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DNA-Loaded PSf Microspheres Used in Environmental Application

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ABSTRACT

DNA-loaded porous polysulfone (PSf) microspheres were prepared using a liquid–liquid phase separation technique. The microspheres were then used in environmental application. The DNA-loaded microspheres were stable in water. They could effectively accumulate the DNA-binding harmful pollutants and endocrine disruptors, such as ethidium bromide (EB), acridine orange (AO), biphenyl (BP), dibenzofuran (DBF), and dibenzo-*p*-dioxin (DBD), in their aqueous solutions. On the other hand, the microspheres were found to selectively remove heavy metal ions

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from their aqueous solutions. The DNA-loaded PSf microspheres have a potential ability to serve as functional materials in environmental application.

Key Words: DNA; Polysulfone; Microspheres; Endocrine disruptors; Heavy metal ions.

1 INTRODUCTION

Functional polymer microspheres are widely used in medical and biochemical fields as absorbents, latex diagnostics, affinity bioseparators, and drug and enzyme carriers. The microspheres are directly prepared by heterogeneous polymerization, e.g., emulsion polymerization, precipitation polymerization, suspension polymerization, and dispersion polymerization.[1] Modification of the existing particles is another method to prepare functional particles.

DNA, the most important genetic material of living organisms, can also be regarded as a naturally occurring and highly specific functional biopolymer. DNA has a double-stranded structure, which allows it to have various specific functions, such as intercalation, groove binding, and electron transfer.[2–4] Although films and fibers can be prepared from DNA, they are water-soluble and have low mechanical strength. Therefore, utilization of DNA as a functional material is limited. In recent reports of Nishi's groups, water-insoluble and nuclease-resistant DNA films were prepared by ultra violet (UV) irradiation,[5,6] and insolubilized DNA immobilized onto porous glass beads was also prepared by treatment with UV irradiation.[5,7] The DNA films and DNA-immobilized glass beads removed DNA-intercalating compounds.

Polysulfone (PSf) is a well-known polymeric material. The PSf and PSf-based membranes show outstanding oxidative, thermal, and hydrolytic stabilities as well as good mechanical and film-forming properties. Almost all the PSf membranes were prepared by using the phase transition technique. In our recent research, DNA was used to modify PSf membranes, the hydrophilicity of the modified membranes increased, and the membranes showed improved blood compatibility.[8,9] The double-stranded DNA was encapsulated in PSf hollow microspheres; the microspheres could remove a DNA-intercalating material—ethidium bromide (EB).[10]

In the present article, we prepare DNA-loaded PSf microspheres using a liquid–liquid phase separation technique. The microspheres were used to remove harmful DNA-intercalating pollutants, endocrine disruptors, and heavy metal ions from their aqueous solutions.

2 MATERIALS AND METHODS

2.1 Preparation of DNA-Loaded PSf Microspheres

The PSf (average Mn ca. 26,000, Aldrich Chemical Company, Inc.) was dissolved in *N*-methyl-2-pyrrolidinone (NMP) [high-performance liquid chromatography (HPLC) grade, 99+%, Aldrich Chemical Company, Inc.] to obtain the PSf solution (10%wt.). Double-stranded DNA from salmon milt (Na salt, molecular weight: 5×10^6) was obtained from the Yuki Fine Chemical Co., Ltd., Tokyo, Japan. The DNA was dissolved in distilled water, and the concentration was 10 mg/mL. The DNA solution was then dropped into the PSf solution to obtain the mixed solution; the weight ratios of DNA to PSf were 0.6%. All the materials were used without further purification.

The mixed PSf solutions were injected into water using a 0.4-mm-diameter syringe needle at room temperature, and stirred at about 300 rpm. The microspheres were then incubated in water for 24 hr at room temperature to elute the solvent in the microspheres.

The amount of DNA incorporated in the PSf porous microspheres was determined by the following procedure: the microspheres were cut into two sections and then hydrolyzed by 6 M HCl solution at 100°C for 1 hr. The amount of DNA in the solution was quantified by the absorption at 260 nm using an UV-VIS spectrophotometer U-200A (Hitachi Co., Ltd., Tokyo, Japan).

2.2 Calculation of the Microsphere Diameter and Porosity and Scanning Electron Microscopy

The diameter and the porosity of the microspheres were calculated from the density of the polymer and the weight change before and after drying, using the following formulas:[11]

$$\text{Diameter} = \left(\frac{6[W_A/\rho_P + (W_B - W_A)/\rho_W]}{\pi} \right)^{1/3}$$

$$\text{Porosity} = \frac{(W_B - W_A)/\rho_W}{W_A/\rho_P + (W_B - W_A)/\rho_W} \times 100\%$$

where W_B is the weight of the membrane before drying, g; W_A is the weight of the membrane after drying, g; ρ_W is the density of water, $\rho_W = 1.0\,\text{g/cm}^3$; and ρ_P is the density of the PSf, $\rho_P = 1.24\,\text{g/cm}^3$.

For the scanning electron microscopy (SEM) observation, the microsphere samples were dried at room temperature, then cut with a single-edged razor blade, attached to the sample supports, and coated with a gold layer. The SEM images were recorded using an S-2500C microscope (voltage = 20 kV, Hitachi, Japan).

2.3 Accumulation and Removal of Harmful DNA-Intercalating Pollutants and Endocrine Disrupters

A 5-mM solution of aqueous EB, a well-studied double-stranded DNA-intercalating reagent, was first used to test the accumulation into the PSf microspheres. Aqueous acridine orange (AO) solution (5 μM) was also used to test the functional utilization. The accumulations of the two reagents were examined by the following procedures: the microspheres (20 mg in dry weight) were put in their aqueous solution (4 mL) and incubated for 24 hr at room temperature. The accumulations were confirmed by the absorption spectra of the aqueous solutions in the absence or presence of the DNA-loaded PSf microspheres using an UV-VIS spectrophotometer U-200A (Hitachi Co., Ltd., Tokyo, Japan).

Biphenyl (BP), dibenzofuran (DBF), and dibenzo-*p*-dioxin (DBD) were used as model endocrine disrupters to test their accumulation into the DNA-loaded microspheres. DBD and DBF are derivatives of dioxin, and BP is a derivative of polychlorobiphenyl (PCB). These reagents show very low solubility in water. Therefore, we dissolved these reagents in ethanol and then diluted in distilled water. All their concentrations were 20 μM. The accumulation of these reagents was examined by the following procedures: DNA-loaded microspheres (20 mg) were put into the respective solutions (10 mL) and incubated for 24 hr at room temperature. The accumulation of these reagents was confirmed by the absorption spectra of their aqueous solutions in the absence or presence of DNA-loaded microspheres.

2.4 Accumulation and Removal of DNA-Intercalating Pollutants by the Microsphere Column

The DNA-loaded PSf microspheres (8 mL) were placed in a 10-mL polypropylene syringe, and the length of the mobile phase was approximately 5 mm. A mixed solution (300 mL) containing AO (5 μM), BP (20 μM), and DBF (20 μM), was used to test the accumulation and removal of DNA-intercalating pollutants by the DNA-loaded PSf microsphere column. The amount of these compounds was determined by the multi-component

determination function of the UV-VIS spectrophotometer U-200A at the wavelength of 492, 280, 268, 256, and 244 nm.

2.5 Accumulation of Metal Ions into DNA-Loaded PSf Microspheres

Accumulation of heavy metal ions by the DNA-loaded PSf microspheres was examined by the following procedure: DNA-loaded PSf 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^{+}, and incubated for 24 hr at room temperature. The microspheres were removed from the aqueous solutions. The amount of metal ions in the aqueous solutions was then determined by an atomic absorption spectrophotometer (Shimadzu SPCA-626D). The initial concentrations of ions in their respective solutions were also determined, and adjusted to about 4 ppm.

3 RESULTS AND DISCUSSION

3.1 DNA-Loaded PSf Microspheres

A liquid–liquid phase separation technique was employed to fabricate the microspheres. When the PSf solution containing DNA was added to the water, liquid–liquid phase separation caused by the rapid exchange of the solvent NMP and water occurred, and a skin layer formed due to the rapid phase separation. With the completion of the exchange between the solvent and the nonsolvent, the porous PSf microspheres were prepared, and many pores presented in the spheres, as shown in Fig. 1.

The diameter of the microspheres was calculated from the formula mentioned above. It was an equivalent diameter, not directly determined. The diameter of the microspheres prepared using 12.5% PSf solution was 1.95 ± 0.2 mm. The porosity was $82.8 \pm 2.0\%$. The diameter and porosity depends on the diameter of the syringe needle and the polymer solution (data not shown). When a large-diameter syringe needle was used, the microsphere diameter was large. By increasing the polymer concentration, the diameter slightly increased. However, the porosity decreased.

3.2 Stability of the DNA-Loaded PSf Microspheres

The stability of the DNA-loaded PSf microspheres was examined in various solutions, including H_2O, NaCl solution, sodium dodecylsulfate

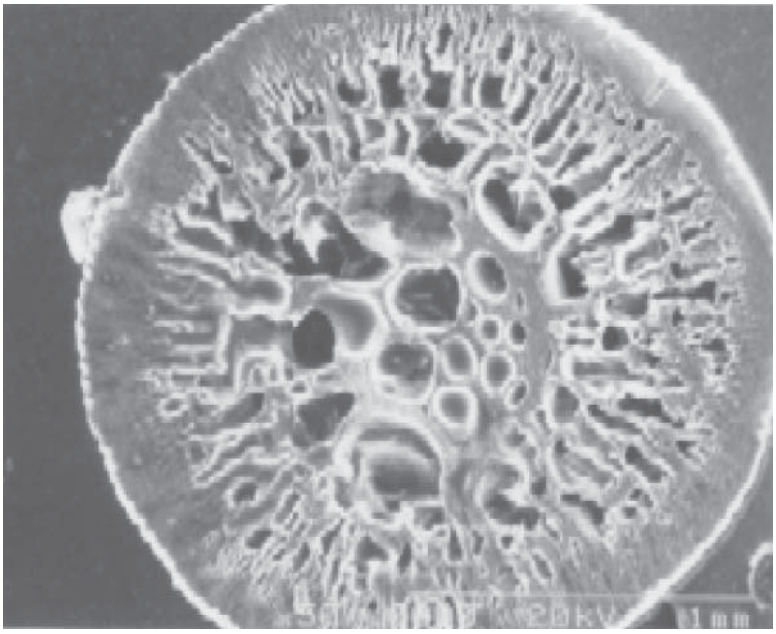

Figure 1. Cross-section of the DNA-loaded PSf microspheres. Voltage: 20 kV; magnification: ×50.

(SDS) solution, NaOH solution, and HCl solution, then the amounts of eluted DNA from the microspheres were determined (Fig. 2). It was shown that the DNA-loaded microspheres were stable in water and NaCl solution, and not more than 4% of the initial incorporated DNA was eluted from the microspheres, even after being incubated for 48 hr. However, a large amount of DNA was eluted into the solution when it was incubated under acidic conditions, and about 30% of the initial incorporated DNA was eluted after being incubated for 48 hr. When it was incubated under alkaline conditions, about 23% of the DNA was eluted.

Approximately 30% of the initial incorporated DNA was eluted into the HCl solution after being incubated for 48 hr, and the amount still increased with time. This indicated that DNA phosphodiester bonds might be hydrolyzed.[12] Also, a large amount of the DNA was eluted into NaOH solution due to the DNA hydrolysis.[13] Part of the DNA was eluted into 2% DNA solution due to the surface activity of the SDS. These results showed that the DNA-loaded PSf microspheres were stable in water and NaCl solution, thus indicating that they should be amenable in environmental applications under neutral conditions.

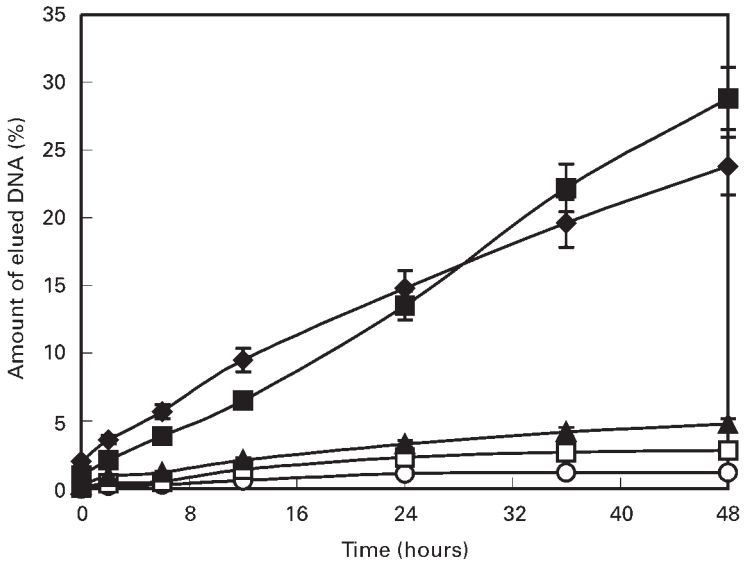

Figure 2. Stability of DNA-loaded PSf microspheres. The microspheres were incubated in the following aqueous solutions at room temperature, and the amount of the eluted DNA from the microspheres was measured at various time intervals. *Key:* ■, 0.1 M HCl solution; ◆, 0.1 M NaOH solution; ▲, 2% SDS solution; □, 25 mM NaCl solution; ○, water. Each bar represents the mean of three experiments (± S.D.).

3.3 Accumulation and Removal of Harmful DNA-Intercalating Pollutants and Endocrine Disrupters

We examined the accumulation and removal of DNA-intercalating pollutants— EB and AO and endocrine disrupters—BP, DBF, and DBD by the DNA-loaded PSf microspheres. The microspheres were incubated in the aqueous solutions of these compounds, respectively. Then the amounts of the resulting compounds in solution were measured by a UV-VIS spectrophotometer. The white PSf microspheres became red when they were incubated in EB solution and changed to orange when incubated in AO solution. Figure 3 shows the absorption spectra of their aqueous solutions in the absence or presence of DNA-loaded microspheres. The absorption peaks of these reagents disappeared following the addition of the microspheres. Over 90% of the EB, BP, and DBF in their solutions were removed by the microspheres; almost all the AO was removed by the microspheres; and approximately 80% of the DBD was removed.

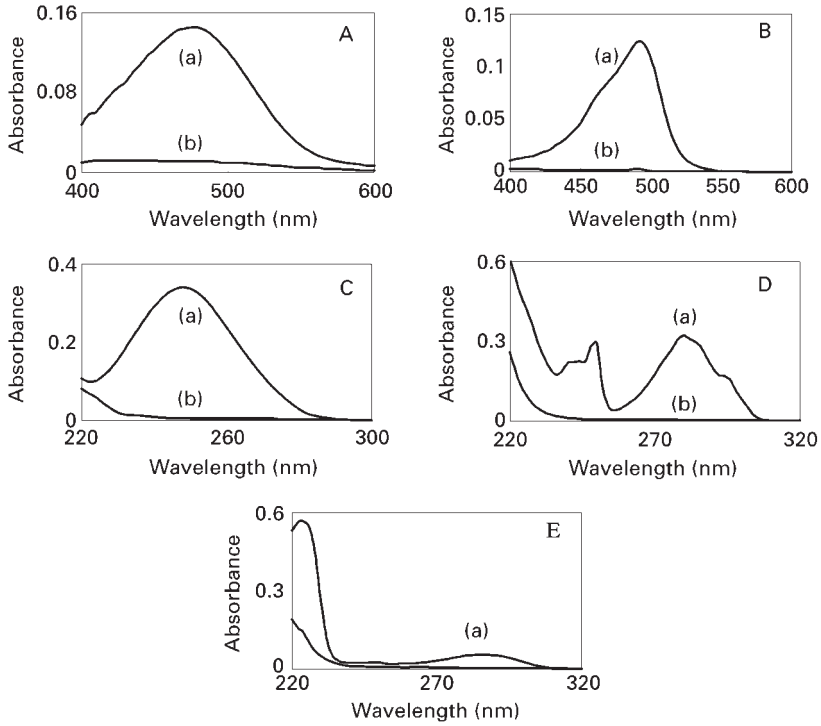

Figure 3. Absorption spectra of aqueous solutions of DNA-intercalating compounds in the absence (a) and presence (b) of DNA-loaded PSf microspheres. (A) EB solution (5 mM), (B) AO solution (5 μM), (C) BP solution (20 μM), (D) DBF (20 μM), and (E) DBD (20 μM) solution.

EB is a well-known double-stranded DNA-intercalating reagent. When the DNA-loaded PSf microspheres were added to the EB solution, they dyed red, due to the binding of EB. The microspheres without DNA also removed a small amount of EB, due to the porosity. However, the white microspheres did not become red. The AO solution also gave similar results. When the PSf microspheres without DNA were incubated in the endocrine disrupters' solutions, the endocrine disrupters bound to the microspheres, due to the hydrophobic interaction between the endocrine disrupters and PSf and the microsphere porosity. However, the removal of the endocrine disrupters was not specific. The removal of endocrine disrupters by double-stranded DNA was specific; only the disrupters with a planar structure were selectively bound. With the increase of the DNA amount incorporated into the PSf microspheres, the removal rate increased.

Additionally, with the increase of the concentrations of endocrine disrupters, the removal ratios increased. However, when a very high or very low concentration was used, there was no difference between the removal ratios for different endocrine disrupters (data not shown).

3.4 Accumulation and Removal of Endocrine Disrupters by DNA-Loaded PSf Microsphere Columns

Since the DNA-loaded PSf microspheres could remove large amounts of DNA-intercalating compounds from their aqueous solutions, we prepared the microsphere column to more effectively remove the DNA-intercalating pollutants and endocrine disrupters. Figure 4 shows the removal rate of these compounds by the DNA-loaded PSf microsphere column for each circulating time, using a mixed solution containing AO, BP, and DBF. The removal rate was defined as:

$$R = \frac{C_{\mathrm{I},n} - C_{\mathrm{F}}}{C_{\mathrm{I}}}$$

where $C_{\mathrm{I},n}$ is the initial concentration of endocrine disrupters for the n times circulation, $n = 1$, 2, 3, 4, and 5, respectively; C_{F} is the final concentration

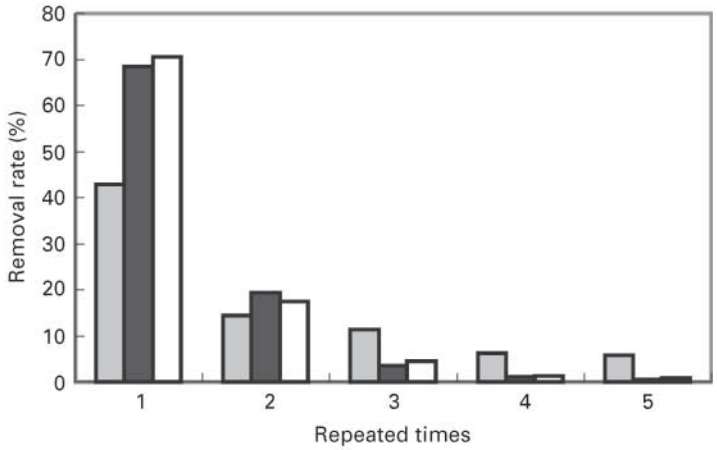

Figure 4. Removal rate of DNA-intercalating compounds by the DNA-loaded PSf microsphere column for each circulating time. *Key:* ■, AO (20 μM); ■, BP solution (20 μM); □, DBF (20 μM).

after DNA-loaded PSf microspheres were injected into the solution; and C_I is the initial concentration of endocrine disrupters.

The white PSf microsphere dyed orange after the solution flew through the column. During the first circulation, about 44% of the AO was removed by the microsphere column; while approximately 70% of the BP and DBF were removed. With the increase of circulating time, the removal rate decreased rapidly. After the third circulation, almost all the BP and DBF were removed.

The removal ratio sharply decreased from the first cycle (70% for BP solution) to the second cycle (20% for BP solution), as shown in Fig. 4. It is easy to understand from the formula $R = (C_{I,n} - C_F)/C_I$. After the first cycle, only about 30% of the BP remained in the mixture solution; thus, the removal ratio was very low.

The removal rate of AO from the mixed solution by the DNA-loaded PSf microspheres was lower than that of BP and DBF. The double-stranded DNA accumulated in BP and DBF; the PSf also bound BP and DBF, due to the hydrophobic interaction and the microsphere porosity. However, the PSf microspheres adsorbed AO only due to the porosity. The mixed solution containing AO, BP, and DBF was applied to the DNA-loaded PSf microsphere column five times, while the solution was incubated with the microsphere for 24 hr. However, the total amounts of these compounds removed by the column five times were almost the same as those by the microspheres. These results suggest that the DNA-loaded PSf microsphere column was an extremely effective method to bind the DNA-intercalating pollutants and endocrine disrupters.

3.5 Accumulation of Metal Ions into DNA-Loaded PSf Microspheres

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

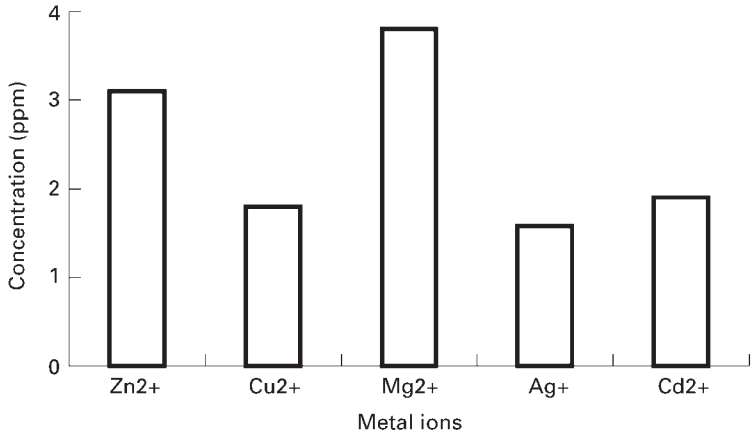

Figure 5. Concentrations of metal ions after accumulation by DNA-loaded PSf microspheres. Aqueous metal ion solutions (4 ppm) were applied.

incorporated into the PSf microspheres could selectively accumulate heavy metal ions.

It has been reported that heavy metal ions could selectively be removed by Cibacron Blue F3GA and chitosan flakes.[14,15] Polyethylene glycol methacrylate gel beads carrying Cibacron Blue F3GA could selectively remove lead ions.[14] Commercial chitosan also adsorbed heavy metals like zinc, copper, cadmium, and lead from their aqueous solutions.[15] The DNA-loaded PSf microspheres also selectively bind heavy metal ions, due to the incorporation of double-stranded DNA. The selective accumulation of heavy metal ions by the double-stranded DNA was proposed through the inter-nucleic acid bases and phosphate groups.[7]

CONCLUSIONS

Double-stranded DNA was incorporated into PSf porous microspheres using a liquid–liquid transition technique. The DNA was stable in water and neutral solutions. The DNA-loaded PSf microspheres and the microsphere column could effectively remove DNA-intercalating harmful pollutants, endocrine disrupters, and heavy metal ions from their aqueous solutions. These results suggest that DNA-loaded PSf microspheres and the column have a potential to serve as a useful biomaterial for medical, engineering, and environmental applications.

As we know, DNA is expensive. However, the amount of DNA in the PSf microspheres is very low, which means that a small amount of DNA could produce a large amount of PSf microspheres. Also, the amount of endocrine disrupters is very low, so only a small amount of the DNA-loaded PSf microspheres is needed to purify tons of water. Additionally, DNA can be purified from either salmon milts or shellfish gonads, which are generally discarded as waste. In fact, the DNA used in our study is not very expensive. So, it is practical to use DNA for the environmental application of endocrine disrupter removal, as well as economically feasible.

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REFERENCES

1. Kawaguchi, H. Functional microspheres. Prog. Polym. Sci. **2000**, *25*, 1171–1210.
2. Saenger, W. *Principles of Nucleic Acid Structure*; Springer: Berlin, 1987.
3. Waring, M.J. DNA modification and cancer. Annu. Rev. Biochem. **1981**, *50* (1), 159–192.
4. Berman, H.M.; Young, P.R. The interaction of intercalating drugs with nucleic acids. Annu. Rev. Biophys. Bioeng. **1981**, *10* (1), 87–114.
5. Yamada, M.; Kato, K.; Nomizu, M.; Ohkawa, K.; Yamamoto, H.; Nishi, N. UV-irradiated DNA matrixes selectively bind endocrine disruptors with a planar structure. Environ. Sci. Technol. **2002**, *36*, 949–954.
6. Yamada, M.; Kato, K.; Nomizu, M.; Sakairi, O.; Ohkawa, K.; Yamamoto, H.; Nishi, N. Preparation of insolubilized-DNA film with three-dimensional network and removal of endocrine disruptors. Nucl. Acids Symp. Ser. **2000**, *44*, 255–256.
7. Yamada, M.; Kato, K.; Nomizu, M.; Sakairi, N.; Ohkawa, K.; Yamamoto, H.; Nishi, N. Accumulation and removal of heavy metal ions by insolubilized-DNA and its interaction. Nucl. Acids Symp. Ser. **2000**, *44*, 221–222.
8. Zhao, C.S.; Liu, X.D.; Rikimaru, S.; Nomizu, M.; Nishi, N. Surface modification of polysulfone membranes modified by DNA immobilization. J. Membr. Sci. **2003**, *179*, 214–225.

9. Zhao, C.S.; Liu, X.D.; Rikimaru, S.; Nomizu, M.; Nishi, N. Blood compatible aspects of DNA-modified polysulfone membrane—protein adsorption and platelet adhesion. Biomaterials **2003**, *24*, 3747–3755.
10. Zhao, C.S.; Liu, X.D.; Rikimaru, S.; Nomizu, M.; Nishi, N. Preparation of polysulfone hollow microspheres encapsulating DNA and its functional utilization. J. Microencapsul. *in press*.
11. Zhao, C.S.; Zhou, X.S.; Yue, Y.L. Determination of pore size and pore size distribution on the surface of hollow fiber filtration membranes—a review of methods. Desalination **2000**, *129*, 107–123.
12. Yamada, M.; Kato, K.; Shindo, K.; Nomizu, M.; Sakairi, O.; Ohkawa, K.; Yamamoto, H.; Nishi, N. UV-irradiation-induced immobilization and functional utilization of DNA on nonwoven cellulose fabric. Biomaterials **2001**, *22*, 3121–3126.
13. Petersen, B.O.; Shuman, S. DNA strand transfer reactions catalyzed by vaccinia topoisomerase: hydrolysis and glycerololysis of the covalent protein–DNA intermediate. Nucl. Acids Res. **1997**, *25* (11), 2091–2097.
14. Buyuktuncel, E.; Tuncel, A.; Genc, O.; Denizli, A. Selective removal of lead ions by polyethylene glycol methacrylate gel beads carrying Cibacron Blue F3GA. Sep. Sci. Technol. **2001**, *36* (15), 3427–3438.
15. Bassi, R.; Prasher, S.O.; Simpson, B.K. Removal of selected metal ions from aqueous solutions using chitosan flakes. Sep. Sci. Technol. **2000**, *35* (4), 547–560.