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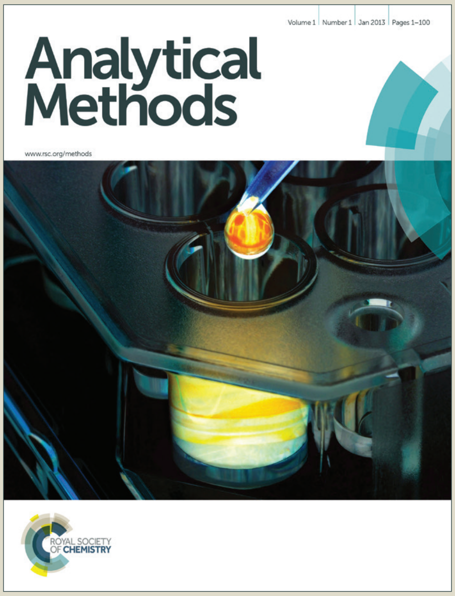

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COMMUNICATION

Colorimetric detection of hypochlorite in tap water based on the oxidation of 3,3’,5,5’-tetramethylbenzidine

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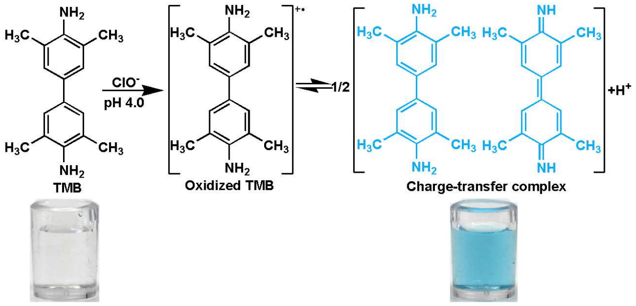
We have developed a simple method for the colorimetric detection of hypochlorite in tap water based on the oxidation of 3,3’,5,5’-tetramethylbenzidine (TMB). Hypochlorite, a kind of oxidant, can rapidly transfer the colorless TMB into the oxidized TMB with blue color in aqueous solution at pH 4.0. The concentration of hypochlorite in tap water can thus be easily detected with the naked eye based on the obvious color change of the solution. The detection system can detect as low as 0.05 μM of hypochlorite with excellent linear relationship and good selectivity. Particularly, the practicability has been successfully applied to the analysis of the tap water.

Introduction

Hypochlorite (ClO^-), the main active ingredient of the chlorinated substance, has been broadly used as bleaching agent and disinfectant in domesticity, wastewater treatment, textile industry and medical field [1,2,3]. ClO^- has brought great convenience to our life, however, highly concentrated ClO^- solution is potentially harmful to the health of humans and animals [4,5,6]. The development of practical analytical platforms to monitor levels of ClO^- is therefore of paramount importance.

A variety of analytical methods are currently available for the detection of ClO^- such as colorimetric, fluorescent, electrochemical and chromatographic methods [6-14]. Among those reported various methods, colorimetric method is widely developed due to its high sensitivity, selectivity, low-cost and simplicity. For example, Yang’s group developed two colorimetric methods for the detection of ClO^- using gold nanopartiles [6,11]. Encouraging results are obtained. But the two methods require the prior synthesis of gold nanoparticles. Lou et al. developed a colorimetric chemosensor for ClO^- by utilization of the oxidation property of ClO^- and different coordinating properties of an azobenzene acid with Cu^+ and Cu^{2+} [9]. However, complex synthetic procedure is needed. Additionally, Zhang and Yang recently described a colorimetric method for sensing ClO^- using 3,3’,5,5’-tetramethylbenzidine (TMB). The detection procedure is conducted in strong acid and the color of the solution changes from colorless to yellow after the addition of ClO^- [12]. But the yellow color is too light to be easily recognized by human eyes when the concentration of ClO^- is very low. Therefore, the detection of ClO^- with TMB needs to be further improved.

To address the problem, we present a simple and fast-response method for the colorimetric detection of ClO^- in tap water under ambient conditions by utilization of the oxidation of TMB by ClO^-. The presence of ClO^- can cause the oxidation of TMB at pH 4.0, leading to the color change of the TMB solution to change from colorless to blue. And the obvious color change can be easily recognized with the naked eye (Scheme 1). Importantly, this method allows the detection of ClO^- at 0.05 μM levels with good selectivity.



Scheme 1 Diagrammatic illustration of the colorimetric detection of ClO^- based on the oxidation of TMB.

Experimental Section

Reagents and apparatus

3,3’,5,5’-tetramethylbenzidine (TMB) was purchased from Aladdin (Shanghai, China). NaClO was obtained from Beijing Chemical Reagent Company. Other reagents were all of analytical reagent grade and used as received. A stock solution of ClO^- was prepared from the commercial NaClO solution and the concentration was assayed based on the absorbance at 292 nm by using its molar extinction coefficient of 391 M^{-1} cm^{-1} before use [15]. The fresh TMB stock solution was prepared in the mixture of ethanol and water (50:50, vol./vol.) and the stock solution of other chemicals was prepared in the double distilled water.

Ultraviolet-visible (UV-vis) absorption spectra were recorded at room temperature on a Lambda 650S UV-vis spectrometer (Perkin-Elmer, USA) in a 1.0 cm path length quartz cuvette. Photographs were taken with a Cannon 700D digital camera.

Colorimetric detection of ClO^-

In the procedure of the detection of ClO^-, acetic acid-sodium acetate buffer (HAc-NaAc, 750 μL, pH = 4.0, 200 mM) and the TMB solution (600 μL, 5 mM) were mixed with different concentrations of NaClO, and water was added to diluted the above mixture to 3 mL. After thorough shaking and incubating for about 1 min, the color changes of the solutions and their corresponding UV-vis absorption spectra were recorded.

Colorimetric detection of ClO$^-$ in tap water

The tap water was directly obtained from our laboratory. In the process of the detection of ClO$^-$ in tap water, HAc-NaAc buffer (750 μL, pH = 4.0, 200 mM), the TMB solution (600 μL, 5 mM) and the tap water (60 μL) were spiked with different concentrations of NaClO, and water was added to ensure that the final volume is 3 mL. After thorough shaking and incubating for about 1 min, the colorimetric response was recorded using the UV-vis absorption spectra.

Results and discussion

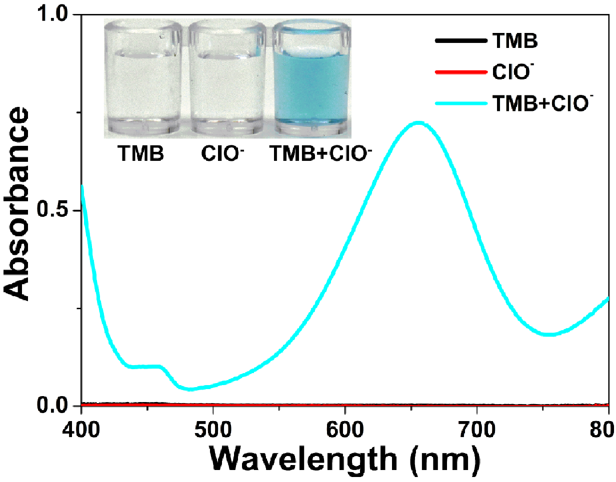


Fig. 1 UV-vis absorption spectra of TMB, ClO$^-$ and TMB with ClO$^-$. Insert is their corresponding pictures (the concentration of TMB is 1 mM, the concentration of ClO$^-$ is 20 μM, 50 mM pH 4.0 HAc-NaAc buffer).

TMB is a kind of colorless peroxidase substrate, which has been broadly utilized in the colorimetric detection of various analytes due to the obvious color difference between the TMB and the oxidized TMB [16,17,18]. And the diluted NaClO solution is also colorless. However, upon mixing TMB with ClO$^-$ at pH 4.0, the mixture would readily change from colorless to blue due to the oxidization of TMB by ClO$^-$. The oxidation process of TMB by ClO$^-$ involves the formation of a semiquinonimine radical cation, which can reversibly form a blue charge-transfer complex with the parent diamine (Scheme 1)[17,18]. The maximum absorption peak of the oxidized TMB solution is centered at about 655 nm. The obvious color change form colorless to blue is easily recognized by the naked eye within visible wavelength (Fig. 1). TMB can thus be employed to detect ClO$^-$ in aqueous solution.

Subsequently, we optimize the assay conditions to achieve better sensing of ClO$^-$. The change of the absorbance at 655 nm (A_{655}) represents the colorimetric response of TMB. The pH value has a great effect on the color change of TMB (1 mM) in the presence of ClO$^-$ (20 μM). The A_{655} of the solution containing TMB and ClO$^-$ at different pH values is used to study the pH effect. The A_{655} of the solution changes significantly at pH 1.0-7.0. And it is the largest when the solution is at pH 4.0 (Fig. S1). We thus chose pH 4.0 as the optimal pH value. We then find that the concentration of TMB has little impact on the detection system, and 1 mM TMB is employed in the following detection (Fig. S2). The oxidation of TMB is also affected by the incubation time. We find that TMB is rapidly oxidized by ClO$^-$ and the oxidation reaction is finished in 1 min. However, the A_{655} of the TMB solution is very low if there is no ClO$^-$ (Fig. S3). Therefore, pH 4.0, 1 mM TMB and 1 min of incubation time are chosen as the detection conditions.

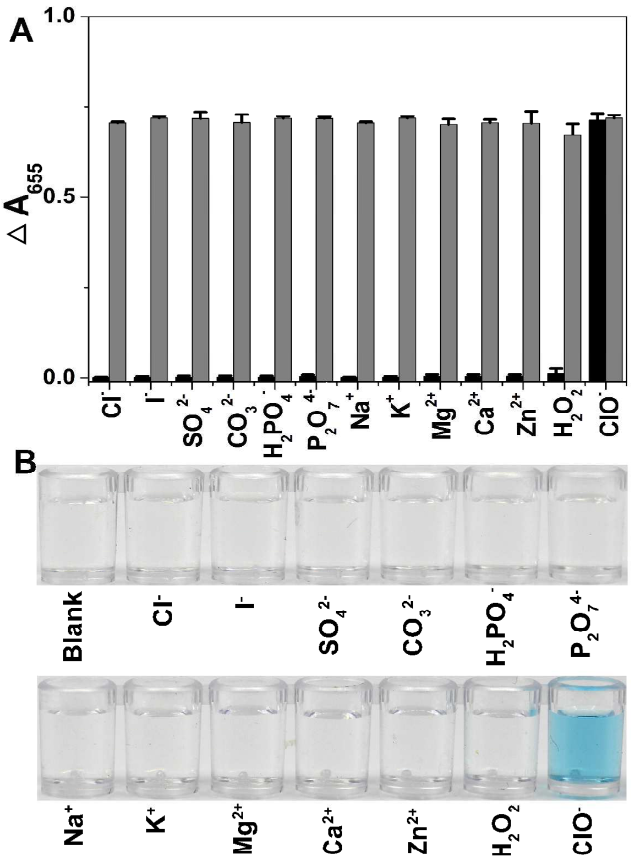


Fig. 2 (A) The plot of $\triangle A_{655}$ of the TMB solution versus different common ions. Black bars represent the addition of a single ion; grey bars represent the addition of ClO$^-$ with another ion. (B) The pictures of the TMB solution in the present of different ions (the concentration of TMB is 1 mM, the concentration of ClO$^-$ is 20 μM, the concentration of these common ions is 200 μM, 50 mM pH 4.0 HAc-NaAc buffer).

Under the optimized detection conditions, we evaluate the selectivity of the assay towards ClO$^-$ with other environmentally relevant ions, including Cl$^-$, I$^-$, SO_4^{2-}, CO_3^{2-}, $H_2PO_4^-$, $P_2O_7^{4-}$, Na$^+$, K$^+$, Mg^{2+}, Ca^{2+}, Zn^{2+} and H_2O_2 at the concentration of 200 μM (Fig. 2). It is notable that the A_{655} of the TMB solution with ClO$^-$ is the largest among these ions. And the A_{655} of the mixture of ClO$^-$ with another ion is observed minor or negligible change (Fig. 2A). Meanwhile, the colorimetric response also confirms this observation. Only ClO$^-$ can cause the color of the TMB to change from colorless to blue (Fig. 2B). The high selectivity of the assay is attributed to the selective oxidation activity of ClO$^-$ to TMB. ClO$^-$ can selectively transfer the colorless TMB in to the oxidized TMB with blue at room temperature. Moreover, it has been reported that Fe^{3+} and Ag^+ can also oxidize TMB at pH 4.0, indicating that Fe^{3+} and Ag^+ may interfere with the assay of ClO^- [19]. However, Ag^+ easily oxidized by O_2 in air is not commonly present in tap water. Fe^{3+} can be masked with pyrophosphate ions and 10 mM pyrophosphate ions have no effect on the assay of ClO^- (Fig. S4) [20]. The experimental results illustrate that the assay can respond selectively toward ClO^-. Consequently, the assay can be further used for the analysis of ClO^- in aqueous solution.

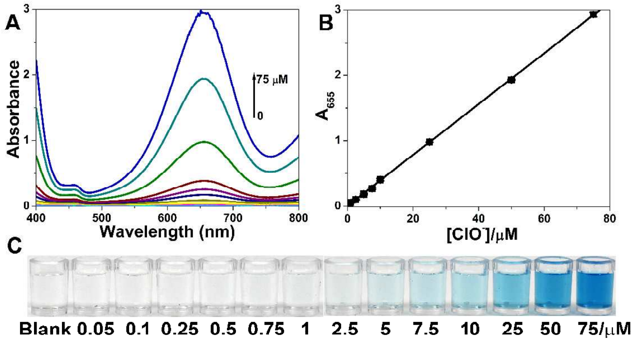


Fig. 3 (A) UV-vis absorption spectra of the TMB solution in the presence of different concentrations of ClO^-. (B) The linear relationship between the A_{655} of the TMB solution and the concentrations of ClO^-. (C) Photographs of the TMB solution in the presence of different concentrations of ClO^- (the concentration of TMB is 1 mM, 50 mM pH 4.0 HAc-NaAc buffer).

To evaluate the minimum concentration of ClO^- detectable by the detection system, the UV-vis absorption spectra of TMB with the addition of different concentrations of ClO^- are recorded. As the concentration of ClO^- gets higher, the absorbance at 655 nm gradually increases (Fig. 3A). The fitting curve shows an excellent linear relationship between the A_{655} and the concentration of ClO^- in the range of 0 to 75 μM with a correlation coefficient of 0.9999. Meanwhile, the proposed method can detect as low as 0.05 μM ClO^-, which is better than those reported colorimetric methods (Fig. 3B) [6,9,12]. Moreover, we can observe a color change from colorless to blue when the concentration of ClO^- increases from 0 to 75 μM. For the colorimetric detection, a sensitivity of 1 μM ClO^- can be achieved with the naked eye, which is lower than the maximum amount of ClO^- (4 μM, calculated by chlorine) allowed in drinking water set by China (Fig. 3C) [11,21]. This result suggests that the proposed assay can be employed for the direct detection of ClO^- with the naked eye.

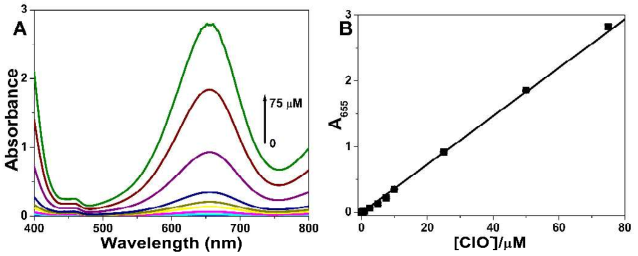


Fig. 4 (A) UV-vis absorption spectra of the TMB solution in the presence of different concentrations of ClO^- in tap water. (B) The linear relationship between the A_{655} of the TMB solution and the concentrations of ClO^- in tap water (the concentration of TMB is 1 mM, 50 mM pH 4.0 HAc-NaAc buffer).

Finally, to demonstrate the potential practical application of the proposed method to measure the ClO^- content of the real water sample, the detection of ClO^- in tap water is carried out. The tap water does not cause the change of the absorption spectra of TMB in comparison with the blank solution, revealing that the concentration of ClO^- in tap water is lower than that of the detection limit of the assay or there is no ClO^- in tap water (Fig. S5). So we spike different concentrations of ClO^- with the tap water to investigate the practicability of the assay. We can observe that the UV-vis absorption curve gradually grows with the increase of the concentration of ClO^-. And the fitting curve exhibits a good linear relationship from 0 to 75 μM and the correlation coefficient is 0.9980 (Fig. 4). The results clearly demonstrated the potential application of the proposed method for the visual determination of ClO^- in the practical sample analysis.

Conclusions

In summary, we have successfully demonstrated a simple approach for the colorimetric detection of ClO^- at pH 4.0 by utilization of the oxidation of a commercially obtained TMB in aqueous media. The obvious color change from colorless to blue is easily recognized by the naked eye. The method exhibits many advantages including simplicity, fast-response, and low-cost. With the method detection, 1 μM ClO^- can be distinguished by the naked eye and even the lowest detection concentration of ClO^- is 0.05 μM with the aid of UV-vis absorption spectra. The method has been also successfully applied in tap water, indicating that the potential application of the methodology in monitoring the quality of water.

Acknowledgments

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Notes and references

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1. M.M. Donnermair, E.R. Blatchley, *Water Res.*, 2003, **37**, 1557-1570.
2. H. Kiura, K. Sano, S. Morimatsu, T. Nakano, C. Morita, M. Yamaguchi, T. Maeda, Y. Katsuoka, *J. Microbiol. Methods*, 2002, **49**, 285-293.
3. J. Van Bueren, R. Simpson, H. Salmax, H. Farrelly, B. Cookson, *Epidemiol. Infect.*, 1995, **115**, 567-579.
4. L.C. Adam, G. Gordon, *Anal. Chem.*, 1995, **67**, 535-540.
5. G. Chen, F. Song, J. Wang, Z. Yang, S. Sun, J. Fan, X. Qiang, X. Wang, B. Dou, X. Peng, *Chem. Commun.*, 2012, **48**, 2949-2951.
6. L. Lu, J. Zhang, X. Yang, *Sens. Actuators B: Chem.*, 2013, **184**, 189-195.
7. X. Chen, X. Tian, I. Shin, J. Yoon, *Chem. Soc. Rev.*, 2011, **40**, 4783-

4804.

8. A. Gallina, P. Pastore, F. Magno, *Analyst*, 1999, **124**, 1439-1442.

9. X. Lou, Y. Zhang, J. Qin, Z. Li, *Sens. Actuators B: Chem.*, 2012, **161**, 229-234.

10. O. Ordeig, R. Mas, J. Gonzalo, F. Del Campo, F.J. Muñoz, C. de Haro, *Electroanalysis*, 2005, **17**, 1641-1648.

11. J. Zhang, X. Wang, X. Yang, *Analyst*, 2012, **137**, 2806-2812.

12. J. Zhang, X. Yang, *Analyst*, 2013, **138**, 434-437.

13. J. Zhu, S. Liu, Z. Liu, Y. Li, M. Qiao, X. Hu, *RSC Adv.*, 2014, **4**, 5990-5994.

14. X. Lou, Y. Zhang, Q. Li, J. Qin, Z. Li, *Chem. Commun.*, 2011, **47**, 3189-3191.

15. Y. Xiao, R. Zhang, Z. Ye, Z. Dai, H. An, J. Yuan, *Anal. Chem.*, 2012, **84**, 10785-10792.

16. Y. Xianyu, K. Zhu, W. Chen, X. Wang, H. Zhao, J. Sun, Z. Wang, X. Jiang, *Anal. Chem.*, 2013, **85**, 7029-7032.

17. D. Xiao, K. Wang, W. Xiao, *Analyst*, 2001, **126**, 1387-1392.

18. X. Xia, J. Zhang, T. Sawall, *Anal. Methods*, 2015, DOI: 10.1039/C5AY00490J.

19. S. Liu, J. Tian, L. Wang, X. Sun, *Sens. Actuators B: Chem.*, 2012, **165**, 44-47.

20. S.-P. Wu, Y.-P. Chen, Y.-M. Sung, *Analyst*, 2011, **136**, 1887-1891.

21. Standardization Administration of China, 2006, GB 5749-2006.

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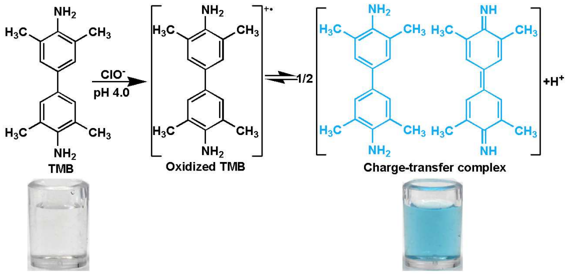

A simple method for the colorimetric detection of hypochlorite in tap water with good selectivity and sensitivity has been developed by utilization of the oxidation of 3,3’,5,5’-tetramethylbenzidine.