Oxidative transformation of thiol groups to disulfide bonds in mesoporous silicas: a diagnostic reaction for probing distribution of organic functional groups†

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A novel reaction route for the oxidation of thiol groups to disulfide bonds in mesoporous silicas with the aid of Cu^{2+} adsorption is reported for the first time, and most importantly it can serve as a diagnostic reaction for probing the spatial proximity and distribution of organic functional groups.

Mesoporous materials functionalized with appropriate organic functional groups have found promising applications in environmental remediation, for example, for the adsorption of heavy metal ions, due to their important structural characteristics such as well-defined pore structures, large surface area, and highly accessible functional groups. Of particular interest is that thiol-functionalized mesostructured silicas MCM-41 and HMS, prepared either by post-grafting or by co-condensation methods, have recently gained recognition as the most exceptional adsorbents for Hg^{2+} ion trapping.[1] Besides the thiol-containing moieties, other organic functional groups such as amines, thiourea, and imidazole have been used as metal ion binding motifs for the efficient removal of toxic heavy metals like Hg^{2+}, Cu^{2+}, and Cd^{2+}.[2] Recently, we have synthesized a series of thiol-functionalized mesoporous silica SBA-1 adsorbents (cubic $Pm\bar{3}n$ mesophase) with uniform porosities and investigated their affinity for Hg^{2+} binding.[3] We have demonstrated that the ^{13}C NMR chemical shift of the carbon adjacent to –SH groups is sensitive to Hg^{2+} binding.[3b] Up to now, however, there is no report on the reactivity of organic functional groups in mesoporous silicas after metal ion adsorption.

Oxidative transformation of thiols to disulfides is important from synthetic and biochemical points of view. For example, disulfide bonds are required for the stability and function of a large number of secreted proteins.[4] The reagents such as metal ions, metal oxides, nitric oxide, hydrogen peroxide and halogens have been utilized for oxidation of thiols to disulfides.[5] Among the various metallic elements, copper has shown promising catalytic ability to convert methanol and ethanol to aldehydes.[6] Chen *et al.* carried out oxidation of a series of alkanethiols (RSH) to disulfides (RSSR) in the presence of Cu nanoparticles, prepared using laser irradiation.[7] Although the effective adsorption of Cu^{2+} ions has been reported using mesoporous materials functionalized with amine or thiol groups, the reactivity of thiol moieties in mesoporous silicas after Cu^{2+} adsorption has not yet been reported. In this letter, a novel reaction route for oxidative transformation of thiol groups to disulfide bonds in mesoporous materials with the aid of Cu^{2+} adsorption is reported. This is the first report on the reactivity of organic functional groups in mesoporous silicas after metal ion adsorption. One of the major interests of organic functionalized mesoporous silicas is the framework distribution of organic functional groups and their spatial proximity. However, such information is often not achievable. In view of two adjacent thiol groups for the formation of a disulfide bond, this reaction route can serve as a diagnostic reaction for probing spatial proximity and distribution of organic functional groups in mesoporous silica materials.

A variety of mesoporous silica materials (SBA-1, SBA-15, and MCM-41) containing variable loadings of mercaptopropyl groups were prepared by following the previously published procedures.[3a,8] Thiol-functionalized SBA-1 and SBA-15 were prepared *via* co-condensation of 3-mercaptopropyl trimethoxysilane (MPTMS, Aldrich) and tetraethoxysilane (TEOS, Aldrich) in aqueous HCl solutions containing cetyltriethylammonium bromide (CTEABr) and triblock copolymer Pluronic P123 as the template agents, respectively. The materials were labeled as S1 (or S15)-x, where x represents the molar ratio of MPTMS/(TEOS + MPTMS) in the initial mixture. The thiol-functionalized MCM-41 was prepared by post-grafting method and denoted as M-0.14, where the number 0.14 represents the percentage of thiol moiety grafted on MCM-41 as determined by ^{29}Si NMR spectroscopy (Fig. S1, ESI†). The structural properties of these functionalized silicas were fully characterized by using X-ray diffraction (XRD), solid-state NMR spectroscopy, and N_2 sorption measurements (Fig. S2 and S3 and Table S1, ESI†). All of the characterization results indicated that the adsorbents exhibited well-ordered silica frameworks with uniform porosities. Both surface area and pore volume decreased with increasing amounts of thiol groups incorporated for the cases of S1-x (Table S1, ESI†) and pure SiO_2 prepared with identical experimental conditions without P123 templating (Fig. S4, ESI†).

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† Electronic supplementary information (ESI) available: Text for DFT calculations and characterization (^{29}Si MAS NMR spectra, XRD patterns and N_2 sorption isotherms). Optimized structures of cyclic-T′-(Q′)$_n$-T′-(Q′)$_{(38-n)}$ (n = 8 and 3) and T^3-$(Q^4)_3$-T^3. See DOI: 10.1039/b9nj00389d

Fig. 1 shows the ^{13}C CPMAS (cross polarization magic angle spinning) NMR spectra of S1-0.2 and S1-0.06 after Cu^{2+} adsorption from the solutions of various Cu^{2+} concentrations. For the sample without Cu^{2+} adsorption, the most prominent peaks arise from mercaptopropyl groups at 28 ppm for the two methylene carbons (C2 and C3 next to –SH) of the propyl chain and at 11 ppm for the carbon (C1) directly bound to the silicon atom.[3] Two additional peaks at 42 and 22 ppm were observed after Cu^{2+} adsorption, and their peak intensities increased progressively with increasing Cu^{2+} concentrations, while the intensity of the peak at 28 ppm from thiol moieties decreased. These two signals result from the formation of disulfide bonds and are attributed to the carbons of a propyl moiety situated, respectively, in α and β positions from the disulfide bond. The same peak at 11 ppm is due to the carbon directly attached to the silicon atom in dipropyl disulfide. In a recent paper, disulfide-containing silane precursors have been prepared and their corresponding ^{13}C NMR signals are consistent with our present results.[9] A small peak at 53 ppm and a shoulder at 18 ppm due to the sulfonic acid groups, another oxidation product of thiol groups, were observed.[1e] The Cu^{2+} adsorption capacity of S1-0.2 (with a sulfur content of 2.39 mmol g^{-1})[3a] was found to be 596 mg g^{-1}, which corresponds to a Cu/S ratio of 3.9. As evident from Fig. 1, ^{13}C CPMAS NMR can also serve as a sensitive probe to detect the trace amounts of Cu^{2+} ions in the solutions by monitoring the characteristic peaks of disulfides at 42 and 22 ppm.

The cyclic voltammograms (CVs) of S1-0.2 after Cu^{2+} adsorption from solutions of Cu^{2+} concentrations of 140 and 1000 ppm are displayed in Fig. S5 (ESI†). The most distinct difference between these two CVs is attributed to the characteristics appearing near 0.2 V. While the sample loaded with an excessive amount of Cu^{2+} (1000 ppm) gave rise to a pronounced peak at 0.2 V, the sample loaded with much less Cu^{2+} (140 ppm) has a nearly featureless current–voltage profile. Thus, the reduction peak near 0.2 V is ascribed to the reduction of Cu^{2+} to Cu^0. This peak was not observed for the sample loaded with 140 ppm Cu^{2+}, possibly because Cu^{2+} ions were used up in the oxidation of thiols to disulfide species. The apparently lower peak current in the anodic branch of the redox couple near 0.2 V indicates the poor reversibility of the redox process of $Cu^{2+/0}$ in the present system. We attribute this result mainly to the difficulty to oxidize Cu atoms trapped in the mesoporous materials, because the lack of a suitable hydration and ionic environment for Cu^{2+} species. This could be in line with the CV result observed for the sample with low Cu^{2+} loading, which reveals no reduction of Cu^{2+} near 0.2 V. The sample with low Cu^{2+} loading (140 ppm) also contains Cu, but mainly in a metallic state. This would explain why there is no paramagnetic broadening of NMR signals. These metallic Cu species were not oxidized even in the air; otherwise, the reduction of Cu^{2+} should have given rise to a peak near 0.2 V.

The same adsorption experiments were also performed on different types of mesoporous silica materials MCM-41 and SBA-15. As shown in Fig. 2, similar NMR spectral phenomena were observed for the cases of thiol-functionalized MCM-41 and SBA-15. For the case of SBA-15, the peaks in the range of 70–75 ppm and at 16 ppm (–CH_3 in PPO) are due to the residual surfactant P123, which was effectively, but not completely, removed, while two peaks at 58 and 16 ppm are due to the surface ethoxy groups (Si–OCH_2CH_3).[10] As evidenced in Fig. 2, the residual P123 did not have significant influence on the oxidation of thiols. Therefore, it can be concluded that formation of disulfide bonds *via* oxidation of the thiol groups incorporated in mesoporous silicas in the presence of copper ions is a general phenomenon. The same NMR signals due to the formation of disulfide bonds were observed when $Cu(NO_3)_2$ was replaced by $CuCl_2$, suggesting that the counter anion has no influence on the oxidation of thiols.

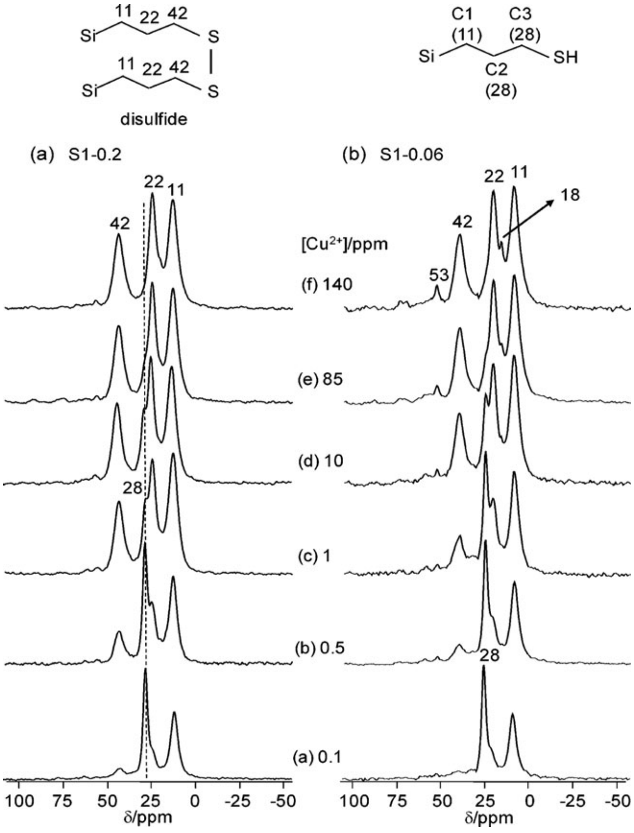

Fig. 1 ^{13}C CPMAS NMR spectra of (a) S1-0.2 and (b) S1-0.06 after Cu^{2+} adsorption from the solutions of various Cu^{2+} concentrations as indicated in the figure. The dashed line represents the peak position of 28 ppm.

One of the major interests of organic-functionalized mesoporous silicas is the framework distribution of organic functional groups and their spatial proximity. Since a disulfide is formed as the result of an oxidation reaction between two adjacent thiol groups, the present reaction route may be utilized to probe the spatial proximity between thiol moieties in mesoporous silicas. Therefore, the same experiments were also performed on the S1-*x* (x = 0.06 and 0.1) samples with a much lower –SH content to ensure the site isolation of thiol moieties in the pore channels. Interestingly, a nearly complete oxidation of the thiol groups to disulfide bonds in S1-0.06 was also observed (Fig. 1B). This observation suggests that there exists two adjacent –SH groups even in the mesoporous silicas

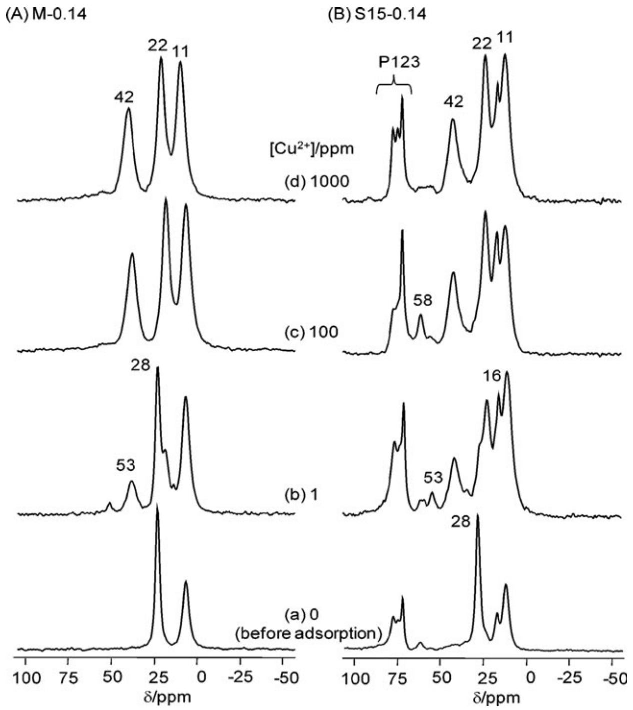

Fig. 2 ^{13}C CPMAS NMR spectra of (A) M-0.14 and (B) S15-0.14 before and after Cu^{2+} adsorption from the solutions of various Cu^{2+} concentrations as indicated in the figure.

with a low –SH content. ^{29}Si MAS NMR also revealed that all the thiol moieties were retained and immobilized on the mesopore surface after Cu^{2+} adsorption. For the samples prepared by the one-pot direct-synthesis route, the assumption of uniform distribution of organic functional groups in mesoporous silicas is often made without direct evidence. To further testify this assumption, molecular modelling is employed to investigate the possible framework distribution of thiol groups, which is critical in their close proximity to form a disulfide bond. Nine molecular models of cyclic-T′-(Q′)$_n$-T′-(Q′)$_{(38\ -\ n)}$ (n = 0–8) are constructed, where T′, a T-like unit of O–SiHR–O (R = $(CH_2)_3SH$), and Q′, a Q-like unit of O–SiH_2–O, are used to model the various T and Q species, respectively. They are energy-minimized using an MM+ molecular mechanics force field in the suite of the HyperChem program.[11] The diameter of these nine models is approximately 30 Å, which is close to the pore size of SBA-1. The energy and S···S distance profiles as a function of the number of Q′ units is shown in Fig. 3. When n is smaller than or equal to three, the structures not only own lower energies, but also have shorter S···S distances (*e.g.*, 4.17 Å for n = 3, Fig. S6, ESI†), which are beneficial to form a disulfide bond. Therefore, the spatial proximity of two T species with three Q species in between may be inferred as the upper limit for the formation of a disulfide bond. Density functional theory (DFT) calculations at the B3LYP/6-31G(d,p) level show that the disulfide bond length in the T^3-$(Q^4)_3$-T^3 motif is 2.08 Å (Fig. S7, ESI†). Since the S···S distance in the n = 3 model is longer than that of a stable disulfide bond, the mesoporous silica framework will suffer more strains after disulfide bond formation than its reduced form. However, the disulfide bond formation is an exothermic reaction which may compensate the unfavorable energy from strains. By contrast, when $n > 3$, the structures have higher energies and longer S···S distances. For the n = 8 model system, its optimized structure has the S···S distance of 11.20 Å (Fig. S8, ESI†), which is too far away to form a disulfide bond, even though the T/(T + Q) ratio in the T'^3-$(Q')_8$-T'^3 motif agrees with that of S1-0.2. Therefore, if the T and Q moieties are uniformly distributed in the final organic functionalized mesoporous silicas as proposed in the one-pot direct-synthesis route, the formation of disulfides is not possible because the thiol groups incorporated will be too far from each other in this case. It should be noted that mesoporous silica SBA-1 exhibits a bimodal pore structure.[12] For the case of S1-0.2, the surface area and pore volume due to micropores is 350 $m^2\ g^{-1}$ and 0.19 $cm^3\ g^{-1}$, respectively. If some portions of the thiol groups were distributed homogeneously in micropores, it is possible that two –SH groups are adjacent to each other to form a disulfide bond. To be consistent with nearly full oxidation of thiol groups to form disulfide bonds as experimentally observed, it can be inferred that there must be heterogeneous distribution of thiol groups in the mesopores. This probably results from the fact that the more hydrophobic nature of MPTMS than that of TEOS tends to aggregate in aqueous solution bringing them closer and thus leads to heterogeneous distribution between the Q and T species throughout the mesopore surface. This situation will favor the formation of disulfide bonds after Cu^{2+} adsorption. Similar adsorption experiments were also conducted for some metal ions such as Cd^{2+}, Pb^{2+}, Fe^{2+}, Co^{2+}, and Ag^+. None of these metal ions can induce the oxidation reaction of thiol groups. Therefore, oxidative transformation of thiol groups to disulfide bonds in mesoporous silica materials is specific on Cu^{2+} ion trapping.

In conclusion, we have demonstrated, for the first time, that oxidation of thiol groups to disulfide bonds can be achieved in a variety of thiol-functionalized mesoporous silicas with the aid of Cu^{2+} adsorption. ^{13}C CPMAS NMR is a sensitive probe to detect the trace amounts of Cu^{2+} ions in the

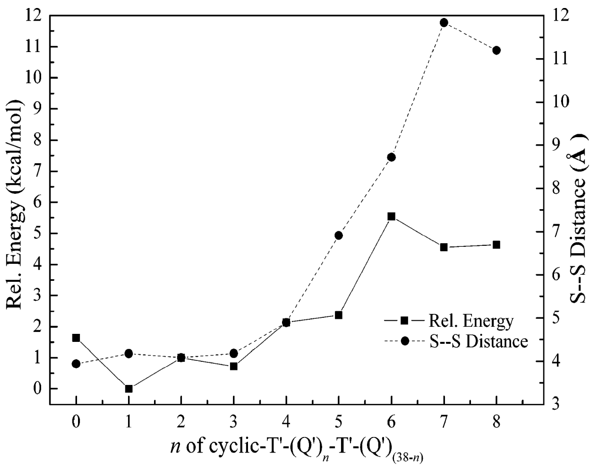

Fig. 3 The energy and S···S distance profiles as a function of the number of the Q′ units in cyclic-T′-(Q′)$_n$-T′-(Q′)$_{(38\ -\ n)}$ model. T′ represents the O–SiHR–O (R = $(CH_2)_3SH$) unit and Q′ is the O–SiH_2–O unit.

solutions. In view of two adjacent thiol groups in order for the formation of disulfide bonds, it can be inferred that there exists heterogeneous distribution of thiol groups in the mesoporous silica materials prepared by the one-pot direct-synthesis route.

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Experimental section

Preparation of thiol-functionalized mesoporous silicas

Thiol-functionalized SBA-1 was synthesized *via* co-condensation of MPTMS and TEOS using CTEABr as the template by following the synthesis procedures described earlier.[3] The molar composition of the reaction mixture was 1 CTEABr : 5 (TEOS + MPTMS) : 460 HCl : 3500 H_2O. The materials obtained were denoted as S1-x, where x (x = 0.06, 0.1, and 0.2) is the molar ratio of MPTMS/(MPTMS + TEOS) in the initial gel mixture. The template was removed from the as-synthesized material by the solvent extraction process. A suspension of 0.35 g as-synthesized sample was stirred in a solution of 5 g HCl (36 wt%) in 220 mL ethanol at 60 °C for 6 h. Thiol-functionalized SBA-15 was prepared with a MPTMS/(MPTMS + TEOS) molar ratio of 0.14 by following the previously reported procedures.[8] The template was removed from the as-synthesized material (1.5 g) by washing with ethanol (400 mL) under reflux for 24 h. The sample was denoted as S15-0.14. The MCM-41 material was synthesized according to a previously described method using sodium silicate and cetyltrimethylammonium bromide (CTMABr, Aldrich) as the source materials for silicon and surfactant, respectively.[1a] The as-synthesized sample was then calcined at 560 °C for 8 h to remove the organic templates. Thiol-functionalized MCM-41 was synthesized by post-grafting 1 g MCM-41 with 5.0 mmol MPTMS in toluene under stirring at 50 °C for 12 h. The solid product was obtained by filtration, washed with toluene and acetone, and dried in air at room temperature. The sample was denoted as M-0.14. Thiol-functionalized MCM-41 *via* co-condensation of TEOS and MPTMS in basic conditions was also prepared and examined by ^{13}C CPMAS NMR. However, small amounts of MPTMS underwent oxidation reactions under such synthesis conditions.[1e]

For Cu^{2+} adsorption, 200 mg of adsorbent was added into 200 mL of $Cu(NO_3)_2$ aqueous solution with various contents of Cu^{2+} and stirred at room temperature for 12 h. The mixture was then filtered and the residual Cu^{2+} in the solution was measured by ICP-AES (inductively coupled plasma-atomic emission spectrometry). The efficient binding ability of the adsorbents reduces the concentration of metals by approximately 99% in nearly all the solutions, resulting in negligible residual Cu^{2+} concentrations. The same experiments were also performed with aqueous solutions containing $CuCl_2$ or other metal nitrates. These adsorbents were then measured by ^{13}C CPMAS NMR.

Characterization methods

Powder X-ray diffraction (XRD) patterns were collected on Wiggler-A beamline (λ = 0.133320 nm) at the National Synchrotron Radiation Research Center in Taiwan. N_2 adsorption–desorption isotherms were measured at 77 K on a Micromeritics ASAP 2020 analyzer. The sample was degassed at 180 °C for 3 h before measurements. Specific surface areas were calculated by using the Brunauer–Emmett–Teller (BET) method. The pore size distribution was obtained from the analysis of the adsorption branch of the isotherm by the Barrett–Joyner–Halenda (BJH) method. Pore volumes were obtained from the volumes of N_2 adsorbed at P/P_0 = 0.95 or in the vicinity. Solid-state ^{13}C and ^{29}Si NMR spectra were recorded on a Varian Infinityplus-500 NMR spectrometer, equipped with 5.0 and 7.5 mm Chemagnetics probes. ^{13}C CPMAS NMR spectra were recorded by using a contact time of 1 ms and at a spinning speed of 6 kHz. Single pulse experiments with a $\pi/6$ pulse of 2 μs and a recycle delay of 200 s were used to acquire the quantitative ^{29}Si MAS NMR spectra. Both ^{13}C and ^{29}Si chemical shifts were externally referenced to tetramethylsilane (TMS) at 0.0 ppm. Electrochemical studies were performed with a three-electrode configuration, consisting of a reversible hydrogen electrode (RHE) as the reference electrode and a Pt wire as the counter electrode. In order to study the electroactivity of the insulating mesoporous powders, 30 mg of the S1-x samples after adsorption of different amounts of Cu^{2+} ions was mixed with carbon black (50 mg), followed by addition of 1 mL methanol and 50 μL Nafion (in 5 wt% solution of lower aliphatic alcohols and H_2O mixture). The resulting mixture was sealed and sonicated for about 2 h to ensure a uniform solution. Afterwards, 5 μL of the resulting mixture was spread onto a glassy carbon electrode and dried in an oven at 60 °C. The electrode was then transferred into the electrochemical cell containing 1 M H_2SO_4. The potentials were cycled with a scan rate of 10 mV s^{-1}.

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