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# A Novel Method to Prepare Magnetic Polymer-Based Anion Exchangers and Their Application

**Yu-Zong Lin, Tzu-Hsien Wang, Yu-Sheng Lin, Wei-Chih Kuan, Wen-Chien Lee**
Department of Chemical Engineering, Systems Biology and Tissue Engineering Research Center, National Chung Cheng University, Min-Hsiung, Chia-Yi 621, Taiwan
Correspondence to: W.-C. Lee (E-mail: chmwcl@ccu.edu.tw)

**ABSTRACT:** Magnetic microspheres with ion-exchange features were prepared by applying a swelling and penetration process using polystyrene–divinylbenzene-based anion-exchange resins as starting materials. The polymeric anion-exchange particles were swollen with an aqueous solution of *N*-methyl-2-pyrrolidone, followed by incubation with superparamagnetic iron oxide nanoparticles to allow them to penetrate into the swollen particles. The pH value in the solution of magnetic nanoparticles could significantly influence the uptake of magnetic nanoparticles by the swollen anion-exchange particles. Higher amounts of magnetic nanoparticles entrapped within anion exchangers could be achieved at pH 10–12. An increase in the concentration of magnetic nanoparticles led to a higher density of magnetic nanoparticles entrapped within the interior of anion exchangers and, thus, higher magnetization of the magnetic anion exchangers. Loading of the magnetic nanoparticles onto the exchanger had no effect on anion-exchange functionality. The utility of the resulting magnetic anion-exchange resins was demonstrated for the isolation of plasmid pEGFP-C1 from *Escherichia coli* cell lysates. The magnetic anion-exchange microspheres could be easily collected within a few seconds in a magnetic field. Thus, automation of the protocol for DNA isolation using these magnetic anion-exchange resins has a high potential. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40725.

**KEYWORDS:** applications; magnetism and magnetic properties; nucleic acids

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## INTRODUCTION

Magnetic polymer microspheres have a wide range of biological applications, including enzyme immobilization, nucleic acid sequencing, and separation of biomolecules (DNA, RNA, and protein) and cells.[1–7] The use of magnetic microspheres has the advantage of allowing quick and one-step purification of biological substances without centrifugation. For their use in biological applications, it is necessary to introduce an active functional group on the surface of magnetic microspheres for covalent or noncovalent binding of biomolecules. Magnetic ion exchange resins are polymer particles with a combination of magnetic and ion-exchange functions. Polymer-based magnetic ion exchangers in the micron size have been used for the isolation and purification of proteins[8–11] and DNA.[12] They are recently used for the extraction of siRNA from human serum.[13]

In general, the preparation of magnetic ion exchangers involves two steps: first, the preparation of magnetic microparticles, and second, the addition of functional groups with ion-exchange function. Magnetic microparticles are commonly produced by two-phase polymerization of monomers with magnetic nanoparticles. In the conventional oil-in-water suspension polymerization method, the system is simply made of monomers, magnetic nanoparticles, an initiator, and a solvent in the organic phase, which form an emulsion suspended in the aqueous phase. After polymerization, magnetic nanoparticles are uniformly distributed in magnetic polymer particles. The resulting magnetic polymer particles are further chemically modified and associate with positively or negatively charged ionic functional groups to form magnetic ion exchange resins.[14] Sometimes, a charged group-containing monomer like methacrylic acid may be involved as one of the monomers and then the polymerized particles without further modification are basically magnetic ion exchangers. For example, carboxylate-modified magnetic particles could be obtained by the polymerization of methacrylic acid, acrylamide, and *N*,*N*′-methylenebisacrylamide in the presence of $Fe_3O_4$ particles.[15]

Although the magnetic polymer particles can be chemically modified and associated with positively or negatively charged ionic functional groups to form magnetic ion exchange resins, these modifications sometime involve the use of a strong acid, base, or solvent, conditions in which magnetic materials may be

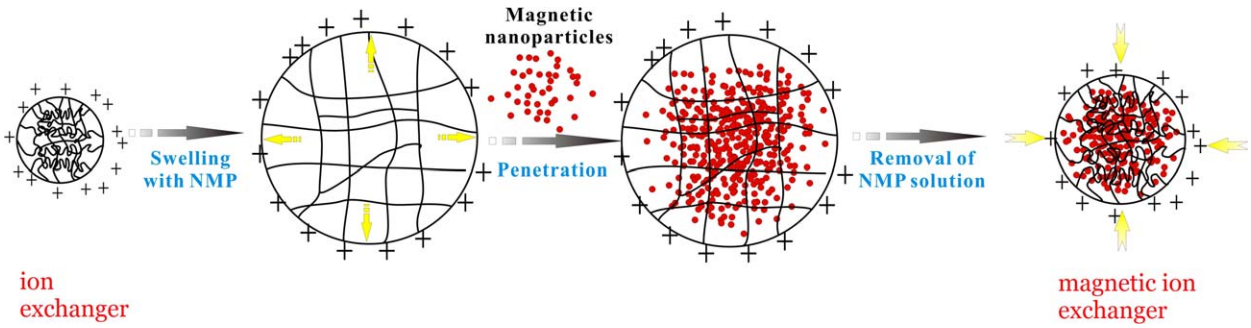


**Figure 1.** Schematic diagram of preparing a magnetic anionic exchanger made from ion-exchange polymer microspheres and magnetic nanoparticles by applying the swelling and penetration method. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

lost due to dissolving or leaking out. To solve this problem and to enable the preparation of magnetic microparticles with functionalities of interest, we here propose a simple way to produce magnetic ion exchangers by changing the order, i.e., preparing the ion exchanger first, followed by addition of the magnetic property. Styrene-based polymer particles with functional groups on the surface are able to incorporate magnetic nanoparticles by applying a swelling and penetration process. According to our previous works,[16,17] microsized styrene-based polymer particles can be swollen in an aqueous solution of *N*-methyl-2-pyrrolidone, which will be then ready for the uptake of superparamagnetic nanoparticles during incubation with them. In the present study, commercially available anion exchangers were used as starting material for the preparation of magnetic anion exchangers, and the latter were tested for their applicability to isolate plasmid DNA.

## EXPERIMENTAL

### Materials

Three anionic exchange resins (polymer-bound diisopropylamine, Lewatit® MonoPlus M500, and Lewatit® MonoPlus MP64) were used as starting materials. Lewatit MonoPlus M500 and MP64, purchased from Fluka, are cross-linked polystyrene–divinylbenzene (PS-DVB) polymer particles. Lewatit MonoPlus M500 with an average particle diameter of 0.62 mm contains surface functionality of -[Ph-$(CH_2)$-$N(CH_3)_3$]$^+$. Lewatit MonoPlus MP64 with an average particle diameter of 0.59 mm is a weak basic anion exchanger containing quaternary amine. Polymer-bound diisopropylamine (Sigma 538736-5G) having a particle size of 100–200 mesh (75–150 μm), purchased from Sigma (St. Louis, MO), is a polymer ion exchanger with PS-DVB base material cross-linked with 1% divinylbenzene.

### Preparation of Magnetic Ion Exchangers

Figure 1 shows the schematic diagram of the preparation of a magnetic anionic exchanger by using a swelling and penetration process. Briefly, 0.25 g of anion-exchange resin was immersed into a solution consisting of *N*-methyl pyrrolidinone (NMP) and water at a volume ratio (v/v) of 3 : 4, i.e., 15 mL of NMP and 20 mL of water. The NMP/water mixture with ion exchangers was left to soak for 24 h at 25°C while stirring (200 rpm).

Subsequently, 2.5 mL of the superparamagnetic nanoparticle dispersion (10 mg/mL or other specified concentrations) was added to the mixture of anion exchangers and NMP/water solution, and the pH of this mixture was adjusted to 3–12, depending on the different experimental settings. The resulting preparation was incubated at 30°C with shaking (at 200 rpm) for 2 days to allow the magnetic nanoparticles to penetrate into the interior of the anion-exchange particles. Afterward, the particles were separated from the solution by filtration, rinsed three times, and air dried to obtain the magnetic anion-exchange resin. Superparamagnetic nanoparticles ($Fe_3O_4$) with an average particle size of about 10 nm were prepared by using a co-precipitation method, as described previously.[17,18]

### Isolation of Plasmid DNA from Cell Lysates

*Escherichia coli* harboring the plasmid pEGFP-C1 was grown in 30 mL of Luria broth (LB) medium containing 100 μg/mL kanamycin, and the resulting culture was placed in a shaker incubator at 150 rpm for 16–18 h at 37°C. Next, the *E. coli* broth was transferred to a conical flask containing 250 mL LB medium and 100 μg/mL kanamycin and incubated for further cultivation of the bacteria at 150 rpm overnight at 37°C. The above broth culture of *E. coli* was scaled-up in a flask containing 3 L LB medium and 100 μg/mL kanamycin and incubated at 150 rpm for 5 h at 37°C. After that, the supernatant was removed by centrifugation at 14,904 × *g* for 20 min at 4°C to harvest the cell pellet. The pellet was resuspended in 84 mL G/T/E buffer (50 m*M* glucose, 10 m*M* EDTA, 25 m*M* Tris-HCl, and 144 mg lysozyme, pH 8.0), and the resulting mixture was incubated on ice for 20 min. Next, 144 mL of freshly prepared NaOH/sodium dodecyl sulfate cold denaturation solution composed of 0.2*N* NaOH and 1% sodium dodecyl sulfate was added, and the resulting solution was thoroughly mixed and incubated on ice for 10 min. Next, 90 mL of 3*M* sodium acetate (pH 5.0) was added, and the resulting solution was mixed and incubated on ice for 20 min. Finally, the supernatant was collected by centrifugation at 4°C at 10,380 × *g* for 20 min yielding the crude cell extract, i.e., cell lysate.

For the isolation of plasmid DNA, 10 mg of magnetic anion-exchange resin (prepared from either Lewatit MonoPlus M500 or polymer-bound diisopropylamine) was thoroughly mixed with 1 mL of crude cell extract and 1 mL of binding buffer (10 m*M* Tris-HCl, 1*M* NaCl, and 1 m*M* EDTA, pH 6.5) for 5 min. DNA in the mixture was selectively adsorbed onto magnetic anion-exchange particles, which were then collected by using the magnetic separator MACSiMAG (Miltenyi Biotec). The supernatant was removed after washing three times with 2 mL of washing buffer (10 m*M* Tris-HCl and 1 m*M* EDTA, pH 8). The purified

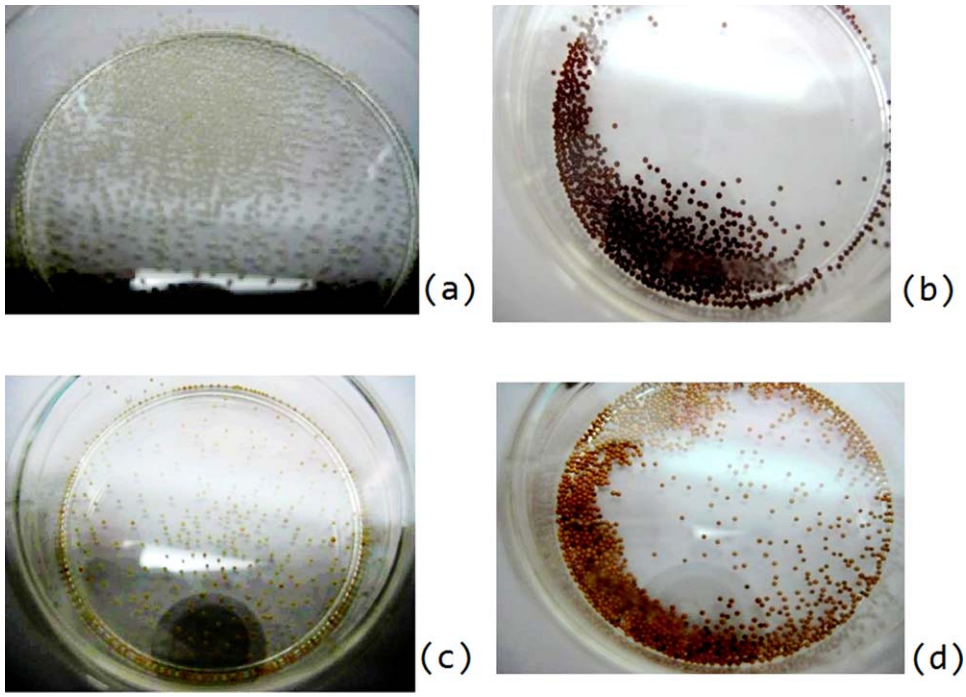


**Figure 2.** Photographs of anion-exchange resins (a and c are Lewatit MonoPlus M500 and MP64, respectively) and magnetic anion-exchange resins prepared from the anion-exchange resins Lewatit MonoPlus M500 (b) and Lewatit MonoPlus MP64 (d). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

plasmid DNA solution was obtained after detachment (twice) by adding 1 mL of desorption buffer (1.5*M* NaCl, 0.01*M* Tris-HCl, and 1 m*M* EDTA, pH 9.0). The $A_{260}/A_{280}$ ratio was determined by using the NanoDrop 1000 spectrophotometer. The RNA concentration was measured by using the Qubit fluorometer, and the protein concentration was measured by applying the Bradford protein assay method. As a control, 10 mg anion-exchange resin Lewatit MonoPlus M500 was used for the isolation of DNA from a crude cell extract by following the same procedure.

## RESULTS

### Preparation of Magnetic Anionic Exchange Microspheres

Figure 2 shows the photographs of the anion-exchange resins Lewatit MonoPlus M500 and Lewatit MonoPlus MP64 before and after treatment according to the proposed method. The two anion-exchange resins were immersed in NMP solution for 24 h and then further immersed and swollen in $Fe_3O_4$ magnetic nanoparticle solution for 2 days [Figure 2(b,d)]. After rinsing and air-drying, the obtained magnetic ion-exchange resins were darker in color, which confirmed that the magnetic nanoparticles have been incorporated into the ion-exchange resin.

To determine the effect of the pH value on the diffusion of $Fe_3O_4$ nanoparticles into the ion-exchange resin, we measured the amounts of $Fe_3O_4$ particles diffusing into the anion-exchange resin Lewatit MonoPlus M500 at various pH values by using atomic absorption spectroscopy, as shown in Figure 3. The amount of $Fe_3O_4$ particles diffusing into the anion-exchange resin changed with the pH values, indicating that the pH value influenced the amount of $Fe_3O_4$ particles incorporated into the interior of the ion-exchange resin. The amount of $Fe_3O_4$ particles contained in the anion-exchange resin increased gradually with pH until pH 7, whereas it became double when

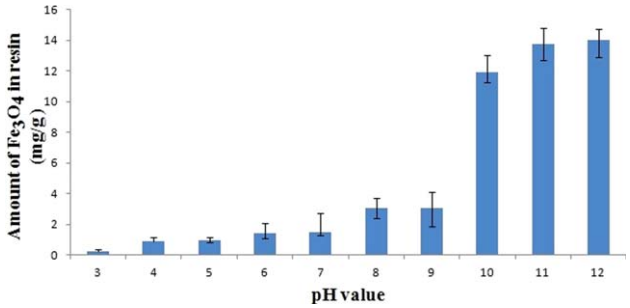


**Figure 3.** Effect of the pH value of the solution on the diffusion of $Fe_3O_4$ nanoparticles into an ion-exchange resin of Lewatit MonoPlus M500. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

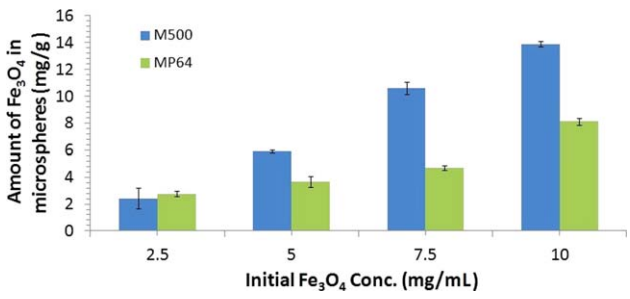


**Figure 4.** The amount of $Fe_3O_4$ entrapped within the ion-exchange resin changes with the initial concentration of $Fe_3O_4$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

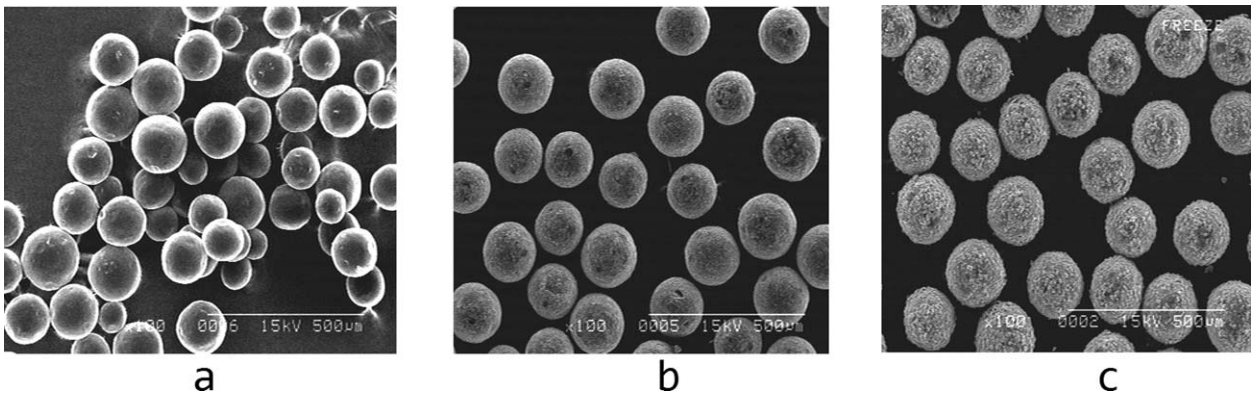


**Figure 5.** Scanning electron microscopy images of anion exchanger polymer-bound diisopropylamine (a) and magnetic anion-exchange resins prepared from this anion exchanger using 5 mg/mL (b) or 10 mg/mL (c) magnetic iron oxide nanoparticles.

the pH raised from 7 to 8–9; at a pH of 10–12, the amount of incorporated $Fe_3O_4$ particles was eight to nine times higher than that at neutral conditions (pH 7). Thus, diffusion occurs under strong alkaline conditions.

To elucidate the relationship between the amount of $Fe_3O_4$ adsorbed by the ion-exchange resin and the initial concentration of $Fe_3O_4$ solution, we measured the amount of $Fe_3O_4$ entrapped within two ion-exchange resins (Lewatit MonoPlus M500 and MP64 were separately used) under various initial $Fe_3O_4$ concentrations by using atomic absorption spectroscopy. Figure 4 shows the amount of $Fe_3O_4$ entrapped within the ion-exchange resin in relation to the initial concentration of $Fe_3O_4$. The amount of $Fe_3O_4$ incorporated into the ion-exchange resin increased with increasing initial concentration of $Fe_3O_4$ incubated with the ion-exchange resin at pH 10. The amount of $Fe_3O_4$ incorporated into the ion-exchange resin was $8.12 \pm 0.38$ mg/g resin (on dry basis) obtained from the anion-exchange resin Lewatit MonoPlus MP64 when an initial concentration of 10 mg/mL $Fe_3O_4$ was used. Under the same conditions, the amount of $Fe_3O_4$ incorporated into Lewatit MonoPlus M500 was $13.9 \pm 0.17$ mg/g resin, and the saturation magnetization was 1.68 emu/g.

This example illustrates that a higher initial concentration of $Fe_3O_4$ resulted in a higher amount of magnetic material incorporated into the magnetic ion-exchange resin and a shorter time required for separating the ion-exchange resin from the solution in the magnetic field. As a result, the time required to separate magnetic Lewatit MonoPlus M500 obtained from the initial concentration of 10 mg/mL $Fe_3O_4$ was the shortest in the magnetic field, i.e., about 1 s using Dynal MPC-E1 magnetic particle separator from Dynal Biotech.

Magnetic anion-exchange resins were also prepared from the anion exchanger polymer-bound diisopropylamine (which has a smaller particle size) using 5 or 10 mg/mL magnetic iron oxide nanoparticles. The resulting magnetic ion-exchange resin particles had a spherical shape with uniform diameter distribution, which could be seen on scanning electron microscopy photographs. According to the observation on scanning electron microscopy images (Figure 5), the average particle diameter of the spherical anion exchanger increased from $144 \pm 23$ to $176 \pm 14$ $\mu$m after entrapment with magnetic nanoparticles by incubating the swollen anion exchanger with a magnetic nanoparticle solution (5 mg/mL $Fe_3O_4$). When the concentration of the $Fe_3O_4$ magnetic nanoparticle solution was 10 mg/mL, the average particle diameter of the resulting magnetic anion exchanger was $194 \pm 16$ $\mu$m. Thus, the particle diameter of the magnetic ion-exchange resin increased with increasing amounts of $Fe_3O_4$ magnetic nanoparticles incorporated into the ion-exchange resin. The saturation magnetization of the magnetic ion-exchange resin manufactured from the 10 mg/mL $Fe_3O_4$ magnetic nanoparticle solution was determined to be $3.60 \pm 0.16$ emu/g. Compared with the nonmagnetic ion-exchange resin, the suspension of the magnetic ion-exchange resin is apt to be separated by using a simple magnetic separator, e.g., Dynal MPC-E1 (Dynal Biotech). Capturing the magnetic ion-exchange resin manufactured from the 10 mg/mL

**Table I.** Results of Plasmid DNA Purification Using 10 mg of Magnetic Anion Exchanger Prepared from Lewatit MonoPlus M500 ($n = 3$)

| Solution | $A_{260}/A_{280}$ | Protein content ($\mu$g/mL) | RNA content ($\mu$g/mL) | DNA ($\mu$g) |
|---|---|---|---|---|
| Supernatant of cell lysate | 2.17 | $114.7 \pm 1.61$ | 648 | $628.27 \pm 8.56$ |
| Residue after adsorption | $2.18 \pm 0.03$ | $80.6 \pm 1.06$ | 579.2 | $547.6 \pm 4.92$ |
| First washing solution | $2.09 \pm 0.09$ | $17.26 \pm 0.53$ | 68.4 | $28.2 \pm 1.31$ |
| Second washing solution | $1.9 \pm 0.59$ | $7.79 \pm 2.15$ | 8.7 | $3.33 \pm 1.74$ |
| Third washing solution | $1.46 \pm 0.32$ | $6.82 \pm 2.11$ | N/A | $2.4 \pm 1.15$ |
| First elution | $1.79 \pm 0.57$ | $0.51 \pm 0.15$ | 0.576 | $30.77 \pm 5.7$ |
| Second elution | $1.8 \pm 0.45$ | $1.21 \pm 0.53$ | 0.5 | $16 \pm 1.87$ |

**Table II.** Results of Plasmid DNA Purification Using 10 mg of Lewatit MonoPlus M500 (Nonmagnetic Microparticles) ($n = 3$)

| Solution | $A_{260}/A_{280}$ | Protein content ($\mu$g/mL) | RNA content ($\mu$g/mL) | DNA ($\mu$g) |
|---|---|---|---|---|
| Supernatant of cell lysate | 2.2 ± 0.02 | 112.56 ± 3.7 | 462 | 635.43 ± 17.82 |
| Residue after adsorption | 2.15 ± 0.03 | 76.62 ± 1.04 | 365.2 | 542.37 ± 11.4 |
| First washing solution | 2.08 ± 0.09 | 18.76 ± 0.3 | 24.2 | 28.7 ± 1.69 |
| Second washing solution | 1.57 ± 0.68 | 6.35 ± 1.29 | 25 | 3.56 ± 1.96 |
| Third washing solution | 0.99 ± 0.37 | 1.34 ± 0.36 | N/A | 2.1 ± 1.01 |
| First elution | 1.94 ± 0.04 | 4.28 ± 0.77 | 5.66 | 27.4 ± 2.29 |
| Second elution | 1.94 ± 0.1 | 4.61 ± 1.27 | 5.74 | 11.9±2.8 |

$Fe_3O_4$ magnetic nanoparticle solution by using the magnetic base took only 4 s. The time required when using the above magnetic ion-exchange resins was shorter than that required when using nonmagnetic ion-exchange resins (average time: 42 s). The reason for this is that nonmagnetic ion-exchange resins settle to the bottom of the test tube only by gravity, and parts of the resin particles are attached to the inner surface of test tube. As demonstrated in this example, using the magnetic ion-exchange resin has advantages because it can be quickly collected and separated from the solution by using the magnetic field.

### Application of Magnetic Anionic Exchange Microspheres for DNA Purification

The prepared magnetic anion exchangers were used for the isolation of plasmid DNA from an *E. coli* extract. Table I shows $A_{260}/A_{280}$ ratio, protein concentration, RNA concentration, and DNA concentration in the supernatant before purification (i.e., the supernatant of the cell lysate), adsorbed residual solution, first wash supernatant, second wash supernatant, third wash supernatant, first desorption solution, and second desorption solution. Absorbance at 260 nm ($A_{260}$) is a measurement of the DNA concentration and absorbance at 280 nm ($A_{280}$) corresponds to the protein concentration; thus, the higher the $A_{260}/A_{280}$ ratio, the higher the DNA purity. With the 10 mg ion-exchange resin Lewatit MonoPlus M500 selected as control group, the results of the purified plasmid DNA separated from the crude cell extract are shown in Table II.

As shown in Table I, the $A_{260}/A_{280}$ ratio of the crude cell extract was 2.17 before purification. The $A_{260}/A_{280}$ ratios of the first and second desorption (elution) solutions were reduced to about 1.80. DNA is considered pure when the $A_{260}/A_{280}$ ratio is in the range of 1.8–1.9.[19] The RNA concentration was reduced to less than 1 $\mu$g/mL, and the protein concentration was reduced to less than 2 $\mu$g/mL. As shown in Table II, which shows the results of purified DNA when the nonmagnetic Lewatit MonoPlus M500 was used, the final $A_{260}/A_{280}$ ratio was 1.94. In that case, the RNA concentration was reduced to less than 6 $\mu$g/mL, and the protein concentration was reduced to less than 5 $\mu$g/mL. A comparison of the data shown in Tables I and II indicates that the purity of DNA was higher when the magnetic anion-exchange resin was used compared with when the nonmagnetic anion-exchange resin was used. As shown in Figure 6, RNA and DNA fragments were separated from the crude cell extract by a series of purification steps. Calculated from the total amounts of the first and second desorption solutions, the amount of plasmid DNA selectively adsorbed by the magnetic anion-exchange resin was 4.68 mg/g resin and that adsorbed by the nonmagnetic anion-exchange resin was 3.93 mg/g resin. The reason for the higher yield when using the magnetic anion-exchange resin is because plasmid DNA could be completely recovered.

Magnetic anion exchangers prepared from polymer-bound diisopropylamine (Sigma 538736-5G) were also suitable for plasmid DNA isolation. As shown in Table III, the magnetic anion-exchange resin could selectively adsorb DNA, as demonstrated by very small amounts of proteins and RNA in the desorption solutions. When combining the total amounts of DNA obtained in the first and second elution solutions, the amount of

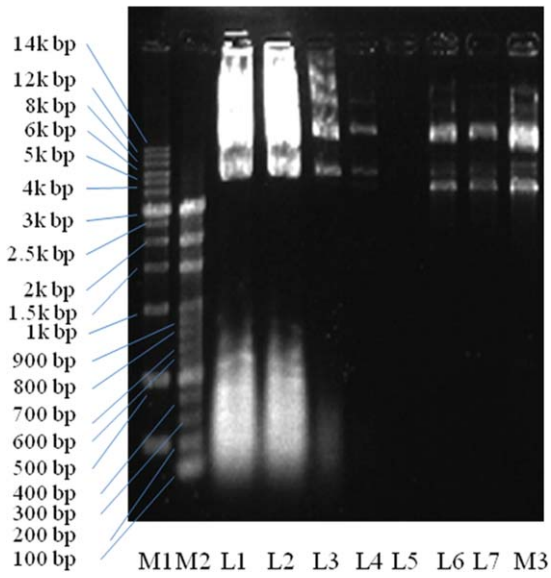


**Figure 6.** Agar gel electrophoresis of DNA obtained from different separation steps by using a magnetic ion-exchange resin (prepared from Lewatit MonoPlus M500), wherein M1: marker (200–14,000 bp), M2: marker (100–3000 bp), L1: crude cell extract, L2: residual adsorption solution, L3: first washing solution, L4: second washing solution, L5: third washing solution, L6: first desorption solution, L7: second desorption solution, and M3: pure plasmid pEGFP-C1. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

**Table III.** Results of Plasmid DNA Purification Using 10 mg of Magnetic Anion Exchanger Prepared from Polymer-Bound Diisopropylamine (Sigma 538736-5G) ($n = 3$)

| Solution | $A_{260}/A_{280}$ | Protein content ($\mu$g/mL) | RNA content ($\mu$g/mL) | DNA ($\mu$g) |
|---|---|---|---|---|
| Supernatant of cell lysate | 2.1 ± 0.01 | 100.47 ± 4.89 | 468 | 626.03 ± 7.64 |
| Residue after adsorption | 1.97 ± 0.01 | 71.09 ± 0.81 | 413.6 | 486.47 ± 13.63 |
| First washing solution | 1.88 ± 0.07 | 18.68 ± 0.7 | 60.8 | 55.73 ± 0.80 |
| Second washing solution | 2.10 ± 0.6 | 3.95 ± 0.48 | 8.02 | 12.80 ± 6.61 |
| Third washing solution | 1.98 ± 0.07 | 1.86 ± 0.48 | N/A | 5.07 ± 1.95 |
| First elution | 1.85 ± 0.03 | 0.70 ± 0.13 | 1.08 | 55.93 ± 4.76 |
| Second elution | 1.85 ± 0.01 | 1.09 ± 0.16 | 0.8 | 31.67 ± 4.88 |

selectively adsorbed plasmid DNA per gram of magnetic anion-exchange resin was 8.76 mg, which was higher than that in the above-mentioned case using magnetic Lewatit MonoPlus M500. The use of magnetic anion exchangers with smaller particle diameter resulted in a larger amount of adsorbed DNA per gram of anion exchanger.

## DISCUSSION

As demonstrated in this study, magnetic ion exchangers could be easily prepared from polymer microparticles with ion-exchange functionality. By applying a swelling and penetration process, styrene-based microparticles were swollen in an aqueous solution of NMP, followed by incubation with superparamagnetic iron oxide nanoparticles to allow them to be incorporated into the swollen particles. In the present work, commercially available PS-DVB-based anion exchangers were used instead of micron-sized, noncross-linked polystyrene or poly(styrene-glycidyl methacrylate) particles used in previous works.[16,17] Furthermore, the ionic functionality of the polymer microparticles could significantly influence the uptake of magnetic nanoparticles by polymer microparticles. The pH in the incorporation step was thus subjected to analysis and optimization. As shown in Figure 3, a higher amount of magnetic nanoparticles entrapped within anion exchangers could be achieved under strong alkaline conditions. The isoelectric point of $Fe_3O_4$ is about pH 6.8. When the pH is higher than 7, $Fe_3O_4$ is negatively charged and easily attaches to the anion-exchange resin, which subsequently allows easy incorporation of the magnetic nanoparticles into the anion-exchange resin.

The results shown in Figure 4 indicate that the amount of iron oxide entrapped within the anionic polymer microparticles increased with increasing amounts of iron oxide incubated with the microparticles. The first step of the proposed method is that polymer-based ion exchange resin particles swell and thereby expand their polymer network structure in a solution of a neutral polar solvent, e.g., NMP. The neutral polar solvent is miscible with water and most of the organic solvents. The magnetic nanoparticles are allowed to diffuse into the polymer network of the ion exchange resin during incubation of the ion exchanger and magnetic nanoparticles. After removal of the solvents, the magnetic nanoparticles are entrapped and the polymeric ion exchange resin becomes magnetic. The basic material of anion-exchange resins is polystyrene cross-linked with a few percentages of divinylbenzene. Because of the cross-linked networks of the ion-exchange resin, magnetic nanoparticles being incorporated into the ion-exchange resin are not apt to leak out thereof. Thus, the magnetic nanoparticles of the magnetic ion-exchange resin are located in the network structure of polymer chains. As shown in Figure 2, the color of the anion exchangers became dark once the magnetic nanoparticles were entrapped within the polymer network. No leakage of nanoparticles from the magnetic anion exchanger was seen during storage and application. Repeated use of the magnetic anion-exchange resins did not cause an increase in collection time in magnetic separator, suggesting that the loss of magnetic nanoparticles from anion-exchange resins was minimal. As shown in transmission electron microscopy images in a previous work, magnetic nanoparticles could be found on the entire body of the microparticles and most of the magnetic nanoparticles were incorporated into the swollen styrene-based polymer and stayed in the interior of the polymeric microparticles.[17]

The magnetic anion-exchange resins prepared using either Lewatit MonoPlus M500 or polymer-bound diisopropylamine were very effective for the isolation and purification of plasmid DNA from *E. coli* cell lysates. Plasmid DNA was selectively adsorbed on the magnetic anion exchanger at pH 6.9 due to ionic interactions. After washing to remove unbound components, adsorbed DNA was eluted from the magnetic anion exchanger by using a buffer of pH 9. The functionality of the anion exchanger was unaffected by loading of the magnetic nanoparticles using the proposed protocol. A comparison of Tables I and II suggests that using the magnetic anion exchanger even led to a better result of DNA isolation. Lesser amounts of RNA and protein were present in the elution solutions. Thus, the $A_{260}/A_{280}$ ratio was lower when a magnetic anion exchanger was used. Typically, pure DNA has an $A_{260}/A_{280}$ ratio of 1.8, whereas RNA has a ratio of 2.0. Thus, an $A_{260}/A_{280}$ ratio closer to 2.0 than 1.8 suggests contamination with RNA. In summary, the prepared magnetic anion-exchange resin was superior to the nonmagnetic anion-exchange resin, loading of magnetic nanoparticles had no effect on the ion-exchange function thereof, and DNA-selective adsorption was applied for separation and purification by using surface functional groups. At the same time, the magnetic anion-exchange resin is stable, and the magnetic material is not apt to leak out in applications.

Tables I and III indicate that anion exchangers of smaller particle size result in higher adsorption density of DNA. The average diameter of the original anion exchanger Lewatit MonoPlus M500 is 0.62 mm, whereas the particle size range for the original anion exchanger polymer-bound diisopropylamine is 75–150 μm. Plasmid DNA binding capacities for these two magnetic anion exchangers were determined to be 4.68 and 8.76 mg/g resin, respectively; thus, the smaller the particle size, the larger the specific surface area. To increase the DNA binding capacity, anion exchangers with smaller particle size can be used. As the particle size decreased to 1 μm, for example, the adsorption capacity of plasmid DNA reached 90 mg/g resin in a buffer solution of pH 6.5 containing 0.5*M* NaCl.[12] Nano-sized magnetic particles have even a higher DNA adsorption capacity. Positively charged poly(hydroxyethyl methacrylate-*N*-methacryloyl-(L)-histidine) magnetic nanoparticles (91.5 nm) can bind 154 mg/g of plasmid DNA.[20] Because of the flexibility of the method proposed in the present work, magnetic ion exchange resins with particle sizes ranging from submicron to millimeter could be effectively prepared in the same way.

Magnetic anion-exchange resins, as demonstrated in present work, are highly applicable for DNA separation because they can be easily collected using an external magnetic field. In contrast to magnetic particles of nanometer size, the time required for collecting micron-sized magnetic particles from a well-mixed suspension using a magnet is much shorter. In the present work, for example, using a magnetic device (Dynal MPC-E1), it took only about 4–9 s to collect the magnetic anion exchanger, depending on the amount of nanoparticles loaded onto the polymer-bound diisopropylamine anion exchanger. If the magnetic anion exchangers were prepared by using Lewatit MonoPlus M500, the collection time could be decreased to just 1 s. Because of the shorter collection times identified for magnetic anion exchangers, the automation of every step in the DNA isolation protocol is highly possible. The whole process of DNA isolation from biological samples can thus be completed quickly in an automatic fashion.

## CONCLUSIONS

This study showed that magnetic anion-exchange microparticles could be prepared in a simple way using off-the-shelf anion-exchange microparticles. The amount of magnetic nanoparticles incorporated into the anion-exchange resins could be controlled by adjusting the pH and the concentration of nanoparticles used for the incubation of magnetic nanoparticles and anion-exchange microparticles. The magnetic anion-exchange microparticles obtained with the proposed method were stable and effective for the purification of plasmid DNA from bacterial lysates. Because the application of a magnetic field resulted in a shorter collection time for these magnetic anion exchangers, their use in the automation of this method for DNA isolation is highly possible.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Lea, T.; Vartdal, F.; Nustad, K.; Funderud, S.; Berge, A.; Ellingsen, T.; Schmid, R.; Stenstad, P.; Ugelstad, J. *J. Mol. Recognit.* **1988**, *1*, 9.
2. Ugelstad, J.; Stenstad, P.; Kilaas, L.; Prestvik, W. S.; Herje, R.; Berge, A.; Hornes, E. *Blood Purif.* **1993**, *11*, 349.
3. Bílková, Z.; Slováková, M.; Lycka, A.; Horák, D.; Lenfeld, J.; Turková, J.; Churácek, J. *J. Chromatogr. B* **2002**, *770*, 25.
4. Safarik, I.; Safarikova, M. *BioMagn. Res. Technol.* **2004**, *2*, 7.
5. Horák, D.; Babic, M.; Macková, H.; Benes, M. J. *J. Sep. Sci.* **2007**, *30*, 1751.
6. Corchero, J. L.; Villaverde, A. *Trends Biotechnol.* **2009**, *27*, 468.
7. Xin, L.; Cao, Z.; Lau, C.; Kai, M.; Lu, J. *Luminescence* **2010**, *25*, 336.
8. Heeboll-Nielsen, A.; Justesen, S. F. L.; Thomas, O. R. T. *J. Biotechnol.* **2004**, *113*, 247.
9. Franzreb, M.; Siemann-Herzberg, M.; Hobley, T. J.; Thomas, O. R. T. *Appl. Microbiol. Biotechnol.* **2006**, *70*, 505.
10. Yang, C.; Xing, J.; Guan, Y.; Liu, H. *Appl. Microbiol. Biotechnol.* **2006**, *72*, 616.
11. Käppler, T. E.; Hickstein, B.; Peuker, U. A.; Posten, C. *J. Biosci. Bioeng.* **2008**, *105*, 579.
12. Zhang, H.-P.; Bai, S.; Xu, L.; Sun, Y. *J. Chromatogr. B* **2009**, *877*, 127.
13. Ye, G.; Beverly, M. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 3207.
14. Lee, Y.; Rho, J.; Jung, B. *J. Appl. Polym. Sci.* **2003**, *89*, 2058.
15. Gai, Q.; Qu, F.; Zhang, T.; Zhang, Y. *Talanta* **2011**, *85*, 304.
16. Lee, W.-C.; Chung, T.-H. (**2010**). US Patent 7,754,278 B2.
17. Chung, T.-H.; Lee, W.-C. *React. Funct. Polym.* **2008**, *68*, 1441.
18. Wang, T.-H.; Lee, W.-C. *Biotechnol. Bioproc. Eng.* **2003**, *8*, 263.
19. Gallagher, S. R. *Curr. Protoc. Mol. Biol.* **2011**, *93*, A.3D.1.
20. Perçin, I.; Karakoç, V.; Akgöl, S.; Aksöz, E.; Denizli, A. *Mater. Sci. Eng. C* **2012**, *32*, 1133.