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A cross-reactive sensor array for the fluorescence qualitative analysis of heavy metal ions

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ABSTRACT

A cross-reactive sensor array using mercaptopropionic acid modified cadmium telluride (CdTe), glutathione modified CdTe, poly(methacrylic acid) modified silver nanoclusters, bovine serum albumin modified gold nanoclusters, rhodamine derivative and calcein blue as fluorescent indicators has been designed for the detection of seven heavy metal ions (Ag^+, Hg^{2+}, Pb^{2+}, Cu^{2+}, Cr^{3+}, Mn^{2+} and Cd^{2+}). The discriminatory capacity of the sensor array to different heavy metal ions in different pH solutions has been tested and the results have been analyzed with linear discriminant analysis. Results showed that the sensor array could be used to qualitatively analyze the selected heavy metal ions. The array performance was also evaluated in the identification of known and unknown samples and the preliminary results suggested the promising practicability of the designed sensor assay.

1. Introduction

The optical sensing arrays, different from the traditional “lock-and-key” principles in sensor design, involve multiple differential binding interactions between multiple cross-reactive indicators and the analytes, and are capable to simultaneously assess multiple chemical information [1,2]. They display many advantages of high selectivity, high sensitivity, low cost and easy operation. In addition, chemometrics, based on a dataset involving hundreds to thousands of variables, and hundreds to thousands of cases or observations, has been widely applied to solve both descriptive and predictive problems in chemistry. Therefore, combining optical sensing arrays with chemometrics analysis technology, the qualitative analysis of various analytes including volatile organic compounds [3], protein [4,5], amine acid [6], aptamers [7], anions [8,9], heavy metal ions [10–12] have been successfully achieved.

Heavy metal ions are ubiquitous in agricultural, industrial and drinking water, thus constituting a serious environmental problem in many countries. Unlike other pollutants, heavy metal ions are difficult to be degraded but easy to be enriched in the human body through the food chain, with the resulting in many disorders in plants and animals [13–16]. Public health concerns are trying to remediate, control and minimize such pollution as much as possible, but water pollution is still serious in the world. From an eco-toxicological point of view, it is imperative to develop sensitive and selective methods for detecting heavy metal ions at low level in the environment. Common techniques for the determination of heavy metal ions include atomic absorption spectrometry [17], plasma spectroscopy [18], atomic fluorescence method [19], ion chromatography [20] and electrochemical methods [21]. Although all of them have acceptable sensitivity and selectivity, they involve rather cumbersome operating conditions, special instruments and high cost, which limit their widespread application. Moreover, the major danger of heavy metal ions in the environment toward humans is attributed to the synergistical consequence of the co-existing heavy metal ions. It is of limited value to selectively determine only one type of heavy metal ion in practical applications and, therefore, it is important to develop new methods for the simultaneous determination of a variety of heavy metal ions in environmental samples.

Fluorescent sensor arrays hold promise to be such a technique due to their superiorities of low cost, high sensitivity and selectivity [22]. Up to now, unfortunately, the most available sensing indicator is commercial or custom organic dyes. Compared with these organic dyes, fluorescent nanomatetials, like semiconductor quantum dots and fluorescent noble metal nanoclusters, with stable fluorescence intensity, high photostability against photobleaching and color tenability, have been adopted for the detection

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of single heavy metal ion [23,24]. To the best of our knowledge, however, there is rare sensing array introduced semiconductor quantum dots and noble metal nanoclusters as sensing indicators though these nanostructured fluorescent materials show high selectivity to heavy metal ions.

In our work, two semiconductor quantum dots (3-mercaptopropionic acid modified cadmium telluride (CdTe@MPA), glutathione modified CdTe (CdTe@GSH)) and two noble metal nanoclusters (poly (methacrylic acid) modified silver nanoclusters (AgNCs@PMAA) and bovine serum albumin modified gold nanoclusters (AuNCs@BSA)) were prepared. Two organic dyes, rhodamine derivative (RHD) and calcein blue (CB), were used as fluorescent indicators in a fluorescent cross-reactive sensor array for the determination of seven heavy metal ions (Ag^{+}, Hg^{2+}, Pb^{2+}, Cu^{2+}, Cr^{3+}, Mn^{2+} and Cd^{2+}). The carboxyl group, amine group and thiol group in the fluorescent indicators are beneficial to discriminate heavy metal ions. The response characteristics were then evaluated with a chemical statistical analysis technology at different pH values and ion concentrations. Experimental results show that the proposed fluorescence cross-reactive sensing array possessed practical applicability and was suitable for the chosen heavy metal ions.

2. Experimental

2.1. Chemicals and solutions

BSA was purchased from Sangon Company (Shanghai, China), PMAA (USA) and CB (Japan) were obtained from Sigma-Aldrich. The other reagents were from the Sinopharm Chemical Reagent Company (Shanghai, China). All chemicals were used without further purification. The ultrapure water (18.2 MΩ) used in all experiments was obtained from a Millipore purification system.

Preparation of 20 mmol L^{-1} 4-(2-hydroxyerhyl) piperazine-1-erhaesulfonic acid (HEPES) buffer solution: 2.408 g HEPES was dissolved with 300 mL ultrapure water and the pH was adjusted to 7.0 with 1 mol L^{-1} NaOH. Finally, the mixture was diluted to 500 mL and kept in a refrigerator at 4 °C.

A 10 mmol L^{-1} stock solution of each heavy metal ion was prepared by dissolving their nitrates with water and the pH of the solutions was adjusted with proper buffer solution following the preparation of standardized metal ions solution. Then solutions of lower concentration were prepared by diluting the stock solutions with water.

2.2. Apparatus

UV–vis absorption and fluorescence spectra were obtained using a UV2300 spectrophotometer (Techcomp) and an F-4500 spectrophotometer (Hitachi), respectively. The solution pH was adjusted with a commercial digital acid meter (CyberScan pH 510). An EOS5D Mark II camera (Canon, Japan) was used to record the color change under an irradiation at 365 nm during the experiment. Ultrapure water was supplied from a WR600A ultrapure water purifier (Millipore Limited Company, USA). An LWMC-205 microwave oven (Nanjing Xuanguang Technology Limited Company, China), and an IKA heating magnetic whisk (Sea Princeton Biotechnology Development Limited Company) were also used for the synthesis of fluorescent indicators in the experiment.

2.3. Synthesis of fluorescent indicators

2.3.1. Synthesis of CdTe@MPA

Based on the Refs. [25,26], 0.6 mmol $CdCl_2 \cdot 6H_2O$ and 1.44 mmol MPA as the stabilizer were dissolved in 50 mL ultrapure water, and then the solution pH was adjusted from 9 to 11 using 1.0 mol L^{-1} NaOH. The solution was degased with N_2 for 30 min. Then 0.3 mmol Te and 0.7 mmol $NaBH_4$ were added, and a colorless NaHTe solution was obtained while stirring. H_2Te was obtained by the addition of H_2SO_4 into the NaHTe solution, and the solution color changed to yellow. The obtained solution was then placed into a microwave digestion furnace (250 W) and refluxed for 10 min to obtain CdTe@MPA. After cooling to room temperature, the solution was stored in a refrigerator at 4 °C.

2.3.2. Synthesis of CdTe@GSH

The carboxyl group, amine group and thiol group in GSH facilitate it to react with metal ions. Herein, 0.6 mmol $CdCl_2 \cdot 6H_2O$ and 2.4 mmol GSH were dissolved in 50 mL ultrapure water, and the following steps were the same as those in Section 2.3.1 except that the refluxing time was 60 min. Then the CdTe@GSH solution was again stored in a refrigerator at 4 °C.

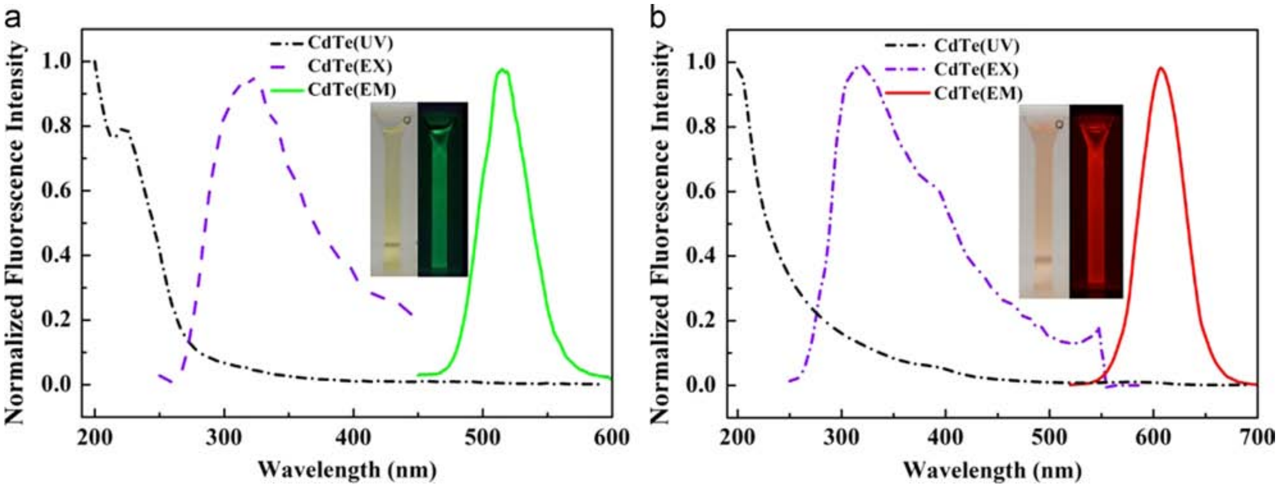

Fig. 1. Normalized absorption (dash dot line), fluorescence excitation (dashed line) and emission (solid line) spectra of (a) CdTe@MPA and (b) CdTe@GSH.

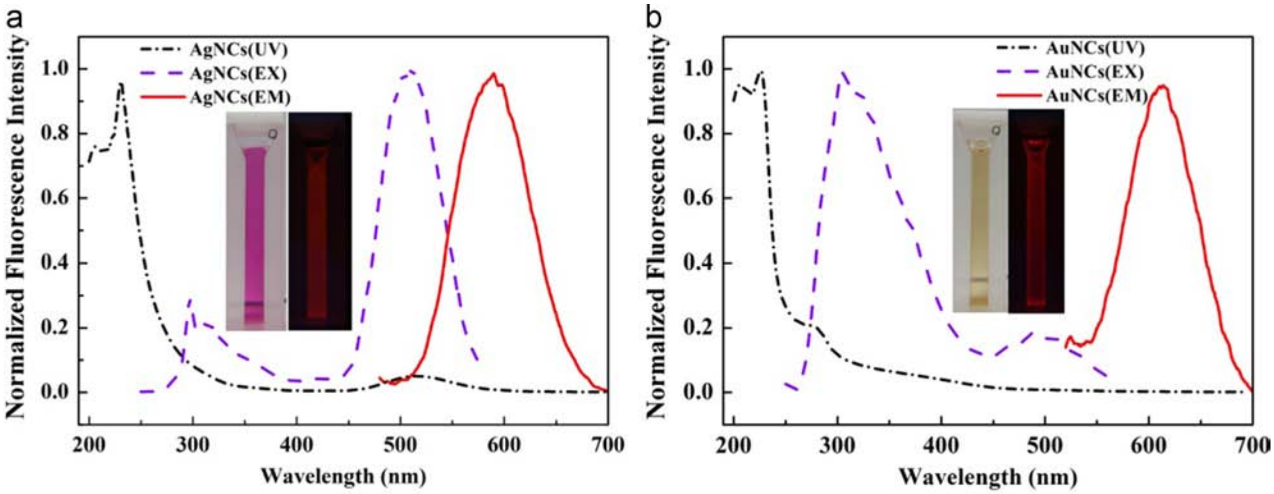

Fig. 2. Normalized absorption (dash dot line), fluorescence excitation (dashed line) and emission (solid line) spectra of (a) AgNCs@PMAA and (b) AuNCs@BSA.

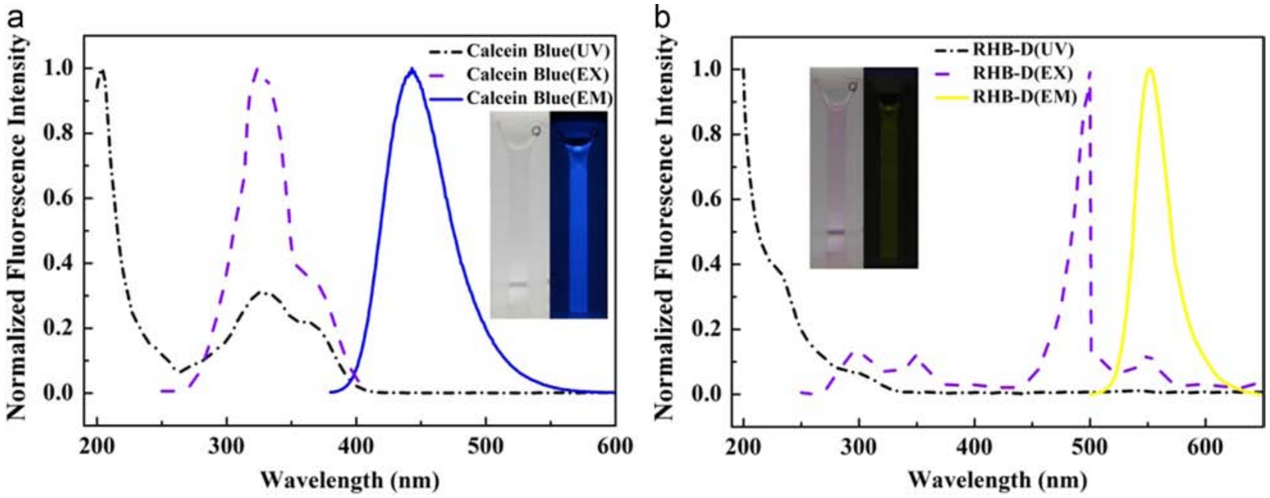

Fig. 3. Normalized absorption (dash dot line), fluorescence excitation (dashed line) and emission (solid line) spectra of (a) CB and (b) RHD.

2.3.3. *Synthesis of AgNCs@PMAA*

In view of the abundant carboxyl group in PMAA, PMAA has been proven to be a versatile template for the preparation of AgNCs, herein, AgNCs@PMAA was prepared according to the previous report [27]. First, 2.5 mL PMAA-Na solution (40%) was added to freshly prepared 50 mL 50 mmol L^{-1} $AgNO_3$ aqueous solution. The mixture was stirred vigorously to acquire a transparent homogeneous solution, and it was then transferred to a reaction tube and placed inside a microwave digestion furnace. Under microwave irradiation (200 W) and keeping the reaction solution refluxing, the reaction was stopped within 70 s. After cooling to room temperature, the solution was stored in a refrigerator at 4 °C. In addition, the pH of the dispersion was adjusted to the required value by adding HNO_3 or NaOH.

2.3.4. *Synthesis of AuNCs@BSA*

According to Xie's work [28], functional protein BSA was used as a scaffold for sequestering and interacting with inorganic ions to form products, and there are much carboxyl groups and amine groups in BSA protein benefit to the recognition of heavy metal ions in the following experiment. Typically, 25 mL 10 mmol L^{-1} $HAuCl_4$ was added to 25 mL 50 mg mL^{-1} BSA aqueous solution with vigorous stirring. 0.5 mL 1 mol L^{-1} NaOH was introduced 2 min later. The reaction was allowed to proceed under vigorous stirring at 37 °C for 12 h.

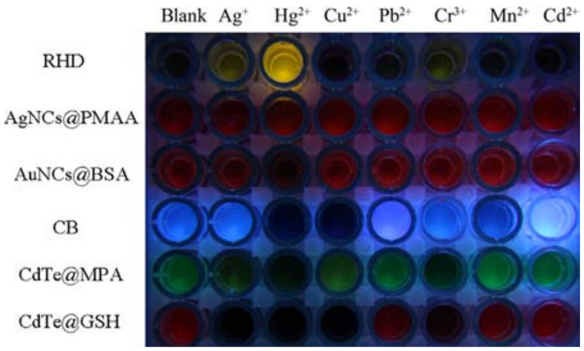

Fig. 4. Color of fluorescent indicators before and after exposure to heavy metal ions under 365 nm UV light in the HEPES buffer solution of pH 7.0 (the concentration of each heavy ion was 10 μmol L^{-1}).

2.3.5. *Synthesis of RHD*

In a typical synthesis [29], 0.10 mL hydrazine monohydrate was added to 300 mg Rhodamine 6G in 2.0 mL MeOH. The reaction solution was refluxed for 6 h and diluted with 30 mL EtOAc. The solution was then washed with 10 mL H_2O and 10 mL 1 mol L^{-1} NaOH. The organic phase was dried over $MgSO_4$, concentrated and column chromatographed on silica-gel (elution with hexanes: CH_2Cl_2:MeOH = 10:2:1) to give Rhodamine 6G hydrazide. Then, 200 mg Rhodamine 6G hydrazide in 1.5 mL DMF was added to a solution of 0.1 mL phenyl isothiocyanate in 1.5 mL DMF. The

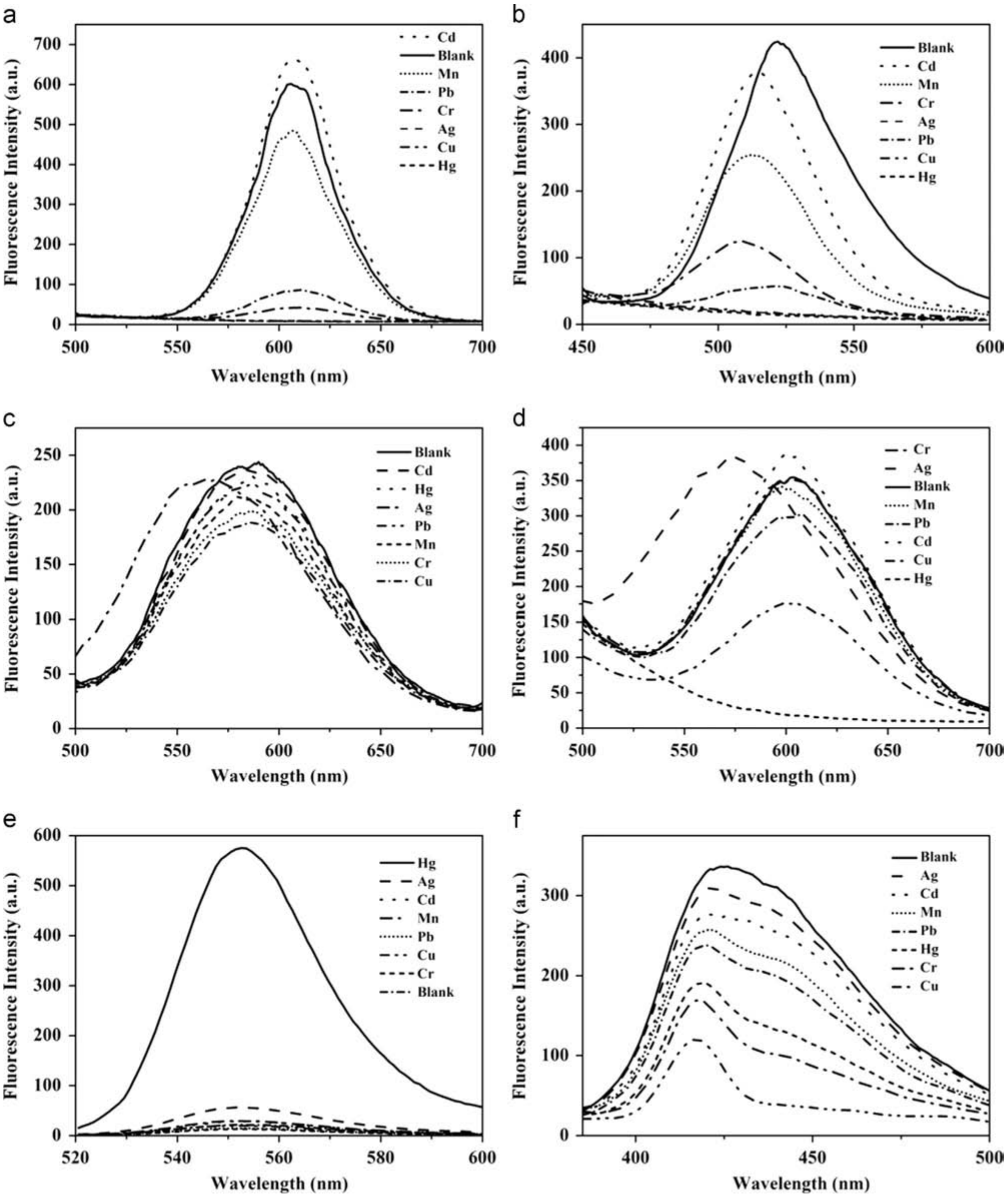

Fig. 5. Fluorescence spectra of fluorescent indicators to heavy metal ions in the HEPES buffer solution of pH = 7.0 ((a) CdTe@GSH, (b) CdTe@MPA, (c) AgNCs@PMAA, (d) AuNCs@BSA, (e) RHD, (f) CB. The concentration of each heavy ion was 10 μmol L^{-1}).

reaction mixture was stirred for 6 h at room temperature. After the solvent was evaporated under reduced pressure, the crude product was column chromatographed on silica-gel (elution with hexanes: EtOAc:CH_2Cl_2=4:1:1) to give RHD.

2.3.6. *Preparation of RHD and CB solution*

10 μmol L^{-1} RHD and CB solution were prepared with HEPES pH 7.0 buffer solution.

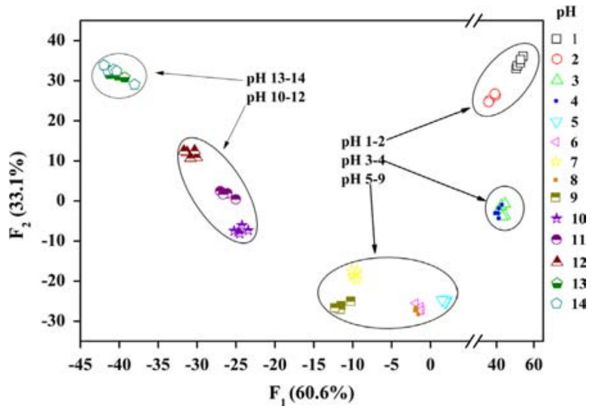

Fig. 6. LDA of sensor array responding to different pHs.

3. Results and discussion

3.1. *Optical properties of fluorescent indicators*

Previous works have reported that CdTe quantum dots possess large Stokes' shift, narrow and tunable emission peak and good stability [30,31]. In our work, the synthesized CdTe@MPA and CdTe@GSH displayed strong absorption in the UV region. They exhibited bright green and red fluorescence under UV-irradiation, with a maximal excitation wavelength/maximal emission wavelength ($\lambda_{ex}/\lambda_{em}$) at 318/515 nm and 316/608 nm, respectively (Fig. 1).

Metal nanoclusters, composed of a few to a hundred atoms, exhibit dramatically different optical, electrical and chemical properties due to their dimensions approaching the Fermi wavelength of the electrons [32]. Compared with semiconductor quantum dots, metal nanoclusters are more suitable for biological labelling and biological imaging. As shown in Fig. 2, the corresponding colors of synthesized AgNCs@PMAA and AuNCs@BSA were rosy red and pale yellow under visible light emission, and both of them emitted red fluorescence under a UV lamp. The $\lambda_{ex}/\lambda_{em}$ of AgNCs@PMAA and AuNCs@BSA were found to be 508/588 nm and 305/610 nm, respectively.

The organic dyes CB and RHD were used to determine heavy metal ions through the fluorescence intensity change due to the self-structure change of the organic dyes caused by the reaction between the dyes and the heavy metal ions. Fig. 3 shows that

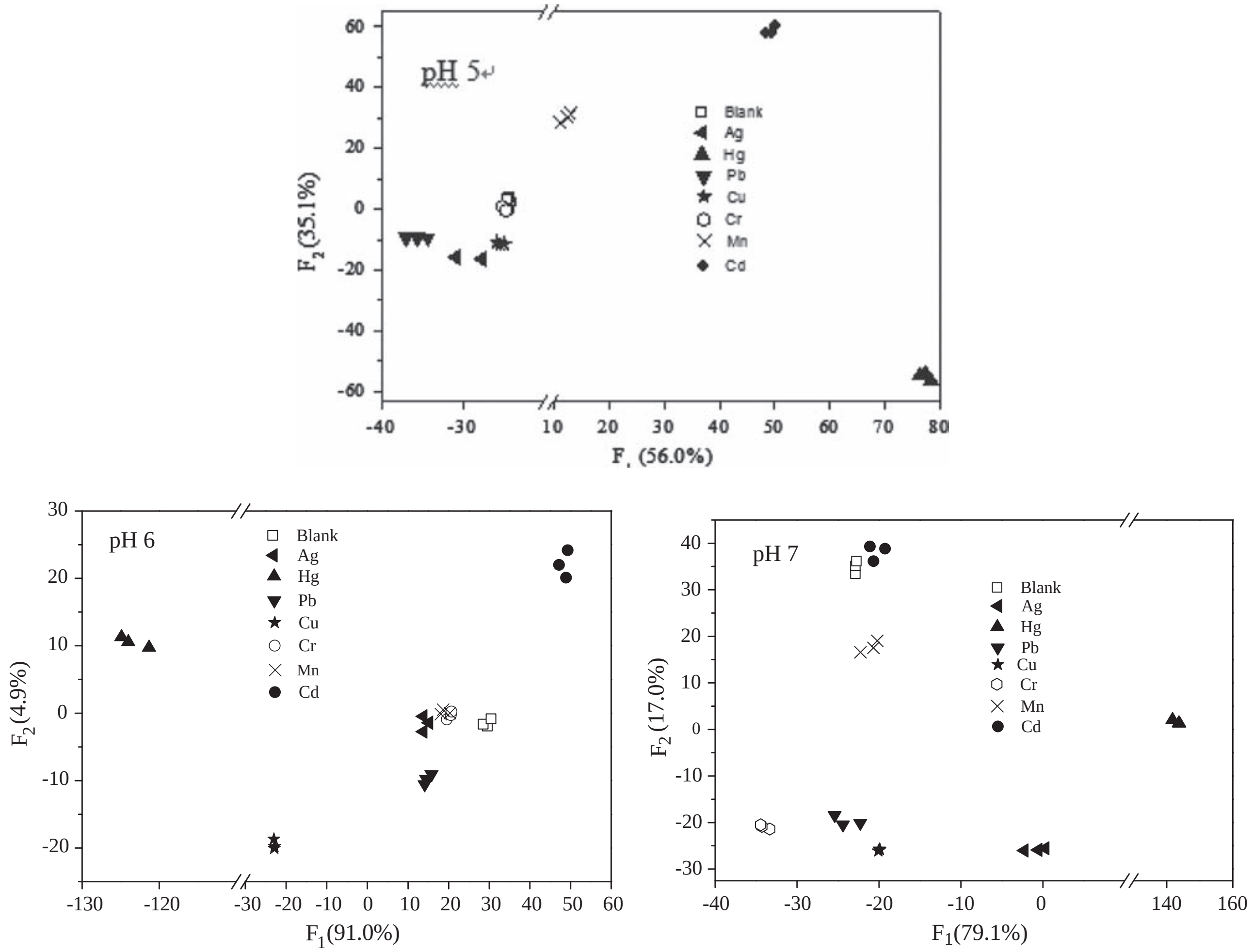

Fig. 7. Response of sensor array to heavy metal ions in pH 5–7 (the concentration of each heavy metal ion was 10 μmol L^{-1}).

CB and RHD emitted blue and pale yellow fluorescence under UV lamp with their corresponding $\lambda_{ex}/\lambda_{em}$ of 326/442 nm and 533/553 nm.

3.2. *Fluorescence spectra of the fluorescent indicators to heavy metal ions*

The selectivity of fluorescent indicators to heavy metal ions is an important factor in the designed sensor array. As shown in Fig. 4, the color change of the sensor array to heavy metal ions under 365 nm UV light could be observed with the naked eyes. In addition, Fig. 5 indicates that most heavy metal ions quenched the fluorescence of the fluorescent indicators while the fluorescence enhancement occurred in the presence of Cd^{2+} for CdTe@GSH (a), Ag^{+} and Cr^{3+} for AuNCs@BSA (d), and Hg^{2+} for RHD (e). Besides, Cu^{2+} and Hg^{2+} quenched the fluorescence of CdTe@MPA, CdTe@GSH, AgNCs@PMAA, AuNCs@BSA and CB, while Hg^{2+} enhanced the fluorescence of RHD. As a result, the designed sensor array could be used to distinguish Cu^{2+} and Hg^{2+}.

3.3. *Effect of pH*

Previous work shows that fluorescent materials are pH-sensitive [33,34], and so, the possible effect of pH ranging from 1 to 14 on the six fluorescent indicators of the sensor array was investigated and analyzed using statistical software SPSS 20.0. Linear discriminant analysis (LDA) is a method usually used for dimensionality reduction and classification to give the similarities and differences in the responses between samples. Also, as a supervised method it provides clear information about clustering of the data. For a defined group classes, LDA aims to maximize the ratio of the between-the-class distance to the within-the-class distance, thus, maximizing the class discrimination. By plotting discriminant scores against the canonical roots, the LDA also provides a graphical output, which shows clusters of similar data, and attests to the degree of discrimination by the array [9]. Therefore, LDA was been used to analyze the responses of the sensor array in pH values ranging from 1 to 14. As indicated in Fig. 6, the effect of pH could be divided into five parts: pH (1 to 2), pH (3 to 4), pH (5 to 9), pH (10 to 12) and pH (13 to 14). The third part covered a large range probably due to the deprotonation of the fluorescent indicators varying with the pH from 5 to 9. When the pH value was higher than 9, the heavy metal ions were in the form of poorly water-soluble metal salts or the hydroxide and the deprotonation of the fluorescent indicators affected the response of the sensitivity of the sensor array, while the sensor array was influenced by the protonation effect when the pH was lower than 4. As a result, a pH value in the range of 5 to 7 was suitable for sensing and was used in the following experiments.

In consideration of its potential applications, the effect of pH ranging from 5 to 7 on the sensor array responding to heavy metal ions was examined and analyzed with statistical software SPSS 20.0. For each pH, a data set corresponding to seven cations (five trials) was generated and LDA was applied separately to each data set (Fig. 7). As shown in Fig. 7, some heavy metal ions such as Cu^{2+}, Cr^{3+} and Mn^{2+} presented similar response to the sensor array at different pHs, while other ions displayed different responses at each pH, especially for Hg^{2+}. In addition, the heavy metal ions were separated obviously from each other in the pH range 5–7, especially at pH 7.

3.4. *Sensing of the heavy metal ions*

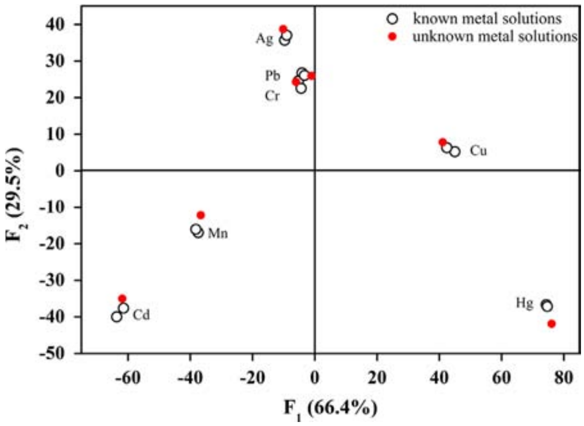

Fig. 8. LDA of known and unknown sample (hollow circle: known sample, solid circle: unknown sample).

Encouraged by the above results, the qualitative analysis ability of the designed sensor array was evaluated by investigating the responses of the sensor array to known and unknown samples. Since this work aims to synchronously qualitative analysis for several heavy metal ions, to reduce the interfering during the detection, for an unknown sample, some pretreatments were carried out before the detection such as removing solid impurities, masking some possible interfering cations and anions previously reported [23,24,27,29]. LDA was used to qualitatively differentiate the fluorescence response of the fluorescent indicators with heavy metal ions. All five replicates of the seven heavy metal ions were grouped and scores of the two factors were plotted. The results showed that the plots ascribed to Pb^{2+} and Cr^{3+} partly overlapped, suggesting that Pb^{2+} could not be distinguished from Cr^{3+}. While the other heavy metal ions were separated from each other, demonstrating the excellent distinguishing ability of the designed sensor array (Fig. 8).

4. Conclusions

A cross-reactive sensor array was designed for the detection of seven heavy metal ions using CdTe@MPA, CdTe@GSH, AgNCs@PMAA, AuNCs@BSA, RHD and CB as fluorescent indicators. The effect of pH on the fluorescent sensor array and its response to the heavy metal ions were studied in this work. The experimental results analyzed with LDA show that the designed sensor array possessed qualitative analysis ability to heavy metal ions. This work offers the possibility of applying the N × N cross-reactive sensor array to detect heavy metal ions in natural water samples.

Acknowledgements

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References

[1] P. Anzenbacher Jr., P. Lubal, P. Bucek, M.A. Palacios, M.E. Kozelkova, Chem. Soc. Rev. 39 (2010) 3954–3979.
[2] K.J. Albert, N.S. Lewis, C.L. Schauer, G.A. Sotzing, S.E. Stitzel, T.P. Vaid, D.R. Walt, Chem. Rev. 100 (2000) 2595–2626.
[3] H. Lin, M. Jang, K.S. Suslick, J. Am. Chem. Soc. 133 (2011) 16786–16789.
[4] H. Kong, D. Liu, S. Zhang, X. Zhang, Anal. Chem. 83 (2011) 1867–1870.
[5] O.R. Miranda, H.T. Chen, C.C. You, D.E. Mortenson, X.C. Yang, U.H. Bunz, V.M. Rotello, J. Am. Chem. Soc. 132 (2010) 5285–5289.
[6] R.K. Pathak, J. Dessingou, C.P. Rao, Anal. Chem. 84 (2013) 8294–8300.
[7] H. Pei, J. Li, M. Lv, J. Wang, J. Gao, J. Lu, Y. Li, Q. Huang, J. Hu, C. Fan, J. Am. Chem. Soc. 134 (2012) 13843–13849.

[8] W. Zhu, W. Li, H. Yang, Y. Jiang, C. Wang, Y. Chen, G. Li, Chem. Eur. J. 19 (2013) 11603–11612.
[9] P. Anzenbacher Jr., Y. Liu, M.A. Palacios, T. Minami, Z. Wang, R. Nishiyabu, Chem. Eur. J. 19 (2013) 8497–8506.
[10] L.Y. Niu, H. Li, L. Feng, Y.S. Guan, Y.Z. Chen, C.F. Duan, L.Z. Wu, Y.F. Guan, C.H. Tung, Q.Z. Yang, Anal. Chim. Acta 775 (2013) 93–99.
[11] G. Aragay, J. Pons, A. Merkoci, Chem. Rev. 111 (2011) 3433–3458.
[12] L.B. Desmonts, F. van der Baan, R.S. Zimmerman, D.N. Reinhoudt, M. Crego-Calama, Sensors 7 (2007) 1731–1746.
[13] E.L. Que, D.W. Domaille, C.J. Chang, Chem. Rev. 108 (2008) 1517–1549.
[14] L. Jarup, Br. Med. Bull. 68 (2003) 167–182.
[15] M. Vahter, M. Berglund, A. Akesson, C. Liden, Environ. Res. 88 (2002) 145–155.
[16] J. Burger, M. Gochfeld, Environ. Res. 99 (2005) 403–412.
[17] B. Chen, I.A. Meinertzhagen, S.R. Shaw, J. Comp. Physiol. A 185 (1999) 393–404.
[18] Y.K. Rui, J. Guo, K.L. Huang, Y.H. Jin, Y.B. Luo, Spectrosc. Spectr. Anal. 27 (2007) 796–798.
[19] Y.K. Rui, J. Guo, K.L. Huang, Y.H. Jin, Y.B. Luo, Spectrosc. Spectr. Anal. 27 (2007) 796–798.
[20] A. Lafaye, C. Junot, B. Ramounet-Le Gall, P. Fritsch, J.C. Tabet, E. Ezan, Rapid Commun. Mass Spectrom. 17 (2003) 2541–2549.
[21] G. Aragay, A. Merkoçi, Electrochim. Acta 84 (2012) 49–61.
[22] M. Schaferling, Angew. Chem. Int. Ed. 51 (2012) 3532–3554.
[23] M.S. Hosseini, A. Pirouz, J. Lumin. (2014), http://dx.doi.org/10.1002/bio.2623.
[24] D. Li, A. Wieckowska, I. Willner, Angew. Chem. Int. Ed. 47 (2008) 3927–3931.
[25] L. Li, H. Qian, J. Ren, Chem. Commun. 4 (2005) 528–530.
[26] Y. He, L.M. Sai, H.T. Lu, M. Hu, W.Y. Lai, Q.L. Fan, L.H. Wang, W. Huang, Chem. Mater. 19 (2007) 359–365.
[27] S.H. Liu, F. Lu, J.J. Zhu, Chem. Commun. 47 (2011) 2661–2663.
[28] J.P. Xie, Y.G. Zheng, J.Y. Ying, J. Am. Chem. Soc. 131, (2009) 888–889.
[29] Y.K. Yang, K.J. Yook, J. Tae, J. Am. Chem. Soc. 127 (2005) 16760–16761.
[30] Z.Y. Tang, N.A. Kotov, M. Giersig, Science 297 (2002) 237–240.
[31] W.W. Yu, L.H. Qu, W.Z. Guo, Chem. Mater. 15 (2003) 2854–2860.
[32] W.W. Yu, L.H. Qu, W.Z. Guo, Chem. Mater. 15 (2003) 2854–2860.
[33] A.S. Susha, A.M. Javier, W.J. Parak, A.L. Rogach, Colloids Surf., A 281 (2006) 40–43.
[34] Z. Deng, Y. Zhang, J. Yue, F. Tang, Q. Wei, J. Phys. Chem. B 111 (2007) 12024–12031.