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Preparation and Characterization of Stimuli-Responsive Magnetic Nanoparticles

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Abstract In this work, the main attention was focused on the synthesis of stimuli-responsive magnetic nanoparticles (SR-MNPs) and the influence of glutathione concentration on its cleavage efficiency. Magnetic nanoparticles (MNPs) were first modified with activated pyridyldithio. Then, MNPs modified with activated pyridyldithio (MNPs-PDT) were conjugated with 2, 4-diamino-6-mercaptopyrimidine (DMP) to form SR-MNPs via stimuli-responsive disulfide linkage. Fourier transform infrared spectra (FTIR), transmission electron microscopy (TEM), and X-ray photoelectron spectroscopy (XPS) were used to characterize MNPs-PDT. The disulfide linkage can be cleaved by reduced glutathione (GHS). The concentration of glutathione plays an important role in controlling the cleaved efficiency. The optimum concentration of GHS to release DMP is in the millimolar range. These results had provided an important insight into the design of new MNPs for biomedicine applications, such as drug delivery and bio-separation.

Keywords Site-specific delivery · Surface chemistry · Nanoparticles · Biomaterials · Conjugation · Controlled release

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Introduction

Recently, stimuli-responsive magnetic nanoparticles (SR-MNPs) have attracted considerable attention in the field of nanotechnology as candidates for drug delivery and bio-separation [1–4]. Owing to their ability to switch on and switch off when necessary, particularly under the action of local stimuli characteristic of the target zone, they have been employed as the carriers of drug in biomedical and bioengineering field. Advantage may also be taken for the magnetic property of MNPs to be attracted toward the target zone in the presence of an external magnetic field. This magnetic motor effect is also attractive for the development of site-specific drug delivery and bio-separation systems. Most of these bio-systems are based on stimuli-responsive polymer, for example, temperature responsive poly(*N*-isopropylacrylamide) [5, 6].

It is well known that pyridyl disulfide conjugates readily with a thiol-containing drug, protein, or other functional molecule, forming a disulfide linkages with such molecules, and simultaneously releasing the byproduct, thiopyridone, under mild reaction conditions [7–9]. The disulfide linkages are chemically labile in nature and have the ability to dissociate in response to various disulfide-reducing agents, especially, glutathione [10–12]. Wang et al. [13, 14] designed a kind of nanoparticles that possess the ability to respond to intracellular glutathione concentrations.

Therefore, the combination of the activated disulfide with magnetic particles is an attractive strategy to improve the functions of MNPs. While the synthesis of MNPs has been well established, not much has been reported in the case of SR-MNPs. We report here the influence of glutathione concentration on the cleavage efficiency of the stimuli-responsive disulfide linkages.

Materials and Methods

Materials

Dichloromethane, ferric chloride hexahydrate, ferrous chloride tetrahydrate, ammonium hydroxide, methanol, ethanol, diethyl ether, *N*,*N*-dimethylformamide (DMF), succine anhydride, acetic acid, and toluene were obtained from China National Medicines Corporation Ltd. and used as received. 3-Aminopropyltriethoxysilane (APTES, 99%), thiopyridyl disulfide, 1, 3-dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), and 2-aminoethylthiol hydrochloride were supplied from Aldrich Chemical Co. 2, 4-Diamino-6-mercaptopyrimidine (DMP) was endowed by MERCK. Reduced glutathione (GSH) was purchased from MERCK.

Methods

Synthesis of S-(2-Aminoethylthio)-2-Thiopyridine Hydrochloride (AE-S-S-Py)

The synthesis of S-(2-aminoethylthio)-2-thiopyridine hydrochloride was described in reference [15]. Thiopyridyl disulfide (1.1 g) was dissolved in 10-mL methanol and 0.2-mL acetic acid. To the solution, 2-aminoethylthiol hydrochloride (0.25 g) in 10-mL methanol was added. The mixture was stirred for 48 h and then evaporated under high vacuum to obtain yellow oil. The product was washed with 50-mL diethyl ether and dissolved in 10-mL methanol, and then was precipitated by addition of 400-mL diethyl ether. Finally, the deposit was collected by vacuum filtration. Yield: 0.4 g, 77%; ^{1}H NMR: δ ppm (in d-chloroform) 8.66 (d, 1H), 7.75 (t, 1H), 7.48 (d, 1H), 7.1(t, 1H), 3.46 (t, 2H), and 2.8 (m, 2H).

MNPs Preparation

MNPs were synthesized by chemical co-precipitation of Fe (II) and Fe (III) ions in alkaline medium. $FeCl_3 \cdot 6H_2O$ (0.02 M) and $FeCl_2 \cdot 4H_2O$ (0.01 M) were dissolved in 120-mL de-ionized water at pH 2 and under N_2 protection. Sixty-milliliter ammonia aqueous solution (28 wt%) was added into this mixture with violently stirring. After 30 min the reaction mixture was heated to 70 °C and kept at this temperature for about 30 min. The obtained MNPs were washed immediately with de-ionized water, ethanol, and toluene five times in turn. Then MNPs were dispersed in 100-mL toluene.

MNPs Functioned with Activated Pyridyldithio

The surface of MNPs was first coated with 3-aminopropyltriethoxysilane by a silanization reaction. 5-mL APTES was added into the MNPs solution and the reaction mixture was kept at 80 °C for 10 h under nitrogen atmosphere with vigorous mechanical stirring. Then, the obtained APTES-immobilized MNPs, defined as MNPs-NH_2, were washed with DMF (5×50 mL^2) and dispersed in 100-mL DMF.

Then, MNPs-NH_2 reacted with succine anhydride. Briefly, 50-mL MNPs-NH_2 and 2 g (excessive) of succine anhydride were added to 50-mL DMF. The mixture was kept at 80 °C for 10 h under nitrogen atmosphere with stirring. After reaction, the mixture was separated under magnetic field. The modified MNPs, defined as MNPs-COOH, were washed with dichloromethane five times and dispersed in dichloromethane. The weight percent of solid was about 3.28%.

Finally, MNPs-COOH reacted with AE-S-S-Py. Five-gram suspension of MNPs-COOH, 150 mg DCC, 150 mg DMAP, and 50 mg AE-S-S-Py were added into 40-mL dichloromethane. After stirring the mixture for 48 h, MNPs modified with pyridyldithio, defined as MNPs-PDT, were washed with methanol and phosphate buffer solution (PBS: 150 mmol/L NaCl, 1.9 mmol/L NaH_2PO_4, 8.1 mmol/L Na_2HPO_4, pH 7.4, 0.01 M) five times, respectively. The concentration of MNPs-PDT dispersed in PBS was 4 mg/mL.

Preparation of SR-MNPs

To form SR-MNPs, 100-μL DMP solutions (3.84×10^{-3} mmol/L) were added to 100-μL MNPs-PDT. The mixture was kept at room temperature for 20 h. After magnetic separation, the obtained nanoparticles were washed five times with PBS.

Stimuli-Responsive Experiment

Hundred-microliter GHS solutions (195.0 mM) were added to SR-MNPs. The total volume was 200 μL. The mixture was shaken for 20 h and separated under magnetic field. UV–Vis spectrometer was adopted to monitor the supernatant.

Characterization

FTIR was recorded in the transmission mode on a Perkin Elmer 2000 FTIR instrument (USA). XPS analyses were performed with an ESCALAB220 i-XL electron spectrometer from VG Company. To determine absorbency spectra of the supernatant Lambda 950 spectrometer (Perkin Elmer) was used. The magnetic properties of nanoparticles were measured with a vibrating sample magnetometer (VSM, Lakeshore 7307) at room temperature. The size and morphology of the particles were examined by a Philips Tecnai 20 transmission electron microscopy (TEM).

Results and Discussion

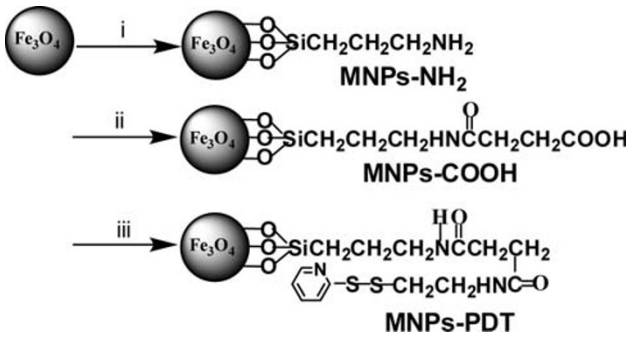
MNPs Modified with an Activated Pyridyldithio

MNPs modified with activated pyridyldithio (MNPs-PDT) are indispensable for the preparation of SR-MNPs. Scheme 1 illustrates the preparation process of MNPs-PDT from MNPs. MNPs were first silanized into amine magnetite nanoparticles (MNPs-NH_2) and further converted into carboxyl magnetite nanoparticles (MNPs-COOH). Then, MNPs-COOH reacted with S-(2-aminoethylthio)-2-thiopyridine (AE-S-S-Py) to form MNPs-PDT.

The morphology and size of MNPs and MNPs-PDT were investigated by TEM. As shown in Fig. 1a, the average diameter of MNPs is 18 ± 3.7 nm. MNPs show either a spherical or ellipsoidal shape with some irregularities. Figure 1b shows clearly that the morphology and size of the modified MNPs almost maintain the original state.

Figure 2 depicts the FTIR spectra of MNPs-NH_2, MNPs-COOH, and MNPs-PDT. The FT-IR spectrum of MNPs-NH_2 (Fig. 2a) reveals absorption peaks of Si–O–Si bond (1,033 cm^{-1}), Si–O groups (1,124 cm^{-1}), and C–H stretching vibration (2,926 cm^{-1}). In FTIR spectra of MNPs-COOH (Fig. 2b), the broad band near 3,400 cm^{-1} and the peak at 1,700 cm^{-1} are characteristic peaks of COOH. The peaks at 1,645 and 1,540 cm^{-1} originate from the amide bond [16, 17]. For MNPs-PDT, FTIR spectra (Fig. 2c) show a new peak at 1,450 cm^{-1}, corresponding to the pyridine ring [18]. The absence of COOH characteristic band of about 1,700 cm^{-1} further confirms that MNPs were functioned with pyridyldithio.

The C 1s core-level spectra (Fig. 3a) of MNPs-NH_2 show the C–C peak at 284.8 eV and the C–N peak at 285.8 eV [19]. The C 1s core-level spectra of MNPs-COOH (Fig. 3b) present two new peaks having binding energies (BE) at about 287.6 and 288.4 eV, assigned to the N–C=O and O–C=O species [20]. The result confirms the



Scheme 1 Preparation of MNPs-PDT: (i) APTES, toluene 80 °C 10 h under N_2; (ii) succine anhydride, DMF, 80 °C 10 h under N_2; (iii) AE-S-S-Py, DCC, DMAP, dichloromethane, room temperature

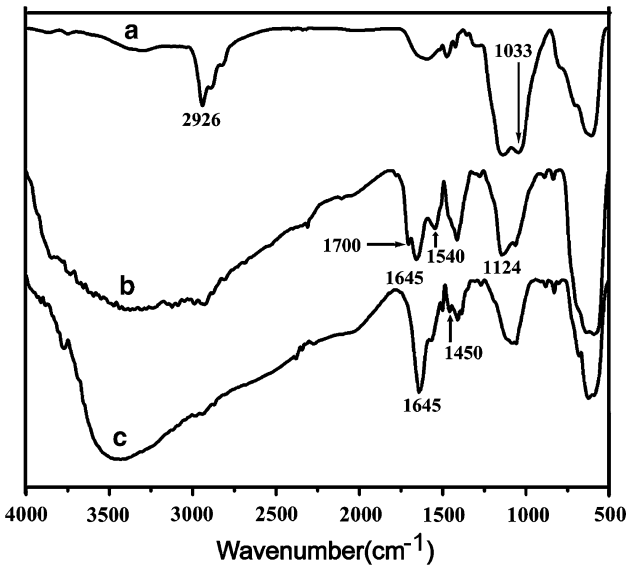


Fig. 2 FT-IR spectra of (**a**) MNPs-NH_2, (**b**) MNPs-COOH, and (**c**) MNPs-PDT

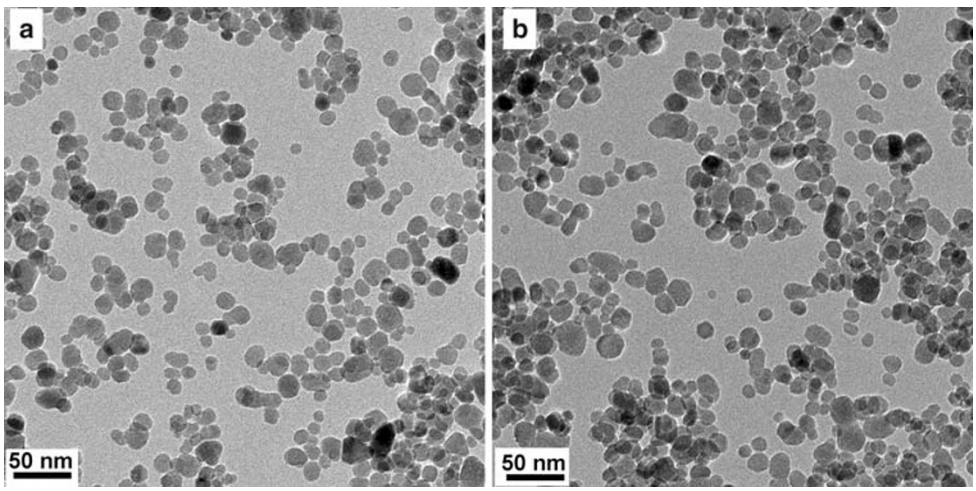


Fig. 1 TEM images of (**a**) MNPs and (**b**) MNPs-PDT

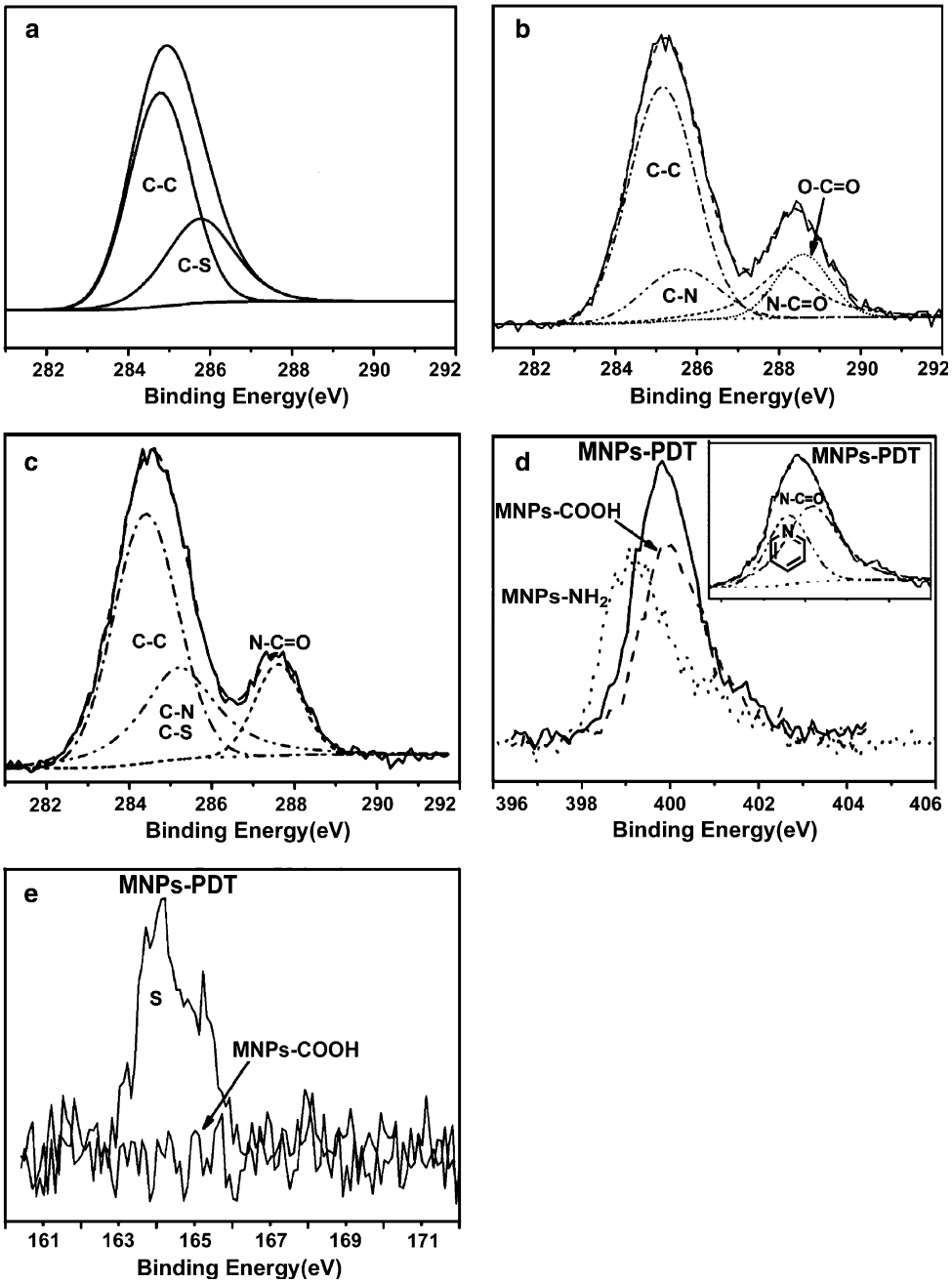


Fig. 3 XPS spectra of (**a**) C 1s of MNPs-NH_2, (**b**) C 1s of MNPs-COOH, (**c**) C 1s of MNPs-PDT, (**d**) N 1s of MNPs-NH_2, MNPs-COOH, and MNPs-PDT, (**e**) S 2p of MNPs-COOH and MNPs-PDT

formation of the amide bond and linkage of succine anhydride. After MNPs-COOH reacted with AE-S-S-Py (Fig. 3c), the peak of 288.4 eV disappears, due to the reduction of O–C=O species. The peak at 285.8 eV is attributed to C–S species. At same time, the intensity of the peak at 287.6 eV increases, indicating increment of the N–C=O species amount. The N 1s core-level spectra of MNPs-NH_2 at 399 eV shift to 399.9 eV after the amidation reaction of MNPs-NH_2 (Fig. 3d). For MNPs-PDT, two kinds of N atoms contribute to the intensity of the N 1s peak at the BE of 399.5 eV: one is attributed to the pyridyldithio groups and the other to amide (Fig. 3d). After MNPs-COOH coupled with AE-S-S-Py, the peak at 164 eV was dedicated to S 2p (Fig. 3e) [21]. The appearance of S 2p peak also suggests that MNPs were functioned with an activated pyridyldithio.

Figure 4 shows the isothermal magnetization curves of MNPs and the modified MNPs as a function of applied magnetic field at room temperature. The saturation magnetization (Ms) value of MNPs is 70.9 emu/g, which is slight lower than 89 emu/g of bulk magnetite. A possible reason why the synthesized MNPs posses lower saturation magnetization may be due to the particle sizes. The energy of a magnetic particle in an external field is proportional to

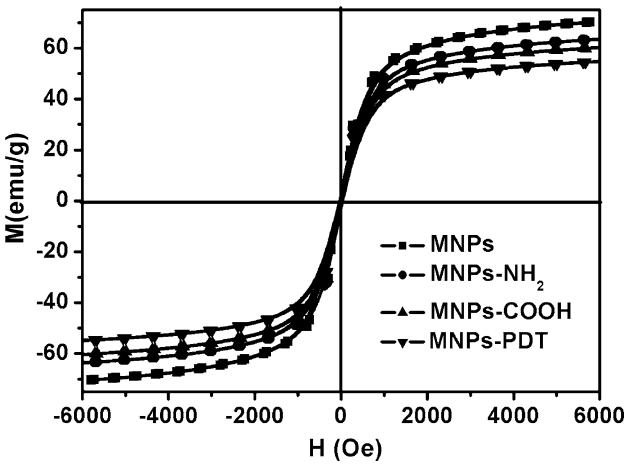


Fig. 4 Magnetization curves of MNPs, MNPs-NH_2, MNPs-COOH, and MNPs-PDT

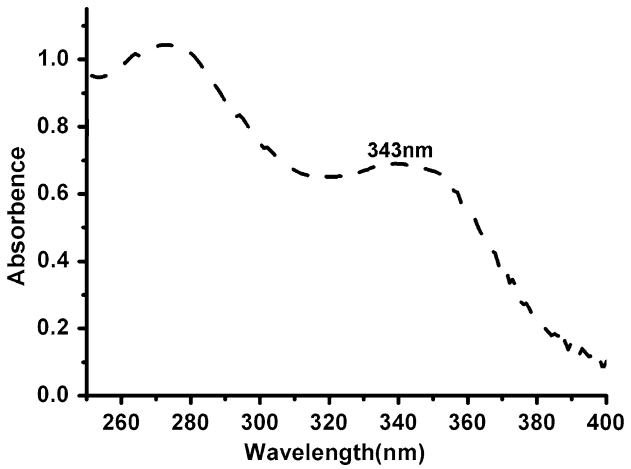
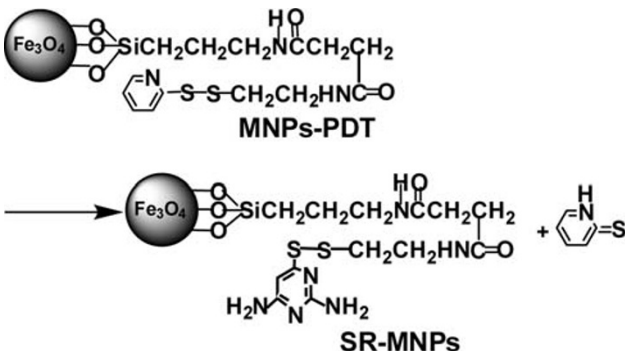


Fig. 5 UV–Vis curve of the supernatant after DMP was added to MNPs-PDT suspension

its size via the number of magnetic molecules in a single-magnetic domain. The saturation magnetizations value decreased during the process of modification. This is duo to that the coatings lead to an increase of the surface spins disorientation and that the amount of nonmagnetic coatings increases. The saturation magnetization value of MNPs-PDT is 54.6 emu/g. This larger saturation magnetization enables them very susceptible to magnetic fields and consequently makes the liquid phase separate easily. Notably, from Fig. 4 it can be found that both MNPs and the modified MNPs exhibit the superparamagnetic behavior because the coercivity and remanence are almost zero. The superparamagnetic behavior prevents particle aggregation and enables their application in biomedical and bioengineering field.

Preparation of SR-MNPs

To prepare SR-MNPs, DMP is selected as a thiol-containing model. DMP conjugated with MNPs-PDT to yield a stimuli-responsive disulfide linkage (Scheme 2). Figure 5 illustrates the UV–Vis curve of the supernatant after the interaction between DMP and MNPs-PDT. The absorbance at 343 nm, assigned to the released 2-pyridinethione [12], confirms that DMP conjugates with MNPs-PDT to form SR-MNPs.



Scheme 2 The preparation of SR-MNPs

Drug Release Behavior

After GHS was added to SR-MNPs suspension and kept at room temperature for 20 h, the absorbance at 343 nm was not observed and a new absorbance at 287 nm appeared (Fig. 6). The new UV absorbance at 287 nm originates from the released DMP. The obtained result shows that the disulfide between DMP and MNPs is stimuli-responsive and cleavable.

More attention is given to investigate the influence of the concentration of GHS on the sensitivity of the stimuli-responsive disulfide linkages. SR-MNPs were mixed with different concentration of GHS in aqueous solution,

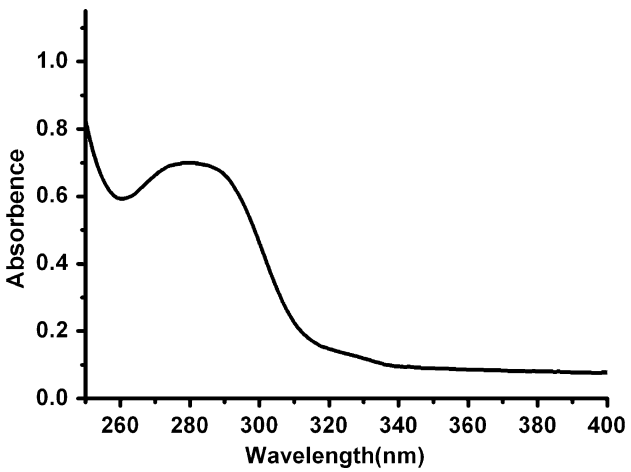


Fig. 6 UV-Vis curve of the supernatant after GHS was added to SR-MNPs suspension

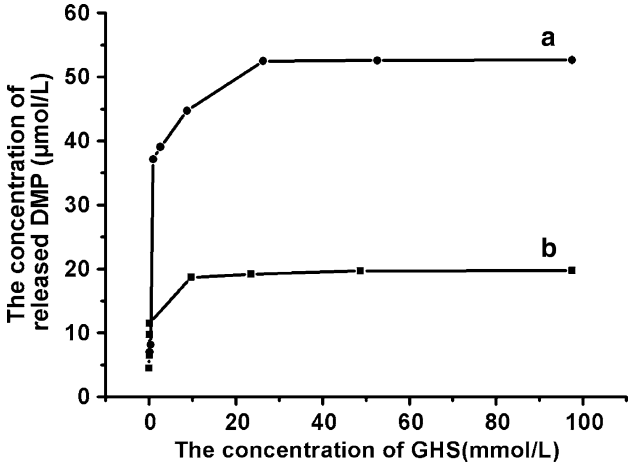


Fig. 7 The plot of the concentration of the released DMP versus the concentration of GHS at the SR-MNPs concentrations of 2 mg/ml (a) and 0.4 mg/ml (b)

respectively. After 20 h, their supernatants were analyzed by UV spectrometer at 287 nm. It was found that the amount of released DMP increases with increasing the concentration of GHS (both at the SR-MNPs concentrations of 0.4 and 2 mg/mL, Fig. 7). This observation indicates that the stimuli-responsive disulfide of SR-MNPs is sensitive to the concentration of GHS [7]. The optimum concentration of GHS to release DMP is in the millimolar range. The concentrations of reducing glutathione are in a millimolar range inside the cells, whereas those in the blood are in a micromolar range [22]. Therefore, SR-MNPs might be stable in the blood stream but be cleaved in the reducing environment of the cytoplasm.

Conclusions

It is clear that SR-MNPs can be synthesized by modifying MNPs with activated pyridyldithio and sequently conjugating thiol-containing molecule (4-diamino-6-mercaptopyrimidine) via stimuli-responsive disulfide linkage. SR-MNPs are sensitive to glutathione concentration: the increase of glutathione concentration is likely to raise the cleavage efficiency of the stimuli-responsive disulfide linkages. The optimum concentration of GHS to cleave the disulfide linkage (smart linkage) is in the millimolar. The synthesized SR-MNPs have the advantages of magnetic motor and selective release for versatile applications, such as drug delivery and bioseparation.

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