization; 1000 (1 part) and 1500 (25 parts)) solution. The mixture was vigorously stirred at 700 rpm under flowing argon for 24 h at room temperature in order to allow the hydrophobic monomer and crosslinker to enter into the pores of the silica gel. The mixture was then heated for 24 h at 90 °C to produce the polymerization. The mixture was cooled to room temperature, and the precipitate was filtered through a sintered-glass filter and washed with 200 mL of water and 100 mL of methanol. The precipitate was added to a mixture of 90 mL of a 5 M NaOH aqueous solution and 60 mL of methanol, and then stirred for 24 h at room temperature to dissolve the template silica gel. The resulting precipitate was washed with water until the solution was neutral followed by 100 mL of methanol, then dried under vacuum at room temperature to give the 2-vinylpyridine–divinylbenzene porous copolymer resins (2P) (2.50 g) in 99% yield. Elemental analysis: C, 85.64; H, 7.43; N, 2.75%.

The 4-vinylpyridine–divinylbenzene porous copolymer resins (4P) (3.47 g) were also obtained in 99% yield in the similar manner from the silanized silica gel (2.96 g), 4-vinylpyridine (0.80 g), 80%-divinylbenzene (1.97 g) and benzoyl peroxide (0.16 g) (molar ratio, 1:2:0.08). Elemental analysis: C, 87.03; H, 7.45; N, 2.60%.

2.3. Microscopy

A JEM-1200EX transmission electron microscope from JEOL (Tokyo, Japan) was used to observe the morphology of the resulting polymer resins. Also, the scanning electron micrographs of the 2-vinylpyridine–divinylbenzene copolymer resins were taken by a JSM-6100 from JEOL.

2.4. Column packing

The 2P and 4P copolymer resins were packed into stainless-steel columns (25 cm × 4.6 mm I.D.) by a conventional slurry packing method using acetonitrile–water (7:3, v/v) as the eluent at a constant pressure of 20 MPa.

2.5. High-performance liquid chromatography

The HPLC analysis was performed with a system consisting of a JASCO (Tokyo, Japan) 880-PU pump, a JASCO 875-UV detector and a Rheodyne (Cotati, CA. USA) 7125 injector. For the data analysis, a SIC (Tokyo, Japan) Chromatocorder 12 was employed.

For the chromatographic analysis, the aromatic amines were dissolved in acetonitrile or a mixture of acetonitrile and water. The samples were eluted with an acetonitrile–aqueous buffer solution (7:3, v/v) at the flow rate of 0.5 mL min^{-1} and were detected at 254 nm. To adjust the ionic strength (I = 0.1), 5 different buffer solutions (pH 2.0, 2.9, 4.1, 7.2 and 11.7) were prepared as in Table 1.

Table 1
Contents of buffer solutions (I^a = 0.1).

	pH				
	2.0	2.9	4.1	7.2	11.7
5.0 M NaCl (mL)	32	32	32	32	32
1.0 M glycine–1.0 M NaCl (mL)	10.6	31.6			19.6
2.0 M HCl (mL)	14.7	4.2			
2.0 M CH_3COONa (mL)			20.0		
3.5 M CH_3COOH (mL)			33.7		
0.5 M Na_2HPO_4 (mL)				22.7	
4.0 M NaH_2PO_4 (mL)				1.6	
2.0 M NaOH (mL)					10.2

[a] I: ionic strength.

3. Results and discussion

3.1. Preparation and characterization of polymer particles

The vinylpyridine–divinylbenzene copolymer resins were prepared by the templating polymerization methods as shown in Fig. 1. The template silica gel, Nucleosil Silica 100-5 (particle size 5 μm), was silanized with chlorotrimethylsilane for the conversion of a hydrophilic silica gel surface into a hydrophobic surface. Using these silanized silica gels as a template, two types of copolymer resins (2P and 4P) were prepared by the copolymerization of 2-vinylpyridine and 4-vinylpyridine as the monomers with divinylbenzene as the crosslinker, followed by dissolution of the template silica gel.

The morphology of these polymer particles was evaluated from their transmission electron micrographs (TEM) and scanning electron micrographs (SEM). Figs. 2 and 3 show that the particles prepared by this method have a spherical morphology with good size monodispersity and are free from any nonspherical by-products. The average particle diameters of 2P and 4P were evaluated to be 4.38 and 4.46 μm, respectively, by measuring the diameter of almost 500 particles as shown in Fig. 4. These results indicate that the size of the particles was well controlled by the diameter of the template silica gel (5 μm). In addition, the scanning electron micrograph of the 2P copolymer resin clearly revealed the porous structure of this copolymer gel (Fig. 3).

For evaluation of the chromatographic performance of these polymer resins, the separation of pyridine was evaluated using a column packed with the 2P copolymer resins and a commercially available ODS column with the mobile phase of pH 5.4. As shown in Fig. 5, the 2P column is 25 cm long and the commercial ODS column is 15 cm long, therefore the retention time of pyridine by ODS column (6.4 min) is faster than that by 2P column (6.7 min). In spite of the long retention time of pyridine by the 2P column, the peak width of pyridine by 2P column was narrower than that by ODS column. The values of the height equivalent to a theoretical plate (HETP) for 2P column and ODS column were calculated as 0.046 and 0.092 mm/plate, respectively. The peak asymmetry factor (A_S) [21] with the 2P column (A_S = 1.0) was also lower than that with the ODS column (A_S = 1.5). These results indicate that polymer supports are advantageous for the separation of basic compounds compared to silica gel supports with the residual silanol groups.

In our repeated experiments under acidic and basic conditions for nearly 6 months, retention time of aromatic amines using 2P stationary phases showed good reproducibility.

3.2. HPLC separation of aromatic amines

Using the 2P and 4P copolymer resins, HPLC separation of aromatic amines was examined with the mobile phase of acetonitrile and a buffer solution of pH 7.2 (7:3, v/v). The retention times for these analytes are shown in Table 2. The 2P copolymer resins showed stronger retentions for the aromatic amines than the 4P copolymer resins. Since the pK_b values of 2-ethylpyridine and 4-ethylpyridine are 8.11 and 8.13, respectively, the basicities of these two components of polymer resins would be almost the same. At pH 7.2, aromatic amines would be separated under the conditions of nearly reversed-phase mode. Therefore, the 2P copolymer resins showed stronger retention than the 4P copolymer resins probably due to hydrophobic effect. The retention times for the aromatic amines in the pH range of 2.0–11.7 were measured using the 2P copolymer resins.

Aromatic amines and the pyridyl groups of the polymers can be protonated under acidic conditions, and this causes an electric repulsion between the cationic forms of these amines and the pyri-

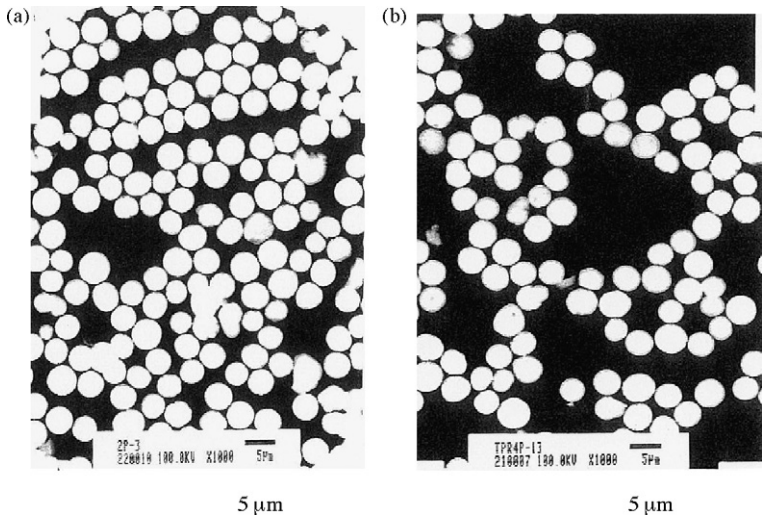

Fig. 2. TEM images of the synthesized copolymer resins: 2P resins (a), 4P resins (b).

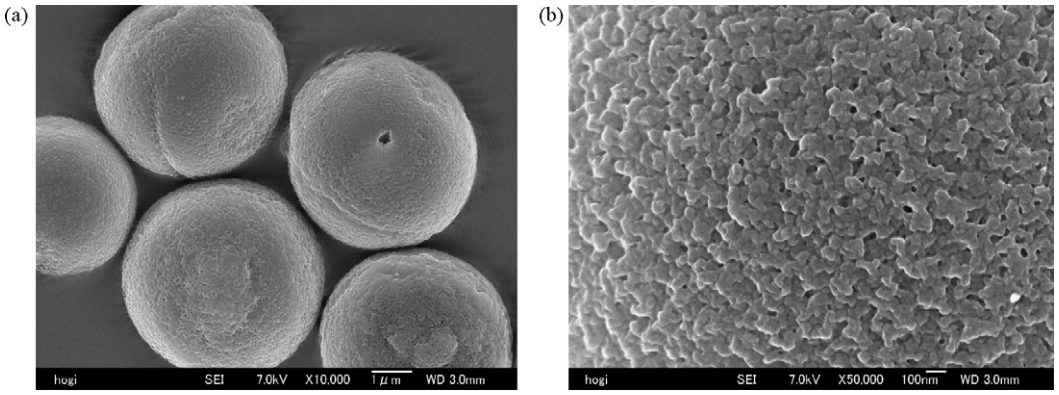

Fig. 3. SEM images of 2P copolymer resins at magnifications of (a) 10,000× and (b) 50,000×.

dinium cations of the polymers. Therefore, the pH change of the mobile phase would affect the separation of the analytes.

Plots of the retention time (t_R) for seven analytes versus the pH value of the mobile phase using the 2P stationary phase are depicted in Fig. 6. The retention time of aniline increased with the pH values of the mobile phase until reaching a plateau value. Similar results were obtained in the case of *N*,*N*-dimethylaniline, *N*-methylaniline and *p*-toluidine. These retention time–pH curves had an inflection point in the pH range of 3–4. However, the retention time of benzylamine gradually increased with the pH value of the mobile phase and showed an inflection point around pH 6–7. On the other hand, the non-basic analyte (acetanilide) showed no change in the retention time in the pH range above 2. These results clearly indicated that the separation profiles depended on the pK_a values (pK_a value of protonated form) of the analytes.

At pH 2.0, aromatic amines, other than acetanilide, eluted at 4.6–5.3 min near the hold-up time. No retention of these analytes at pH 2.0 is obviously due to the repulsion between the positively charged analytes and the positively charged pyridyl groups in the stationary phases. On the other hand, acetanilide, a non-basic ana-

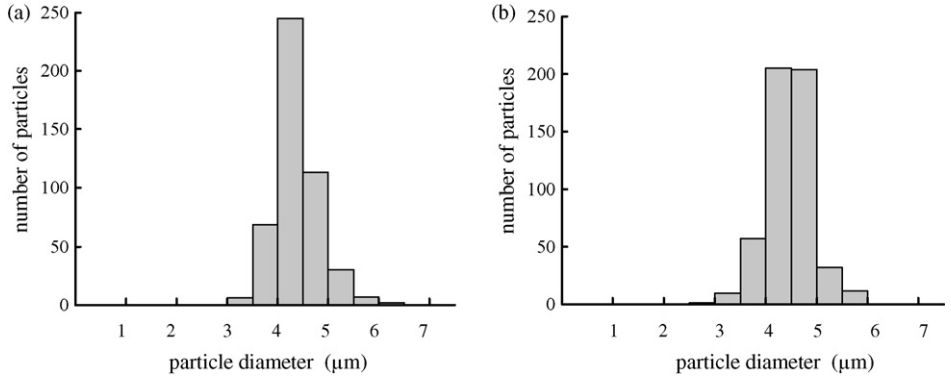

Fig. 4. Particle size distributions of 2P resins (a) and 4P resins (b), calculated from TEM images. Mean particle diameter: 4.38 μm (a), 4.46 μm (b) and total numbers of particles: 472 (a), 521 (b).

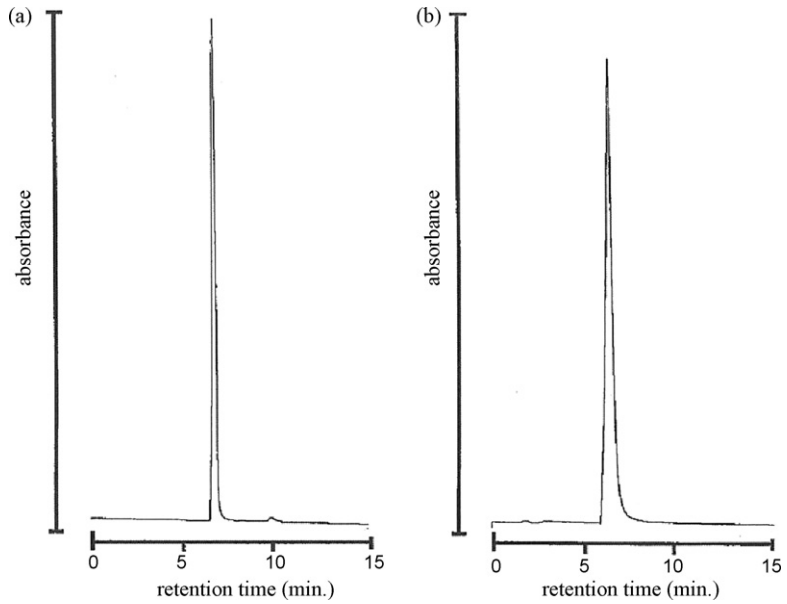

Fig. 5. Pyridine peak profiles: (a) with a 2P column (25 cm × 4.6 mm I.D.), and (b) with a commercial ODS column (15 cm × 4.6 mm I.D.). HPLC conditions: mobile phase, CH_3CN–H_2O (pH 5.4) (7:3, v/v) at flow rate 0.5 mL/min; detection, UV at 254 nm; sample concentration, 0.01%(v/v)-pyridine/CH_3CN solution; the injection volumes, (a) 3 μm and (b) 10 μm.

Table 2
Retention time of aromatic amines separated with 2P and 4P polymer resins at pH 7.2[a].

Amines	pK_a values of protonated amines	Retention time (min)	
		2P	4P
Pyridine	5.17	6.71	6.51
N,*N*-Dimethylaniline	5.15	17.02	14.21
p-Toluidine	5.08	9.36	8.81
N-Methylaniline	4.85	12.46	11.37
m-Toluidine	4.71	9.62	9.03
Aniline	4.60	8.73	8.35
o-Toluidine	4.45	9.74	9.21

[a] HPLC conditions: column, 4.6 mm I.D. × 25 cm; mobile phase, CH_3CN–buffer solution (pH 7.2) (7:3, v/v) at flow rate 0.5 mL/min; detection, UV at 254 nm.

lyte, eluted around 7 min under both the acidic and basic conditions. This result indicates that acetanilide was eluted in the reversed-phase separation mode.

Except for benzylamine, the retention time–pH curves of all the analytes showed plateaus above pH 4.1. The ionized form of *N*,*N*-dimethylaniline (pK_a 5.15) at pH 4.1 and 2.9 was calculated to be 91.7% and 99.4%, respectively. On the other hand, the ionized form of 2-methylpyridine (pK_a 5.96), which is assumed to be a functional group in the stationary phase, was calculated to be 98.6% and 99.9% at pH 4.1 and 2.9, respectively. These results showed that almost complete ionization of both analytes and functional groups in the stationary phase is necessary for the repulsion. On the other hand, the inflection points for benzylamine (pK_a 9.35) were observed at pH 6–7. Benzylamine, the strongest base among these examined amines, is positively charged even at pH 4.1 and therefore would not be retained. Under basic conditions, the aniline derivatives eluted in the order of aniline, *p*-toluidine, *N*-methylaniline, and *N*,*N*-dimethylaniline. These results indicated that more hydrophobic aromatic amines eluted with a longer retention time, and these analytes would be separated in the reversed-phase mode.

The retention times of the chloroanilines and nitroanilines with a low basicity were also evaluated in mobile phases of different pH and the results are shown in Figs. 7 and 8. At pH 2.0, these amines were strongly retained when compared to aniline. The retention time–pH curves of three isomers of the chloroanilines, *o*-chloroaniline (pK_a 2.7), *m*-chloroaniline (pK_a 3.5) and *p*-chloroaniline (pK_a 3.4) showed an appreciable degree of repul-

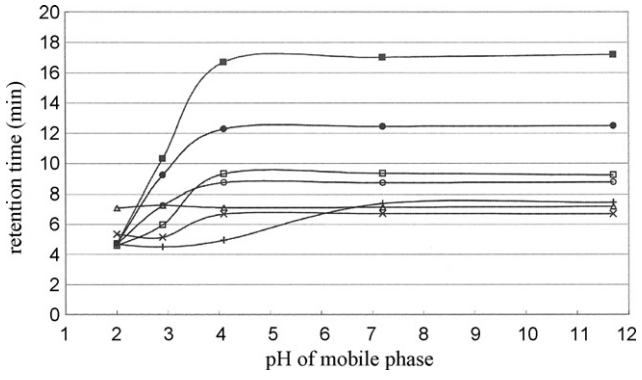

Fig. 6. Separation of aromatic amines with 2P polymer resins in the pH range of 2.0–11.7. HPLC conditions: column, 4.6 mm I.D. × 25 cm; mobile phase, CH_3CN–buffer solution (7:3, v/v) at flow rate 0.5 mL/min; detection, UV at 254 nm ■ = *N*,*N*-dimethylaniline; ● = *N*-methylaniline; □ = *p*-toluidine; ○ = aniline; + = benzylamine; △ = acetanilide; × = pyridine.

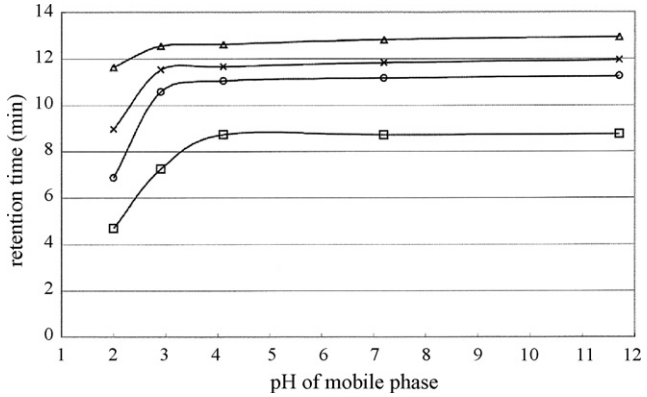

Fig. 7. Separation of chloroanilines with 2P polymer resins. HPLC conditions are shown in Fig. 6. △ = *o*-, × = *m*-, ○ = *p*-chloroaniline; □ = aniline.

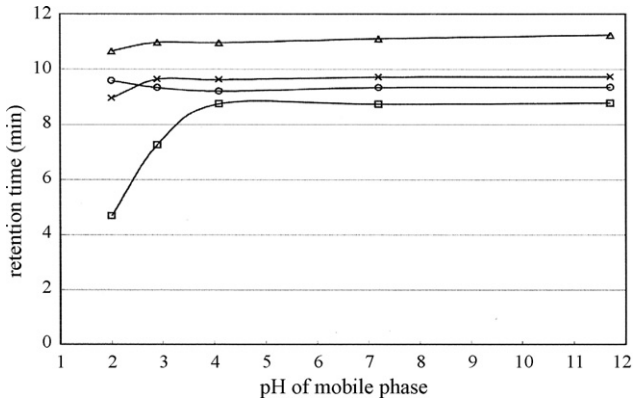

Fig. 8. Separation of nitroanilines with 2P polymer resins. HPLC conditions are shown in Fig. 6. △ = *o*-, × = *m*-, ○ = *p*-nitroaniline; □ = aniline.

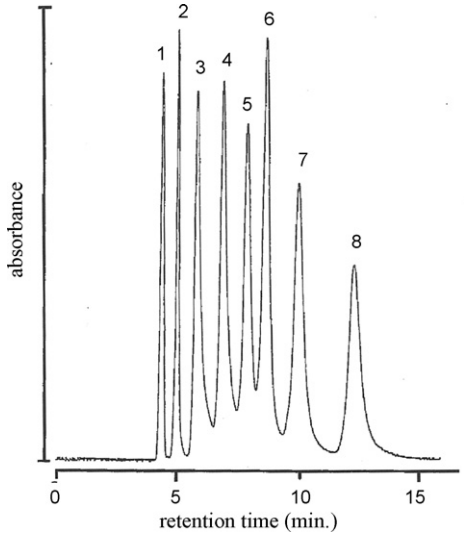

Fig. 9. Chromatogram of aromatic amines with 2P polymer resins. Mobile phase: CH_3CN–buffer solution (pH 2.9) [7:3, v/v] at flow rate 0.5 mL/min, detection: UV at 254 nm. Peaks: 1 = benzylamine, 2 = pyridine, 3 = *p*-toluidine, 4 = aniline, 5 = *o*-toluidine, 6 = *N*-methylaniline, 7 = *N*,*N*-dimethylaniline, 8 = *o*-chloroaniline.

sion at pH 2.0. On the other hand, the *o*- and *p*-nitroanilines, of which basicity are extremely low (pK_a −0.28 and 1.01), showed constant retention times under acidic conditions due to the absence of repulsion. These aniline derivatives with a low basicity would be mainly separated in the reversed-phase mode.

As described above, the separation of the aromatic amines on these polymer particles showed an electric exclusion mode under acidic conditions and typical reversed-phase mode separation under neutral and basic conditions. Hence, by changing the pH value of the mobile phase, the separation patterns of the aromatic amines are effectively tunable. A good separation of the eight aromatic amines was achieved using the 2P stationary phase at pH 2.9, and is shown in Fig. 9. The theoretical plate number of 2P polymer resins was calculated to be 10,042 plates, from the peak No. 2 in the chromatogram of Fig. 9.

4. Conclusion

The monodispersed vinylpyridine–divinylbenzene porous copolymer resins were prepared by polymerization in the presence of template porous silica gel particles (particle size: 5 μm, pore size: 10 nm), followed by dissolution of the template silica gel in an alkaline solution. The transmission electron micrographs of these polymer products revealed a good monodispersity and the scanning electron micrograph showed fine porous structures. The aromatic amines were analyzed by an HPLC system using these monodispersed polymer resins. The separation of the aromatic amines showed the typical reversed-phase mode separation under neutral and basic conditions, and also the exclusion mode under acidic conditions (around pH 2.0). Therefore, the separation patterns of the aromatic amines are effectively tunable by changing the pH of the employed eluent.

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