Analyst

COMMUNICATION

View Article Online
View Journal | View Issue

Visual detection of biological thiols based on lightening quantum dot–TiO_2 composites†

Cite this: *Analyst*, 2014, **139**, 996

Received 17th October 2013
Accepted 13th December 2013

DOI: 10.1039/c3an01957h

www.rsc.org/analyst

Qin Mu, Yan Li,* Yunfei Ma and Xinhua Zhong*

The quenched fluorescence of quantum dots (QDs) attached to TiO_2 nanoparticles was selectively switched on by biothiols through ligand replacement, which makes it feasible for facilely sensing biothiols based on the fluorescence turn on mechanism. The present sensor exhibited excellent selectivity and high sensitivity. Furthermore, a novel fluorescent indicating paper was constructed by immobilizing the probe on filter paper to visually detect biothiols in which only a UV lamp was used.

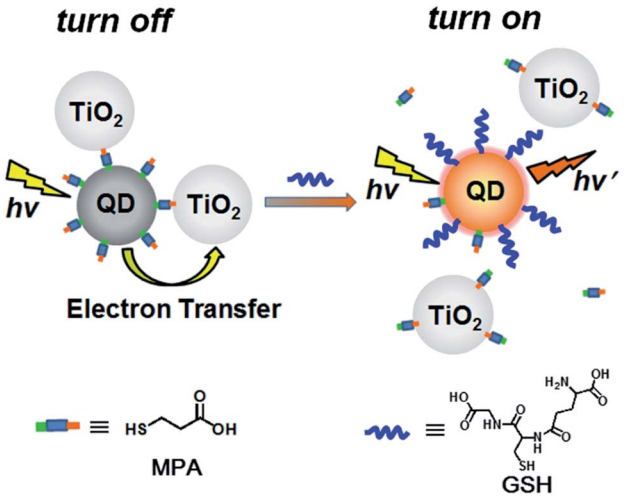
Due to the superior size-tunable optical properties, fluorescent quantum dots (QDs) have attracted increasing interest in many interdisciplinary applications from energy to health.[1] In the past decade, the electron transfer between QDs and TiO_2 semiconductors or graphene was extensively investigated for the fabrication of optoelectronic devices[2] and solar cells.[3] When low band gap QDs are coupled to TiO_2 substrates, electrons can be injected from QDs into TiO_2 substrates because of the lower conduction band edge of QDs compared to that of TiO_2. This is a foundation for the QD sensitized solar cell (QDSC). During the electron injection process, QDs usually serve as an electron-donor and TiO_2 serves as an electron-acceptor.[3] It is well known that QDs are highly sensitive to electron transfer processes, which can bring forward the variation of photoluminescence (PL) intensity, thus generating interest in electron-transfer based biosensing.[4,5] Despite the comprehensive development in the fabrication of solar cells, the application of the electron transfer process between QDs and TiO_2 nanomaterials for sensing biomolecules, to the best of our knowledge, has seldom been described in the literature. In this work, we designed a fluorescent sensor for the detection of biothiols on the basis of the electron transfer between QDs and TiO_2 nanoparticles (NPs).

Shanghai Key Laboratory of Functional Materials Chemistry, Institute of Applied Chemistry, East China University of Science and Technology, Shanghai 200237, P.R. China. E-mail: yli@ecust.edu.cn; zhongxh@ecust.edu.cn; Fax: +86 21 6425 0281

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3an01957h

Biothiols, playing a crucial role in biological systems, are involved in various diseases such as rheumatoid arthritis, heart disease and AIDS.[6] A sensitive and selective detection of thiols in biological fluids therefore is important in both biological processes and medical treatment. Until now, a variety of instrumental methods including high-performance liquid chromatography (HPLC), capillary electrophoresis (CE), electrochemistry (EC), and mass spectrometry (MS) for quantitative detection of biothiols have been reported.[7] These conventional instrumental methods can provide a high degree of specificity and a low detection limit, but usually require expensive and bulky equipment, and the detection procedure is time-consuming. In recent years, chemical techniques especially fluorescent methods have attracted increasing interest due to their high sensitivity, selectivity and simplicity.[8] Most of the fluorescent probes for thiols were designed based on the fluorescent response of organic dye molecules, which involves specific reactions between the probes and the thiol group of the analytes, such as Michael addition, cyclization with aldehydes, cleavage of sulfonamide and sulfonate esters by thiols and others. Although most of the organic fluorescent probes exhibited high selectivity and sensitivity, they also suffer from some shortcomings including low fluorescence quantum yield, easy photobleaching, poor water solubility, and complicated synthesis and purification procedures. Therefore, facile routes with simplicity and high selectivity are still highly desirable in terms of the inherent limitation of the methods available.[5,9]

By the consideration that the quenched fluorescence of QD–TiO_2 NPs can be selectively switched on by biothiols through ligand replacement, herein, we report a novel probe based on turning on the fluorescence of the QD–TiO_2 composite for facilely sensing biothiols including glutathione (GSH), cysteine (Cys) and homocysteine (Hcy). After further immobilization of the probe on a piece of filter paper, an unprecedented indicating paper with theoretical simplicity and low technical demands was developed for the visual detection of biothiols according to the brightness of the indicating paper.



Scheme 1 Schematic illustration of fluorescence quenching of CdTe/CdS QDs by TiO_2 NPs and subsequent turn on with the replacement of MPA ligands by GSH.

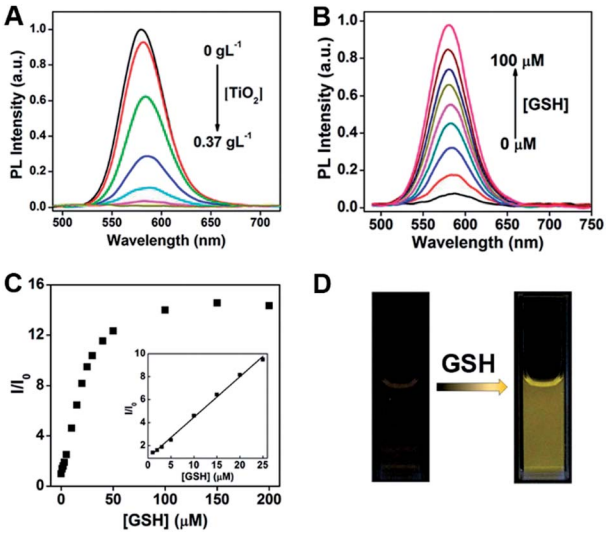


Fig. 1 (A) Fluorescence quenching of CdTe/CdS QDs with the addition of TiO_2 NPs. (B) Fluorescence enhancement of the CdTe/CdS QD–TiO_2 probe with the addition of GSH under the optimal conditions. (C) The relationship between I/I_0 and GSH concentration (where I_0 and I are the fluorescence intensities of QDs in the absence and presence of GSH, respectively). The inset presents the linear relationship between I/I_0 and the concentration of GSH. (D) Colorful images of the QD–TiO_2 probe solution under a 365 nm UV lamp before and after the addition of 100 μM GSH.

Scheme 1 shows the fluorescence switch mechanism of the detection of GSH. When an aqueous solution of mercaptopropionic acid (MPA) capped QDs is mixed with TiO_2 NPs, QDs will be bonded onto TiO_2 NPs through the covalent bonding between the terminal carboxyl group of MPA ligands and the Ti atom on the surface of TiO_2 NPs. This self-assembly strategy has been widely applied in the immobilization of QD sensitizers on TiO_2 film electrodes for the construction of QDSCs.[3] The coupling of QDs with TiO_2 leads to the fluorescence quenching of QDs due to the electron injection from QDs to TiO_2. When GSH was introduced into the QD–TiO_2 system, the MPA ligands on the surface of QDs may be replaced by GSH due to the stronger coordination capacity of GSH with metal ions on the QD surface,[10] and the distance between QDs and TiO_2 NPs is consequently enlarged.[11] As a result, the initially quenched fluorescence of QDs is effectively recovered by the interruption of the electron transfer pathway,[11] making it feasible to construct a selective and sensitive fluorescence probe for GSH.

In order to prove the working principle mentioned above, the fluorescence response of the probe for GSH was recorded, with the results shown in Fig. 1. Firstly, MPA capped CdTe/CdS QDs of high fluorescence with emission wavelength at 580 nm and TiO_2 NPs were synthesized according to the literature procedures, respectively, (details described in the ESI†). Upon the addition of TiO_2, the fluorescence intensity of MPA capped CdTe/CdS QDs decreased remarkably in 20 min and then was constant in 4 h (Fig. S1†), but no obvious change in the emission peak position could be found (Fig. 1A). This indicates that the presence of TiO_2 NPs had no effects on the electronic properties of QDs and just reduced the fluorescence intensity *via* electron transfer. After the subsequent addition of GSH to the solution of QD–TiO_2 composites, the fluorescence of QDs restored gradually along with the increase in the amount of GSH (Fig. 1B). The optimal pH condition (Fig. S2†) was found to be 6.0 probably due to the isoelectric point of GSH (5.93). Since GSH is almost neutral at pH 6.0, there is little electrostatic repulsion between GSH and MPA on the QD surface, which makes GSH effectively displace the MPA ligands. 15 min of the reaction time (Fig. S3†) for the detection of GSH were used. In this case, the fluorescence intensity was linear with the GSH concentration ranging from 1.0 to 25.0 μM with a standard deviation of 0.995 (Fig. 1C). According to the IUPAC 3σ criterion, as the concentration of analyte that produces an analytical signal is equal to three times the standard deviation of the background fluorescence signal, the limit of detection (LOD) of the present assay was calculated to be 0.17 μM.[12] Meanwhile, the originally lightless solution changed to bright yellow-orange under the illumination using a 365 nm UV lamp which could be observed with the naked eye (Fig. 1D). Similar results were obtained for other structurally related biothiols Cys (Fig. S4†) and Hcy (Fig. S5†). The restoration capability of Cys and Hcy, however, was much lower than that of GSH following the order of GSH > Hcy > Cys, which is in accordance with their varied steric hindrance effects. The recovery order agrees with that of the QD-based fluorescent probe for biothiols reported previously.[11,13]

Being combined with QDs, TiO_2 NPs provide a favorable non-radiative channel for the fluorescence quenching of QDs *via* electron transfer, a process could be verified by the shortened exciton lifetime of the QDs from 32.29 to 8.67 ns (Fig. 2 and Table S1†).[14] With the addition of GSH to the QD–TiO_2 system, the remarkable PL recovery was accompanied by the elongation of the exciton PL lifetime to 21.76 ns. The elongation of QD PL lifetime after the addition of GSH demonstrates the shutting off of the electron transfer pathway, which is ascribed to the larger steric hindrance effect resulting from the displacement of MPA

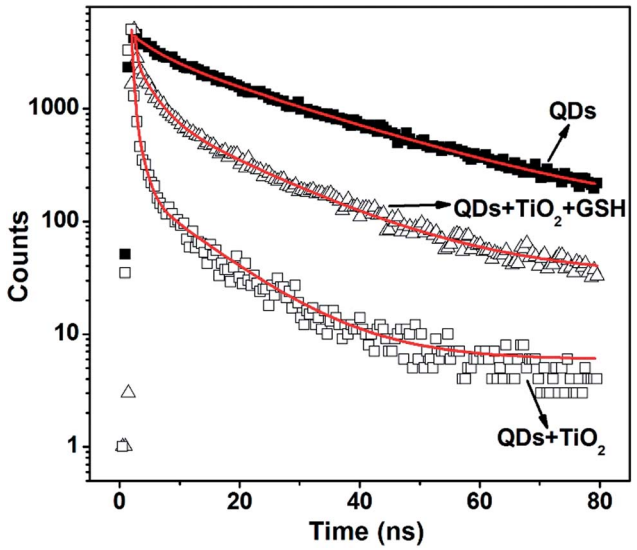


Fig. 2 Normalized PL decay curves of native QDs, and the QD–TiO_2 system in the absence and presence of GSH. The red lines represent the corresponding fitting curves.

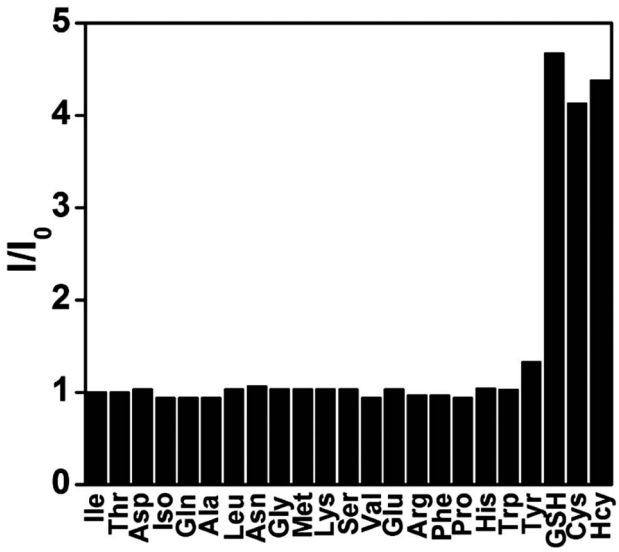


Fig. 3 The fluorescence turn-on selectivity of the present probe for biothiols (10 μM) with other natural amino acids (1 mM) except Tyr with saturation concentration (incubation time 15 min, 1 mM citrate buffer with pH 6.0).

ligands by GSH with stronger coordination capability. A similar elongation of the exciton PL lifetime of the QD–TiO_2 system by Cys and Hcy was also observed (Fig. S6 and Table S1†). In order to further illustrate the fluorescence recovery mechanism, the evolution of PL intensities of MPA-QDs with the addition of GSH was examined (Fig. S7†). The fluorescent intensity of QDs exhibits a negligible change (less than 5%) in 3 h after the addition of 20 μM GSH, indicating that GSH has no effect on the electronic properties of QDs and just acts as a stabilizer or capping ligands for QDs.

The present fluorescence probe exhibits high selectivity for biothiols over other 19 natural amino acids. In the presence of other natural amino acids even at 100-fold higher concentrations, the fluorescence of QDs was still quenched, suggesting that these natural amino acids have little effect on the fluorescence intensity of the QD–TiO_2 probe (Fig. 3). To further evaluate the performance of the designed probe for biothiols in practical applications, the selective PL response with the co-existence of 19 natural amino acids was also measured, as shown in Fig. S8.† Experiment results indicated that the co-existence of the other 19 amino acids (each at 100 μM) did not induce an evident fluorescence response of the present probe, while the fluorescence was obviously restored by GSH as well as Cys and Hcy, similar to the response of those systems in which the corresponding examined biothiols were added alone. Thus, the designed fluorescence probe exhibited excellent selectivity for biothiols. After that, human urine from healthy adult volunteers was used for GSH detection in biologic fluids (details in the ESI†). The recovery of the added known amount of GSH to the urine samples was found to be in the range of 97.41–101.57% (Table S2†), demonstrating that the co-existence of the components containing many inorganic cations and anions in human urine has no significant interference with the detection of biothiols in urine samples. The outstanding selectivity and reliability of the probe for detecting biothiols in biological fluids therefore can be confirmed.

The conventional methods for the detection of GSH usually are restricted, to some extent, by the dependence on expensive instruments or operational complexity. For practicality, we further immobilized the probe on filter paper to prepare GSH-indicating paper for the facile detection of GSH. The GSH detection can be simply applied as a procedure illustrated in Fig. 4A in which only a UV lamp is used. The immobilization was accomplished by the sequential immersion of a piece of filter paper in a QD solution and a TiO_2 NP solution, followed by drying at room temperature. No lighting can be observed for the as-prepared indicating paper under a 365 nm UV lamp (Fig. 4B). After dropping the GSH solution even in trace concentrations

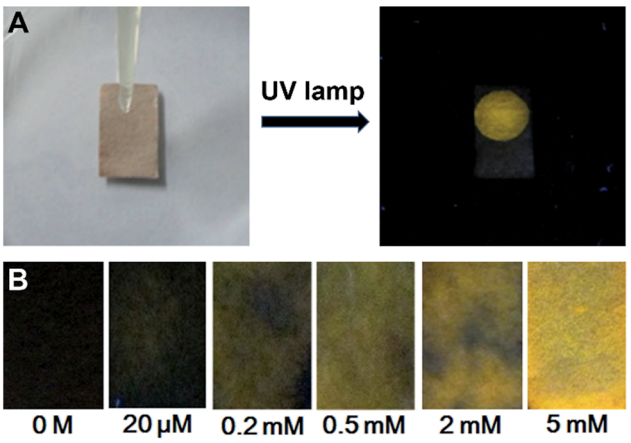


Fig. 4 (A) Facile operational approach for visual detection of GSH by using the designed indicating paper. (B) Color images of the designed GSH-indicating paper after dropping various concentrations of GSH under the illumination of a 365 nm UV lamp.

on the indicating paper, one may find that the QD fluorescence against the dark background is steadily recovered, as shown in Fig. 4. Furthermore, the intensity of QD fluorescence depends on the amount of GSH on the paper. Since the detection limit is defined as the least amount of GSH capable of producing a differently colored fluorescence spot that can be noted by the independent observers, the current detection limits for GSH thus are found to be *ca.* 20 μM.

Conclusions

In summary, we demonstrated here a novel fluorescence probe based on QD–TiO_2 composites for sensing biothiols with high sensitivity and excellent selectivity. This strategy took advantage of the fluorescence quenching of QDs by TiO_2 *via* electron transfer and a subsequent fluorescence turn-on *via* the surface ligand replacement by biothiols. Notably, the indicating paper was further constructed for visual detection of biothiols based on the brightness of the indicating paper. Therefore, this reported indicating paper is a very simple and inexpensive strategy for sensing biological thiols. This sensing method provides a general platform for the visually sensing species based on the QD–TiO_2 system.

Acknowledgements

This work was supported by the Chenguang Program of the Shanghai Education Commission (11CG31), the National Natural Science Foundation of China (no. 21175043 and 21301059), the Science and Technology Commission of Shanghai Municipality (11JC1403100 and 12NM0504101), and the Fundamental Research Funds for the Central Universities.

Notes and references

1 (*a*) P. Zrazhevskiy, M. Sena and X. H. Gao, *Chem. Soc. Rev.*, 2010, **39**, 4326; (*b*) P. V. Kamat, K. Tvrdy, D. R. Baker and J. G. Radich, *Chem. Rev.*, 2010, **110**, 6664; (*c*) C. X. Guo, Y. Q. Dong, H. B. Yang and C. M. Li, *Adv. Energy Mater.*, 2013, **3**, 997; (*d*) R. Freeman and I. Willner, *Chem. Soc. Rev.*, 2012, **41**, 4067; (*e*) Z. X. Pan, H. Zhang, K. Cheng, Y. M. Hou, J. L. Hua and X. H. Zhong, *ACS Nano*, 2012, **6**, 3982.

2 (*a*) X. L. Li, Y. Jia and A. Y. Cao, *ACS Nano*, 2010, **4**, 506; (*b*) P. Bhattacharya and Z. Mi, *Proc. IEEE*, 2007, **95**, 1723.

3 (*a*) Z. X. Pan, K. Zhao, J. Wang, H. Zhang, Y. Y. Feng and X. H. Zhong, *ACS Nano*, 2013, **7**, 5215; (*b*) P. V. Kamat, *J. Phys. Chem. C*, 2008, **112**, 18737; (*c*) A. J. Nozik, M. C. Beard, J. M. Luther, M. Law, R. J. Ellingson and J. C. Johnson, *Chem. Rev.*, 2010, **110**, 6873; (*d*) J. Tang and E. H. Sargent, *Adv. Mater.*, 2011, **23**, 12; (*e*) C. X. Guo, H. B. Yang, Z. M. Sheng, Z. S. Lu, Q. L. Song and C. M. Li, *Angew. Chem., Int. Ed.*, 2010, **49**, 3014.

4 (*a*) I. L. Medintz, M. H. Stewart, S. A. Trammell, K. Susumu, J. B. Delehanty, B. C. Mei, J. S. Melinger, J. B. Blanco-Canosa, P. E. Dawson and H. Mattoussi, *Nat. Mater.*, 2010, **9**, 676; (*b*) L. Jia, J. P. Xu, D. Li, S. P. Pang, Y. Fang, Z. G. Song and J. Ji, *Chem. Commun.*, 2010, **46**, 7166; (*c*) P. Wu and X. P. Yan, *Biosens. Bioelectron.*, 2010, **26**, 485; (*d*) K. Zhang, H. B. Zhou, Q. S. Mei, S. H. Wang, G. J. Guan, R. Y. Liu, J. Zhang and Z. P. Zhang, *J. Am. Chem. Soc.*, 2011, **133**, 8424; (*e*) M. Shamsipur, M. Shanehasz, K. Khajeh, N. Mollania and S. H. Kazemi, *Analyst*, 2012, **137**, 5553; (*f*) C. Yuan, K. Zhang, Z. P. Zhang and S. H. Wang, *Anal. Chem.*, 2012, **84**, 9792; (*g*) R. Freeman, T. Finder, L. Bahshi, R. Gill and I. Willner, *Adv. Mater.*, 2012, **24**, 6416; (*h*) Y. F. Liu, M. Luo, J. Yan, X. Xiang, X. H. Ji, G. H. Zhou and Z. K. He, *Chem. Commun.*, 2013, **49**, 7424.

5 (*a*) S. Banerjee, S. Kar, J. M. Perez and S. Santra, *J. Phys. Chem. C*, 2009, **113**, 9659; (*b*) Y. Zhang, Y. Li and X. P. Yan, *Anal. Chem.*, 2009, **81**, 5001–5007; (*c*) G. Garai-Ibabe, L. Saa and V. Pavlov, *Anal. Chem.*, 2013, **85**, 5542; (*d*) S. Chen, J. N. Tian, Y. X. Jiang, Y. C. Zhao, J. N. Zhang and S. L. Zhao, *Anal. Chim. Acta*, 2013, **787**, 181.

6 (*a*) E. Weerapana, C. Wang, G. S. Simon, F. Richter, S. Khare, M. B. D. Dillon, D. A. Bachovchin, K. Mowen, D. Baker and B. F. Cravatt, *Nature*, 2010, **468**, 790; (*b*) P. K. Sudeep, S. T. S. Joseph and K. G. Thomas, *J. Am. Chem. Soc.*, 2005, **127**, 6516; (*c*) S. Sreejith, K. P. Divya and A. Ajayaghosh, *Angew. Chem., Int. Ed.*, 2008, **47**, 7883; (*d*) Z. Z. Huang, F. Pu, Y. H. Lin, J. S. Ren and X. G. Qu, *Chem. Commun.*, 2011, **47**, 3487; (*e*) K. S. Park, M. I. Kim, M. A. Woo and H. G. Park, *Biosens. Bioelectron.*, 2013, **45**, 65.

7 (*a*) T. Toyo'oka, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2009, **877**, 3318; (*b*) M. Rafii, R. Elango, G. Courtney-Martin, J. D. House, L. Fisher and P. B. Pencharz, *Anal. Biochem.*, 2007, **371**, 71; (*c*) F. Carlucci and A. Tabucchi, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2009, **877**, 3347; (*d*) J. C. Harfield, C. Batchelor-McAuley and R. G. Compton, *Analyst*, 2012, **137**, 2285.

8 (*a*) X. Q. Chen, Y. Zhou, X. J. Peng and J. Y. Yoon, *Chem. Soc. Rev.*, 2010, **39**, 2120; (*b*) Y. Li, P. Wu, H. Xu, H. Zhang and X. H. Zhong, *Analyst*, 2011, **136**, 196; (*c*) H. Xu, Y. W. Wang, X. M. Huang, Y. Li, H. Zhang and X. H. Zhong, *Analyst*, 2012, **137**, 924; (*d*) K. H. Leung, H. Z. He, V. P. Y. Ma, D. S. H. Chan, C. H. Leung and D. L. Ma, *Chem. Commun.*, 2013, **49**, 771.

9 (*a*) Q. Mei and Z. P. Zhang, *Angew. Chem., Int. Ed.*, 2012, **51**, 5602; (*b*) B. L. Ma, F. Zeng, X. Z. Li and S. Z. Wu, *Chem. Commun.*, 2012, **48**, 6007; (*c*) X. Ran, H. J. Sun, F. Pu, J. S. Ren and X. G. Qu, *Chem. Commun.*, 2013, **49**, 1079; (*d*) H. Y. Cao, M. H. Wei, Z. H. Chen and Y. M. Huang, *Analyst*, 2013, **138**, 2420.

10 M. S. Diaz-Cruz, F. Tauler and M. Esteban, *J. Inorg. Biochem.*, 1997, **66**, 29.

11 B. Y. Han, J. P. Yuan and E. K. Wang, *Anal. Chem.*, 2009, **81**, 5569.

12 (*a*) IUPAC, *Spectrochim. Acta, Part B*, 1978, **33**, 241; (*b*) ACS committee on environmental improvement, *Anal. Chem.*, 1980, **52**, 2242.

13 J. F. Liu, C. Y. Bao, X. H. Zhong, C. C. Zhao and L. Y. Zhu, *Chem. Commun.*, 2010, **46**, 2971.

14 (*a*) P. K. Santra, P. V. Nair, K. G. Thomas and P. V. Kamat, *J. Phys. Chem. Lett.*, 2013, **4**, 722; (*b*) W. J. Zhang and X. H. Zhong, *Inorg. Chem.*, 2011, **50**, 4065.