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Research Article

Facile Method for Preparation of Silica Coated Monodisperse Superparamagnetic Microspheres

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This paper presents a facile method for preparation of silica coated monodisperse superparamagnetic microsphere. Herein, monodisperse porous polystyrene-divinylbenzene microbeads were prepared by seeded emulsion polymerization and subsequently sulfonated with acetic acid/H_2SO_4. The as-prepared sulfonated macroporous beads were magnetized in presence of Fe^{2+}/Fe^{3+} under alkaline condition and were subjected to silica coating by sol-gel process, providing water compatibility, easily modifiable surface form, and chemical stability. FE-SEM, TEM, FT-IR, and TGA were employed to characterize the silica coated monodisperse magnetic beads (~7.5 μm). The proposed monodisperse magnetic beads can be used as mobile solid phase particles candidate for protein and DNA separation.

1. Introduction

Manipulation of biological objects at the cellular scale is necessary for both fundamental studies and the purification of biological products. For example, optical tweezers [1, 2] allow studying the strength of interactions between single molecules with piconewton accuracy such as DNA and protein. Due to the advantage of noncontact with biomolecules or cell during separation, the magnetic manipulation has been attracted in bioapplication over the several decades [3–9].

The dimension of the magnetic nano-/microparticles, the key factor for the successful magnetic separation, is usually less than 10 μm to ensure perfect and selective interaction with biological objects. These particles' surface must be modified with biocompatible inorganic or organic materials to both prevent aggregation caused by hydrophobic or magnetic attraction and facilitate the immobilization of specific ligands such as antibody, aptamer, and DNA [3, 10].

The magnetic microparticles have been attracted for use in bioapplication for many years, due to the advantages that they offer easily scalable, time-efficient, cost-effective, and gentle separation of biological compounds by using external magnetic field gradient [11–17]. The magnetic microspheres offered many exciting applications in biomedical field as a solid support for protein purification [18–20], targeted drug delivery [21–24], cell separation [25–27], medical diagnosis [28–30], immunoassays [31], DNA sequencing [32], and cell analysis [33]. Various materials have been used for matrices embedding superparamagnetic nanoparticles in the form of microbeads including mesoporous silica, polysaccharides, poly(ethylene glycol), and many other synthetic polymers [26, 34–39]. By doing so, the resulting microbeads retain superparamagnetic property with tunable size.

The porous PS-DVB (polystyrene-divinylbenzene) beads usually give a very rigid structure resulting from their highly cross-linked matrix and, thus, they had less possibility of collapsing in most solvents, which allows the solvents and

reagents to freely access the internal reactive sites through the interconnected pore networks [40–43]. The microbeads with pore networks can act as monodisperse core structures and provide the space within the beads where in situ forming nanomagnetite resides. Recently, we reported on the use of porous PS-DVB based monodisperse magnetic beads with SERS (surface-enhanced Raman scattering) active property (magnetic SERS beads) in protein separation [44, 45].

Despite extensive efforts made by many research groups, the demand for magnetic polymeric particles having uniform size, superparamagnetic property, chemical stability, user-defined magnetic contents, biocompatibility, and the readily tailorable surface is still increasing in potential application to biochips and bioseparation. In this paper, we demonstrate our strategy to prepare monodisperse porous PS-based superparamagnetic beads (~7.5 μm), which on surface were coated with silica that can result in improving biocompatibility and capability for facile surface modification [46–48].

2. Experimental

2.1. Materials. Tetraethylorthosilicate (TEOS), ammonium hydroxide (NH_4OH), styrene, 2,2′-azobis-isobutyronitrile (AIBN), dibenzoyl peroxide (BPO, including 97% of active compound), dibutyl phthalate (DBP), sodium silicate, $Fe_3Cl_3{\cdot}6H_2O$, $FeCl_2{\cdot}4H_2O$, ethylene glycol, *N,N*-dimethylformamide (DMF), sodium dodecyl sulfate (SDS), polyvinylpyrrolidone-40 (PVP-40, Mr: 40,000), *N,N*-diisopropylethylamine (DIEA), and (3-aminopropyl)triethoxysilane (APTS) were purchased from Sigma-Aldrich (MO, USA).

2.2. Methods

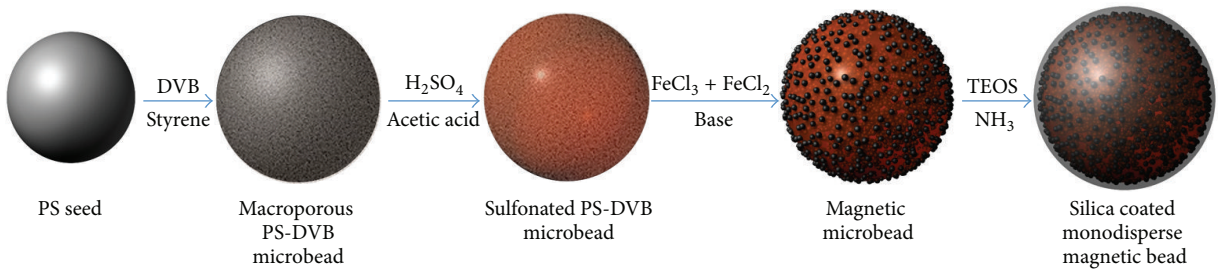
2.2.1. Preparation of Monodisperse Macroporous PS-DVB Beads. Monodisperse macroporous PS-DVB (polystyrene-divinylbenzene) beads were prepared by seeded polymerization methods. Monodisperse PS seeds (4 μm) were prepared using dispersion polymerization method [49]. Dispersion medium was ethanol/2-methoxyethanol (3 : 2) which contains 1 g of PVP-40 as a steric stabilizer (90 mL). AIBN (150 mg) was dissolved in styrene (15 mL) in which inhibitor was removed and then was added to the as-prepared dispersion medium. After sonication for 10 min, dispersion polymerization was performed in a cylindrical reaction chamber with shaking (120 cpm) at 70°C for 20 h. The suspension was centrifuged and the precipitates were washed intensively with distilled water by repeating centrifugation and vortexing. Finally, the PS seeds were washed with ethanol and vacuum-dried overnight affording PS seeds (4 μm, 8.3 g).

Swelling and polymerization processes were performed in a glass reactor equipped with overhead stirrer and a reflux condenser in an oil bath equipped with a temperature controller. The two-step seeded polymerization method was employed for preparation of monodisperse macroporous poly(styrene-co-DVB) (PS-DVB) beads. The PS seeds (4 μm, 700 mg) were dispersed in DBP (0.7 mL) emulsified aqueous medium (100 mL) containing 0.25% (w/w) SDS. The resultant dispersion medium was stirred (400 rpm) at room temperature for 20 h for swelling the PS seeds with DBP. Mixture of styrene (4.6 mL) and DVB (2.3 mL) in which BPO (240 mg) was dissolved was emulsified in an aqueous medium (100 mL) containing 0.25% (w/w) SDS by using homogenizer for 1 min. The emulsified monomer solution was added to the DBP-swollen PS seeds dispersion medium being stirred. Monomer swelling was performed for 20 h at room temperature with continuous stirring (400 rpm). After swelling process, an aqueous solution of 10% (w/v) PVA in deionized water (10 mL) was added to the dispersion medium and the medium was purged with nitrogen stream for 30 min. The seeded polymerization was performed at 70°C for 20 h with continuous stirring (200 rpm) to obtain monodisperse PS-DVB beads. The polymer beads were intensively washed with warm deionized water (50°C) by repeating washing and centrifugation. Subsequently, the collected beads were washed with ethanol and THF intensively to remove DBP and linear polymer. Finally, the beads were dried in vacuum at 30°C for 24 h to obtain macroporous PS-DVB beads (7.5 μm, 2.5 g).

2.2.2. Sulfonation of Macroporous PS-DVB Beads. Sulfonation of the PS-DVB beads was performed with reference to the reported method [50]. Briefly, monodisperse PS-DVB beads (1 g) were added to 5 mL of acetic acid in an ice bath. Sulfuric acid (50 mL) was then slowly added to the beads at room temperature and the temperature was increased up to 90°C and the resin mixture was stirred for 30 min to 2 hr. The dispersion was poured into iced water (400 mL) to quench the reaction and the sulfonated PS-DVB beads were collected by centrifugation. The beads were extensively washed with deionized water by repeating centrifugation-decantation. Subsequently, the sulfonated microspheres were washed three times with ethanol and dried under vacuum (1.1 g).

2.2.3. Magnetization of Sulfonated Macroporous PS-DVB Beads. The sulfonated macroporous PS-DVB beads (500 mg) were dispersed in deionized water (10 mL) at room temperature with mechanical stirring (200 rpm) and purging with nitrogen. A freshly prepared mixture of $FeCl_3{\cdot}6H_2O$ (618 mg, 2.26 mmol) and $FeCl_2{\cdot}4H_2O$ (257 mg, 1.28 mmol) in deionized water (10 mL) was added to the dispersion for adsorption. After 2 h, with continuous stirring 28% ammonium hydroxide (50 mL) was added dropwise to the beads suspension for 40 min. The magnetized microspheres were isolated from the mixture by centrifugation and briefly washed with 25% TFA and then extensively with deionized water and ethanol. Finally, the magnetized microspheres were dried under vacuum (magnetized microspheres, 7.5 μm, 653 mg).

2.2.4. Preparation of Silica Coated Magnetic Beads. APTS solution (1% (v/v), 100 mL) was added to 100 mg of magnetic sulfonated PS-DVB beads and was shaken for 10 min at room temperature. Thereafter, ammonium hydroxide (28%, 2 mL) was added to the bead dispersion and was shaken for 20 min at room temperature. To the dispersion TEOS (2 mL) was added and vigorously shaken for 12 h at room



Scheme 1: Synthetic scheme of silica coated superparamagnetic microbead.

temperature. The resulting beads (silica coated magnetic beads) were collected by magnet and washed with ethanol five times.

2.2.5. Streptavidin Separation of Silica Coated Magnetic Beads. APTS (240 μL) and ammonium hydroxide (40 μL) were added to 4 mL absolute ethanol containing 200 mg of silica coated magnetic beads and incubated for 6 h at room temperature. The resulting beads (amine-silica coated magnetic beads) were collected by magnet, washed with ethanol five times, and dispersed into PBS, pH 7.2, to obtain 10 mg/ml of amine-silica coated magnetic beads.

Biotin (5 mg) was activated with EDC (40 mg) and NHS (20 mg) in PBS, pH 7.2, for 1 h at room temperature. Then, this solution was added into amine-silica coated magnetic beads (1 mg, 100 μL) to modify biotin on its surface. The biotinylated silica coated magnetic beads were collected by magnet, washed with ethanol five times, and dispersed into 100 μL PBS, pH 9.0. The biotinylated silica coated magnetic beads were passivated with 5% BSA in PBS, pH 9.0, for preventing nonspecific binding.

Streptavidin-FITC (100 μg) in PBS pH 9.0 was added into the biotinylated silica coated magnetic beads and incubated for 30 mins at room temperature. The biotinylated silica coated magnetic beads after incubation with streptavidin-FITC were collected by magnet, washed with PBS, pH 9.0, containing 0.1% Tween 20 for five times, and dispersed into 100 μL PBS, pH 9.0. The fluorescent images were collected by the Olympus BX-UCB at room temperature with 200 ms exposure.

2.2.6. Characterization. Cross-sectional images of the beads were obtained using transmission electron microscopy (TEM, Jeol Inc., JEM 1010). The slices for the TEM analysis were prepared by means of an ultramicrotome (RCM, MTX). The surface and cross-sectional images of the beads were obtained using field emission scanning electron microscopy (FE-SEM, Carl Zeiss, SUPRA 55VP). The existence of the magnetic NP particles was confirmed by means of an energy dispersive X-ray spectrometer (EDX, Bruker, EDX). The amount of magnetic particles embedded in the beads was measured by a thermogravimetric analyzer (TGA, PerkinElmer Pyris 6).

3. Results and Discussion

For preparing magnetic beads, we employed a method using magnetization of uniform porous microbeads. As shown in Scheme 1, monodisperse superparamagnetic microbead has a spherical core-shell structure with silica layer over magnetized core PS-DVB bead.

Monodisperse polystyrene-divinylbenzene (PS-DVB) beads (7.5 μm, cross-linked with 33% DVB) were prepared using the seeded polymerization method that was previously reported by Ugelstad [49]. The seeded polymerization is a useful method for preparation of monodisperse microbeads which is essential for bioapplications including relative quantitative flow cytometry and biochip applications. The PS seeds were prepared in the range of 4.0~4.2 μm (61% solid yield) (Figure 1(a)) and the molecular weight was 29,000 (determined by GPC analysis). The final PS-DVB beads were prepared with repolymerization of the seeds (4 μm, 2.5 g, 47% solid yield) using DBP as a porogen and activated swelling agent.

The prepared PS-DVB microbeads showed a macroporous surface (Figure 1(b)), which enables the magnetite particles to form easily inside the microbeads upon magnetization from iron salts adsorbed in the porous structures. However, the PS-DVB microbead itself was not compatible in aqueous solution to adsorb the ferrous and ferric salts. Thus, we introduced sulfonate groups onto the PS-DVB microbeads to make them compatible in aqueous solution and the sulfonate group might have the effect that the iron salt is drawn into the microbeads and is considerably bound therein.

Sulfonated PS-DVB polymers have been used for many years in cationic exchange chromatography, as well as other analytes [51–53]. Recently, Chambers and Fritz have reported on the simple preparation of sulfonated PS-DVB with the -SO_3H loading range of 0.27~2.63 mmol/g using sulfonic acid and acetic acid [50]. To demonstrate the effect of sulfonation loading level of beads to magnetization, various loading levels of sulfonate group were prepared by controlling temperature and reaction time (data not shown). As a result from applying Chamber's method to the as-prepared PS-DVB microbeads, we obtained high loaded sulfonated PS-DVB (PS-DVB-SO_3H) of 4.1 mmol/g by elemental analysis. FT-IR analysis showed that the sulfonation of the phenyl groups of the PS-DVB was successfully achieved (Figure 2). In the FT-IR spectra of PS-DVB before (Figure 2(a)) and

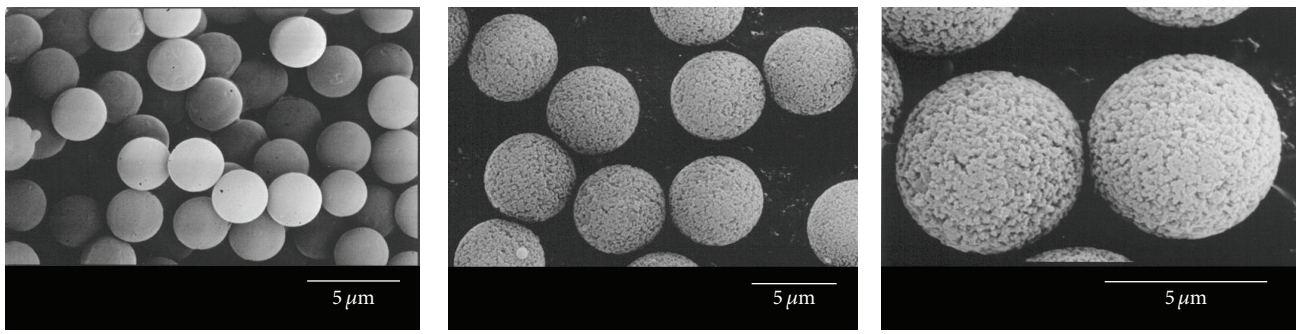


Figure 1: SEM images of (a) PS seeds and (b) PS-DVB microbeads prepared by seeded emulsion polymerization at low magnification (left) and high magnification (right).

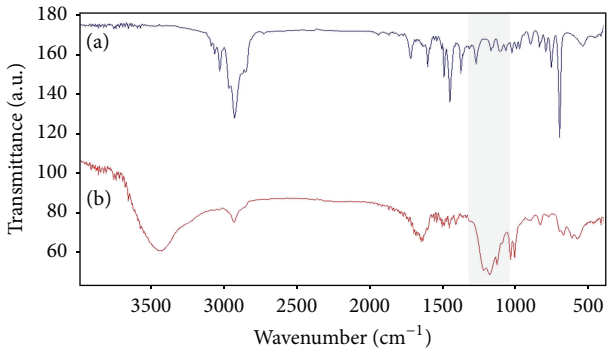


Figure 2: FT-IR spectra of PS-DVB microbeads (a) before and (b) after sulfonation. Shaded area represents the characteristic peak of sulfonate groups. Resolution setting, 4 cm^{-1}, 16 scans.

after sulfonation (Figure 2(b)), the changes in the combination vibrations (finger bands) between 1950 and 1650 cm^{-1} particularly characterize the phenyl group in the microbeads. The band centered around 1200 cm^{-1} (Figure 2(b)) represents the characteristic peak of the O_ySy_O asymmetric stretching vibration. The absorbance peaks at 1005 and 1126 cm^{-1} result from the vibrations of phenyl ring substituted with a sulfonate group and sulfonate anion, respectively, that are attached to phenyl ring [54]. A broad band centered around 3450 cm^{-1} is associated with OH stretching vibration of the sulfonic acid group (Figure 2(b)).

Direct polymerization of ion-binding monomers during the seeded emulsion polymerization process can be an alternative method of preparing functional macroporous microbeads. However, formation of uniformly macroporous structures using the ionic monomers is often challenging. In contrast, the postmodification of highly cross-linked macroporous beads as described above can preserve the structure of monodisperse microbeads.

After magnetization of sulfonated PS-DVB microbeads with a mixture of ferrous ions and ferric ions (2 : 1 molar ratio) in the excess basic solution of ammonium hydroxide, precipitates of magnetite were observed in the solution as well as in the beads as they turned blackish. The surface morphology of microbeads was not significantly changed after magnetization, meaning that the magnetite was formed and precipitated mostly inside the porous structure of the microbeads (Figures 3(a) and 3(b)). After purifying the magnetized beads from the mixture by centrifugation, we confirmed that the precipitate is indeed iron oxide from energy dispersive X-ray spectroscopy (Figure 3(c)). These magnetized microbeads indeed responded to external magnetic field (Figure 3(d), inset). The magnetic property was maintained even after keeping the beads for 4 h in 100% TFA.

The saturation magnetization value of the resulting beads was 7.6 emu/g (Figure 3(d)), which is relatively lower than that of commercial magnetites (~70 emu/g). However, the magnetized beads showed strong magnetic property enough in that they can be separated from the solvent by 4,000 gauss magnet in 4 sec. The magnetized sulfonated PS-DVB microbeads consist of many isolated, around a few tens nm, iron oxide grains that are embedded in a polymer matrix which can lead to their superparamagnetic behavior. The sulfonation of the beads and the subsequent magnetization successfully provided a means for preparing magnetic microbeads in straightforward and cost-effective way.

Iron oxide contents of the magnetized beads were measured by TGA (Figure 4). Iron oxide content was in the range of 18–26% whose value was suitable for further application of the magnetic beads comparing with the conventional magnetic beads. In the magnetization process, we attempted to simplify the purification by washing the beads after iron salts adsorption to prevent the formation of the magnetite in the solution after precipitation of iron salts using ammonium hydroxide (MAG 1). The iron oxide content of the magnetized beads was lower than that of beads prepared by in situ precipitation method without additional washing step (MAG 2).

Finally, the magnetic beads were coated with a silica shell using APTS and tetraethoxyorthosilicate (TEOS) for their surface to be water-compatible and amenable to chemical modification using various types of functional silane compounds. The amino silane compound APTS is prone to promote the adhesion of silane layer on the negatively charged sulfonated beads, thereby facilitating the further base-catalyzed condensation of TEOS from the surface. We found

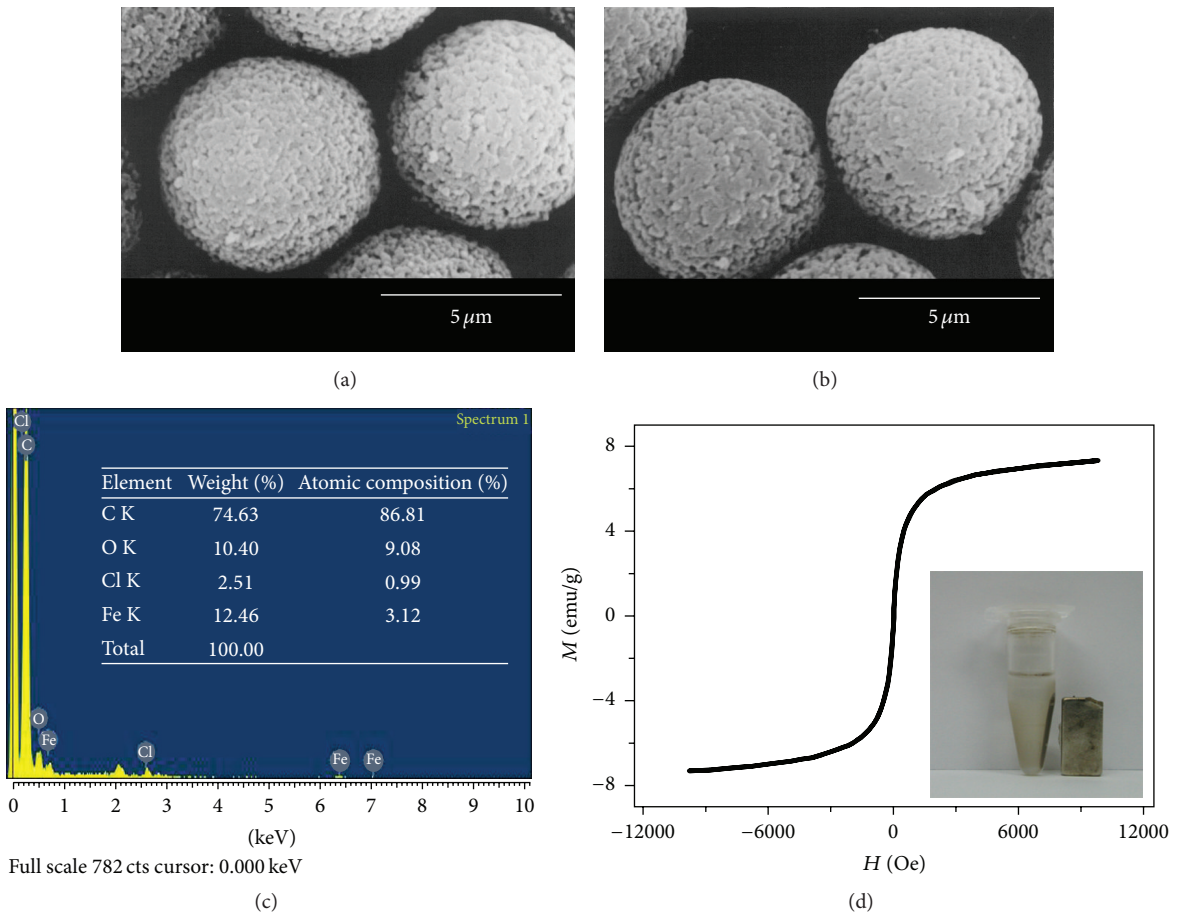


Figure 3: Characteristics of magnetized sulfonated microbeads. SEM images of sulfonated PS-DVB microbeads (a) before and (b) after magnetization. (c) Representative EDS analysis result of magnetized microbeads. (d) Hysteresis loops of magnetic beads. Inset: the beads were attracted to one side of the tube using a magnet.

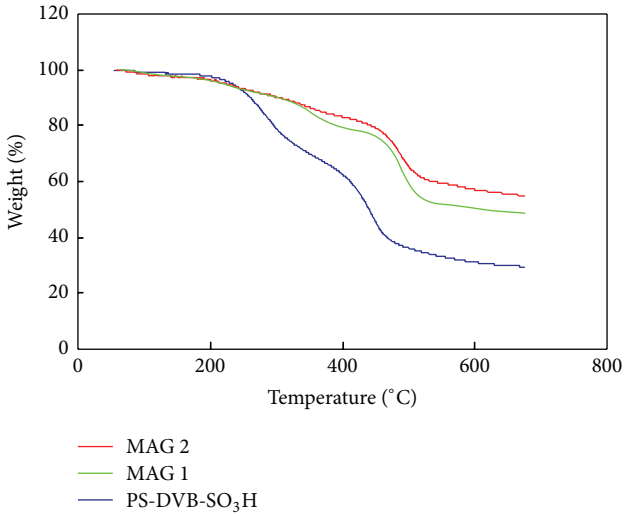


Figure 4: TGA thermograms of sulfonated PS-DVB microbeads (PS-DVB-SO_3H) before and after magnetization: (MAG 1) magnetization after washing the iron salt adsorbed beads; (MAG 2) magnetization without washing (in situ magnetization); iron oxide content: 18% (MAG 1) and 26% (MAG 2).

that this pretreatment greatly enhanced the silica coating efficiency as compared to no pretreatment (data not shown) and pretreatment with (3-mercaptopropyl)triethoxysilane solution [44]. The surface morphology of the beads significantly changed after the sol-gel process (Figures 5(a)–5(d)) revealing formation of silica grains on the surface (Figures 5(b) and 5(d)). From the cross-sectional analysis, the silica shell of 200–300 nm was observed around the core magnetic beads. The silica coated magnetic beads were also homogeneous in size and could be obtained in gram scale.

To support the presence of silica coating on the magnetic beads, we carried out EDX analysis of the sulfonated PS-DVB, magnetic PS-DVB, and silica coated magnetic PS-DVB beads. The result was shown in Figure 6. The sulfonated PS-DVB beads contained C, O, and S elements with the atomic composition of 86.5%, 9.1%, and 4.4%, respectively, in Figure 6(a). After magnetic nanoparticles were deposited on the sulfonated PS-DVB beads, C element decreased dramatically to 67.2% while O element increased up to 20.3%. Particularly, the presence of Fe element with 5.7% confirmed the successful deposition of magnetic nanoparticles on the sulfonated PS-DVB beads. When the silica shell was coated

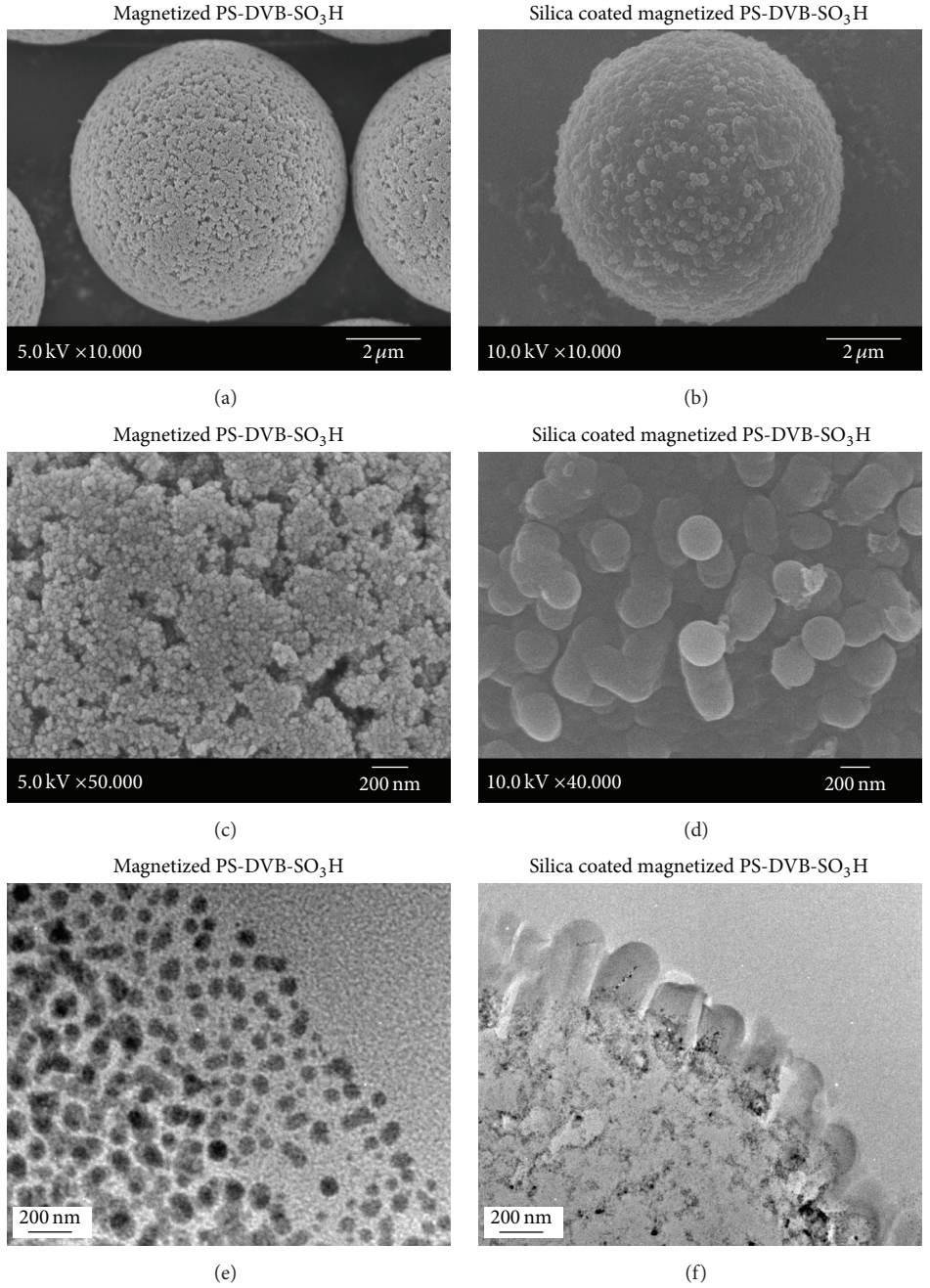


Figure 5: Silica coating of magnetized PS-DVB-SO_3H microbeads. SEM images of magnetized PS-DVB-SO_3H microbeads (a, c) before and (b, d) after silica coating at low magnification and high magnification. Cross-sectioned TEM images of (e) magnetized PS-DVB-SO_3H and (f) silica coated magnetized PS-DVB-SO_3H microbeads.

on the magnetic PS-DVB beads, the silica coated magnetic PS-DVB beads contained C (50.3%), O (33.3%), S (5.9%), Fe (3.8%), and Si (6.7%) element as shown in Figure 6(c). Comparing to that of the magnetic PS-DVB beads, O element of the silica coated magnetic beads increased by 13%, which is about two times Si element that demonstrated the presence of SiO_2 on the surface of magnetic PS-DVB beads. Therefore, EDX data was consistent with TEM images and evidenced the successful deposition of silica shell on the magnetic PS-DVB beads surface.

To demonstrate the application of the silica coated magnetic beads, we used streptavidin-FITC as model biomolecules for bioseparation. Briefly, the silica coated magnetic beads surface was modified with amine group and conjugated with biotin through EDC/NHS. The fluorescent images were shown in Figures 6(d)–6(f). The fluorescence of the biotinylated silica coated magnetic beads without reaction with streptavidin-FITC was negligible comparing to the biotinylated silica coated magnetic beads treated with streptavidin-FITC as shown in Figure 6(e). Also we carried

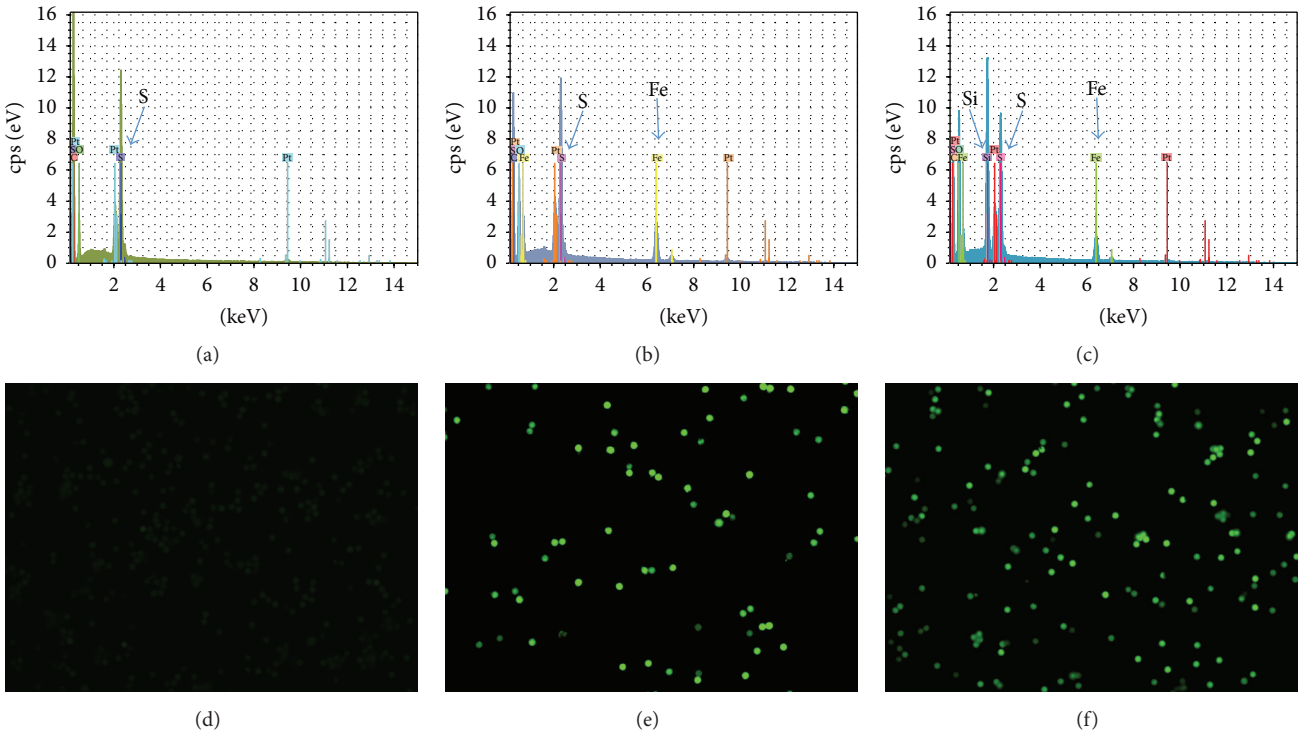


FIGURE 6: Chemical composition and bioapplication of silica coated magnetized PS-DVB-SO_3H microbeads. EDX data of (a) PS-DVB-SO_3H, (b) magnetized PS-DVB-SO_3H, and (c) silica coated magnetized PS-DVB-SO_3H microbeads. Fluorescent images of silica coated magnetized PS-DVB-SO_3H microbeads without (d) and with (e) streptavidin-FITC. (f) Nonspecific adsorption of streptavidin-FITC to silica coated magnetized PS-DVB-SO_3H beads.

out the nonspecific binding test of silica coated magnetic beads without biotinylation and BSA blocking in Figure 6(f). Streptavidin-FITC easily adsorbed into the silica coated magnetic beads. Therefore, BSA blocking step played an important role in our protocol.

4. Conclusions

In conclusion, we successfully prepared monodisperse silica coated superparamagnetic magnetic beads using the macroporous polystyrene microbeads as template materials. The sulfonation of the porous PS-based beads and the subsequent magnetization successfully provided a means for preparing magnetic microbeads in straightforward and cost-effective way, which can be readily modified with silica shell as a water-compatible protective layer and surface modification site for various functional silane compounds. When used with flow cytometry or in a microfluidic system, the monodisperse magnetic microbeads may have great potentials for drug screening, medical diagnostics, and combinatorial chemical synthesis in more simple, convenient, and cost-effective way.

Abbreviations

FE-SEM: Field emission scanning electron microscopy
TEM: Transmission electron microscopy
FT-IR: Fourier transform infrared spectroscopy
TGA: Thermogravimetric analysis.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

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