A Flexible Chemosensor Based on Colorimetric and Fluorescent Dual Modes for Rapid and Sensitive Detection of Hypochlorite Anion

Qin Wu 1, Tao Tao 1,2,*, Yunxia Zhao 1 and Wei Huang 2

1 Institute of Advanced Materials and Flexible Electronics (IAMFE), School of Chemistry and Materials Science, Nanjing University of Information Science & Technology (NUIST), Nanjing 210044, China; 20191221038@nuist.edu.cn (Q.W.); nlgzyx@nuist.edu.cn (Y.Z.)
2 School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210093, China; whuang@nju.edu.cn
* Correspondence: taotao@nuist.edu.cn

Abstract: A flexible chemosensor has been developed based on colorimetric and fluorescent dual modes using tetraphenylethylene-centered tetraaniline (TPE4A) for rapid and sensitive detection of hypochlorite anion. The fluorescent probe TPE4A exhibits a unique aggregation-induced emission (AIE) character which is proved by a blue shift of the fluorescent peak from 544 to 474 nm with the water equivalents increasing. With the addition of hypochlorite in solution, the absorbance of the probe changes and the responding fluorescence color can be observed to change from light green to purple. The detection limit of hypochlorite is $1.80 \times 10^{-4}$ M in solution, and the visual detection limit is 1.27 µg/cm² with the naked eye for the flexible paper-based chemosensor. The proposed flexible chemosensors show a good selectivity and sensitivity which has great potential for effective detection of hypochlorite anions without any spectroscopic instrumentation.

Keywords: aggregation-induced emission; chemosensor; tetraphenylethylene; hypochlorite; test paper

1. Introduction

Hypochlorite anion (ClO$^-$) plays an essential role in biological organisms and environmental monitors, which not only is produced by H$_2$O$_2$ and chloride ions in activated neutrophils [1–5] but is also a kind of disinfector which can kill the coronavirus [6–8]. However, excessive hypochlorite could lead to diseases and even cancer including cardiovascular diseases, neuron degeneration, and arthritis [9,10]. Therefore, quantitative detection of hypochlorite with highly sensitive and selective methods becomes more and more crucial.

Many various analytical methods are available for the detection of hypochlorite (for example, colorimetric and fluorescent chemosensors) [11,12]. Recent reviews have summarized the advances of hypochlorite probes [13,14]. There are some successful designs for quantitative detection of hypochlorite based on the principle of colorimetric, chromatographic, electrochemical, and luminescent methods. Specifically, the fluorescent probe is a promising method with advantages such as low cytotoxicity, high selectivity, and fast response time for ClO$^-$ detection. Moreover, understanding visual detection is an unceasingly thorough process for rapid and sensitive detection of hypochlorite anions [15–24].

Recently, dual modes [25–27] bioanalysis has become a popular research area, wherein researchers are studying scrambly as well. When the environment changes in the direction of complexity, single-switch optical detection may no longer meet the reliability of data and the diversity of application scenarios. Therefore, it is very important to construct a dual-mode optical detection method. The optical detection based on colorimetric and fluorescent dual modes could not only improve the accuracy of results but also lead to
higher efficiency. In recent years, flexible thin-film devices [28,29] have been developed vigorously, especially in material, chemical, biological, physical fields, due to their unique advantages such as their low-cost, porosity, ready availability, mechanical flexibility, etc. Therefore, the design of paper-based chemosensors for simple and rapid detection is of great significance. In this work, we present a commercially available material TPE4A for fabricating a flexible paper-based chemosensor based on colorimetric and fluorescent dual modes in Scheme 1.

Scheme 1. The chemical structure of tetraphenylethylene-functionalized molecule TPE4A.

2. Results and Discussion

2.1. NMR Spectra

A commercially available material TPE4A, namely 4′,4‴,4′′′,4‴,4‴,4‴,4‴,4‴-(ethene-1,1,2,2-tetrayl)-tetrakis((1,1′-biphenyl)-4-amine)) [30,31], has been purchased where the tetraphenylethylene (TPE) group shows an aggregation-induced emission (AIE) character [32,33]. The chemical structure of TPE4A has double checked by NMR spectra in Figure S1. 

Scheme 1. The chemical structure of tetraphenylethylene-functionalized molecule TPE4A.

2.2. Aggregation-Induced Fluorescent Behavior

With the enhancement of volume fraction of water phase (f_w), UV–Vis and fluorescent spectra have been shown for compound TPE4A in Figure 1. A step-by-step fluorescent turn-on is observed from f_w = 0 to 70%, due to the controlled rotational motion in the molecule. Interestingly, a remarkable color change occurs from orange when f_w = 70% to green when f_w = 80% mainly due to the formation of aggregates. Simultaneously, the maximum emission wavelength λ_max is from 544 nm when f_w = 70% to 474 nm when f_w = 80%. Absorption and emission spectra show a clear AIE character for compound TPE4A as expected. The dominant luminescence is from the emissive solution to solid fluorescence. The same phenomenon is observed in the different concentration measurements. According to restriction of intramolecular vibrations (RIV) and restriction of intramolecular rotations (RIR), the possess of twisted intramolecular charge transfer (TICT) also is blocked, resulting in decreasing red-shift and increasing blue-shift.

2.3. Titration Experiment of Probe TPE4A

Compared to other chemical anions, molecule TPE4A has been designed as a highly selective fluorescent probe for ClO^- anion in Figure 2. After adding 1.00 mM of analytes, such as SCN^- , CO_3^{2-}, S_2^- , NO_2^-, H_2O_2, F^-, and ClO^-, UV–Vis absorption, and fluorescent intensities of probe TPE4A (40 µM) have been shown in mixed solution (f_w = 80%). The absorption peak of TPE4A is from 398 nm to 429 nm (also from 3.12 eV to 2.89 eV) in the presence of ClO^-, which is consistent with the results of calculated energy gaps.
Figure 1. UV–Vis absorption (a) and fluorescent emission (b) spectra and their visual photograph (c) for compound TPE4A with different $f_w$ in their THF/water solutions with the same concentration of 10 µM.

Figure 2. UV–Vis absorption (a) and fluorescent quenching percentages at 480 nm (b) of probe TPE4A (40 µM) in mixed solution ($f_w = 80\%$) upon exposure to other analytes for 60 s. Inset: a colorimetric and fluorescent change photograph for ClO$^-$ and other analytes (SCN$^-$, CO$_3^{2-}$, S$_2^{2-}$, NO$_2^-$, H$_2$O$_2$, and F$^-$).

More importantly, a fluorescent quenching occurs from light green to dark, while the emissive color of this probe remains unchanged after the adding of other chemical species. Quantitative titration experiment shows a clear fluorescent turn-off in the presence of different concentrations of hypochlorite anion from 0 to 25 equivalent in mixed solution ($f_w = 80\%$) with 100% of quenching percentage in Figure 3. It is noted that the intensity of
TPE4A at 480 nm does not significantly change under H$_2$O$_2$ or ClO$_4^-$ oxidants, since this compound avoids being oxidized in this case.

![Absorption spectra](image.png)

**Figure 3.** Absorption (a) and emission (b) titration spectra of the probe TPE4A (40 µM) in the presence of various concentrations of ClO$^-$ from 0 to 25 equivalent in mixed solution ($f_w = 80\%$). Inset: the contrastive pictures before and after the addition of ClO$^-$. 

2.4. Paper-Based Sensor

To effective detection of ClO$^-$ in the state of aggregation, we prepared a test paper as the paper-based sensor. Then, we dipped the test paper into a solution containing chromophore test paper. As shown in Figure 4, the SEM diagram indicates that TPE4A can be attached to paper-based fibers. The diameter of particulate matter is approximately 100 µm, while the diameter of melt-blown fibers is 20~50 µm, showing a size matching on a micron scale. We carried out the naked-eye recognition matrix of paper-based sensor TPE4A for different concentrations of ClO$^-$ under visible and UV light in Figure 5. The fluorescence of the paper-based sensor is quenched gradually with the increase of the concentration of ClO$^-$. At the same time, the color of the sensor changes from white to yellow under visible light, while from green to purple with the light of the UV lamp at 365 nm. Quantitative analysis demonstrates that the detection limit of hypochlorite is 1.80 × 10$^{-4}$ M in solution based on the IUPAC definition 139 ($C_{DL} = 3$ Sb m$^{-1}$) [34], and the visual detection limit is 1.27 µg/cm$^2$ with the naked eye. Therefore, tetraphenylethylene-centered tetraaniline materials have a rapid and highly sensitive character for more convenient and visual detecting hypochlorite in a few seconds.

2.5. Possible Mechanism of Probe TPE4A

According to the references [16,35,36], as well as experimental and theoretical calculations [37], we have investigated the possible mechanism of TPE4A in Scheme 2. First of all, the tetraaniline structure may be oxidized to the azo counterpart under an appropriate oxidant in this case. It is noted that perchloride is a stronger oxidant meant it could oxidize the aniline group to form the azo intermediate. However, the solubility of perchloride is limited in $f_w = 80\%$. The mechanism of fluorescent turn-off quenching is a reaction from strong chromophore TPE4A to weak chromophore TPE4A-NNCI, which could be speculated that owing to the oxidation of TPE4A. Furthermore, the C–N bond could be easy to cleavage in the azo intermediate and further to form a radical which could combine with chlorine radicals to form the compound TPE4A-NNCI. The energy gap is 3.44 eV for TPE4A, while the energy gap is 3.17 eV for TPE4A-NNCI (see Supporting Information).
Figure 4. SEM images in different sizes of paper-based sensor TPE4A: 200 μm (a,b), 100 μm (c), and 50 μm (d).

Figure 5. The naked-eye recognition matrix diagram of paper-based sensor TPE4A for different concentrations of ClO\(^-\) under visible and UV light. Original 0 M, (a) \(5.0 \times 10^{-4}\) M, (b) \(1.0 \times 10^{-3}\) M, (c) \(5.0 \times 10^{-3}\) M, (d) \(1.0 \times 10^{-2}\) M, (e) \(5.0 \times 10^{-2}\) M, (f) \(1.0 \times 10^{-1}\) M.

In addition, we carried out the ESI-MS analysis to explore the complete reaction mixture of the probe with ClO\(^-\) in Figure S3. ESI-MS analysis that a peak at \(m/z = 837.1954\) corresponding to \([\text{TPE4A-NNCl}-2\text{Cl}+\text{Na}]^+\) (calcd for \([\text{C}_{50}\text{H}_{32}\text{Cl}_2\text{N}_4\text{Na}]^+\): 837.2019) is clearly observed with other identical isotopic peaks. This promoted a possible method to design fluorescent probes for hypochlorite with the inorganic/organic composites containing amino groups.
Scheme 2. Possible mechanism of chemosensor TPE4A for ClO⁻.

3. Conclusions

In summary, we have designed and developed a flexible paper-based material for hypochlorite detection. The quantitative analysis demonstrates that with the enhancement of hypochlorite equivalents, the UV–Vis peaks have been decreasing from 398 nm to 429 nm, while the fluorescent peak at 480 nm has been decreasing more remarkably. Furthermore, the detection limit of hypochlorite is 1.80 × 10⁻⁴ M in solution, and the visual detection limit is 1.27 μg/cm² with the naked eye. The mechanism of fluorescent turn-off quenching is a reaction from strong chromophore TPE4A to weak chromophore TPE4A-NNCl, which has been confirmed by experimental and theoretical results, as well as support from references. This study can provide a flexible paper-based portable chemosensor based on colorimetric and fluorescent dual modes for hypochlorite before conventional chemical synthesis.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/s21238082/s1. Figure S1: ¹H NMR of TPE4A; Figure S2: The selectivity of TPE4A for ClO⁻, SCN⁻, CO₃²⁻, S²⁻, NO₂⁻, H₂O₂, F⁻ and ClO₄⁻; Figure S3: Fitting plot for compound 1 with the addition of different contentions of TPE4A in THF solution to calculate the limit of detection; Figure S4: The experimental and theoretical ESI-MS of the product obtained by mixing probe NaOCl; Table S1: The HOMOs and LUMOs of compounds TPE4A and TPE4A-NNCl.

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References
1. Gross, S.; Gammon, S.T.; Moss, B.L.; Rauch, D.; Harding, J.; Heinecke, J.W.; Ratner, L.; Piwnica-Worms, D. Bioluminescence imaging of myeloperoxidase activity in vivo. Nat. Med. 2009, 15, 455–461. [CrossRef]
2. Reja, S.I.; Bhalla, V.; Sharma, A.; Kaur, G.; Kumar, M. A highly selective fluorescent probe for hypochlorite and its endogenous imaging in living cells. Chem. Commun. 2014, 50, 11911–11914. [CrossRef]
3. Gui, S.L.; Huang, Y.Y.; Hu, F.; Jin, Y.L.; Zhang, G.X.; Yan, L.S.; Zhang, D.Q.; Zhao, R. Fluorescent turn-on chemosensor for highly selective and sensitive detection and bioimaging of Al^3+ in living cells based on ion-induced aggregation. *Anal. Chem.* 2015, 87, 1470–1474. [CrossRef] [PubMed]

4. Guo, Z.R.; Hu, T.L.; Sun, T.; Li, T.D.; Chi, H.; Niu, Q.F. A colorimetric and fluorometric oligothiophene-indenedione-based sensor for rapid and highly sensitive detection of cyanide in real samples and bioimaging in living cells. *Dyes Pigments* 2019, 163, 667–674. [CrossRef]

5. Xu, H.; Wu, S.L.; Lin, N.J.; Lu, Y.; Xiao, J.; Wang, Y.W.; Peng, Y. A NIR fluorescent probe for rapid turn-on detection and bioimaging of hypochlorite anion. *Sens. Actuators B Chem.* 2021, 346, 130484. [CrossRef]

6. Patterson, D.I.; Davies, M.J. Evidence for rapid inter- and intramolecular chlorine transfer reactions of histamine and carnosine chloramines: Implications for the prevention of hypochlorous-acid-mediated damage. *Biochemistry* 2006, 45, 8152–8162. [CrossRef]

7. Kohler, A.T.; Rodtoff, A.C.; Labahn, M.; Reinhardt, M.; Truyen, U.; Speck, S. Efficacy of sodium hypochlorite against multidrug-resistant Gram-negative bacteria. *J. Hosp. Inf.* 2018, 100, E40–E46. [CrossRef] [PubMed]

8. Chatterjee, A. Use of Hypochlorite Solution as Disinfectant during COVID-19 Outbreak in India: From the Perspective of Human Health and Atmospheric Chemistry. *Aerosol Air Qual. Res.* 2020, 20, 1516–1519. [CrossRef]

9. Wang, L.; Long, L.L.; Zhou, L.P.; Wu, Y.J.; Zhang, C.; Han, Z.X.; Wang, J.L.; Da, Z.L. A ratiometric fluorescent probe for highly selective and sensitive detection of hypochlorite based on the oxidation of N-alkylpyridinium. *RSC Adv.* 2014, 4, 59535–59540. [CrossRef]

10. Sun, Y.; Gao, Y.; Tang, C.; Dong, G.; Zhao, P.; Peng, D.; Wang, T.; Du, L.; Li, M. Multiple rapid-responsive probes towards hypochlorite detection based on dioxetane luminescence derivatives. *J. Pharm. Anal.* 2021, in press. [CrossRef]

11. Jin, L.; Xu, M.Y.; Jiang, H.; Wang, W.L.; Wang, Q.M. A simple fluorescein derived colorimetric and fluorescent ‘off-on’ sensor for the detection of hypochlorite. *Anal. Methods* 2018, 10, 4562–4569. [CrossRef]

12. Dongare, P.R.; Gore, A.H. Recent advances in colorimetric and fluorescent chemosensors for ionic species: Design, principle and optical signalling mechanism. *ChemistrySelect* 2021, 6, 5657–5669. [CrossRef]

13. Ma, C.G.; Zhong, G.Y.; Zhao, Y.; Zhang, P.; Fu, Y.Q.; Shen, B.X. Recent development of synthetic probes for detection of hypochlorous acid/hypochlorite. *Sensors* 2020, 20, 6151–6160. [CrossRef]

14. Song, Z.-G.; Yuan, Q.; Lv, P.; Chen, K. Research progress of small molecule fluorescent probes for detecting hypochlorite. *Sensors* 2021, 21, 6326. [CrossRef]

15. Zhu, B.C.; Xu, Y.H.; Liu, W.Q.; Shao, C.X.; Wu, H.F.; Jiang, H.L.; Du, B.; Zhang, X.L. A highly selective colorimetric probe for fast and sensitive detection of hypochlorite in absolute aqueous solution. *Sens. Actuators B Chem.* 2014, 191, 473–478. [CrossRef]

16. Li, J.F.; Huo, F.J.; Yin, C.X. A selective colorimetric and fluorescent probe for the detection of ClO− and its application in bioimaging. *RSC Adv.* 2014, 4, 44610–44613. [CrossRef]

17. Yu, S.Y.; Hsu, C.Y.; Chen, W.C.; Wei, L.F.; Wu, S.P. A hypochlorous acid turn-on fluorescent probe based on HOCl-promoted oxime oxidation and its application in cell imaging. *Sens. Actuators B Chem.* 2014, 196, 203–207. [CrossRef]

18. Venkatasean, P.; Wu, S.P. A turn-on fluorescent probe for hypochlorous acid based on the oxidation of diphenyl telluride. *Analyst* 2015, 140, 1349–1355. [CrossRef]

19. Chen, W.C.; Venkatasean, P.; Wu, S.P. A highly selective turn-on fluorescent probe for hypochlorous acid based on hypochlorous acid-induced oxidative intramolecular cyclization of boron dipyromethene-hydrazone. *Anal. Chim. Acta* 2015, 882, 68–75. [CrossRef]

20. Zhu, H.; Zhang, Z.; Long, S.R.; Du, J.J.; Fan, J.L.; Peng, X.J. Synthesis of an ultrasensitive BODIPY-derived fluorescent probe for detecting HOCl in live cells. *Nat. Protoc.* 2018, 13, 2348–2361. [CrossRef]

21. Hwang, S.M.; Kim, A.; Kim, C. A simple hydrazine-based probe bearing anthracene moiety for the highly selective detection of hypochlorite. *Inorg. Chem. Commun.* 2017, 101, 1–5. [CrossRef]

22. Hu, Y.; Liu, J.; You, X.; Wang, C.; Li, Z.; Xie, W. A light-up probe for detection of adenosine in urine samples by a combination of an AIE molecule and an aptamer. *Sensors* 2017, 17, 2246. [CrossRef]

23. Rha, C.J.; Lee, H.; Kim, C. Development of an azo-naphthol-based probe for detecting hypochlorite (ClO−) via color change in aqueous solution. *Inorg. Chem. Commun.* 2021, 121, 108244. [CrossRef]

24. Lee, S.C.; Park, S.; So, H.; Lee, G.; Kim, K.-T.; Kim, C. An acridine-based fluorescent sensor for monitoring ClO− via color change in aqueous solution. *Inorg. Chem. Commun.* 2020, 20, 4764. [CrossRef]

25. Zhou, Y.Y.; Zhuang, Y.P.; Li, X.; Agren, H.; Yu, L.; Ding, J.D.; Zhu, L.L. Selective dual-channel imaging on cyanostryyl-modified azulene systems with unimolecularly tunable visible-near infrared luminescence. *Chem. Eur. J.* 2017, 23, 7642–7647. [CrossRef]

26. Fang, H.; Gan, Y.T.; Wang, S.R.; Tao, T. A selective and colorimetric chemosensor for fluoride based on dimeric azulene boronate ester. *Inorg. Chem. Commun.* 2018, 95, 17–21. [CrossRef]

27. Kim, D.; Jeong, K.; Kwon, J.E.; Park, H.; Lee, S.; Kim, S.; Park, S.Y. Dual-color fluorescent nanoparticles showing perfect color-specific photoswitching for bioimaging and super-resolution microscopy. *Nat. Commun.* 2019, 10, 3089. [CrossRef]

28. Zhu, Q.B.; Li, B.; Yang, D.D.; Liu, C.; Feng, S.; Chen, M.L.; Sun, Y.; Tian, Y.N.; Su, X.; Wang, X.M.; et al. A flexible ultrasensitive optoelectronic sensor array for neuroimaging visual systems. *Nat. Commun.* 2021, 12, 1798. [CrossRef]

29. Kim, S.; Yun, T.G.; Kang, C.; Son, M.J.; Kang, J.G.; Kim, L.H.; Lee, H.J.; An, C.H.; Hwang, B. Facile fabrication of paper-based silver nanostructure electrodes for flexible printed energy storage system. *Mater. Des.* 2018, 151, 1–7. [CrossRef]
30. Jiao, J.J.; Li, Z.J.; Qiao, Z.W.; Li, X.; Liu, Y.; Dong, J.Q.; Jiang, J.W.; Cui, Y. Design and self-assembly of hexahedral coordination cages for cascade reactions. *Nat. Commun.* **2018**, *9*, 4423. [CrossRef]

31. Liu, Y.Z.; Diercks, C.S.; Ma, Y.H.; Lyu, H.; Zhu, C.H.; Alshimimri, S.A.; Alshihri, S.; Yaghi, O.M. 3D covalent organic frameworks of interlocking 1D square ribbons. *J. Am. Chem. Soc.* **2019**, *141*, 677–683. [CrossRef]

32. Huang, L.; Tao, H.; Zhao, S.J.; Yang, K.; Cao, Q.Y.; Lan, M.H. A tetraphenylethylene-based aggregation-induced emission probe for fluorescence turn-on detection of lipopolysaccharide in injectable water with sensitivity down to picomolar. *Ind. Eng. Chem. Res.* **2020**, *59*, 8252–8258. [CrossRef]

33. Zhang, S.S.; Huang, Y.P.; Kong, L.; Zhang, X.J.; Yang, J.X. Aggregation-induced emission-active tetraphenylethylene derivatives containing arylimidazole unit for reversible mechanofluorochromism and selective detection of picric acid. *Dyes Pigments* **2020**, *181*, 108574. [CrossRef]

34. Ding, Y.; Li, X.; Li, T.; Zhu, W.; Xie, Y. α-Monoacylated and α,α′- and α,β′-diacylated dipyrrins as highly sensitive fluorescence “turn-on” 

35. Xiong, K.M.; Huo, F.J.; Yin, C.X.; Chu, Y.Y.; Yang, Y.T.; Chao, J.B.; Zheng, A.M. A novel recognition mechanism supported by experiment and theoretical calculation for hypochlorites recognition and its practical application. *Sens. Actuators B Chem.* **2016**, *224*, 307–314. [CrossRef]

36. Cheng, X.H.; Jia, H.Z.; Long, T.; Feng, J.; Qin, J.G.; Li, Z. A “turn-on” fluorescent probe for hypochlorous acid: Convenient synthesis, good sensing performance, and a new design strategy by the removal of C=N isomerization. *Chem. Commun.* **2011**, *47*, 11978–11980. [CrossRef]

37. Frisch, M.J.; Trucks, G.W.; Schlegel, J.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Schlegel, H.B.; Scalmani, G.; Barone, V.; Mennucci, B.; et al. *Gaussian 09, Revision C.01*; Gaussian, Inc.: Wallingford, CT, USA, 2010.