COMMUNICATION

Check for updates

Silver nanoclusters with enhanced fluorescence and specific ion recognition capability triggered by alcohol solvents: a highly selective fluorimetric strategy for detecting iodide ions in urine†

Luping Feng, Zongzhao Sun, Huan Liu, Min Liu, Yao Jiang, Chuan Fan, Yuanyuan Cai, Sheng Zhang, Jihong Xu and Hua Wang [ID] *

Alcohol solvents especially isopropanol were demonstrated for the first time to endow silver nanoclusters (AgNCs) in water with dramatically enhanced red fluorescence. More importantly, the specific iodide recognition capability of the AgNCs could thus be obtained towards a highly selective fluorimetric assay for detecting iodide ions in urine.

It is well established that iodide (I^-) ions can play vital roles in a series of human physiological activities such as mental development and basic metabolism, in which the I^- levels may be closely indicative of some serious diseases like hypothyroidism, hyperthyroidism and carcinogenesis.[1,2] In particular, I^- ions in urine are recognized to be the most sensitive biomarkers for the diagnosis and monitoring of these clinical diseases.[3] In the past few decades, many analysis technologies have been proposed for the detection of I^- ions, such as ion chromatography,[4] atomic absorption spectrometry,[5] colorimetric assay,[6,7] and chemi-luminescence analysis.[8] Nevertheless, most of these classic detection methods may encounter some common disadvantages, either being time-consuming and not cost-effective, or having low detection selectivity. Therefore, exploring a simple, rapid, selective, and sensitive analysis method is of great interest for probing I^- ions, especially those with low levels in complicated media like blood and urine.

Fluorimetric assays have arisen to be one of the fast and sensitive analytical methods widely applied for detecting medical biomarkers including some important anions like I^- ions.[9,10] For example, Leclerc's group reported fluorimetric and colorimetric methods for the detection of μM-scale I^- ions using a cationic polythiophene derivative.[9] A fluorescent chemosensor with a benzimidazole tripodal receptor was also developed for the

detection of I^- ions in aqueous solution.[10] Yet, most of the current fluorimetric methods may focus on the synthesis of fluorescent probes with organic materials. In recent years, increasing effort has been made to introduce noble metal nanoclusters (NCs) as new fluorescent probes for fluorimetric assays and biomedical imaging, most of them being gold (Au) and silver (Ag) NCs, due to their unique properties like strong luminescence and low toxicity.[11–17] As a result, they have been widely employed for the detection of some small molecules like cysteine and heavy metal ions like Cu^{2+} and Hg^{2+} ions.[15–17] For example, Xie *et al.* employed fluorescent AuNCs for the ultra-sensitive detection of Hg^{2+} through Hg^{2+}-induced fluorescence quenching of the AuNCs.[14] Our group also developed some fluorescent sensors for probing Hg^{2+} and/or Cu^{2+} ions using AgNCs or Au–Ag NCs.[15,16] Nevertheless, there are few fluorimetric methods with noble metal NCs for probing inorganic anions like I^- ions of meaningful medical importance. What is more, most of the current fluorimetric protocols may feature fluorescence quenching-based detection of targets, which may suffer from low detection selectivity and sensitivity with limited biomedical applications. Furthermore, the fluorescence intensity and recognition specificity of current fluorescent probes are still desirable to improve, especially probes with blue fluorescence that can be additionally challenged by other fluorophores like proteins in complicated sample backgrounds.

In recent years we have witnessed increasing attention focused on the synthesis and wide applications of Ag nanomaterials with special structures and exotic catalytic and luminescence properties.[18,19] However, the inherent instability of AgNCs in water has limited their applications on a large scale.[20] As a result, much effort has been devoted to the enhancement of the stability and especially the recognition capability of AgNCs typically by surface functionalization or passivation with some functional ligands or polymers.[12,15,21] For example, 3-amino-phenylboronic acid was used to cap CuNCs to achieve the selective detection of glycoproteins.[21] More recently, AgNCs were passivated with glutathione showing the specific recognition

Institute of Medicine and Materials Applied Technologies, College of Chemistry and Chemical Engineering, Qufu Normal University, Qufu City, Shandong Province 273165, P. R. China. E-mail: huawangqfnu@126.com; Fax: +86 5374456306; Tel: +86 5374456306

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c7cc04924b

modulated from Hg^{2+} ions to Cu^{2+} ions.[15] Moreover, various solvents were introduced to alter the luminescence properties and/or the specific recognition of some fluorescent probes.[22,23] These alterations are known as general solvent effects that induce changes in the Stokes shifts.[22] For example, Qu *et al.* discovered that polyethyleneimine-encapsulated AgNCs could display different fluorescence emissions when dispersed in various solvents through solvatofluorochromism.[22] A quinoline-based smart probe was demonstrated with different recognition abilities for Al^{3+} and Cu^{2+} ions in methanol and acetonitrile, respectively.[23]

Inspired by these pioneering studies, in the present work, several kinds of organic solvent were separately introduced to disperse the AgNCs newly synthesized in water. To our surprise, alcohol solvents, especially isopropanol (IPA), were shown to enable the dramatically enhanced red fluorescence of the AgNCs in water. More importantly, IPA could further trigger the highly specific recognition of I^- ions by the AgNCs as a result of fluorescence quenching, which otherwise might not occur in the absence of an alcohol solvent. Scheme 1 illustrates the AgNC-based fluorometric mechanism and procedure for detecting I^- ions by the IPA improved fluorescence enhancement and triggered specific recognition of the I^- ions. To the best of our knowledge, this is the first report on the solvent-dependent fluorescence enhancement and specific ion recognition capability of AgNCs toward the fluorimetric analysis of I^- ions.

The investigation was first conducted on the fluorescence properties of the AgNCs that were synthesized in water and then dispersed separately with different commonly used solvents (Fig. 1). It was noted that the AgNCs could exhibit dramatically enhanced fluorescence intensities (FIs) if various alcohol solvents were introduced, especially with IPA, but they showed different degrees of red shifts following the order: $FI_{IPA} > FI_{n\text{-propanol}} > FI_{ethylene\ glycol} > FI_{ethanol} > FI_{methanol} > FI_{water}$ (Fig. 1A). In contrast, the FI of the AgNCs could be weakened and even quenched in other common solvents such as DMF and THF. Herein, the FI enhancement of AgNCs in different alcohols was
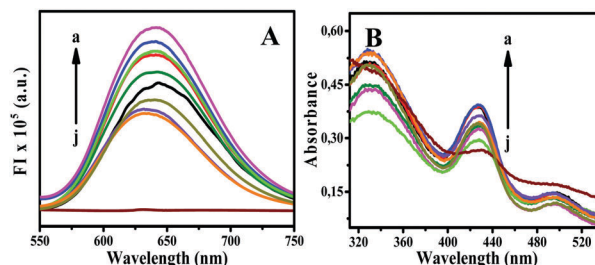

Fig. 1 (A) Fluorescence spectra and (B) UV-vis spectra of AgNCs dispersed in water containing different kinds of solvent. From a to j: IPA, *n*-propyl alcohol, ethylene glycol, ethanol, methanol, water, acetone, DMF, DMSO, and THF with AgNC-to-solvent volume ratios of 50%. The fluorescence spectra were recorded at the excitation wavelength of 425 nm.

thought to result from the well known "electron-donor effects" of the hydroxyl groups of alcohols, as observed for amine groups elsewhere.[24,25] In particular, the "solvent effects" of alcohols, such as the refractive indices, dielectric constant, and polarity, might also contribute to the changes in the Stokes shifts of the AgNCs.[22] What is more, differing from other alcohols and water, IPA with its branched chain might additionally endow AgNCs with greatly improved FI and the detailed mechanism would be explored in the future. It is noteworthy that Fig. 1B reveals that the UV-vis absorption spectra of the AgNCs in these solvents, except for THF, display similar absorption peaks at about 330 nm, 430 nm, and 490 nm that are characteristic of AgNCs, indicating that these solvents might not change the morphological properties of AgNCs like size and structure.

Moreover, it is well recognized that AgNCs in water are notorious for their inherent instability and oxidation susceptibility,[19] yet, they could achieve greatly improved aqueous stability upon the addition of IPA, which could be stored in the dark for up to 12 months with no significant change in FI (Fig. S1A, ESI†). More importantly, alcohols, especially IPA, could surprisingly trigger the specific FI quenching of AgNCs by I^- ions. Fig. S1B (ESI†) shows the comparison of the I^--induced quenching of fluorescence efficiencies of the AgNCs dispersed in water separately containing various alcohols, which are defined as $(F_0 - F)/F_0$, where F_0 and F refer to the FIs of the AgNCs in the absence and presence of I^- ions, respectively. Accordingly, IPA could endow AgNCs with the highest quenching of fluorescence efficiency induced by I^- ions, suggesting that IPA should be chosen ideally for dispersing AgNCs so as to trigger the specific I^- recognition, in addition to enhanced FI and aqueous stability.

A comparable study on the fluorescence responses to I^- ions was further carried out for AgNCs in water with and without IPA (Fig. 2). As shown in Fig. 2A, AgNCs could, as expected, exhibit much stronger FIs in the IPA-containing water (curve c) than the IPA-absent one (curve a), for which the quantum yields are about 5.3% and 3.1%, respectively. Importantly, when I^- ions were added, only a little bit of fluorescence quenching could occur for the AgNCs in water without IPA (curve b). In contrast, the IPA-dispersed AgNCs could exhibit a dramatic FI decrease upon the addition of I^- ions (curve d), for which the FI change is more than 10 times higher than that for the nanoclusters
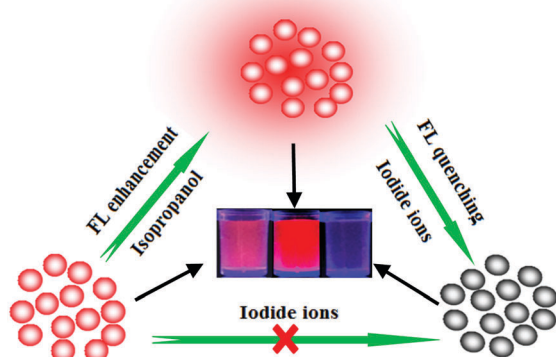
Scheme 1 Schematic illustration of the AgNC-based fluorometric detection mechanism and procedure for iodide ions, showing the IPA-triggered fluorescence enhancement of the AgNCs and then the fluorescence turning-off induced by the iodide ions.
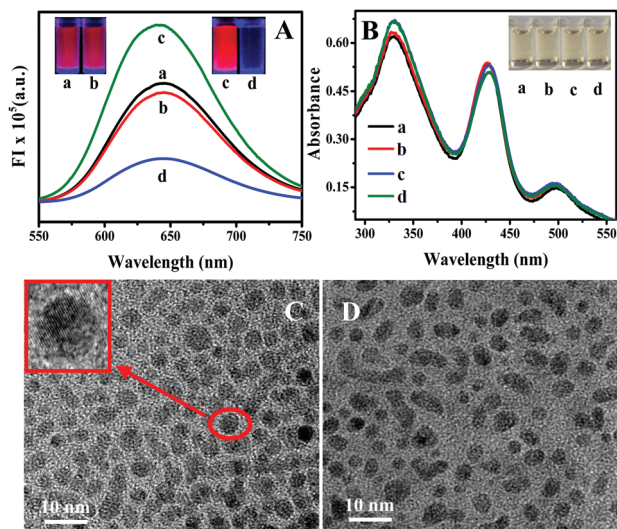
Fig. 2 (A) Fluorescence spectra (inset: photographs under UV light) and (B) UV-vis spectra (inset: photographs under visible light) of the AgNCs in (a) water and (c) IPA-containing water, and (b) and (d) their solutions with I^- ions (5.0 μM), respectively. TEM images of the AgNCs in IPA-containing water in the (C) absence and (D) presence of I^- ions. The fluorescence spectra were recorded at an excitation wavelength of 425 nm.

without IPA. The IPA-triggered specific responses of the AgNCs for the I^- ions could also be visually seen in the corresponding photographs under UV light (Fig. 2A, inset). Furthermore, the IPA-to-water ratios were examined, showing an optimal ratio of 50% (Fig. S2, ESI†). Again, IPA was demonstrated to trigger the enhanced fluorescence as well as specific ion recognition ability of the AgNCs for the I^- ions toward the highly selective and sensitive analysis of I^- ions.

Previous studies have reported that AgNCs can interact with I^- ions to form AgI showing UV absorbance at about 420 nm.[26] In order to explore whether the I^--induced fluorescence quenching of the AgNCs might result from the formation of AgI, the investigation of the UV-vis spectra was performed for AgNCs in IPA-containing water before and after adding I^- ions, in comparison to the ones without IPA (Fig. 2B). One can see that no significant color change was observed among all of the test solutions under visible light (Fig. 2B, inset). As shown in Fig. 2B, the AgNCs could display basically consistent characteristic peaks of UV absorbance in the absence and presence of I^- ions. It should be pointed out that the addition of oppositely charged I^- ions might reduce the surface charges of the AgNCs and lead to their partial aggregation as disclosed in the TEM images afterwards. Yet, they show no obvious absorption around the characteristic wavelength bands that AgI could have (i.e., 420 nm), implying that AgI might not be formed by way of common chemisorption of I^- ions on the AgNCs.

Moreover, the topological structures of the AgNCs before and after adding I^- ions were characterized by transmission electron microscopy (TEM) imaging (Fig. 2). It was found that the AgNCs could enjoy uniform mono-dispersion showing nearly spherical shapes in IPA-containing water (Fig. 2C). After I^- ions were introduced, a slight change in the topological

structure and shape was witnessed for the products of the AgNCs (Fig. 2D). The average hydrodynamic diameters of the AgNCs in the presence and absence of I^- ions were calculated to be about 4.9 nm and 6.5 nm, respectively (Fig. S3, ESI†). Notably, once the I^- ions were introduced, some spherical AgNCs might change to elongated structures, presumably due to the oppositely charged I^- ions that might reduce the surface charges of the AgNCs leading to their partial aggregation. Hence, the evidence from the TEM imaging, together with the UV-vis spectra above, manifest that the I^- ion-induced fluorescent quenching of the AgNCs might not result from the formation of AgI precipitation, which is quite different from the reports describing the I^- ion-induced fusion or topologic transformation of AuNPs in water.[6,27]

On the basis of our experimental results and referring to the previous studies,[24,25,27–30] a possible mechanism is thus proposed for the IPA-triggered fluorescence enhancement and highly specific I^- recognition of the AgNCs leading to the fluorescence quenching. On the one hand, IPA might conduct the famous "electron-donor effects",[24,25] so as to facilitate the enhanced red fluorescence of the AgNCs. On the other hand, I^- ions, as the nucleophile reagents, would readily perform reductive chemisorption onto the surface of the AgNCs to conduct the specific "energetic injection" leading to the fluorescence quenching of the AgNCs, as described previously for the interaction between I^- ions and AuNPs or AgNCs.[6,27–29] Yet, the detailed mechanism currently remains unclear, and will be explored in future work.

Furthermore, the selectivity of the fluorimetric responses of the AgNCs for the I^- ions was probed by comparing them to some other inorganic anions, typically including Cl^-, HPO_4^{2-}, $H_2PO_4^-$, SO_4^{2-}, Ac^-, $C_2O_4^{2-}$, I^-, CO_3^{2-}, HCO_3^{2-}, Br^-, IO_3^-, NO_3^-, S^{2-}, and F^- ions (Fig. 3). Obviously, these inorganic anions alone, especially the other similar kinds of halide ion like F^-, Cl^- and Br^- ions, could induce no significant change in the fluorescence of the IPA-dispersed AgNCs (Fig. 3A). Also, they could exert no effect on the fluorescent responses of the AgNCs for the I^- ions when separately mixed with I^- ions (Fig. 3B). Therefore, the introduction of IPA could endow AgNCs with
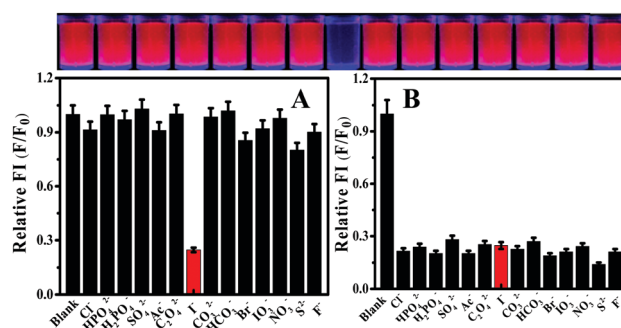

Fig. 3 Relative fluorescence intensities of the AgNCs in IPA-containing water with (A) different anions alone (top: photographs of testing solutions under UV light, from the left to right: Cl^-, HPO_4^{2-}, $H_2PO_4^-$, SO_4^{2-}, Ac^-, $C_2O_4^{2-}$, I^-, CO_3^{2-}, HCO_3^{2-}, Br^-, IO_3^-, NO_3^-, S^{2-}, and F^- ions (1.0 μM)); (B) the intensities of the AgNCs in the presence of different anions co-existing with I^- ions.

high selectivity for sensing I⁻ ions in addition to the afore-mentioned enhanced fluorescence.

Under the optimal conditions (Fig. S4, ESI†), the developed fluorimetric method was applied for the detection of I⁻ ions using AgNCs in water containing IPA (Fig. S5, ESI†). Fig. S5A (ESI†) shows the change in the fluorescence spectra of the AgNCs with different I⁻ concentrations. It was observed that the FIs would decrease gradually as the I⁻ concentration increased, as also visibly revealed in the photographs of corresponding products (Fig. S5B, Inset, ESI†). Fig. S5B (ESI†) exhibits the relationship between the logarithms of the concentrations of I⁻ ions and the fluorescence quenching efficiencies. Accordingly, I⁻ ions with concentrations from 0.0010 to 10.0 μM could be analyzed, with a limit of detection (LOD) of about 0.50 nM as estimated by the 3σ rule.

Furthermore, the application feasibility of the AgNC-based fluorimetric method was investigated by probing urine spiked with different levels of I⁻ ions (Fig. S5C and S5D, ESI†). It is noted that I⁻ ions in urine could be detected with concentrations linearly ranging from 0.010 to 10.0 μM, with the LOD being about 7.50 nM. Obviously, it can ensure the clinical evaluation of I⁻ ions in urine with normal levels over 0.79 μM.[31] Furthermore, a comparison of detection performances was conducted among the developed method and other fluorimetric ones by using different fluorescent probes (Table S1, ESI†). Accordingly, the developed fluorimetric method could enjoy better or comparable analysis ability for I⁻ ions. Besides, the red fluorescence of the AgNCs might additionally help to circumvent any formidable inter-ference from complex media like urine and blood.

In summary, AgNCs were discovered initially with dramatically enhanced red fluorescence and improved aqueous stability once dispersed in water containing alcohols, especially IPA. The introduction of IPA could enable the specific recognition of I⁻ ions by the AgNCs through the unique ''energetic injection'' process resulting in their fluorescence quenching. In particular, the as developed fluorimetric analysis method could facilitate the discrimination of I⁻ ions from other kinds of iodide components like IO₃⁻ ions and other similar kinds of halide ion like F⁻, Cl⁻, and Br⁻ ions. Moreover, the powerful red fluorescence of the AgNCs could be advantageous for probing I⁻ ions in complicated media like blood and urine, with the LOD of urinary I⁻ ions being 7.50 nM. The developed fluorimetric strategy should find wide practical applications for the highly selective and sensitive monitoring of I⁻ ions in clinical, food, and environmental samples. More importantly, it may open a new door towards the solvent-enabled improvement of fluores-cence and especially triggering specific ion recognition with noble metal NCs like AgNCs serving as the fluorescent probes for the biological sensing and imaging of various targets like

ions of great interest in complicated media such as blood and urine.

Notes and references

1 F. Azizi, M. Hedayati, M. Rahmani, R. Sheikholeslam, S. Allahverdian and N. Salarkia, *J. Endocrinol. Invest.*, 2005, **28**, 23–29.
2 R. Chopra, P. Kaur and K. Singh, *Dalton Trans.*, 2015, **44**, 16233–16237.
3 T. Ohashi, M. Yamaki, C. Pandav, M. Karmarkar and M. Irie, *Clin. Chem.*, 2000, **46**, 529–536.
4 M. Wang, Z. Huang, J. Liu, J. He, J. Zhang and Y. Zhu, *Chin. Chem. Lett.*, 2015, **26**, 1026–1030.
5 P. Bermejo-Barrera, M. Aboal-Somoza, R. Anllo-SendíN and A. Bermejo-Barrera, *Microchem. J.*, 2001, **69**, 205–211.
6 J. Zhang, X. Xu, C. Yang, F. Yang and X. Yang, *Anal. Chem.*, 2011, **83**, 3911–3917.
7 L. Chen, W. Lu, X. Wang and L. Chen, *Sens. Actuators, B*, 2013, **182**, 482–488.
8 T. Fujiwara, I. Mohammadzai, H. Inoue and T. Kumamaru, *Analyst*, 2000, **125**, 759–763.
9 H. Ho and M. Leclerc, *J. Am. Chem. Soc.*, 2003, **125**, 4412–4413.
10 D. Lee, N. Singh, M. Kim and D. Jang, *Org. Lett.*, 2011, **13**, 3024–3027.
11 Z. Luo, K. Zheng and J. Xie, *Chem. Commun.*, 2014, **50**, 5143–5155.
12 X. Song, N. Goswami, H. Yang and J. Xie, *Analyst*, 2016, **141**, 3126–3140.
13 Y. Liu, D. Ding, Y. Zhen and R. Guo, *Biosens. Bioelectron.*, 2017, **92**, 140–146.
14 J. Xie, Y. Zheng and J. Ying, *Chem. Commun.*, 2010, **46**, 961–963.
15 Z. Sun, S. Li, Y. Jiang, Y. Qiao, L. Zhang, L. Xu, J. Liu, W. Qi and H. Wang, *Sci. Rep.*, 2016, **6**, 20553.
16 N. Zhang, Y. Si, Z. Sun, L. Chen, R. Li, Y. Qiao, H. Wang and A. Chem, *Anal. Chem.*, 2014, **86**, 11714–11721.
17 X. Yuan, Y. Tay, X. Dou, Z. Luo, D. T. Leong and J. Xie, *Anal. Chem.*, 2013, **85**, 1913–1919.
18 Z. Sun, N. Zhang, Y. Si, S. Li, J. Weng, X. Zhu and H. Wang, *Chem. Commun.*, 2014, **50**, 9196–9199.
19 R. Huang, Y. Wei, X. Dong, X. Wu, C. Du, S. Zang and T. Mak, *Nat. Chem.*, 2017, **9**, 689–697.
20 C. P. Joshi, M. S. Bootharaju and O. M. Bakr, *J. Phys. Chem. Lett.*, 2015, **6**, 3023–3032.
21 X. G. Li, Z. Fei, Y. Gao, Q. M. Zhou, Z. Ye, L. Yan, J. Z. Huo and X. J. Zhao, *Biosens. Bioelectron.*, 2016, **86**, 270–276.
22 F. Qu, L. Dou, N. Li and H. Luo, *J. Mater. Chem. C*, 2013, **1**, 4008–4013.
23 A. Ghorai, J. Mondal, S. Chowdhury and G. K. Patra, *Dalton Trans.*, 2016, **45**, 11540–11553.
24 S. Li, Z. Sun, R. Li, M. Dong, L. Zhang, W. Qi, X. Zhang and H. Wang, *Sci. Rep.*, 2015, **5**, 8475–8483.
25 Y. Qiao, J. Shang, S. Li, L. Feng, J. Yao, Z. Duan, X. Lv, C. Zhang, T. Yao and Z. Dong, *Sci. Rep.*, 2016, **6**, 36494–36502.
26 D. B. Pedersen, S. Wang, E. J. S. Duncan and S. H. Liang, *J. Phys. Chem. C*, 2007, **111**, 13665–13672.
27 J. Wang, Y. Li and C. Huang, *J. Phys. Chem. C*, 2008, **112**, 11691–11695.
28 W. Cheng, S. Dong and E. Wang, *Angew. Chem., Int. Ed.*, 2003, **42**, 449–452.
29 F. Qu, N. B. Li, H. Q. Luo and A. Chem, *Anal. Chem.*, 2012, **84**, 10373–10379.
30 M. Brust, C. J. Kiely, D. Bethell and D. J. Schiffrin, *J. Am. Chem. Soc.*, 2005, **120**, 12367–12368.
31 M. Haldimann, B. Zimmerli, C. Als and H. Gerber, *Clin. Chem.*, 1998, **44**, 817–829.