

A Facile Controlled Preparation Method of Multifunctional Core-Shell Magnetic Nanoparticles, and Their Potential Use in Microfluidic Separations

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Abstract

Organotriethoxysilanes bearing carboxylic acid, ester, and amine functional groups were synthesized via a facile photoinitiated catalyst-free thiol-ene click reaction. We demonstrate these systems to be excellent candidates to make core-shell magnetic nanoparticles composed of a single-core structure and a variety of surface functionalities via a simple synthesis in a reverse microemulsion system. Compared to the most commonly used surface modification strategies, this method avoids complicated multi-step procedures and tedious removal of metal catalysts from the nanoparticle surface. Also, the density of surface functionality is more predictable and tunable. The results show that the morphology, size, and colloidal stability can be well predicted and amply controlled. The organotriethoxysilanes were confirmed by ¹H-NMR and ¹³C-NMR spectra. The surface functionalized nanoparticles were characterized by a variety of methods, including TEM, FTIR, UV-Vis. The resulting nanoparticles have been shown to be monodisperse core-shell structures with a chemically active silica coating onto a single magnetic core, a system with great potential for a broad range of applications, such as biomedicine, catalysis, purification and separation. Notably, we have demonstrated the potential applicability of these systems for use in a closed-cycle magnetic purification (CCMP) microfluidic device by studying the reversible and quantitative affinity of resulting IONPs towards ionic compounds.

Keywords: Single core, Thiol-ene click chemistry, Magnetic separation, Ionic dyes, Organotriethoxysilane.

Introduction

A simple approach for the preparation of highly monodisperse and size controlled core-shell iron oxide magnetic nanoparticles (IONPs) with diverse functionality have drawn ever-increasing attention because of their potential applications in various fields, such as biomedicine [1], diagnosis applications [2], catalysis [3,4], magnetic purification and separation [5-7]. Over the past decade, due to the incredible progress in understanding the thermodynamic and kinetic mechanism of metal oxide

nanocrystal growth [8,9], the synthesis of precise particle size- and shape- controlled IONPs is no more a challenge by using advanced technologies such as thermal decomposition [10,11] and hydrothermal reduction [12-14]. Therefore, a proper surface modification strategy has become the key to maintain the chemical and physical stability of nanoparticles over a long period, as well as providing an avenue for the binding of application-specific surface functionalities. Encapsulation with small molecules, polymers, and lipids is widely employed for this purpose [15]. For example, Liu et al, [16]. Developed a method to directly convert hydrophobic IONPs into hydrophilic IONPs using 3,4-dihydroxyhydrocinnamic acid under alkaline conditions without any complicated organic synthesis. Basuki et al, [17]. Prepared diblock copolymers, poly(pentafluorophenyl acrylate) (P(PFPA)) and poly(oligoethylene glycol acrylate) (P(OEGA)), and then grafted them onto IONPs surfaces in the presence of various amines and acrylates to introduce a variety of surface functionalities and fluorescence in situ. Each method presents its own problem. Small molecules tend to detach from the surface of IONPs resulting in insufficient protection of iron oxide nanoparticles from oxidation and harsh conditions, whereas polymeric synthesis or lipids encapsulation involves tedious multiple-step reactions, incomplete conversion, side reactions, and the unwanted association between surface and ligands. These techniques can also result in nanoparticles having very broad size distributions [18,19].

Silica coating in reverse microemulsion systems is another popular approach for surface modification of IONPs. Particularly, the synthesis of core-shell structures with a single IONP core loaded into silica shell is attractive but challenging. The benefit of this synthesis technique is that it offers nanoparticles with excellent monodispersity, controllable size, high surface-to-volume ratio, and low decay of magnetic properties that could occur due to multi-core loading. On the other hand, the surface energy of sub-50-nm IONPs is so high that aggregation can easily be triggered either in the process of pre-treatment of IONPs or by a change of the microemulsion composition. Nevertheless, much research has recently focused on the formation of silica-stabilized IONPs

in microemulsions. Lemyre [20] determined that the formation of reverse micelles is enthalpy-driven. A new geometrical model was developed, using dynamic light scattering (DLS) analysis, to evaluate the contribution of parameters including free surfactant concentration, micellar molar composition, surfactant interfacial area, and aggregation numbers to the micelle size and stability. This model provides a better understanding of the synthesis of nanoparticles in non-ionic reverse micellar systems. A ligand exchange mechanism of silica coating in reverse microemulsion system has also been investigated by several research groups [21-23]. For the preparation of core-shell nanoparticles, the formation of core-free silica beads and multi-core loading are the two main obstacles to obtaining uniform single-cored structures. The theory behind these issues is that the energy barrier for heterogeneous nucleation is lower than for homogeneous nucleation. Thus, heterogeneous nucleation competes favorably with homogeneous nucleation throughout the reaction. It is believed that core-free silica beads will form if the concentration of hydrolyzed TEOS accumulates above the concentration threshold where homogeneous nucleation starts [21].

Multi-loading phenomenon may also be caused by the aggregation of IONPs before being injected into the reverse microemulsion system, or by the loss of microemulsion stability during the reaction due to the evaporation of organic solvent and ammonia [24]. To achieve the goal of forming only single-loaded IONPs, Ding [21] found that the number of magnetic cores must match the number of reverse micelles which can be achieved by altering the proportions of TEOS, ammonia, surfactant, and IONP cores. Also, Chen [25] demonstrated that the rate of addition is vital for the reproducible formation of single-cored nanoparticles, so addition of TEOS using a syringe pump affords better control of the final nanoparticle structures. Overall, the achievement of single-cored nanoparticles is not determined by one factor alone but by a combination of many factors interacting with each other.

Regarding surface functionalities, amines and carboxylic acids are high-affinity functional groups and present remarkable adsorption properties [19]. Nanoparticles with charged carboxylic acids and amines are excellent candidates for the purification and adsorption of molecules and noble metals such as gold and silver [26]. Unfortunately, there are few commercially available anchor molecules containing amine and carboxylic acids, likely due to complicated and low-yield syntheses. Thus, intense research activities have been devoted to amine and carboxyl functionalized ligands.

Over the past decade, click chemistry has emerged as a powerful tool to tailor a variety of functionalities on material surfaces. Click reactions in material chemistry has proven to be unique and superior due to its versatile mechanisms, mild reaction conditions, rapid reaction rates, clean products, high yields, excellent selectivity, and good biocompatibility. A highly effective and most widely used click reaction is the Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC) [27-29]. But using copper as the catalyst can be disadvantageous for some materials, including our systems. For example, strong coordination between copper and silica, precludes complete removal of the copper species from the surface under mild conditions. Highly acidic or basic solutions are undesirable for many applications such as catalyst loading and biomolecule binding [30]. Some groups reported catalyst-

free click reactions such as strain-promoted cycloaddition by using cyclooctyne and derivatives [31]. The catalyst-free click reaction works well for biological applications, but results in low reaction selectivity and functionality loading due to the stereo hindered effect [32].

The radical-mediated thiol-ene click reaction has become increasingly attractive to material scientists. It contains all the desirable features of a click reaction as mentioned above, in addition to the advantages of metal-free and easy accessibility to numerous starting materials [33,34]. Examples of surface modification via thiol-ene click reactions found in the literature mostly involve a two-batch reaction: the synthesis of surface modified nanomaterials with terminated alkene (or thiol), and the reaction between them and functionalized thiol (or alkene) under UV light in the presence of the photoinitiator. Nevertheless, such two-batch procedures cannot guarantee high surface coverage, and again it requires multiple steps for obtaining the final products. Very recently, several groups have been focusing on the thiol-ene synthesis of functional water-sensitive organotrialkoxysilanes for surface coating [35,36]. The thiol-ene reaction is tolerant of a broad range of functional groups using fewer organic synthesis steps, and it is of significance to provide access to a diversity of commercially-unavailable-functionalized-trialkoxysilanes with quantitative yields and high purity even on large scale [26].

Detailed studies concerning the fabrication of well-defined nanoparticles using these trialkoxysilanes is necessary for future applications. In our previous work, we developed a facile method of making single-loaded and amine terminated core-shell magnetic nanoparticles with tunable shell thickness [37]. This approach demonstrates the potential for making a large scope of functionalized and single-core loaded core-shell magnetic nanoparticles, using functionalized triethoxysilanes as a second siloxane source.

In this contribution, we, for the first time, report a facile one-pot method for making single-loaded core-shell magnetic nanoparticles terminated with a variety of functionalities using thiol-ene clicked triethoxysilanes. These triethoxysilanes contain functional groups including carboxylic acid, ester, and amine. None of them are commercially available but easy to synthesize using inexpensive and readily accessible materials such as triethoxyvinylsilane, cysteamine, 3-mercaptopropionic acid, and ethyl mercaptoacetate. The products are clean, high yielded, and ready to use for surface coating without any post-synthesis work-up steps. The merits of this strategy are that we provided a less tedious but more efficient one-pot synthetic route, to make size- and morphology-controlled nanoparticles with terminated amine and carboxyl groups, but not limited to the functional groups we discussed in this manuscript, using the commercially unavailable organotriethoxysilanes. These highly-uniform functionalized nanoparticles show the potential of fulfilling a broad range of highly demanded applications, such as microfluidic separations, biomolecule adsorption, drug targeting, and catalyst loading.

Experimental

Chemicals

Ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) was purchased from

Fisher, ammonium hydroxide (NH₄OH, 28%~30%) was obtained from Caledon. Tetraethyl orthosilicate (TEOS), triethoxyvinylsilane (98%), cysteamine (95%), 3-mercaptopropionic acid (99%), ethyl mercaptoacetate (97%, 2,2-Dimethoxy-2-phenylacetophenone (DMPA, 99%), IGEPAL®CO-520 (average Mn 441), cyclohexane, and all other solvents were purchased from Sigma-Aldrich.

Characterization Techniques

High temperature reactions were carried out in a Welmet FT3034 or Carbolite STF 16/180 tube furnace. High-speed centrifugation was performed using a Baxter Biofuge 17R centrifuge. Sonication was performed using a Branson 450 Digital Sonifier. Morphologies and particles sizes were investigated by a transmission electronic microscopy (TEM). The samples were prepared by depositing a drop of a diluted colloidal solution on carbon-film-coated copper grid (200 mesh). The surface functionalities were determined by a Fourier transform infrared spectroscopy (FTIR). The samples were prepared by making pellets with desiccated KBr powder. Nuclear magnetic resonance (NMR) spectra for all synthesized triethoxysilanes were recorded on Bruker AV 400 MHz spectrometer. UV-VIS spectroscopy was performed on a NanoDrop 2000c/2000 UV-Vis spectrophotometer. A sintered Nd₂Fe₁₄B permanent supermagnet was used for magnetically-assisted precipitation of nanoparticles.

Thiol-ene Synthesis of Functional triethoxysilanes (see Scheme 1)

Thiol-ene Synthesis of 3-(2-(triethoxysilyl)ethylthio)propanoic Acid, Compound 1: Compound 1 was synthesized via a modification of the radical mediated photochemical click reaction described by Tucker-Schwartz [36]. Typically, triethoxyvinylsilane (5.22 mL, 25 mmol) and 3-mercaptopropionic acid (2.18 mL, 25 mmol) were added to a flame dried Pyrex tube flask, and mixed with photoinitiator DMPA (128.15 mg, 2mol%). The flask was sealed with a plastic septum and the mixture was thrice evacuated and backfilled with argon. The mixture was then stirred until all solid starting materials dissolved. A thiol-ene reactor, equipped with an ACE photochemical UV medium pressure quartz mercury lamp (CN, PC451050, 450 watt) inside a stainless steel cabinet, was placed in a water-reflux-quartz-well. The flask was secured onto the water-reflux-quartz-well using elastic bands. The reaction mixture was UV irradiated at 0 °C for 24h. The reaction was run neat in the absence of any solvent. The product was obtained as a light yellow oil with quantitative yield. The structure was confirmed by ¹H-NMR (Figure S1) and ¹³C-NMR (Figure S2) spectra.

3-(2-(triethoxysilyl)ethylthio)propanoic acid (1): (>95% yield). The spectra for the product are: ¹H-NMR (400 MHz, CD₃OD) δ 3.85 (q, J = 6.8 Hz, 6H), 2.80 (t, J = 7.2 Hz, 2H), 2.68-2.60 (m, 4H), 1.22 (t, J = 7.2 Hz, 9H), 0.95 (t, J = 8.4 Hz, 2H). ¹³C-NMR (100 MHz, CD₃OD): 174.20, 58.10, 34.08, 26.08, 25.46, 17.16, 11.19.

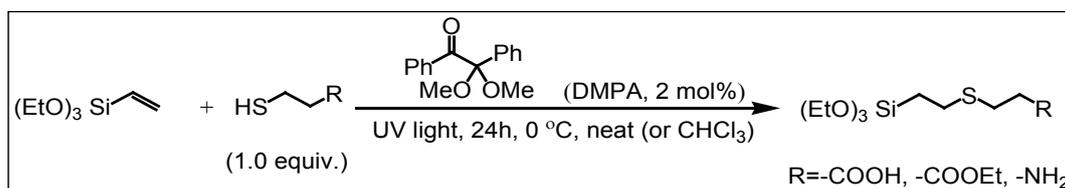
Thiol-ene Synthesis of Ethyl 2-(2-(triethoxysilyl)ethylthio)acetate, Compound 2: The synthesis procedure of compound 2 was similar to that described above. The reaction was initiated by mixing equimolar amounts of triethoxyvinylsilane and ethyl mercaptoacetate in the presence of 2 mol% of DMPA and running under neat condition at 0 °C for 24 h. After the reaction was complete, a clear oil was obtained with quantitative yield. This product was stored in a glass vial filled under argon. The structure was confirmed by ¹H-NMR (Figure S3) and ¹³C-NMR (Figure S4) spectra.

Ethyl 2-(2-(triethoxysilyl)ethylthio)acetate. (2) (>95%) The spectra for the product are: ¹H-NMR (400 MHz, CDCl₃) δ 4.16 (q, J = 6.8 Hz, 2H), 3.80 (q, J = 6.8 Hz, 6H), 3.21 (s, 2H), 2.73 (t, J = 8.8 Hz, 2H), 1.26 (t, J = 7.2 Hz, 3H), 1.20 (t, J = 6.8 Hz, 9H), 0.97 (t, J = 8.4 Hz, 2H). ¹³C-NMR (100 MHz, CDCl₃): 170.40, 61.08, 58.38, 33.39, 27.00, 18.11, 14.01, 11.14.

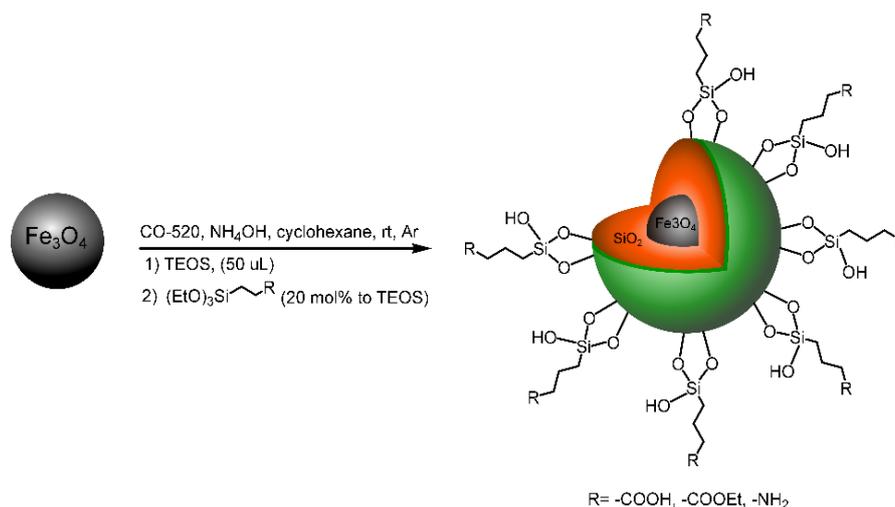
Thiol-ene Synthesis of 2-(2-(triethoxysilyl)ethylthio)ethanamine, Compound 3: Compound 3 (Figure S5, S6) was synthesized via the photoinitiated click reaction mentioned above. Typically, cysteamine (192.88 mg, 2.5 mmol) and DMPA (12.82 mg, 2 mol%) were added into a flame dried pyrex tube flask. The flask was sealed with a plastic septa and the mixture was vacuumed and purged with argon for 3 times. After that, triethoxyvinylsilane (0.522 mL, 2.5 mmol) and anhydrous chloroform (5 mL) were added into the mixture with an argon-purged-syringe. The mixture was stirred until all starting materials dissolved. The tube flask was placed in the thiol-ene reactor by attaching it onto the water-reflux-quartz-well. The reaction mixture was irradiated 0 °C for 24h. The product obtained was clear oil with quantitative yield. The product was kept in a glass vial filled with argon and ready to use for the surface modification. The structure was confirmed by ¹H-NMR (Figure S5) and ¹³C-NMR (Figure S6) spectra.

2-(2-(triethoxysilyl)ethylthio) ethanamine(3): (>95%) The spectra for the product is: ¹H-NMR (400 MHz, CDCl₃) δ 3.80 (q, J = 6.4 Hz, 6H), 2.85 (t, J = 6.4 Hz, 2H), 2.62 (m, 4H), 1.35 (bs, 2H), 1.20 (t, J = 7.2 Hz, 9H), 0.92 (t, J = 8.4 Hz, 2H). ¹³C-NMR (100 MHz, CDCl₃): δ 58.34, 40.93, 35.97, 25.87, 18.11, 11.83.

Synthesis of Monodisperse Hydrophobic Fe₃O₄ Nanoparticles: Monodisperse hydrophobic Fe₃O₄ IONPs were prepared by a modification of literature procedures [10,21,37]. FeCl₃·6H₂O was recrystallized from distilled water immediately before use, then 20 mmol, 5.41 g was dissolved in 280 mL of a solvent mixture of distilled water, ethanol and hexane (3:4:7 v/v). Oleic acid (60 mmol, 19 mL) was added and the mixture was stirred at room temperature for 30 min. Then 2.4 g NaOH was added to the above solution. The reaction mixture was stirred and refluxed at 70 °C for 4 h. The product Fe(oleate)₃ complex was obtained by separation of the organic layer, washed with distilled water 3 times, and removal of the organic solvent to dryness



Scheme 1: Synthesis of functionalized triethoxysilanes



Scheme 2: Synthesis of functionalized IONPs in reverse microemulsion

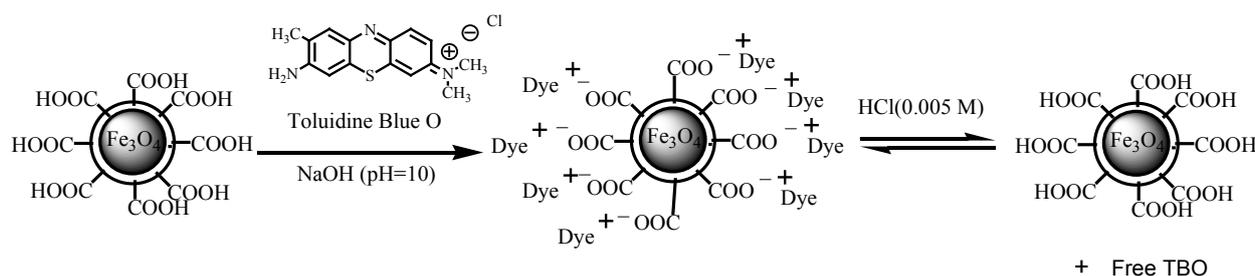
under reduced pressure in a rotary evaporator. 4.5 g (5 mmol) Fe(oleate)₃ was transferred into a 80 mL tube flask containing 1-octadecane (32 mL) and oleic acid (2.5 mmol, 0.79 mL), and magnetically stirred to homogeneity. The tube flask was then placed in a vertical tube furnace, flushed with argon, heated to 320 °C, and refluxed at this temperature for 30 min. The resulting black solution was cooled to room temperature and precipitated by adding excess ethanol. The precipitates were collected by centrifugation, then re-dispersed in hexane under sonication and precipitated with ethanol several times in order to purify the resulting Fe₃O₄ nanoparticles. The purified Fe₃O₄ nanoparticles were stored under Ar as a suspension in cyclohexane (25 mg/mL) containing a few drops of oleic acid to stabilize the colloid.

One-pot Synthesis of Surface Functionalized Core-shell Magnetic Nanoparticles Using Thiol-ene Synthesized Triethoxysilanes (see Scheme 2): The surface functionalization of magnetic nanoparticles using our thiol-ene synthesized triethoxysilanes were prepared using a reverse microemulsion method based on our previous work with some modification: 0.25 mL surfactant Igepal CO-520 was dissolved in 10 mL cyclohexane and subjected to sonication for 15 min, and then 100 μL NH₄OH was added followed by adding 3.0 mg Fe₃O₄ (2.5 mg/mL in cyclohexane). After 30 min of continuous magnetic stirring, 50 μL TEOS was added into the mixture using a syringe pump at rate of 2.5 mL/h. After 24 h, 20 mol% thiol-ene synthesized triethoxysilanes (relative to TEOS) was added. When the reaction was complete, 2 mL of ethanol was added to break the reverse microemulsion. The products precipitated in the layer of ethanol and were obtained by washing with EtOH and distilled water alternatively. The resulting products were named according to surface functionality as Fe₃O₄@SiO₂-

COOH, Fe₃O₄@SiO₂-COOEt, and Fe₃O₄@SiO₂-NH₂.

Characterization of Surface Functionalities: We previously demonstrated that ionic dye molecules can act as excellent models for testing the potential adsorption ability of the amine functionalized Fe₃O₄@SiO₂-NH₂ IONPs [37].

Here we chose toluidine blue O (TBO) to analyze the adsorption activity of surface -COOH groups of thiol-ene synthesized Fe₃O₄@SiO₂-COOH magnetic nanoparticles (see Scheme 3). First, 10 mg of Fe₃O₄@SiO₂-COOH were homogeneously dispersed in 2.95 mL MQ water by sonication. The pH was then adjusted to 10 by adding 50 μL NaOH solution (0.1 M). Then 2 mL of TBO (1 mM) was added to the suspension of deprotonated IONPs. The mixture was incubated for 1h to allow for the formation of ionic complex between the surface carboxyl groups and cationic dye molecules. After association, the complex was collected either magnetically or by centrifugation. The supernatant was separated, and the percentage of TBO loaded onto the IONPs was determined by its UV absorbance. The IONP-TBO complex was rinsed with acetone once and flow dried with air. The bound dye moieties were cleaved from the nanoparticles by the addition of 5 mL of HCl (0.005 M) solution, then subjecting the mixture to sonication for 15 min. After the adsorption process, the supernatants were removed with a pipette, and the IONP-TBO complex was rinsed with acetone once and dried in air. 5 mL of HCl solution (0.005 M) was added to release the electrostatically bound TBO molecules from IONPs surface. The release procedure was repeated 3 runs for each sample. Then the amount of TBO released from the IONPs was determined by measuring the UV absorbance of supernatants at wavelength 590 nm⁻¹. The



Scheme 3: Association of TBO with Fe₃O₄@SiO₂-COOH IONPs at high pH and dissociation of TBO at low pH

percentage of recovered TBO for 3 runs were added up together to determine the total recovery of TBO.

Results and Discussion

In our previous work, the one-pot synthesis has highlighted the potential of silanes with terminal functionalities for surface modifications of magnetic nanoparticles in a reverse microemulsion reaction system. Here, the synthesis of the multifunctional magnetic nanoparticles has been achieved through triethoxysilane precursors via the thiol-ene click reaction. With such a one-pot, two step procedure, the preparation of functionalized core-shell IONPs proved facile, and produced highly monodisperse products.

Synthesis of Functionalized Core-shell Magnetic Nanoparticles in Reverse Microemulsion: Carboxyl-, ester-, and amino-substituted triethoxysilylthioethers were synthesized by a thiol-ene click reaction as shown in Schemes 1 and 2, and were

characterized by $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra. The reaction conditions and results are summarized in Table 1.

The presence of the surface functional groups of these magnetic nanoparticles was demonstrated by FTIR spectra (Figure 1). In all cases, there are absorption peaks near 579 cm^{-1} , representing the stretch vibration mode of Fe-O bonds. Peaks near 1090, 798, and 474 cm^{-1} can be ascribed to the unsymmetrical stretching vibration, symmetrical stretching vibration and bending vibration modes of Si-O-Si, respectively. In Figure 1, the absorbance at 3420, 1710, and 1420 cm^{-1} is indexed to -OH, C=O, C-O-H of carboxylic acids, peaks at 3420, 1650, 1380 and 947 cm^{-1} are corresponding to -OH, C=O, C-O of esters, bands at 3350, 3270, and 1620 cm^{-1} can be derived from the stretch vibration and bending vibration of amines.

Energy dispersive X-ray spectra (EDX) also show strong evidence of successful surface modification (Figure 2). Peaks

Table 1: Summary of the thiol-ene click synthesis of functionalized triethoxysilanes

Entry	R	Molar ratio	Time (h)	DMPA (mol%)	Solvent	Temperature ($^{\circ}\text{C}$)	Yield (%)
1	-COOH	1:1	24	2	neat	0	>95
2	-COOH	1:1	24	2	CHCl_3	0	>95
3	-COOEt	1:1	24	2	neat	0	>95
4	$-\text{NH}_2$	1:1	24	2	CHCl_3	0	>95

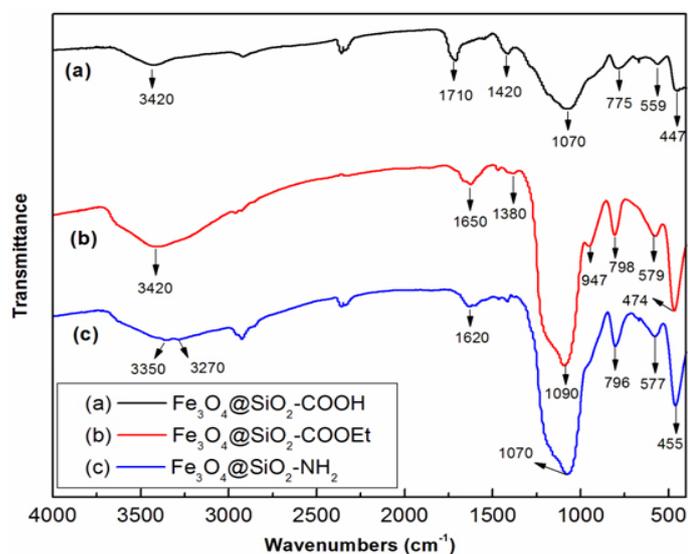


Figure 1: FTIR spectra of core-shell functionalized IONPs with thiol-ene clicked triethoxysilanes

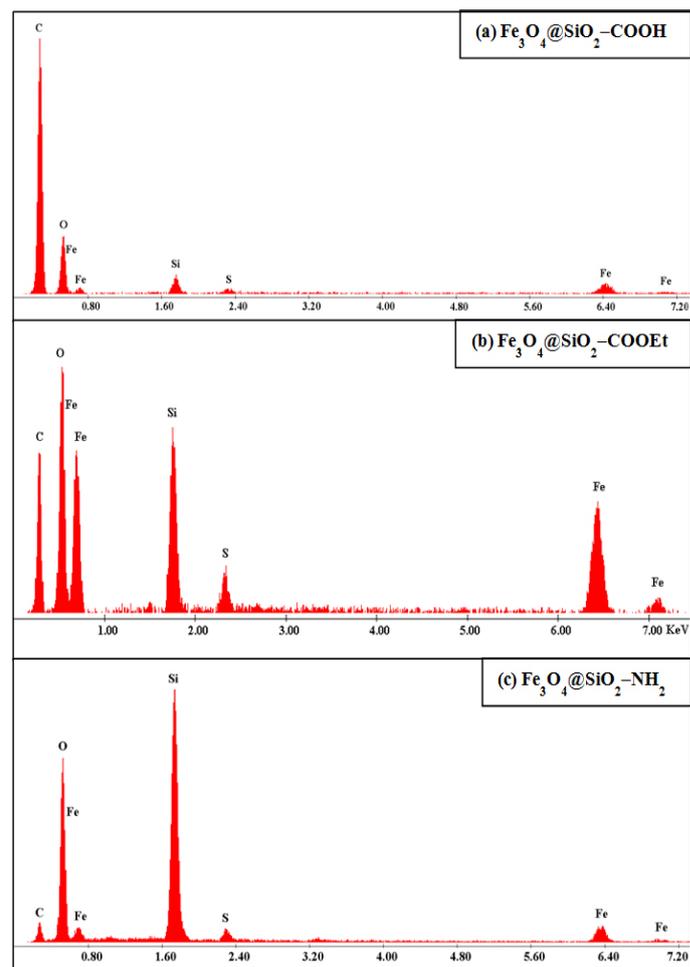


Figure 2: EDX spectra of core-shell functionalized IONPs with thiol-ene clicked triethoxysilanes: a) $\text{Fe}_3\text{O}_4@SiO_2\text{-COOH}$, b) $\text{Fe}_3\text{O}_4@SiO_2\text{-COOEt}$, c) $\text{Fe}_3\text{O}_4@SiO_2\text{-NH}_2$

referring to sulfur were found in EDX spectra for sample $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOH}$, $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOEt}$, and $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-NH}_2$. With the adding of 20 mol% of triethoxysilanes to TEOS, the S/Si ratio of functionalized IONPs were 3.2%, 2.8% and 6.0% for $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOH}$, $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOEt}$, and $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-NH}_2$, respectively. Comparing to the theoretical S/Si ratio of 19.0%, these measured values were much lower. One reason is that the packing surfaces of sample were not flat because of such small spherical size of IONPs, which can affect the sensitivity of measurement.

The TEM images (Figure 3) show that the iron oxide magnetic cores after surface modification with the thiol-ene clicked triethoxysilanes were single loaded monodisperse core-shell structures. The average size measured by ImageJ with 50 counts were 30 nm, 75 nm, 79 nm and 94 nm for Fe_3O_4 , $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOH}$ (Figure 3, Image B), $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOEt}$ (Figure 3, Image C), and $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-NH}_2$ (Figure 3, Image D). The results indicate that this one-pot method of fabricating single-loaded core-shell magnetic nanoparticles terminated with a variety of functionalities using thiol-ene clicked triethoxysilanes is a facile technique for creating size- and -morphology controlled nanoparticles with a broad range of functional groups.

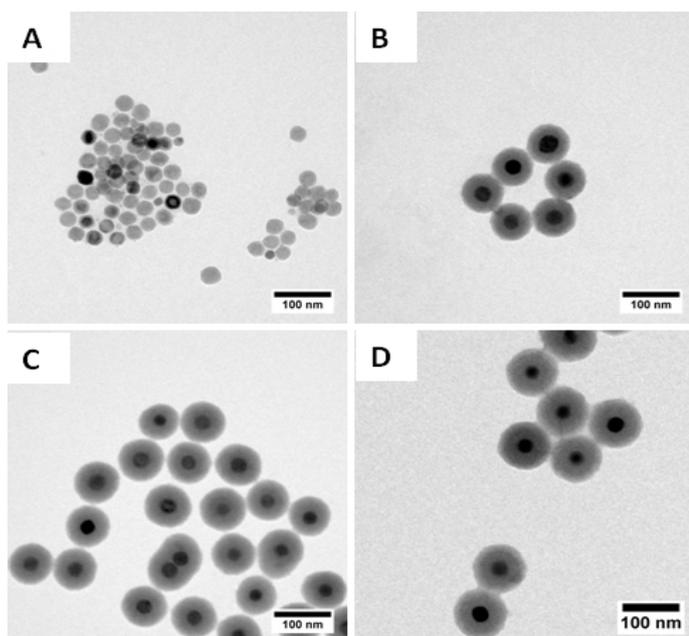


Figure 3: TEM images of (A) Fe_3O_4 cores, (B) $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOH}$, (C) $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOEt}$, (D) $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-NH}_2$

The magnetic properties of iron oxide cores and functionalized IONPs after surface coating were investigated. The saturation magnetization curves of Fe_3O_4 core and -COOH , -COOEt , and -NH_2 functionalized IONPs are presented in Figure 4, and were determined to be 36.0, 10.8, 6.9, and 6.1 emu/g, respectively. The decrease in the saturation magnetization after surface coating is in accordance with the increase of the shell thickness. All nanoparticles in this study were found to be superparamagnetic at 300K, as no hysteresis loop was observed. As a consequence of the superparamagnetism, the functionalized IONPs can be collected by an external magnetic field as shown.

We found that the reproducibility of monodisperse single-loaded core-shell IONPs is crucially dependent on the control

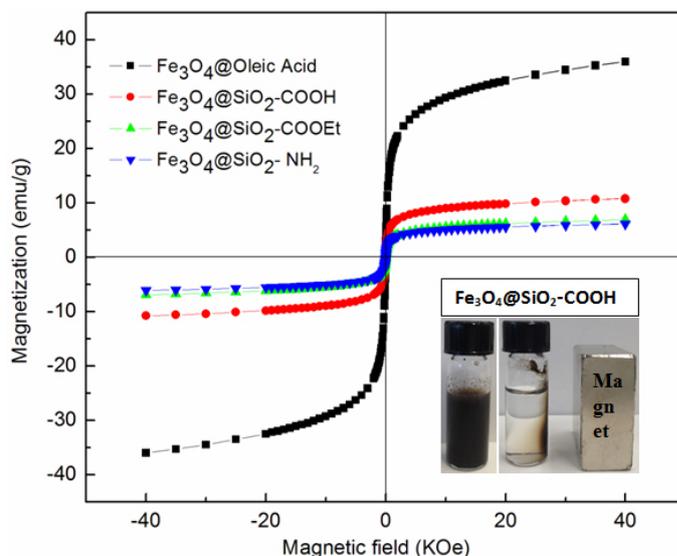


Figure 4: Field-dependent magnetization curve

of the addition rate of triethoxysilanes. The morphology of functionalized IONPs varied from batch to batch when the addition of the reagent triethoxysilane as conducted manually. However, reproducible functionalized magnetic nanoparticles were obtained from each batch by adding triethoxysilanes using a syringe pump at a controlled rate. For example, in the preparation of $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOH}$, at low pumping rate 0.5 mL/h (Figure 5, Image B) and 1 mL/h (Figure 5, Image C), homogeneous nucleation was dominated and a large amount of core-free structures were observed. At rate 2.5 mL/h (Figure 5, Image D), the control of size and morphology of functionalized IONPs were optimized, and single-loaded core shell structures were obtained. Therefore, due to the complexity of the reverse microemulsion system, and contrary to popular belief, it is not always the case that the lower addition rate of silanes results in a greater percentage of single-cored structures. To be more specific, although the reverse microemulsion system is very stable in sealed flask, some amount of organic solvent could

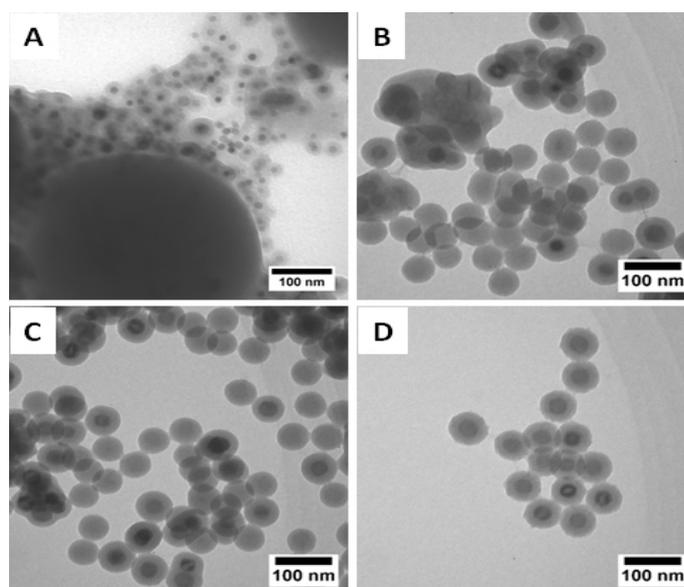


Figure 5: TEM images of $\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-COOH}$ by adding silanes by hand (A), and by syringe pumping at different rate: 0.5 mL/h (B), 1 mL/h (C), 2.5 mL/h (D)

possibly evaporate from the flask, resulting in very slight change of the emulsion stability. So at low pumping rate, it might give the magnetic nanoparticles a chance to precipitate.

The amount of triethoxysilanes used for surface modification was studied in order to gain the desired density of surface functional groups without sacrificing the morphology. The result shows that, at low mole ratio of 3-(2-(triethoxysilyl)ethylthio)propanoic acid (compound 1), core-shell structures were maintained (Figure 6, Image A, B). But when the mole ratio was raised in excess of 30 mol% (Figure 6, Image C), compound 1 began to polymerize between core-shell nanoparticles, resulting in and a bulk block of polymer encapsulating the nanoparticles.

Adsorption Ability of Surface Functionalized IONPs Using a Chromophoric Probe: We conducted cycling experiments to investigate the practical utility of these functionalized core-shell systems as potential separation media. The result shows that the functionalized IONPs are promising for the application of purification and adsorption of target compounds. Here we used the $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ as an example, to analyze the reversible adsorption ability of carboxylic-acid-functionalized $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ magnetic nanoparticles for cationic compounds. Toluidine blue O, was chosen as a model cationic compound. The result shows that the association and dissociation between $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ and TBO was reversible by changing pH of the solution (Figure 7). The maximum loading of TBO molecules onto IONPs via electrostatic binding was spectrophotometrically determined to be $1.98 \times 10^{-4} \text{ mol/g}$ (8.70×10^3 molecules/nanoparticle). The recovery percentage of TBO dropped dramatically with increasing cyclical usage, as shown in Figure 8, from 81.1% to 49.8%, at the 5th cycle. Although there was some mass loss of nanoparticles during the wash steps, the most likely reason for this observation is the gradual degradation of

the silica shell by repeated exposure to high pH conditions, and evidenced in Figure 9.

Binding Capacity of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ to a Bioactive Amine:

We also investigated the affinity of our carboxyl terminated IONPs towards pharmaceutical amines. Ephedrine hydrochloride was used as a model compound to test the binding capacity of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ to drug molecules. The result shows that 94.0% of ephedrine hydrochloride molecules were bound to nanoparticles and 76.9% were recovered from the solution after 3 repeated release procedures, as shown in Figure 10.

Conclusion and Prospects

A quick, inexpensive, convenient high-yield click reaction was successfully applied for making functionalized core-shell magnetic nanoparticles in a facile synthesis strategy. By synthesizing organotrialkoxysilanes precursors via thiol-ene click reaction, a variety of commercially unavailable silica nanoparticle precursors are readily created with high yield and purity. Also, the number and complexity of steps required to fabricate functionalized magnetic nanoparticles were largely reduced using this one-pot procedure. Tedious wash steps and grafting stages were avoided, and the resulting uniform single-loaded core-shell magnetic nanoparticles terminated with a variety of functionality show promise for a wide range of applications like microfluidic separations, biomolecule adsorption, drug targeting, and catalyst loading.

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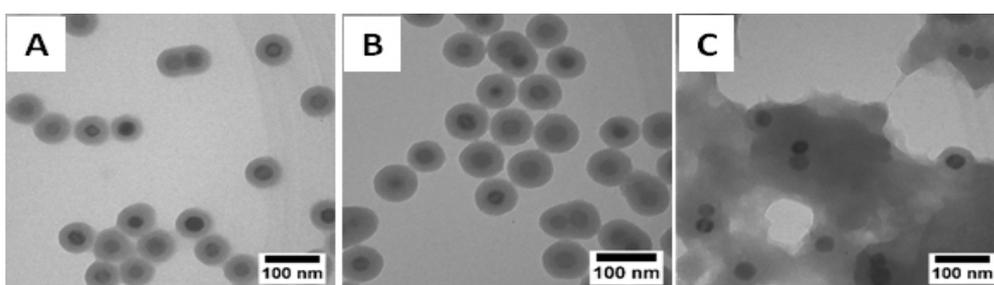


Figure 6: TEM images of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ with different mole ratio of compound 1 to TEOS: 5 mol%(A), 20 mol%(B), 30 mol%(C)

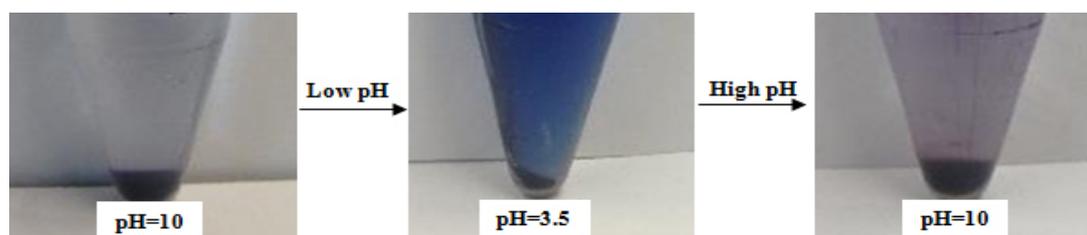


Figure 7: Reverse association and dissociation between $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ and TBO by changing pH of solution

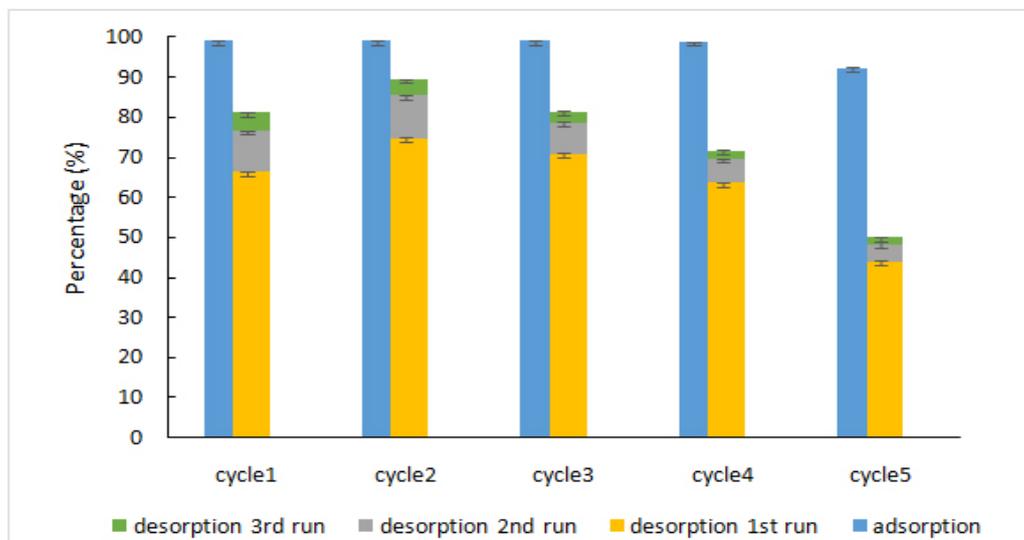


Figure 8: Percentage loading and recovery of TBO of 5 cycles^a (a: The percentage numbers of each column were obtained from the average value of two parallel samples. And the measurement for each sample was repeated 3 times.)

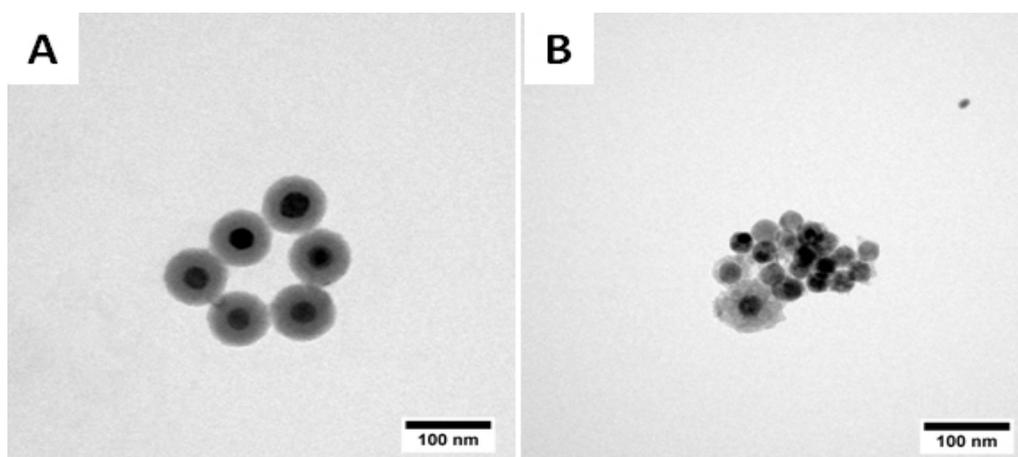


Figure 9: TEM images of $\text{Fe}_3\text{O}_4@\text{SiO}_2\text{-COOH}$ IONPs before (A) and after (B) 5 cycles

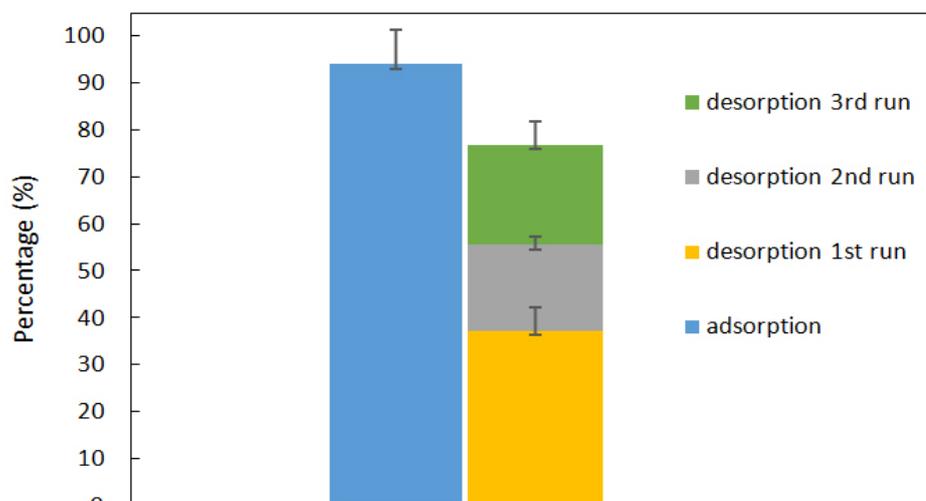


Figure 10: Percentage loading and recovery of ephedrine hydrochloride

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