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Quantitative Determination of Thiol Groups Modified Mesoporous Silica Nanoparticles by Ellman's Reagent

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Abstract

Ellman's reagent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) is found to be a sensitive tool for the assay of thiol groups. DTNB reacts with aliphatic thiols to form fluorescent derivative "2-nitro-5-thiobenzoate (TNB$^-$)". In this work, Ellman's reagent was used to determine mercapto groups modified Mesoporous Silica Nanoparticles (MSNs). Mesoporous silica materials were prepared with a pore size of 5-6 nm, high surface area ca. 930 m^2/g and a large pore volume of 1.3 cm^3/g. MSNs were fabricated with 3-mercaptopropyl groups in both inner and outer surfaces. Such materials were reacted with DTNB in PBS solution to determine the concentration of 2-nitro-5-thiobenzoate (TNB$^-$) by colorimetric method. The concentration of thiol groups in the outer surface was estimated to be ca. 0.02 mmol/g, while the total amount of mercapto groups coated outer/inner surfaces was calculated to be ca. 0.11 mmol/g. The results indicate that thiol groups in the inner surface Fare almost five times higher than the outer surface.

Keywords

Ellman's reagent; Nanoparticles; Mesoporous silica; Surface modification

Introduction

Nanostructured materials can be fabricated with different size, shape, and surface functionality, which allow for the adjustment of their unique properties over a broad range. There has been an increase in attention to mesoporous nanomaterials during the last decades [1-6]. Mesoporous Silica Nanoparticles (MSNs) have been used for various applications in technology and pharmaceutics, due to their high surface area, excellent physicochemical stability and facile modification [4-9]. The surfaces of silica nanoparticles can be modified through the attachment of silane reagents or coating with polymers [10-14]. Silane reagents can be covalently attached to the surface of silica nanoparticles through a condensation reaction, such as (3-aminopropyl)triethoxysilane or (3-mercaptopropyl) trimethoxysilane [15,16].

3-Mercaptopropyltrimethoxysilane (MPTMS) is often used to functionalize silica surfaces with thiol groups or to deposit silica shells on metallic surfaces [17,18]. Huang and co-workers reported the fabrication of mesoporous silica materials functionalized with MPTMS, followed by conversion of a thiol group to sulfonic acid to be used as a catalyst [19]. Shen et al. have reported the fabrication of PEGylated organosilica-capped mesoporous silica nanoparticles by employing organosilica coated with MPTMS as a gatekeeper to block the mesopores of MSNs [20]. Mosquera et al. developed an efficient coating strategy to stabilize gold nanoparticles (Au NPs) with silica monolayers after immobilization of MPTMS on a gold surface and hydrolyzed under basic conditions [21]. The polymeric silica monolayer on the outer surface of Au NPs can be readily further functionalized using silane coupling agents.

Ellman's reagent [5,5'-dithiobis-(2-nitrobenzoiaccid)] (DTNB) has been used for the determination of mercapto groups by nucleophilic substitution of the reagent [22]. This reagent is usually used to quantify thiol groups in enzymes and proteins under a wide variety of experimental conditions with satisfactory results [22,23]. Woeppel et al. reported the immobilization of thiolated nanoparticles onto glass and silicon substrates via silane chemistry [24]. Ellman's reagent was used to quantify the surface thiol concentration grafted on the nanoparticles, which is estimated to be 12.4 nmol of MPTMS per 0.2 mg of thiolated nanoparticles.

In this work, mesoporous silica nanoparticles (MSNs) were fabricated with a surface area of ~900 m^2/g, and an average pore size of ~5.5 nm. MSNs were modified with mercapto groups on both inner and outer surfaces. 5,5'-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, DTNB) was used to quantify mercapto groups on the surface. When DTNB reacted with materials in PBS solution, the concentration of 2-nitro-5-thiobenzoate (TNB$^-$) increased as the reaction time passes. When the reaction completed, the concentration of 3-mercaptopropyl groups grafted on MSNs was quantified by measuring TNB$^-$ via the colorimetric method. This hypothesis is examined using, BET, FT-IR and UV-vis.

Experimental section

Materials: Elga Pure Nanopore was used to obtain deionized water. N-cetyltrimethylammonium bromide (CTAB, 98%), tetraethylorthosilicate (TEOS, 98%), n-hexane (HPLC grade), (3-mercaptopropyl)trimethoxysilane (MPTMS, 95%), methanol (99.8% HPLC grade), ethanol (99.8%, HPLC grade), toluene (analytical grade) and ammonium hydroxide (28 wt%), phosphate buffered saline (PBS) were purchased from Sigma-Aldrich. 5,5'-Dithiobis(2-nitrobenzoic acid) (Ellman's reagent, DTNB) was obtained from vwr. Ammonium nitrate (95%) was obtained from Fisher Scientific. All the chemicals were used as received.

Mesoporous silica preparations and functionalization: Synthesis of mesoporous silica nanoparticles: 1 g of CTAB was dissolved in 160 mL of deionized water under stirring, followed by addition of 7 mL of concentrated ammonia water (28 wt%). A mixture solution of n-hexane (20 mL) and TEOS (5 mL) was added dropwise to the

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reaction flask under stirring at 35°C. After 12 h, the final product was centrifuged and washed with deionized water and ethanol and dried in an oven at 100°C for 2 h.

Synthesis of 3-mercaptopropyl-functionalized MSNs (MP-MSNs): Mesoporous silica nanoparticles coated with a 3-mercaptopropyl group was obtained in two steps. First, CTAB templates were removed from prepared mesoporous silica nanoparticles using a solution of ammonium nitrate in ethanol (10 mg/mL) at 80°C for 12 h under stirring. The sample was centrifuged, washed with ethanol, and dried in oven. Secondly, thiol-modified MSNs surface was obtained by suspending the nanoparticles (1 g) in a solution of MPTMS (2.5 mmol) in toluene (40.0 mL). The mixture was heated under reflux overnight. The final product was collected by centrifugation, washed with toluene and ethanol, and dried under vacuum.

Synthesis of the 3-mercaptopropyl-functionalized outer surface of MSNs (MP(outer)-MSNs): 3-Mercaptopropyl group coated outer surface of MSNs was obtained in two steps. First, the thiol-modified outer surface of MSNs surface was obtained by suspending the as-prepared nanoparticles (1 g) in a solution of MPTMS (2.5 mmol) in toluene (40.0 mL). The mixture was refluxed overnight. The final product was collected by centrifugation, washed with toluene and ethanol, and dried under vacuum. Secondly, CTAB templates were removed from prepared mesoporous silica nanoparticles using a solution of ammonium nitrate in ethanol (10 mg/mL) at 80°C for 12 h under stirring. The sample was centrifuged, washed with ethanol, and dried in an oven.

Determination of thiol groups functionalized MSNs: Ellman's reagent was used for the determination of thiol groups grafted MSNs. A 0.1 mM solution of DTNB was freshly prepared by dissolving 10 mg of in 250 cm^3 phosphate buffer solution (pH=8). To ensure the accuracy of the method used, 0.1 mM Ellman's reagent was reacted with different concentration of an aqueous solution of mercaptoethanol at 25°C. The possible presence of 2-nitro-5-thiobenzoate (TNB$^-$) in sampled solutions was tested using a colorimetric method of determination. Prior to measurement, the solution was allowed to react for 2 h to ensure complete the reaction. Derivatized solutions were measured using a 1 cm absorption cell at an absorbance wavelength of 412 nm by Shimadzu (UV-2600) UV-vis spectrophotometer. Linear regression analysis of concentration vs. absorption gave regression lines of $y=0.0035x+0.0057$ with a correlation coefficient of 0.9974, as shown in Figure S1.

To determine the quantity of thiol group functionalized MSNs, each functionalized material was conducted to Ellman's reagent solution at different times. Ellman's solutions were freshly prepared with a concentration of 0.1 mM (5 cm^3) and stirred with 10 mg of each material. Each mixture was shaken at 25°C for (5, 10, 20, 30, 40, 60, or 90) min. After each time period, solutions were separated by centrifugation and washed two times with 5 cm^3 buffer solution. All fragments of each experiment were combined to determine the concentration of 2-nitro-5-thiobenzoate (TNB$^-$) in the final solution by colorimetric method, and the concentration was calculated on the basis of a calibration curve.

Measurement and characterization

Surface area analysis: The surface area of fabricated nanoparticle was measured using nitrogen physisorption isotherms on a Micromeritics Gemini 2375 volumetric analyzer. Prior to analysis, each sample was degassed for 5 h at 120°C. The Brunauer-Emmett-Teller (BET) surface areas were determined by experimental points at a relative pressure (P/P°) of 0.05-0.25. The pore volume of each sample was determined from N_2 adsorbed at the P/P° of 0.99. MSNs average pore size distribution was calculated using the Barrett-Joyner-Halenda (BJH) model. Scanning Electron Microscopy (SEM): JEOL JSM-6380 LA was used to collect SEM images. The dried samples were used for imaging without any treatment. Transmission Electron Microscopy (TEM): A JEOL JEM-1230 was used for TEM imaging. A drop of diluted sample suspension in HPLC ethanol was placed on a copper grid and dried at room temperature the measurement. FTIR Spectroscopy: Thermo Scientific Nicolet iS10 instrument was used to obtain the IR spectra of all samples. The spectra were obtained in KBr pellets in the 4000-400 cm^{-1} region with a resolution of 4 cm^{-1}. Thermogravimetric analysis (TGA): SII TGA 6300 instrument was used to determine the decomposition of materials, with a heating rate of 10°C/min under N_2.

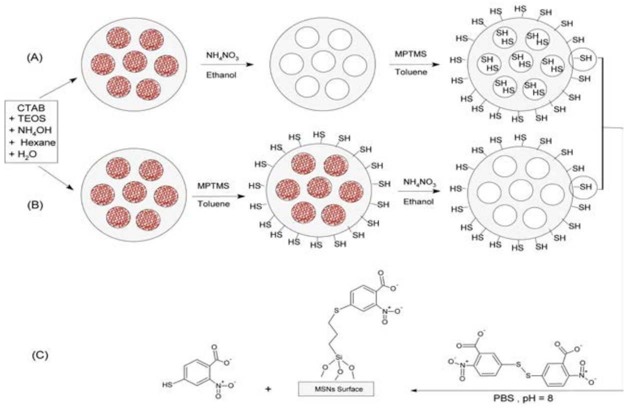
Results and Discussion

MSNs were prepared by mixing TEOS with a template (CTAB) in the presence of pore expander (n-hexane), as shown in Scheme 1. 3-Mercaptopropyltrimethoxysilane (MPTMS) can be conveniently anchored onto MSNs to functionalize the internal and external mesopore surfaces.

Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM) was used to study the morphology of the MSNs nanoparticles. SEM image reveals that the cleaned MSNs are almost nanospheres, and the particle size is polydisperse, ranging from 150 nm-250 nm, as shown in Figure 1A. TEM image shows that the obtained MSNs are dispersed without aggregation, with a diameter ranging from 150 nm to 250 nm, which agrees with the SEM results (Figure 1B). From TEM images, it can be clearly seen that highly ordered arrays mesopores were formed and pores size was estimated to be ca. 6 nm, due to the pore expanding effect of n-hexane (Table 1).

All samples of the cleaned MSNs, MSN-SH (outer surface), and MSN-SH (outer and inner surface) were characterized using Brunauer-Emmett-Teller (BET). Table 1 summarized the physicochemical properties of all nanomaterials. After surface modification, there were reductions in surface area, and pore volumes were observed. All fabricated materials have a pore size of about (4-6) nm, which similar to the pore size estimated by the TEM image. In Figure 2, the N_2 adsorption/desorption isotherms are Type IV for all prepared samples, typically for the ordered mesoporous materials. At higher relative pressures, small different capillary condensation steps were noted for MSN-SH (outer surface) and MSN-SH (outer and inner surface) compared with cleaned surfaces. The hysteresis loop was broader for cleaned MSNs, compared to 3-mercaptopropyl-functionalized MSNs, which confirming the modification process.

FTIR was used to characterize the cleaned MSNs, MSN-SH (outer surface), and MSN-SH (outer and inner surface), as shown in Figure 3. FTIR spectra show that wide bands at 1250 cm^{-1}-1050 cm^{-1} were attributed to siloxane groups (Si-O-Si) bands. The peak at ~800 cm^{-1} was attributed to Si-O stretching vibration. Peak was noted at ~1640 cm^{-1}, which attribute to the bending vibration of water. These features were noted in all materials. The absorption peaks of 3-mercaptopropyl-functionalized MSNs were noted at ~1490 cm^{-1}, which assigned to C-H as bending vibration in -CH_2-, and peaks around ~2930 cm^{-1} ascribed to C-H as stretch vibration. These



Scheme 1: Illustration of surface modification process of Mesoporous Silica Nanoparticles (MSNs). **(A):** MSNs totally covered with 3-mercaptopropyl groups; **(B):** MSNs outer surface coated with 3-mercaptopropyl groups; and **(C):** Surface reaction of MSNs with Ellman's reagent..

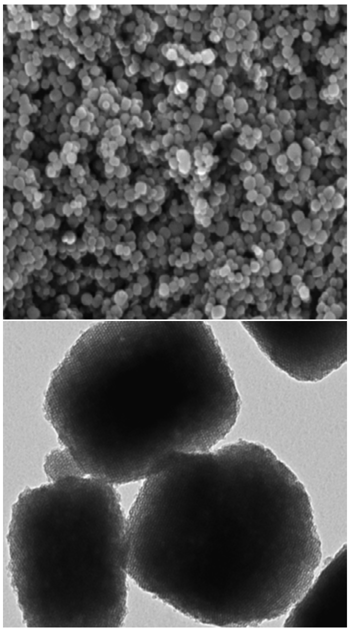


Figure 1: (A): SEM images of mesoporous silica nanoparticles; **(B):** TEM images of mesoporous silica nanoparticles.

Table 1: Physiochemical data obtained for the cleaned MSNs, MSN-SH (outer surface), and MSN-SH (outer and inner surface).

Material	BET surface area ($m^2.g^{-1}$)	Pore volume ($cm^3.g^{-1}$)	Average pore size (nm)
Cleaned MSNs	930	1.16	~5
MSN-SH (outer surface)	753	0.97	~5
MSN-SH (outer and inner surface)	691	0.87	~5

characters provide evidence of the successful functionalization of 3-mercaptopropyl groups.

In our studies, to quantify the number of accessible thiol groups on the outer and inner MSNs surface, Ellman's reagent (DTNB) was used. When DTNB reacts with the surfaces, 2-nitro-5-thiobenzoate (TNB^-) is generated this absorbs at 412 nm. Figure 4 illustrates the UV-vis spectra of the solution after the reaction between MSN-SH (outer and inner surface) and DTNB at different time. It can be seen that the amount of produced TNB^- increased as the reaction time increased which the thiol concentration was calculated based on the calibration curve.

Figure 5 shows the concentration of TNB^- in the solution which is corresponding to the concentration of mercapto groups grafted on MSNs at different times. MSN-SH (outer surface) requires about 10 min to reach the maximum amount of released TNB^-, with ca. 0.02 mmol/g. For the MSN-SH (outer and inner surface), the amount of released TNB^- dramatically increased to 0.08 mmol/g in 30 min and then remains to 0.11 mmol/g after 40 min. It indicates that outer surface samples display a fast reaction rate and fewer thiol groups, while outer and inner surface samples exhibiting a lower reaction rate and higher thiol groups, due to their thiol groups in opened mesopores and the diffusion of DTNB/TNB^-.

The successful surface reaction between marcapto groups and Ellman's reagent was evaluated by thermogravimetic analysis (TGA) when heating in an N_2 atmosphere to 600°C (Figure 6). After surface modification with MPTMS in both materials (-SH in outer and outer/inner surfaces), ca. 8 wt% of weight loss was observed. When the MSN-SH (outer surface) was reacted with Ellman's reagent, the weight loss was about 10 wt%. The weight loss in the MSN-SH (outer/inner surface) after reaction with Ellman's reagent was ca. 17%.

Conclusion

MSNs were prepared with a surface area of ~900 m^2/g, the large pore volume of 1.3 cm^3/g and an average pore size of ~5.5 nm, using the Stöber method in the presence of expander reagent (hexane). MSNs were fabricated with 3-mercaptopropyl groups in both inner and outer surfaces. FT-IR spectra show peaks at ~1490 cm^{-1} and ~2930 cm^{-1}, which assigned to C-H. Ellman's reagent was successfully reacted with fabricated materials. The intensity of the absorption peak of TNB^- increased as the reaction time passes. The reaction in the outer surface was noticed to be 4 times faster, comparing to the fully coated surface. Thiol groups in the outer surface were calculated to be ca. 0.02 mmol/g, while the total amount of marcapto groups coated outer/inner surfaces was estimated to be ca. 0.11 mmol/g. The amount

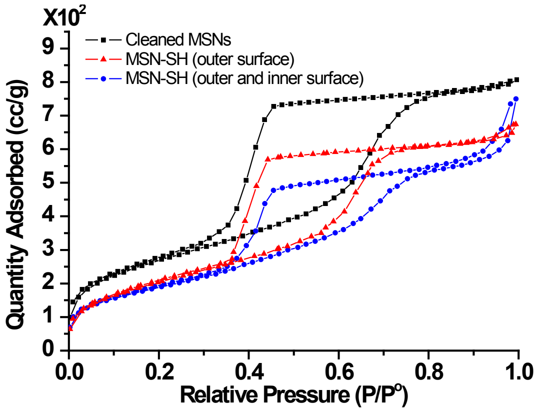


Figure 2: Nitrogen adsorption isotherms of the cleaned MSNs (■ black), MSN-SH (outer surface) (▲ red), and MSN-SH (outer and inner surface) (● blue).

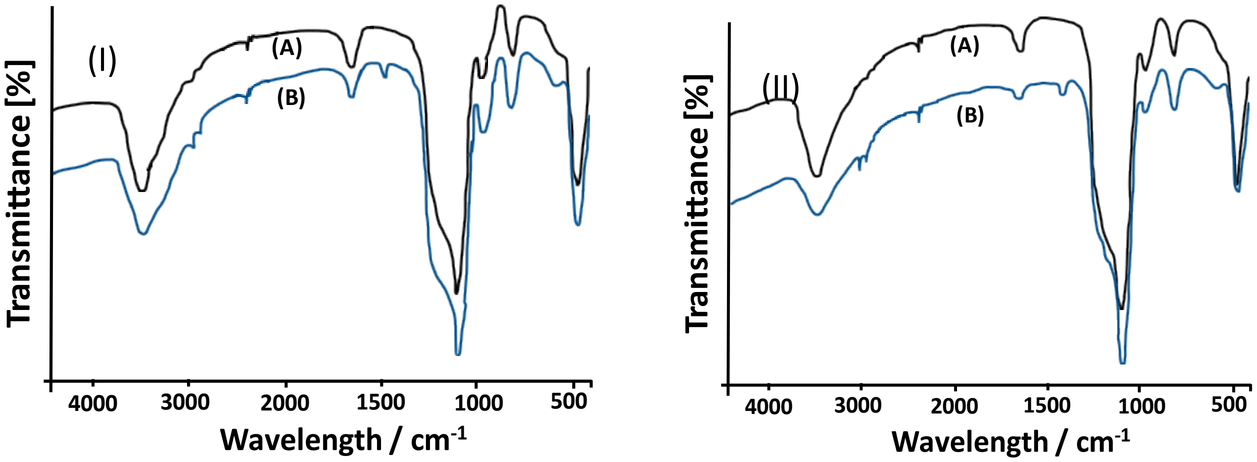


Figure 3: **(I):** FTIR spectra obtained for **(A):** the cleaned MSNs; **(B):** MSN-SH (outer surface); **(II):** FTIR spectra obtained for **(A):** the cleaned MSNs; **(B):** MSN-SH (outer and inner surface).

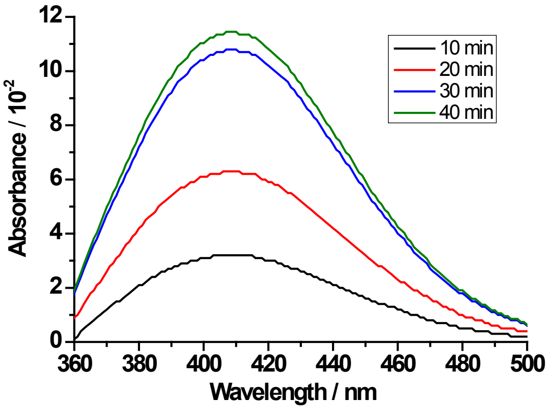


Figure 4: UV-vis spectra of the PBS solution after the reaction between MSN-SH (outer and inner surface) and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) at different time.

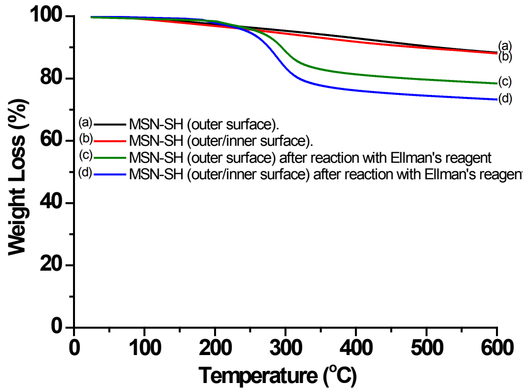


Figure 6: Thermogravimetric analysis (TGA) of **(A):** MSN-SH (outer surface); **(B):** MSN-SH (outer/inner surface); **(C):** MSN-SH (outer surface) after reaction with Ellman's reagent, and **(D):** MSN-SH (outer/inner surface) after reaction with Ellman's reagent.

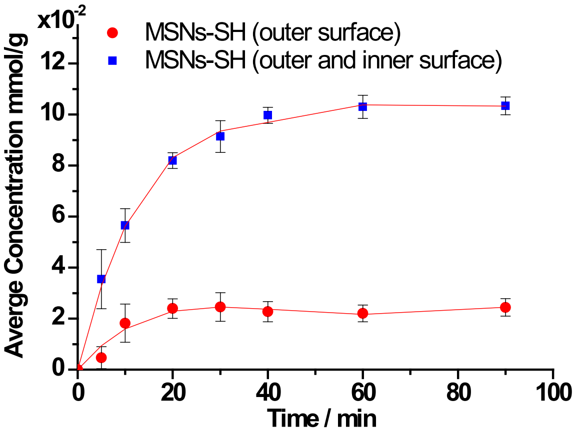


Figure 5: Thiol concentration grafted on MSNs at different time.

of thiol groups in the inner surface is almost five times higher than the outer surface.

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