Preparation of Normal-Phase HPLC Stationary Phase Based on Monodisperse Hydrophilic Polymeric Beads and Their Application

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ABSTRACT: The monodisperse, 5.0 μm hydrophilic macroporous poly(glycidymethacrylate-*co*-ethylenedimethacrylate) beads were first prepared based on monosized linear poly(glycidylmethacrylate) beads as seed by using a single-step swelling and polymerization method. The seed beads prepared by dispersion polymerization exhibited good absorption of the monomer phase. The pore size distribution of the beads was evaluated by mercury instrusion method. The surface area was calculated from the BET isotherms of nitrogen adsorption and desorption. The beads were modified to be a normal-phase liquid chromatographic (NPLC) stationary phase for high performance liquid chromatography (HPLC) in the following steps. First, the beads were completely hydrolyzed. Second, hydrolyzed particles were reacted with epichlorihydrin followed by another hydrolysis of the newly introduced epoxide groups. The retention properties of the NPLC stationary phase were easily modulated by changes in the composition of the mobile phase. The performance of theses beads was demonstrated with the separation of a variety of polar compounds. The satisfactory results were obtained. © 2007 Wiley Periodicals, Inc. J Appl Polym Sci 106: 2730–2735, 2007

Key words: monodisperse hydrophilic poly(glycidylmethacrylate-*co*-ethylenedimethacrylate) beads; normal-phase liquid chromatography; polar compounds

INTRODUCTION

The first chromatographic separations, carried out a century ago, involved a stationary phase that was more polar than the mobile phase. This separation mode, called normal-phase liquid chromatography (NPLC), is well suited for the separation of organic compounds that differ only slight in their structures for which the more common reversed-phase LC mode is not suitable.[1–3] Today, NPLC is employed in about 20% of all high performance liquid chromatography (HPLC) separations.

Silica as well as aminopropyl-silica and cyanopropyl-silica are the most common adsorbents in NPLC. Silica-based stationary phases provide a large surface area and high column efficiency while affording excellent mechanical resistance.[4] However, silica-based packings are less stable under high pH conditions; they can not, sometimes, satisfy the requirement for the separation of various compounds. Polymer-based packings can be employed for various compound separation even in the pH range from 1 to 14; this led us to the develop polymer-based resins in HPLC.

In most cases, polymer-based packings can be commonly employed for ion exchange chromatography,[5] hydrophobic interaction chromatography,[6] and affinity chromatography[7] of biopolymer separations. The testing of the organic polymer matrixes under normal-phase conditions is seldom reported.[8,9] Petro et al.[10] and Xu et al.[11] reported based on linear polystyrene beads (preparation by emulsification polymerization method) as seeds for preparation of macroporous poly(glycidymethacrylate-*co*-ethylenedimethacrylate) ($P_{GMA/EDMA}$) beads by using a two-step swelling and polymerization; then the hydrolysis of the beads were used as the NPLC stationary phase for the separation of a variety of polar compounds.

We have reported a preparation of PGMA/EDMA beads by a single-step swelling and polymerization method in the presence of linear polystyene, solvents as a porogens, and chemical modification of the beads for the preparation of stationary phases of hydrophobic interaction chromatography,[12] weak

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cation exchange chromatography,[13] strong anion exchange chromatography,[14] and affinity chromatography[15] and their application for the separation of biopolymers.

In this article, to increase the density of hydroxyl groups in the polymer beads and hydrophilicity, we report a preparation of novel macroporous hydrophilic $P_{GMA/EDMA}$ beads in the presence of linear poly(glycidylmethacrylate) as seeds, solvents as a porogens by using single-step swelling and polymerization method, and chemical modification of the beads for the preparation of stationary phase of NPLC. The NPLC stationary phase was also used for the separation of a variety of polar compounds including positional isomers and aniline derivatives, satisfactory results were obtained.

EXPERIMENTAL

Materials

Glycidyl methacrylate (GMA) (Aldrich, USA) was distilled under vacuum. Ethylene dimethacrylate (EDMA) (Aldrich, USA) was extracted three times with 10% aqueous sodium hydroxide and distilled water, and then dried with anhydrous magnesium sulfate. Poly (vinylpyrrolidone, k-30) (PVP, k-30) was purchased from Aldrich (USA). Azobisisobutyronitrile (AIBN) was bought from Shanghai Chemical Reagent Co. Ltd. Benzoyl peroxide (BPO) was brought from Shanghai Chemical Reagent Co. Ltd. Toluene and cyclohexanol were gained from Shanghai Experimental Reagent Co. Ltd. Polyvinyl alcohol (PVA) and sodium dodecyl sulfonate (SDS) were obtained from Beijing Chemical Reagent Co. Ltd. Terahydrofuran (THF) was brought from Tianjin Kermel Chemical Reagents development centre. Heptane and hexane were purchased from Tianjin Chemical reagent Co. Ltd. All other chemicals were of analytic grade.

All chromatographic tests were carried out by using a chromatographic system (Agilent 1100) including a pump and a multiple-wavelength UV detector. GY-100 model high-pressure pump (Beijing Fusiyuan Machine Manufacturer) was used for column packing. Samples were injected through an autosampler (G1313A) and detected at 254 nm.

Dispersion polymerization for preparation of polyglycidymethacrylate seed beads

Monodisperse linear poly(glycidylmethacrylate) beads with low molecular weight were prepared by dispersion polymerization.[16] Around 2.5 mL of GMA as monomer, 0.05 g of AIBN as initiator, and 0.5 g of PVP as stabilizer were admixed in 22.5 mL of ethanol media under a nitrogen atmosphere. The polymerization was carried out at 70°C for 24 h with stirring at 300 rpm. After reaction, the seeds were obtained by centrifugal separation. The size of the prepared beads was measured to be 1.8 μm.

Preparation of uniform macroporous hydrophilic $P_{GMA/EDMA}$ beads

About 0.3 g of 1.8 μm dispersed linear poly(glycidylmethacrylate) seed beads and 20 mL of 0.1% SDS (w/w) of aqueous solution were placed in a 250-mL flask, and the mixture was stirred slowly by a mechanical stirrer. Then 8.0 mL of the mixture consisting of 2.0 mL GMA, 2.0 mL EDMA, 2.0 mL cyclohexanol, 2.0 mL toluene, and 3.0% (w/w) BPO initiator in terms of the total monomers were added into 75 mL aqueous solution of 0.1% (w/w) SDS and 1.0% (w/w) PVA and then emulsified under ultrasonic condition until the size of oil drops became at the most 0.5 μm (observed by optical microscope). The emulsion was sequently added into the dispersion solution of the seed beads. The mixture was stirred for 10–15 h at room temperature so that all the emulsified organic phase was completely absorbed by the polymer seeds. This whole process was monitored by an optical microscope until the organic liquid drops completely disappeared. The mixture was degassed by purging with nitrogen for 20 min. The polymerization was carried out at 70°C for 24 h with continuous stirring. The beads obtained were washed with hot water and methanol. The porogens were removed by extraction with THF for 48 h. The beads were washed with methanol again and dried in vacuum at 50°C, affording 3.6 g of monodisperse beads for a 90% yield. The size and dispersion coefficient of the prepared monodisperse $P_{GMA/EDMA}$ beads were measured to be 5.0 μm and 0.02, respectively.

Modification of the $P_{GMA/EDMA}$ beads for NPLC stationary phase

The $P_{GMA/EDMA}$ beads were modified to be a NPLC stationary phase in following steps. First, 3.0 g of hydrophilic macroporous $P_{GMA/EDMA}$ (beads I) were suspended in 100 mL of 0.1 mol/L sulfuric acid, stirred, and heated at 60°C by water bath for 10 h. After that, the beads were filtered, washed with water for three times, then dipped into distilled water overnight, washed with water again, dried under vacuum condition, obtaining the hydrolyzed beads (beads II). Second, the beads II were redispersed in the mixture of dimethyl sulfoxide and aqueous sodium hydroxide and stirred for 1 h, and the beads were transferred to 3 mL of an epichlorohydrin and stirred at room temperature for 4 h. Then epoxided beads III were obtained. Third, the

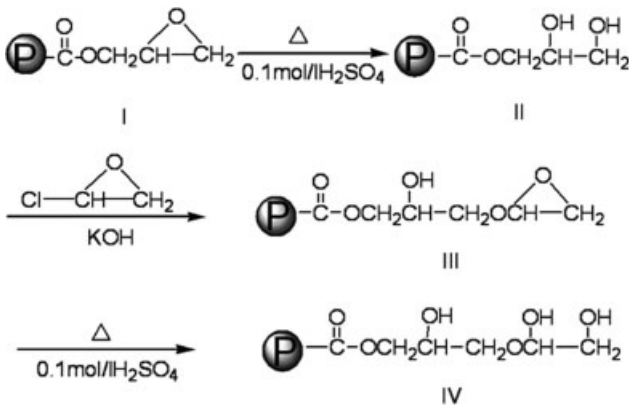

Figure 1 Chemical modification scheme for the preparation of the NPLC packings.

beads III were then hydrolyzed and worked up using the same procedure as described above to afford beads with three hydroxyl groups (beads IV). The obtained beads were filtered and washed with a large amount of water, acetone, and dried under vacuum condition. Thus, a new hydrophilic NPLC stationary phase for HPLC was obtained (beads IV). Figure 1 shows the chemical modification scheme for the preparation of the NPLC packings. (The ''P'' in the scheme denotes the polymer frame.)

Characterization of polymeric beads

The particle size and surface morphology, and the specific surface area and pore distribution of the synthesized $P_{GMA/EDMA}$ resins were measured by scanning electron microscopy and the mercury intrusion method, respectively. The surface area was calculated from the BET isotherms of nitrogen adsorption and desorption using ASAP 2010.

Determination of epoxy groups

The $P_{GMA/EDMA}$ beads were dispersed in 0.1 mol/L tetraethylammonium bromide in acetic acid solution and titrated with 0.1 mol/L perchloric acid solution until the crystal violet indicator changed to blue-green.

High performance liquid chromatography

About 1.6 g of the NPLC packings were dispersed under sonication in 15 mL of a 60 : 40 cyclohexanol/acetone mixture and then slurry packed into a 150 × 4.6 mm I.D. stainless steel column using acetone as the packing solvent at a constant pressure of 20 MPa for 30 min. The packed column was conditioned using a 50 : 50 heptane/THF mixture for 30 min followed by heptane for 1 h at a flow rate of 1.0 mL/min.

RESULTS AND DISCUSSION

Preparation of monodisperse macroporous hydrophilic $P_{GMA/EDMA}$ beads

To obtain monodisperse final beads, uniformly sized latex seed particles must be used as shape templates. Generally, the seed-swelling polymerization is considered one of the most effective methods for the preparation of monosized polymer resins. The monosized seeds mostly used were linear polystyrene, which were preswollen by small molecular

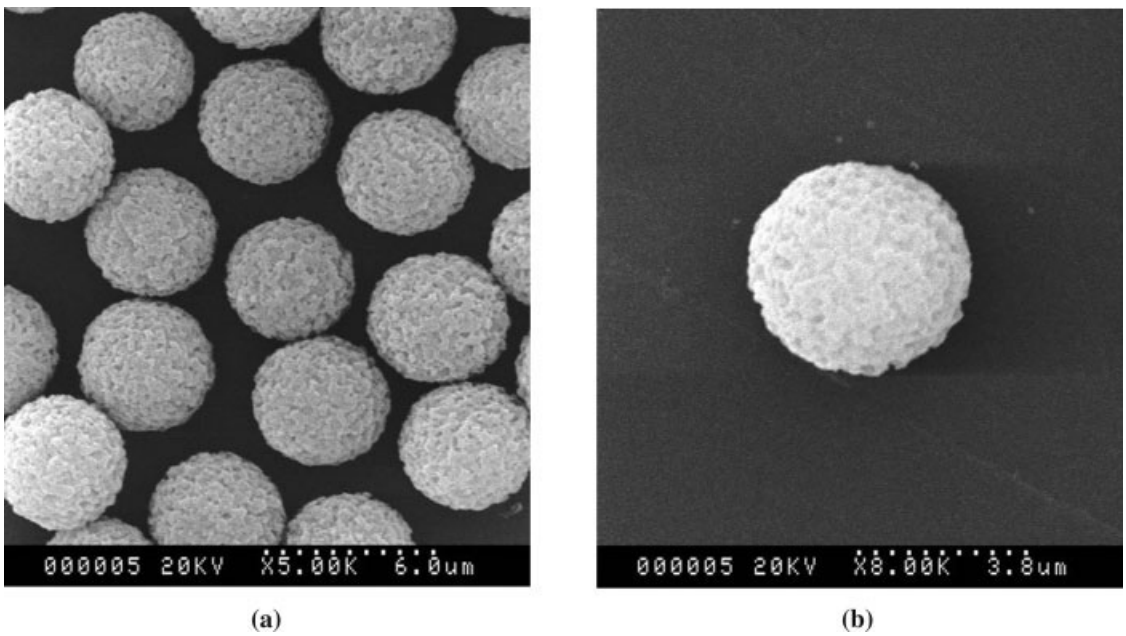

Figure 2 Scanning electron micrographs of the monosized porous beads (a) and their surface structure (b).

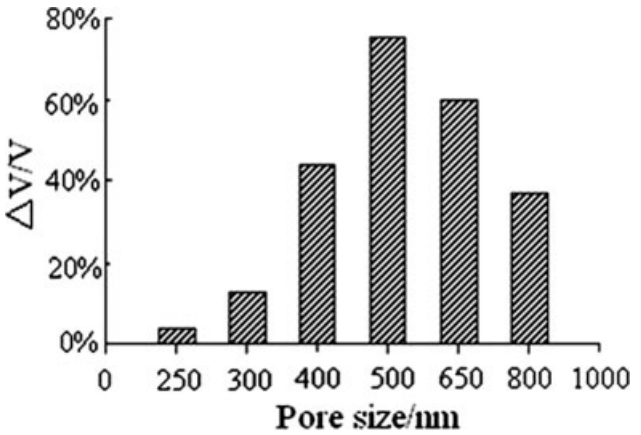

Figure 3 Pore size distribution of a poly(GMA-*co*-EDMA) bead (measured by mercury intrusion method).

compounds and then swollen by mixtures of monomer and crosslinker. However, the linear polystyrene beads in the final uniform sized beads are hard to extract completely, which will produce irreversible adsorption to separated compounds.

In this article, to increase the density of hydroxyl groups in the polymer beads and hydrophilicity, a new 1.8 μm monosized hydrophilic linear polyglycidylmethacrylate seed beads with lower molecular weight were synthesized by dispersion polymerization in alcohol medium; then macroporous hydrophilic $P_{GMA/EDMA}$ beads were prepared based on the beads as seed by using a single-step swelling and polymerization method. This method is simple, efficient, and suitable for the preparation of a series of hydrophilic $P_{GMA/EDMA}$ resins with different particle diameter, porous structure, and crosslinking degree. In comparison with the reported methods, this article has attained further development for preparation of monodisperse beads. Figure 2(a,b) shows the scanning electron micrographs of the prepared beads, illustrating that the prepared beads in this study are uniform in size and have macroporous structure.

Characterization of the beads

Cyclohexanol and toluene-solvents with large differences in their solvency for the $P_{GMA/EDMA}$ beads were chosen as coporogenic diluents in this study.[11] Toluene is a thermodynamically poor solvent for the polymer, which produces large pores, whereas cyclohexanol is a better solvent. Therefore, it is important to select the appropriate mixture ratio of cyclohexanol and toluene for preparation of the beads.

Effect of cyclohexanol/toluene ratios (1 : 1, 1 : 2, 1 : 3) on median pore diameter, specific surface area, and surface of $P_{GMA/EDMA}$ beads was studied. The experimental results show that the optimized ratio of cyclohexanol to toluene (1/1, v/v) was used to avoid formation of surface holes and simultaneously obtain beads with a smoother surface. Figure 3 shows the pore size distribution of the synthesized resin in dry state, which was measured by a mercury intrusion method and adsorption method. It was obvious that super-macroporous (diameter > 400 nm) and macroporous (diameter > 100 nm) structures were dominant with the macroporous beads. Table I shows the properties of the beads.

TABLE I
Properties of Macroporous $P_{GMA/EDMA}$ Beads

Particle size (μm)	5.0
Epoxide groups (mmol/g)	3.0
Pore volume (mL/g)[a]	1.92
Median pore diameter of mercury porosimetry (nm)	500.0
BET surface area (m^2/g)	11.2

[a] According to the mercury intrusion method.

Preparation of NPLC stationary phase

Many reactions can be used for the chemical modification of the epoxide groups existing on the surface of the $P_{GMA/EDMA}$ resin. The beads were modified to be a NPLC stationary phase for HPLC as following steps. As shown in Figure 1, first, the particles were completely hydrolyzed. Second, hydrolyzed particles were reacted with epichlorihydrin followed by another hydrolysis of the newly introduced epoxide groups. This chemical modification method not only results in a better shielding of the hydrophobic main chains of the polymer thereby preventing them from contact with the separated compounds, but also obtains three hydroxyl groups that are advantageous

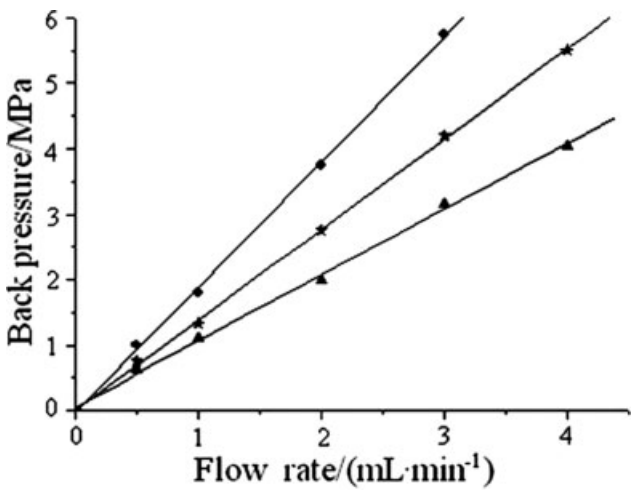

Figure 4 Effect of the mobile phases on column backpressure. Column: 150 × 4.6 mm I. D. Mobile phases: THF(●), 1 : 1 THF/heptanes (□), and heptanes (▲).

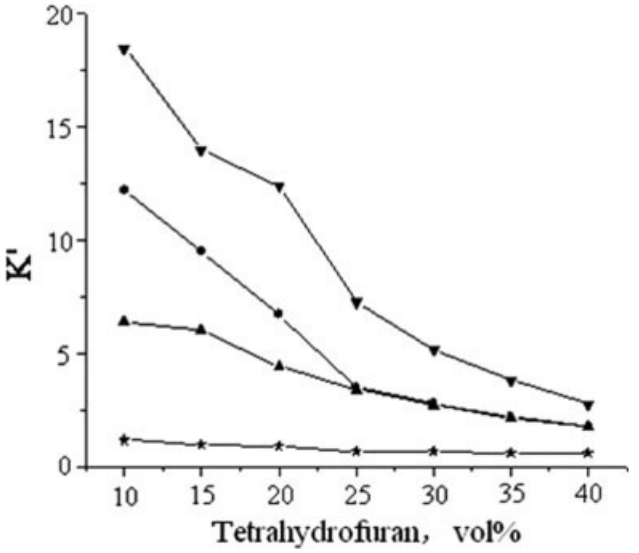

Figure 5 Influence of the mobile phase composition on the retention factors. Column: 150 × 4.6 mm^2 I. D. The isocratic elution: THF : heptane ratio varied from 10 to 40%; flow rate 1.0 mL/min; UV detection at 254 nm. Analytes: toluene (★), nitrobenzene (▲), phenol (●), and 3-nitrophenol (▼).

to NPLC stationary phase. The content of hydroxyl groups was determined to be 2.1 mmol/mL, which was much more than the surface coverage of 0.16 mmol/mL measured early for the typical bare silica stationary phase Nucleosil.[17] IR spectrum of the modified polymer exhibited a large broad adsorption peak at 4330 cm^{-1}, corresponding to hydroxyl, and 900 cm^{-1} epoxide groups were disappeared. These results prove that the newly introduced epoxide groups were really bound to the surface of the prepared polymer.

Column backpressure

Back pressure in the column is closely related to the quality of the packing. Figure 5 shows back pressure versus flow rate plots for a 150 × 4.6 mm I.D. column packed with the NPLC packings and for different mobile phases typical of NPLC. Since no solvation of the NPLC beads occurs in heptane, column permeability is highest when this solvent is used.[11] In contrast, THF swells the beads, leading to a decrease in the size of interparticular voids and a concomitant increase in back pressure. Values obtained for the 1 : 1 heptane/THF mixture are between those for the pure solvents.

The straight lines observed in Figure 4 confirm the high mechanical stability of the NPLC beads and back pressure is only about 6.0 MPa at the flow rate of 4.0 mL/min. This is much lower than the reported method.[11] This result demonstrates that the beads possess high permeability.

Effect of composition of the mobile phase on retention

Figure 5 shows the influence of the mobile phase composition on the retention factors of the test compounds in an experiment on the synthesized NPLC column for four model analytes—toluene, nitrobenzene, phenol, and 3-nitrophenol. It can be seen from Figure 5 that the increase of the polarity of the

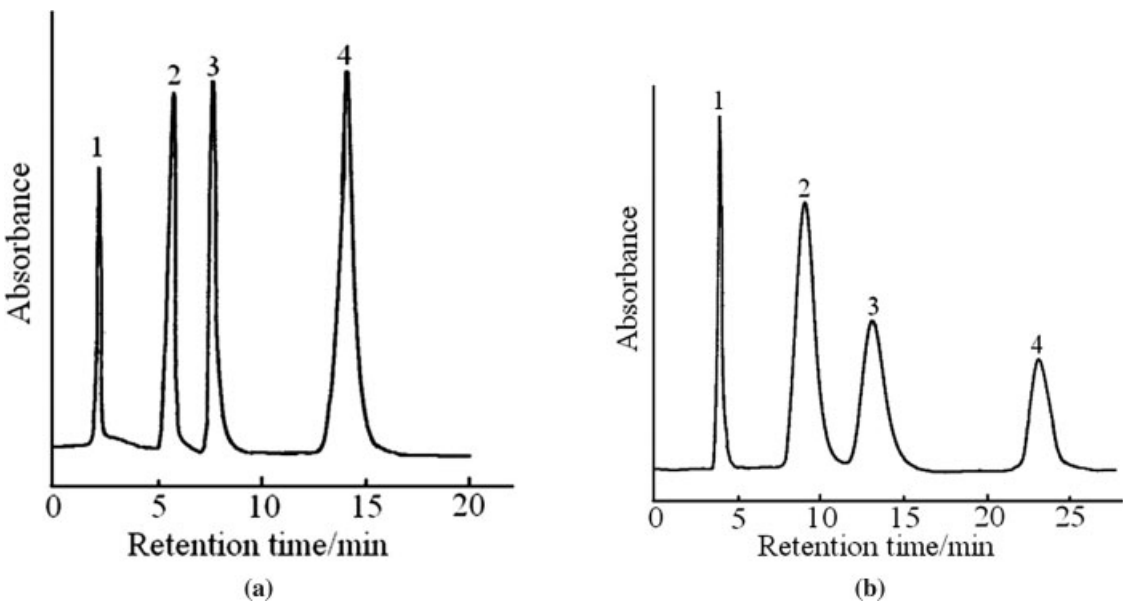

Figure 6 Chromatogram of analytes separated by the NPLC column. Column: 150 × 4.6 mm^2 I. D. (a) mobile phase, heptane/THF (80 : 20); flow rate 1 mL/min; UV detection at 254 nm. Peaks: 1, toluene; 2, nitrobenzene; 3, phenol; 4, 3-nitrophenol. (b) mobile phase, heptane/THF (75 : 25); flow rate 1 mL/min; UV detection at 254 nm. Peaks: 1, 4-nitrotoluene; 2, 4-nitrobenzoic acid; 3, 4-nitrophenol; 4, 4-hydroxy-benzoic acid.

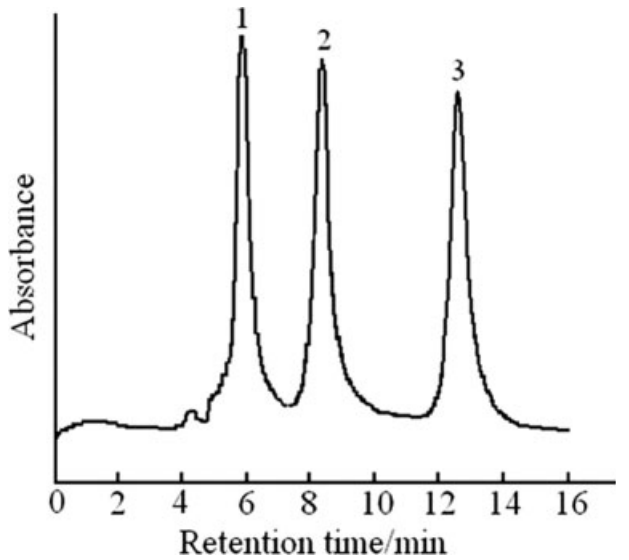

Figure 7 Chromatogram of three positional isomers of nitroaniline separated by the NPLC column. Column: 150 × 4.6 mm^2 I. D. Mobile phase, linear gradient of 25–35% THF′ in hexane′ in 15 min; flow rate 1.5mL/min; UV detection at 254 nm. (THF′ is a 99.6 : 0.4 mixture of THF and triethylamine, hexane′ is a 99.6 : 0.4 mixture of hexane and triethylamine). Peaks: 1, 2-nitroaniline; 2, 3-nitroaniline; 3, 4-nitroaniline.

mobile phase decreased the retention factors of the more retained compounds dramatically, as expected for normal-phase, but not for reversed-phase chromatography experiments. Figure 5 also documents that the retention of a column packed with the synthesized NPLC stationary phase can easily be modulated through the composition of the mobile phase.

Normal phase HPLC separations

To test the resolution property of the synthesized NPLC column, experiments was performed to resolve a model mixture consisting of toluene, nitrobenzene, phenol, and 3-nitrophenol [Fig. 6(a)]. All analytes were baseline separated with very high resolution. Another model mixture consisting of 4-nitrotoluene, 4-nitrobenzoic acid, 4-nitrophenol, and 4-hydroxy-benzoic acid was also baseline separated [Fig. 6(b)].

The immense selectivity typical of normal-phase HPLC that allows the separation of compounds with only very small structural differences is well known. Figure 7 shows the baseline separation of three positional isomers of nitroaniline. Baseline separation is achieved using gradient elution.

CONCLUSION

Around 1.8 μm of linear poly(glycidylmethacrylate) seed beads were synthesized by dispersion polymerization; then 5.0 μm of uniform-size hydrophilic poly(glycidymethacrylate-*co*-ethylenedimethacrylate) beads with macroporous were obtained by single-step swelling and polymerization method. The physical properties of the beads were measured and discussed in detail. The results show that the beads have uniformity in particle size, strong particle rigidity, and the desired macroporousity. By using this medium, one kind of NPLC stationary phase was synthesized by a chemical modification method. Compared with the conventional material like typical bare silica,[17] the advantages of the synthesized new NPLC stationary phase are: (1) hydrophilic, (2) easily derivatizable, (3) high surface coverage, (4) homogeneous. The NPLC stationary phase was used for the separation of a variety of polar compounds including positional isomers and aniline derivatives. The satisfactory results were obtained.

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