DNA-Loaded Porous Polyethersulfone Particles for Environmental Applications II. Utilization

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ABSTRACT: DNA-loaded porous polyethersulfone (PES) particles are fabricated using a liquid–liquid phase separation technique. The particles are then used for environmental applications. Both the DNA-loaded porous PES particles and the DNA-loaded PES porous particle column could accumulate and remove DNA intercalating pollutants, such as ethidium bromide, acridine orange, endocrine disruptors, and heavy metal ions. The microsphere column shows more high removal efficiency. These results proved that the DNA-loaded porous particles have the potential to serve as absorbents for environmental applications. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 1674–1678, 2005

Key words: DNA; poly(ether sulfones); particle column; endocrine disruptors; heavy metal ions

INTRODUCTION

DNA is the most important material for the genetic process of living organisms. In this study, DNA is regarded as a naturally occurring and highly specific functional biopolymer and is used to prepare DNA-loaded porous polyethersulfone (PES) particles. The porous particles have the potential to be used for environmental applications.[1]

Functional polymer microspheres or particles are widely used in the medical and biochemical fields as absorbents, latex diagnostics, affinity bioseparators, and drug and enzyme carriers, and microspheres are directly prepared by heterogeneous polymerization.[2] In the first study, we prepared DNA-loaded porous PES particles by a liquid–liquid phase separation technique and characterized the particles. DNA-loaded porous PES particles could accumulate and remove DNA-intercalating compounds—ethidium bromide (EB). In the present study, we focused on the functional utilization of the particles as absorbents. We prepared a microsphere column for the removal of ethidium bromide and acridine orange (AO). The accumulation and removal of endocrine disruptors such as biphenyl and dibenzofuran as well as heavy metal ions was also investigated.

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METHODS

Preparation of the DNA-loaded PES particles

PES (Ultrason E 6020P, CAS No. 25,608–63-3, BASF Aktiengesellschaft) was dissolved in *N*-methyl-2-pyrrolidinone (NMP; HPLC grade, 99+%, Aldrich Chemical) to obtain the PES solution (12.5%). Double-stranded DNA from salmon milt (Na salt, molecular weight: 5×10^6) was obtained from the Yuki Fine Chemical (Tokyo, Japan). The DNA was dissolved in distilled water (20 mg/mL) and then dropped into the PES solution to obtain the mixed solution; the weight ratios of DNA to PES were controlled at 0.015. All materials were used without further purification.

The mixed PES solution was injected into water using a 0.4-mm-diameter syringe needle and stirred at about 300 rpm. The injection speed was controlled at 60–100 drop/min. The air gap from the needle to the water was 8–15 cm. The microspheres were then incubated in water to elute the solvent from the particles as mentioned in the first part.[1]

Preparation of the particle column

The DNA-loaded porous PES particles (about 500 mg in dry weight) were placed in a 10-mL polypropylene syringe, and the length of the mobile phase was approximately 50 mm. The flow rate of the DNA-loaded PES particle column ranged from 0.5 to 2.0 mL/min.

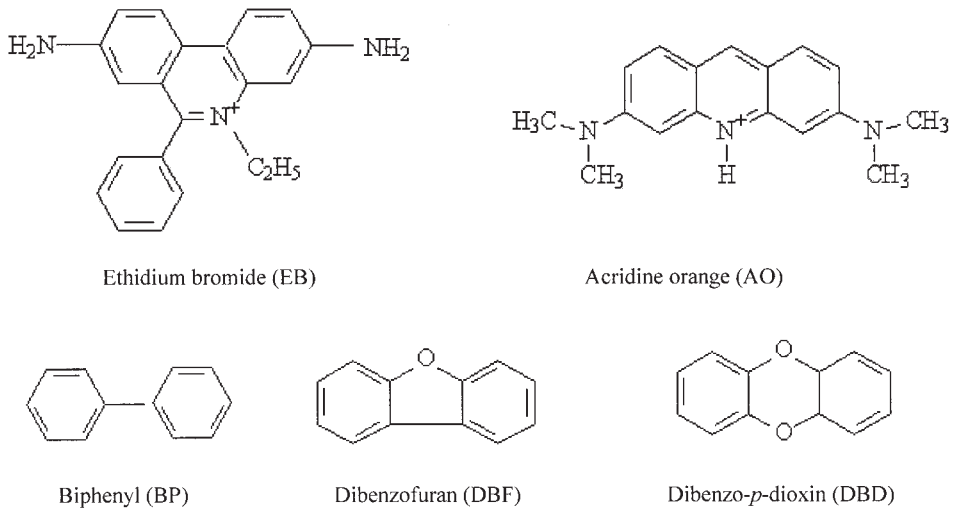

Figure 1 Structure of harmful compounds and endocrine disruptors.

Accumulation and removal of ethidium bromide and acridine orange by the microsphere column

EB and AO were obtained from Tokyo Kasei Industries (Osaka, Japan) and were used to test the functional utilization of the DNA-loaded PES microsphere column. Their structures[3] are shown in Figure 1. The amount of the EB and AO in the solution was quantified by the absorption at 480 and 492 nm, respectively, using a UV–vis spectrophotometer U-200A (Hitachi, Tokyo, Japan).

The removal of harmful DNA-intercalating compounds (EB and AO) by the DNA-loaded PES porous particle column was examined by the following procedures. In Method 1, the aqueous solution (10 mL, 80 μM) of harmful compounds was applied to the DNA-loaded PES particle column; the concentration of the eluted solution was then determined by the spectra of the eluted solution. The eluted solution was reapplied, and this process was repeated four times. In Method 2, the aqueous solution (10 mL, 80 μM) of harmful compounds was applied to the particle column; the concentration of the eluted solution was determined. Then, a fresh aqueous solution (10 mL, 80 μM) was applied to the same column, and the concentration of the eluted solution was also determined. This process was repeated three times.

Accumulation and removal of endocrine disruptors by the particles

Biphenyl (BP), dibenzofuran (DBF), and dibenzo-*p*-dioxin (DBD) were obtained from Wako Pure Chemical Industries (Osaka, Japan). Their structures[3] are shown in Figure 1. These compounds were used as the model endocrine disruptors to test their accumulation into the DNA-loaded microspheres. Dibenzo-*p*-dioxin and dibenzofuran are derivatives of dioxin, and biphenyl is a derivative of polychlorobiphenyl. These reagents show very low solubility in water. Therefore, we dissolved these reagents in ethanol and then diluted them in distilled water. The concentrations of all the reagents above were 20 μM.

The accumulation of these reagents was examined by the following procedures: DNA-loaded PES porous particles (30 mg) were put into the respective aqueous solutions (8 mL) and incubated for 24 h at room temperature. The accumulation of these reagents was confirmed by the absorption spectra of their aqueous solutions in the absence or presence of the DNA-loaded PES porous particles.

Accumulation and removal of metal ions by the particles

Accumulation of heavy metal ions by the DNA-loaded PES porous particles was examined by the following procedure: DNA-loaded PES porous microspheres (40 mg) were put into the aqueous solution (25 mL) containing metal ions, such as Zn^{2+}, Cu^{2+}, Mg^{2+}, Cd^{2+}, and Ag^{+}, respectively. The microspheres were put out from the aqueous solutions after incubation for 24 h at room temperature. Then, the amount of the metal ions in the aqueous solutions was determined by an atomic absorption spectrophotometer (Shimadzu SPCA-626D, Japan). The initial concentrations of the irons in their respective solutions were also determined and adjusted to about 4 ppm.

RESULTS AND DISCUSSION

Removal of pollutant organic compounds by the DNA-loaded PES particle column

Since DNA-loaded porous polyethersulfone particles could remove about 80% DNA-intercalating com-

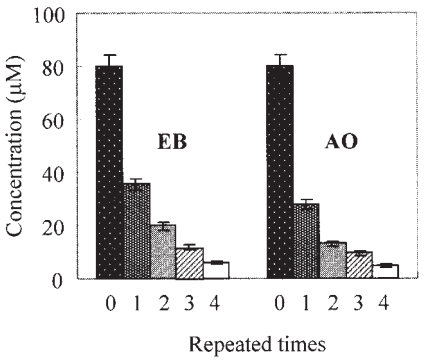

Figure 2 Concentration change with repeated times when the eluted solutions were reapplied to the DNA-loaded PES particle column. Flow rate: 2 mL/min. Data are expressed as means ± SD of three independent experiments.

pound—EB from its aqueous solution, we prepared DNA-loaded porous PES particle columns to more effectively remove these kinds of harmful compounds. Besides EB, AO was also used to test the functional utilization of the DNA-loaded particle columns.

DNA-loaded PES porous particles were packed into a polypropylene syringe, and the chemical compounds (EB and AO) containing aqueous solution were applied. The removal of these compounds was determined by the absorption spectra of the eluted solution. When aqueous ethidium bromide was applied to the DNA-loaded PES particle column, ethidium bromide was bound to the DNA-loaded PES particle column, and the absorption spectra (absorption peak at the wavelength of 480 nm) of the eluate disappeared. The white DNA-loaded PES porous particles were dyed red by the binding of ethidium. A similar result was obtained when an aqueous acridine orange solution was applied to a DNA-loaded particle column. The absorption peak at the wavelength of 492 nm of the eluate disappeared, and the white PES particles were dyed orange due to the binding of the acridine orange.

Aqueous solutions (10 mL, 80 μM) of EB or AO were applied to the DNA-loaded PES particle column, respectively. The eluted solution was reapplied four times; each time the concentration was determined by the spectra of the eluted solution. The results are shown in Figure 2.

As shown in Figure 2, when the EB solution was applied to the DNA-loaded PES porous particle column for the first time, the concentration sharply decreased from about 80 to about 35 μM. A similar result was obtained when the AO solution was applied to the column, and the concentration decreased from about 80 to about 27 μM. These mean that about 56% of EB and 66% of AO in their respective solutions was removed when their solutions were applied to the particle column, respectively. When the eluate was reapplied for the fourth time, the concentrations for EB and AO decreased to about 6 and 4.8 μM, respectively, indicating that over 93% of the harmful compounds were removed by the DNA-loaded PES porous particle column, and the harmful DNA-intercalating compounds could be removed completely by reapplied the eluate to the column.

Next, we determined the accumulation and removal of EB and AO when the eluted solutions were not reapplied. Aqueous solutions (10 mL, 80 μM) of harmful compound solution were applied to the DNA-loaded PES particle columns, and the concentration of the eluate was then determined by the spectra of the eluted solution. Then, a fresh aqueous solution (10 mL, 80 μM) was applied to the same column, and the concentration of the eluted solution was also determined. This process was repeated three times. The results are shown in Figure 3.

As shown in Figure 3, when an aqueous EB solution (10 mL, 80 μM) was applied to the DNA-loaded PES porous particle column for the first time, the concentration decreased to about 35 μM. The second and third times, the concentrations decreased from about 80 to about 36 and 37 μM, respectively. A similar result was obtained when the AO solution was applied to the column, and the concentrations decreased from about 80 to about 27, 28, and 29 μM, respectively. This means that the DNA-loaded PES porous particle columns could treat a very large volume of these kinds of pollutant solutions, while there is no significant decrease in the removal ratios.

Furthermore, we investigated the effect of the flow rate on the removal of EB and AO. The concentration of the EB or the AO in the eluted solution decreased when the flow rate of the DNA-loaded PES porous particle column decreased. When the flow rate decreased from 2 to 0.5 mL/min, the EB concentration in the eluate decreased from about 35 to about 22 μM,

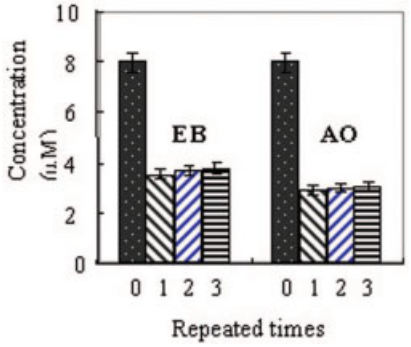

Figure 3 Concentration change with repeated times when the new solutions were applied to the DNA-loaded PES particle column. Flow rate: 2 mL/min. Data are expressed as means ± SD of three independent experiments. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

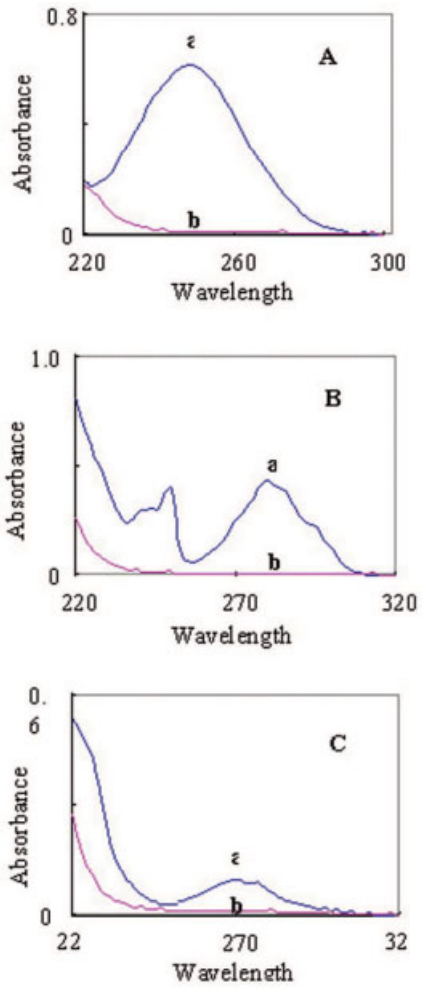

Figure 4 Absorption spectra of aqueous solutions of endocrine disruptors in the absence (a) and presence (b) of DNA-loaded porous PES microspheres (25 mg). (A) BP solution; (B) DBF solution; (C) DBD solution. Duplicate experiments gave similar results. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

which means that the removal ratio increased from about 56 to about 73%. The AO concentration decreased from about 27 to 16 μM, and the removal ratio increased from 66 to about 80%. This suggested that a high removal ratio could be obtained not only by reapplying the eluted solution but also by decreasing the flow rate.

Removal of endocrine disruptors by the DNA-loaded PES particles

The DNA-loaded PES porous particles were also used for the accumulation and removal of other DNA intercalating materials, such as the endocrine disruptor, BP, DBF, and DBD. Figure 4 shows the absorption spectra of their aqueous solutions before and after application to the DNA-loaded microspheres for 24 h, respectively. The absorption peaks of these compounds disappeared by the application to the particles. The biphenyl and dibenzofuran were almost completely removed by the microspheres, respectively. About 78% of the dibenzo-*p*-dioxin was removed by the microspheres.

The double-strand DNA film and DNA immobilized porous glass beads selectively bind endocrine disruptors with planar structure.[3–5] Of course, the DNA-loaded PES porous microspheres bind endocrine disruptors with planar structure. However, the PES microspheres without DNA also adsorb endocrine disruptors (biphenyl, dibenzofuran, and dibenzo-*p*-dioxin) due to the hydrophobic interaction between PES and endocrine disruptors and the large specific surface areas.[6] Additionally, the amount of the incorporated DNA in the microspheres and the microsphere structure also affects the removal of the endocrine disruptors as those affected to the EB removal.[1] When the particle columns were used, they could more effectively remove the endocrine disruptors.

Evidence is growing that a serious health link exists between chemicals that mimic the hormone estrogen and damage to the human reproductive system. Known as endocrine disruptors, these chemicals are widely distributed in the environment. As mentioned in this study, the DNA-loaded PES porous particle microspheres could remove endocrine disruptors from water; they might have environmental clean-up applications.

Removal of heavy metal ions by the DNA-loaded PES particles

Figure 5 shows the accumulation of metal ions from their aqueous solutions by DNA-loaded PES porous particles. The concentration of Ag^+ decreased from 4 to about 1.2 ppm after the incubation with the particle. The concentration of Cu^{2+} decreased from 4 to about 1.5 ppm after the incubation. Cd^{2+} and Zn^{2+} were also found to accumulate into the DNA-loaded PES porous particles. However, the accumulation amounts were smaller than that of Ag^+ and Cu^{2+}. Also, the accumulation amounts were different between them. When large amounts of the DNA-loaded particles were used, the amounts of the accumulated heavy metal ions were large. On the other hand, Mg^{2+} was not accumulated into the DNA-loaded PES porous particles. We examined three kinds of magnesium salts, magnesium chloride, magnesium sulfate, and magnesium nitrate, but magnesium salts were not accumulated into the microspheres. We also examined the removal of metal ions by the DNA-loaded PES particle column. The results were similar to that of the microspheres. These results indicated that the incorporated DNA into the PES porous particles could selectively accumulate heavy metal ions.

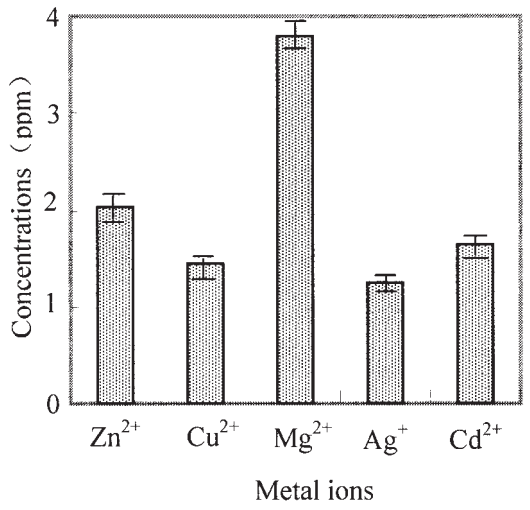

Figure 5 Concentrations of metal ions after the accumulation by the DNA-loaded PES porous particles (40 mg). Aqueous metal ion solutions (4 ppm, 25 mL) were applied. Data are expressed as means ± SD of three independent experiments.

It has been reported that heavy metal ions could selectively be removed by water-insoluble DNA film, and the metal ion selectivity could be measured by infrared spectrometry.[7] Mg^{2+} did not affect on the absorption band of phosphate group (1230 cm^{-1}) of the DNA, and almost no Mg^{2+} accumulated in the DNA-loaded particles in this study (as shown in Fig. 5). The infrared spectrum of DNA-film with copper chloride largely changed the vibration of not only the nucleic acid base but also the phosphate group. Cu^{2+} has been reported to bind strongly with internucleic acid bases rather than the phosphate group from another study.[8] Therefore, the selective accumulation of the heavy metal ions by the double-strand DNA incorporated into the polyethersulfone porous particles was proposed through the internucleic acid bases and phosphate groups.

CONCLUSIONS

DNA-loaded porous PES particles, prepared by a liquid–liquid phase separation technique, could be used for environmental applications, such as accumulation and removal of harmful pollutants, endocrine disruptors, and heavy metal ions. The DNA-loaded PES porous microsphere column could more effectively remove harmful compounds and heavy metal ions. These results prove that the DNA-loaded porous particles have the potential to serve as absorbents for environmental applications.

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