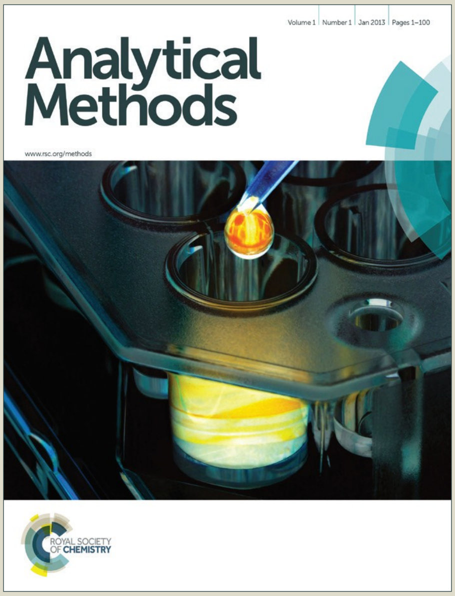
Analytical Methods



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A pyrene-based fluorescent and colorimetric chemodosimeter for detection of ClO^- ions

Yang Yang [a, b*], Chaoying Gao [a, b], Jing Chen [b], Ning Zhang [a] and Dewen Dong [a*]

[a] *Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, 130022, PR China*

[b] *College of Chemistry and Chemical Engineering, Inner Mongolia University for the Nationalities, Tongliao 028000, PR China*

E-mail: yangyang-000@163.com(Y. Yang), dwdong@ciac.jl.cn (D. Dong)

Abstract

A bi-functional colorimetric and fluorescent chemodosimeter PYCN has been designed and synthesized by simple condensation of 1-pyrenecarboxaldehyde and 1,2-diaminomaleonitrile. In aqueous media, the chemodosimeter PYCN exhibits remarkably enhanced absorbance and color change from yellow to colorless for hypochlorite anion, and shows significant “off-on” fluorescence accompanied with a blue emission to ClO^- anion. Based on the ClO^- promoted de-diaminomaleonitrile reaction, the application of this dye as a ratiometric optical chemodosimeter for ClO^- has been investigated in detail.

Keywords: colorimetric; fluorescence; chemodosimeter; hypochlorite; ROS

1. Introduction

The study of reactive oxygen species (ROS) is attracting increasing attention due to their essential roles in cell signaling and homeostasis [1,2]. Among these ROS, hypochlorite ion (ClO^-) is one of the most powerful natural important oxidants serve as a pathogens killer and anti-inflammation regulation [3,4]. Hypochlorite likely functions to injure microorganisms oxidatively and also have detrimental effects on host tissue [5]. An abnormal level of ClO^- would cause a wide range of diseases, such as cancer, atherosclerosis, arthritis and renal disease [6-8]. Therefore, a suitable way for selective and quantitative determination of hypochlorite in drinking water and organism is especially significant.

Compared with traditional methods for the detection of hypochlorite, such as electrochemistry and chromatography, fluorescent probes are the most powerful tools due to their high sensitivity, fast response and simplicity of implementation [9,10]. Recently, a number of fluorescent probes have been developed based on ClO^- or HClO promoted oxidation reactions and C=N isomerization mechanism that give highly fluorescent products for ClO^- detection [11-15]. These probes showed excellent selectivity, highly photosensitive, and pH dependency [16-18], but still face some challenge for detecting ClO^- like the interference of other ROS [19,20].

Recently, pyrene-based chemosensors have attracted intense attention for selective emission response to specific analytes [21-24]. In this paper, we have successfully synthesized a pyrene-based Schiff base derivative PYCN, which is used as a bi-functional colorimetric and fluorescent chemodosimeter for ClO^- in aqueous media.

It exhibits remarkably color change and significant “off-on” fluorescence with blue emission for ClO^-. The spectroscopic properties of PYCN reveal that the diaminomaleonitrile group in the probe can be selectively hydrolyzed by OCl^- instead of the other ions or ROS.

2. Experimental

2.1 Apparatus

^{1}H and ^{13}C NMR (DMSO-d_6) were recorded using a Bruker Avance III 500 spectrometer with tetramethylsilane (TMS) as internal standard. IR spectra were recorded in diffuse reflection with a Thermo Nicolet 5700 FT-IR spectrophotometer. The ESI mass spectra were recorded with a Thermo Finnigan Deca XP MAX LC/MSn spectrometer system. The UV/Vis spectra were recorded on a Shimadzu UV-3600 spectrophotometer. Fluorescence spectra measurements were performed on a JASCO FP-6500 fluorescence spectrophotometer. The pH measurements were made with a Model pHS-3TC pH meter.

2.2 Reagents

All reagents were purchased from commercial suppliers and used without further purification. Solvents for chemical synthesis and analysis were purified according to standard procedures. Nitric oxide radical (NO·) was generated from SNP (sodium nitrofer-ricyanide (III) dihydrate). SNP was added to deionized water and then stirred for 1 h at room temperature. Hydroxyl radical (·OH) was generated by the Fenton reaction. To prepare ·OH solution, $FeSO_4$ was added in the presence of 50 μM of H_2O_2. The superoxide radical ($\cdot O_2^-$) was generated from improved pyrogallol

(1,2,3-trihydroxybenzene) autoxidation method, the pyrogallol solution (in 1 M HCl) was thoroughly mixed with pH 7.4 Tris-HCl buffer. Peroxynitrite ($ONOO^-$) was prepared by the reaction of hydrogen peroxide with sodium nitrite. ROO· was generated from TBHP and CH_3CO_3H by reaction with $FeSO_4$ at room temperature for 1 h.

2.3 Synthesis

To a stirring solution of corresponding 1-pyrenecarboxaldehyde (0.23 g, 1.0 mmol) in absolute ethanol (20 ml), 2,3-diaminomaleonitrile (0.11g, 1.1 mmol) was added and the reaction mixture was refluxed for 10 h under N_2 atmosphere. After cooling it to room temperature, a yellow precipitate formed, which was filtered and washed with ethanol for three times. The product was purified by column chromatography with petroleum ether/ethyl acetate (1:1, v/v), gave a yellow solid in 65% yield. ^1H NMR (500MHz, DMSO-d_6): δ 9.30 (s, 1H), 9.05 (d, J = 8.2 Hz, 1H), 8.83 (d, J = 9.4 Hz, 1H), 8.46–8.30 (m, 5H), 8.26 (d, J = 8.9 Hz, 1H), 8.15 (t, J = 7.6 Hz, 3H); ^{13}C NMR (125MHz, DMSO-d_6): δ 152.5, 133.5, 131.3, 130.5, 130.3, 129.8, 129.7, 128.0, 127.9, 127.3, 127.2, 127.0, 126.8, 126.7, 125.6, 115.1, 114.5, 104.2; IR (KBr, cm^{-1}): ν = 3441, 3314, 2239, 2197, 1599, 1377, 1257, 1232, 1179, 846, 714; ESI-MS: *m/z* = 320.1.

3. Results and discussion

3.1 Characterization of chemodosimeter PYCN

The chemodosimeter 2-amino-3-(pyren-1-ylmethyleneamino)maleonitrile (PYCN) was synthesized by condensation of 1-pyrenecarboxaldehyde and

1,2-diaminomaleonitrile to form an imine bond as shown in Scheme 1 [25]. Due to the poor water solubility, the spectroscopic characteristics of PYCN with ClO^- were studied at the ratio of 8:2 (v/v) in EtOH–H_2O solvents.

3.2 Photophysical responses of PYCN to ClO^-

The main absorption band of PYCN lies at 420 and 446 nm in EtOH–H_2O (8:2, v/v) solution (Fig. 1a). Upon addition of a constant amount (10 equiv.) of ClO^- ions into PYCN solution, the main absorption band (420 nm) disappeared and a significant enhancement in absorbance at 343 nm was observed, which induced a clear color change from yellow to colorless. In contrast, the absorbance intensity of PYCN was found to be almost unaffected by the addition of Cl^-, Br^-, I^- ClO_3^-, BrO_3^-, IO_3^-, NO_2^-, NO_3^-, S^{2-}, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, CO_3^{2-}, CH_3COO^-, H_2O_2 and a blank solution. The results demonstrated that PYCN was characteristic of high selectivity toward ClO^- over other competitive anions.

To further investigate the sensitivity of chemodosimeter PYCN towards the hypochlorite anions, we also studied the fluorescence response of chemodosimeter PYCN in EtOH–H_2O solution (Fig. 1b). To our delight, only ClO^- incurs a dramatic fluorescence enhancement with the maximum wavelength centered at about 420 nm. Other competitive species, including H_2O_2, ClO_3^-, BrO_3^-, IO_3^-, NO_2^-, NO_3^-, S^{2-}, SO_3^{2-}, SO_4^{2-}, $S_2O_3^{2-}$, CO_3^{2-} and CH_3COO^- elicited almost no changes in the fluorescence spectra. Fig. 2 exhibits the naked-eye colorimetric changes (top) and the fluorescent (bottom) changes of PYCN with different anions. A clear and selective color change from yellow to colorless was observed for ClO^- and striking “turn-on” blue fluorescence was observed by introducing of ClO^-. These results indicate that chemodosimeter PYCN can be used conveniently for hypochlorite detection by simple visual detection.

We also did an investigation to evaluate the optical response of PYCN toward ClO^- in the presence of other ROS/RNS, including NO·, $\cdot O_2^-$, $ONOO^-$, BuOO·, AcOOH, and ·OH. It was found that the strong blue fluorescence emission only occurred upon addition of ClO^- to the PYCN solution; other ROS/RNS induced nearly no optical responses even after 30 min (Fig. 2). The emission bands at 420 nm were recorded in the presence of several ROS, but ClO^- was the only reactive species to cause an obvious fluorescence enhancement. Therefore, we consider PYCN was a highly selective probe to HClO over other ROS.

3.3 Spectral titration experiment

In order to obtain a better insight into the response mechanism of PYCN toward ClO^-, spectroscopic titration was carried out. As shown in Fig. 3a, after addition of different equivalents of ClO^-, the absorbance band at 420 nm gradually decreased with the simultaneous appearance of a new blue-shifted band centered at 343 nm, which can be ascribed to the transformation of hydrazone to aldehyde promoted by the oxidant hypochlorite anions. With the concentration of ClO^- up to 10 equivalents, the maximum absorbance changes ($|A_0-A|$) were 0.24 and 0.42 respectively (inset of Fig. 3a). The fluorescence titration of the ClO^- ion was carried out under the same working conditions. As shown in Fig. 3b, when excited at 355 nm, the fluorescence band at 420 nm increased more than 200 fold on increasing the concentration of ClO^-

from 0 to 10 equivalents. A satisfactory Boltzmann correlation existed between the fluorescence intensity and the concentration of hypochlorite within the range from 0 to 10.0 equivalents (Fig. 3b). The fluorescence changes at 420 nm increases with the concentrations of hypochlorite, and then remains unchanged at last, indicating the saturated oxidization of imine group to aldehyde by hypochlorite.

3.4 Selectivity and tolerance of PYCN to ClO^-

To evaluate the selectively respond of PYCN to OCl^- under simulated physiological conditions, competition experiment was carried out according to the reported procedures [26]. As shown in Fig. 4a, ClO^- could be distinguished from other chemical species by monitoring the absorbance band centered at 343 and 420 nm. Except for $S_2O_3^{2-}$ and H_2O_2, other anions did not exhibit obvious interference for ClO^- detection, which demonstrates that the absorbance of PYCN is enhanced effectively by ClO^- with these anions as backgrounds. It is interesting to note that the examination of other chemical species/ClO^- coexisted systems by fluorescence spectra also showed the same tendencies (Fig. 4b).

Because ClO^- in living organisms is generated from the oxidation of chloride ion and hydrogen peroxide catalyzed by myeloperoxidase [27], another experiment was conducted by alternated adding ClO^- and H_2O_2 ($S_2O_3^{2-}$) in PYCN system (Fig. S4). The absorbance intensity changed dramatically upon the addition of $ClO^-+H_2O_2$ ($ClO^-+S_2O_3^{2-}$), whereas a weak change was detected in $H_2O_2+ClO^-$ ($S_2O_3^{2-}+ClO^-$) system. Since ClO^- can react with H_2O_2 and $S_2O_3^{2-}$, part of hypochlorite was consumed in the solution resulting in the reaction between PYCN and ClO^- being less effective.

3.5 pH dependence

In order to investigate the influence of the different acid concentration on the spectra of PYCN and find a suitable pH span in which PYCN can selectively detect ClO^- efficiently, the pH dependence experiments were performed. In the absence of OCl^-, the free probe PYCN is stable over a wide range of pH values from 2.0 to 12.0 (Fig. 5). However, the addition of ClO^- led to the fluorescence enhancement over a comparatively wide pH range (4.0~8.0). The pH value that gave the highest different fluorescence signal of PYCN+ClO^- was pH 6.0~8.0. Therefore, neutral pH was chosen as the optimum experimental condition.

3.6 Time-dependence in the detection of ClO^-

Time-dependent variations in the UV–vis and fluorescence emission spectroscopes were monitored in the presence of 10 equivalents of ClO^- (Fig. S5). The kinetic study showed that the reaction was complete within 15 s for ClO^-, thus indicating that the probe PYCN shows good sensitivity to hypochlorite, and the detection limit is as low as 2.83 μM according to the reported formula LOD=3σ/S [28].

For practical applicability of PYCN to real samples, different amounts of ClO^- were measured in real water sample (by adding ClO^- ions into the tap water). PYCN (10 μmol/L) was treated with various concentrations of ClO^- (10^{-6}~10^{-2} mol/L) and the emission intensity at 420 nm plotted as a function of the ClO^- concentration. It can be seen that a concentration of ClO^- as low as 10^{-5} mol/L can be detected (Fig. 6).

3.7 Sensing mechanism

FT-IR and ^{1}H NMR spectra of PYCN and PYCN+ClO$^-$ were also employed to investigate the sensing mechanism (Fig. S6, S7). The absorption peaks attributed to the strong vibration of -NH$_2$ (3441, 3313 cm^{-1}), -CN (2229 cm^{-1}) and -C=N (1599 cm^{-1}) disappeared after addition of ClO$^-$. The emergence of a new band at 1678 cm^{-1} which attributed to the stretch vibration of C=O was formed during the hypochlorite sensing process (Scheme 2). After reaction with ClO$^-$ in aqueous EtOH, A fluorescence product was obtained by chromatography and subjected to NMR analysis. We found that after addition of ClO$^-$, the proton (δ = 9.3 ppm) corresponding to -CH=N shifted to high field and a new proton (δ = 10.7 ppm) appeared due to the formation of 1-pyrenealdehyde. These observations supported the mechanism that OCl$^-$ induced de-diaminomaleonitrile reaction and an aldehyde derivative was formed [29].

4. Conclusions

In summary, we have developed a pyrene-based bifunctional colormetric and fluorescent chemodosimeter PYCN, which exhibited a rapid, highly selective, and sensitive response to ClO$^-$ over other chemical species. We observed prominent absorption spectra enhancement resulting in a change of solution color from yellow to colorless and significant “off–on” fluorescence enhancement accompanied with blue emission after reaction with ClO$^-$. The optimal pH range for ClO$^-$ detection by PYCN is 4~8 and the detection limit is as low as 10^{-5} mol/L in real tap water. The sensing mechanism is based on OCl$^-$ promoted de-diaminomaleonitrile reaction, and form 1-pyrenealdehyde. Owing to the simplicity and sensitivity to the analysis, the sensor would be found a lot of practical applications in environmental and biological systems.

Acknowledgments

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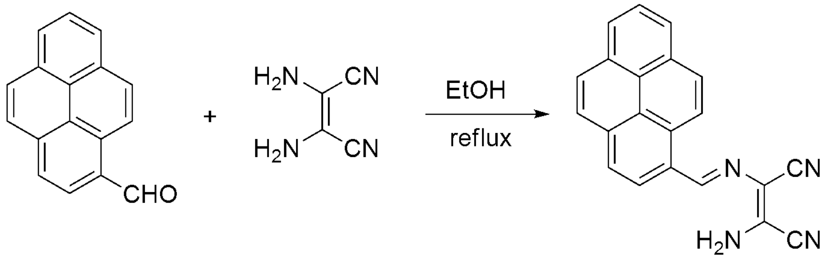
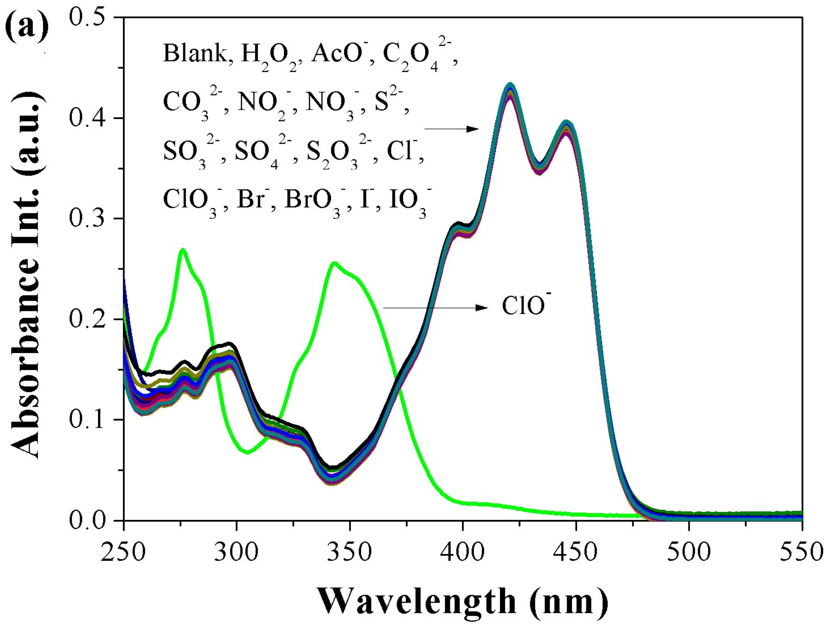


Scheme 1. Synthesis and structure of chemodosimeter PYCN.



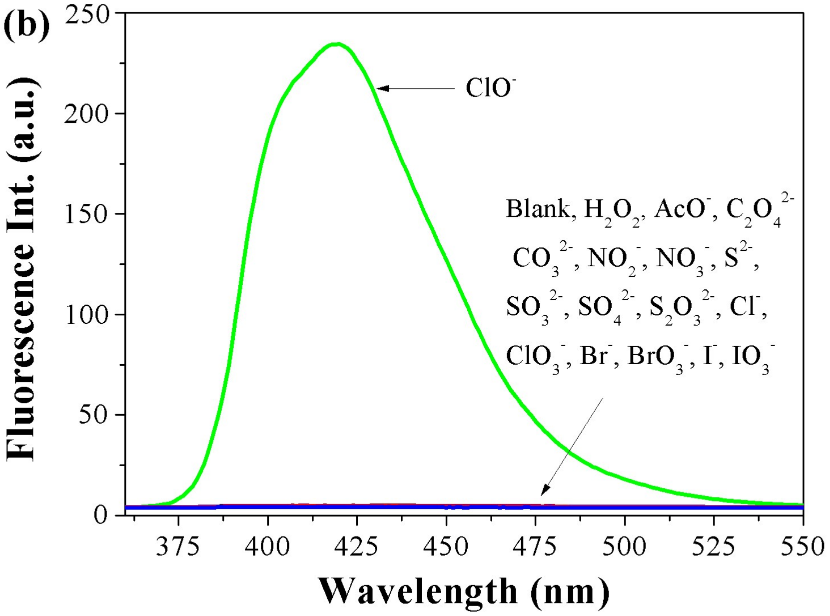


Fig. 1. (a) Absorption spectra of PYCN (10 μM) in EtOH–H_2O (4:1, v/v) in the presence of various anions (10 Eq). (b) Fluorescence spectra of PYCN (10 μM) in EtOH–H_2O solution in the presence of various anions (10 Eq).

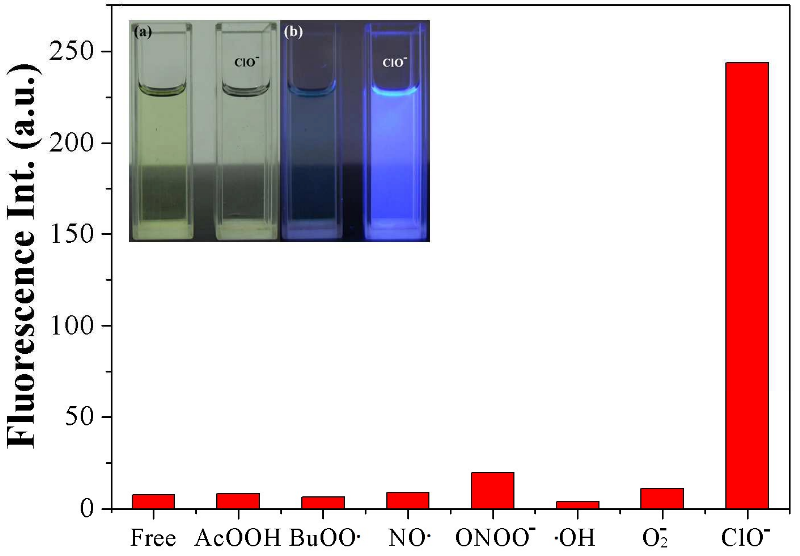


Fig. 2. Fluorescence responses of PYCN (10 μM) in EtOH–H_2O solution in presence

of NaOCl, other ROS, and RNS (10 eq).

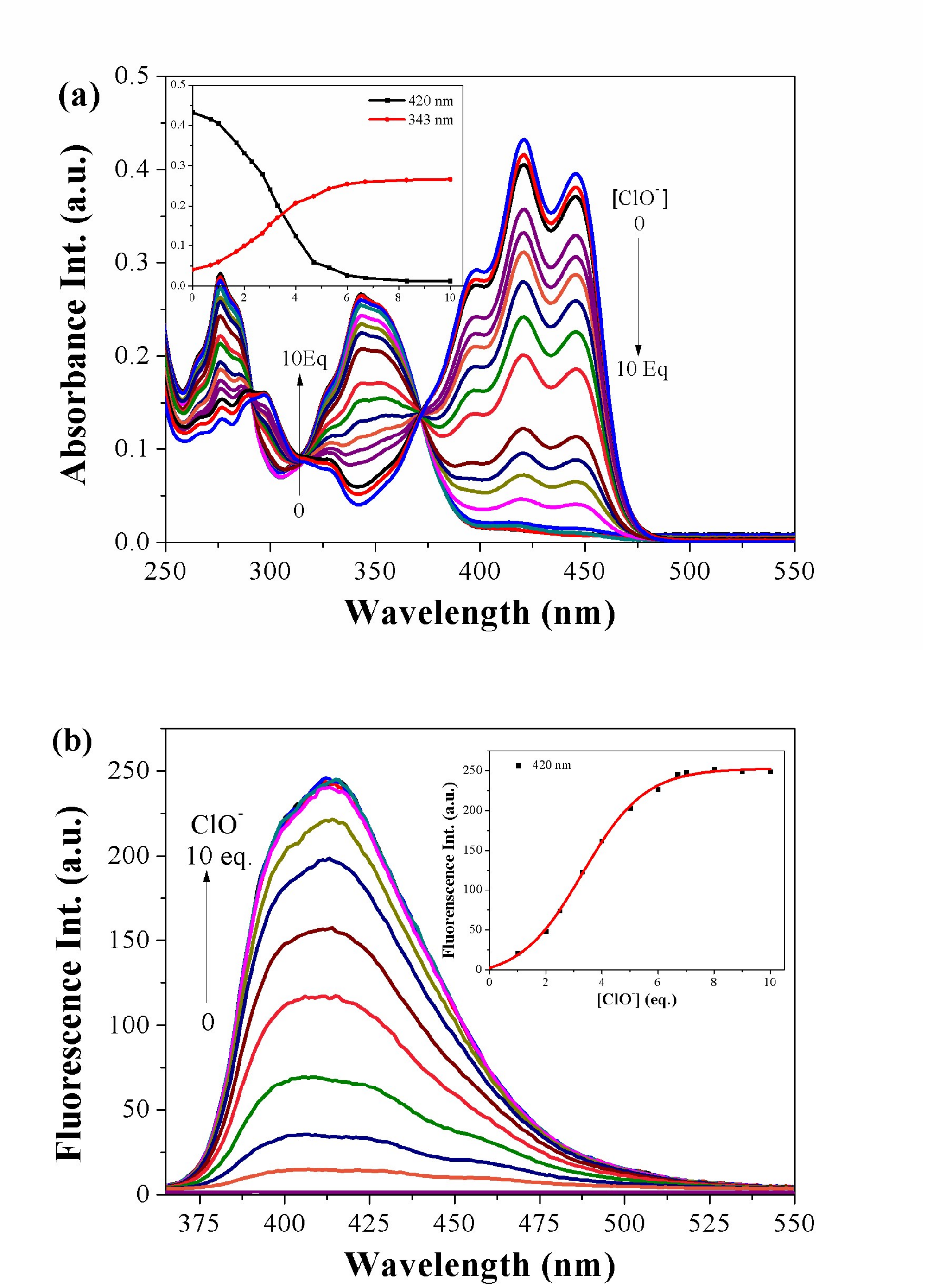


Fig. 3. (a) Changes in the absorption spectra of PYCN (10 μM) as the titration of ClO^-(0~10 Eq) in EtOH–H_2O solution. The inset shows the relative absorption value at 343 and 420 nm as a function of added ClO^-. (b) Changes in the fluorescence

intensity of PYCN (10 μM) in the presence of various equivalents of ClO^- in EtOH–H_2O solution.

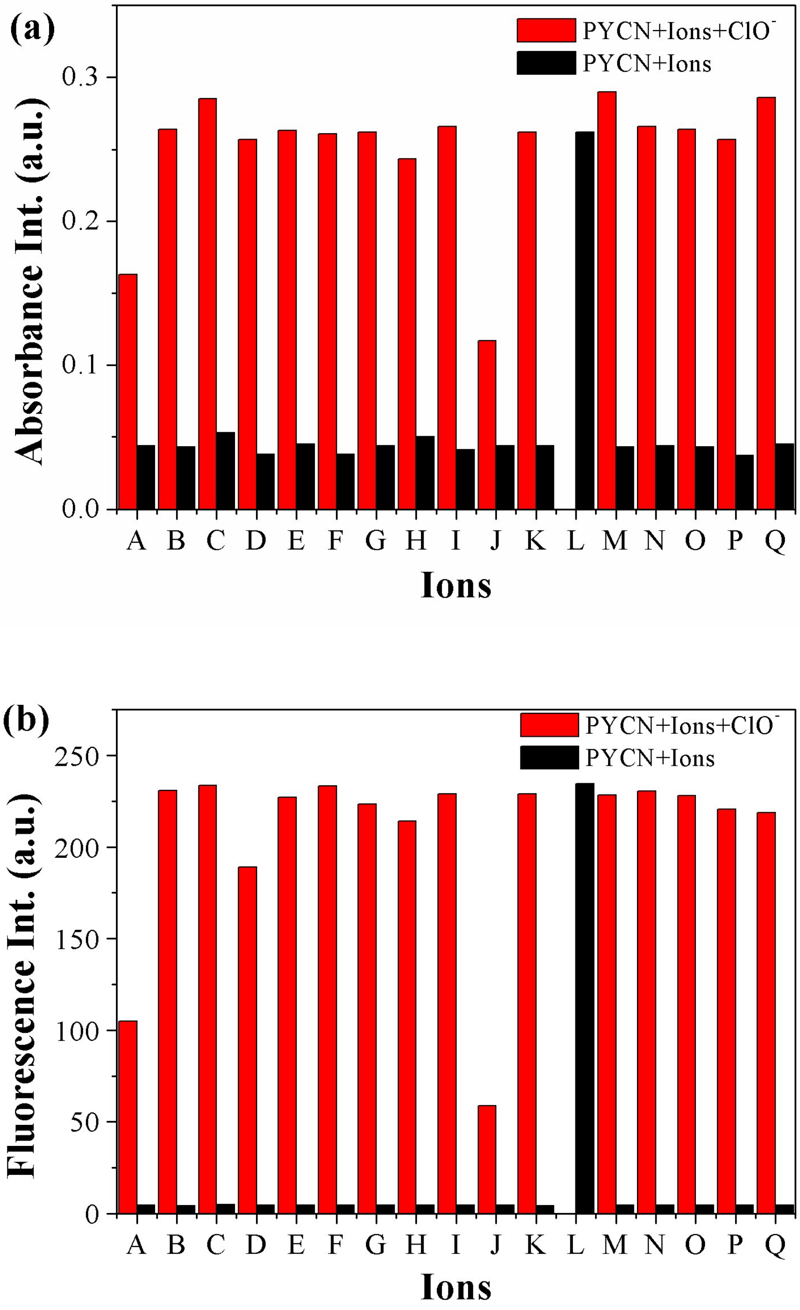


Fig. 4. The absorption (a) and fluorescence (b) changes of PYCN in the presence of competitive anions (A~Q: H_2O_2, AcO^-, $C_2O_4^{2-}$, CO_3^{2-}, NO_2^-, NO_3^-, S^{2-}, SO_3^{2-}, SO_4^{2-},

$S_2O_3^{2-}$, Cl^-, ClO^-, ClO_3^-, Br^-, BrO_3^-, I^- and IO_3^-, 10 Eq). The black bars represent the intensity of PYCN in the presence of different anions (10 Eq); the red bars represent the intensity of PYCN in the presence of the indicated anions and ClO^- ion.

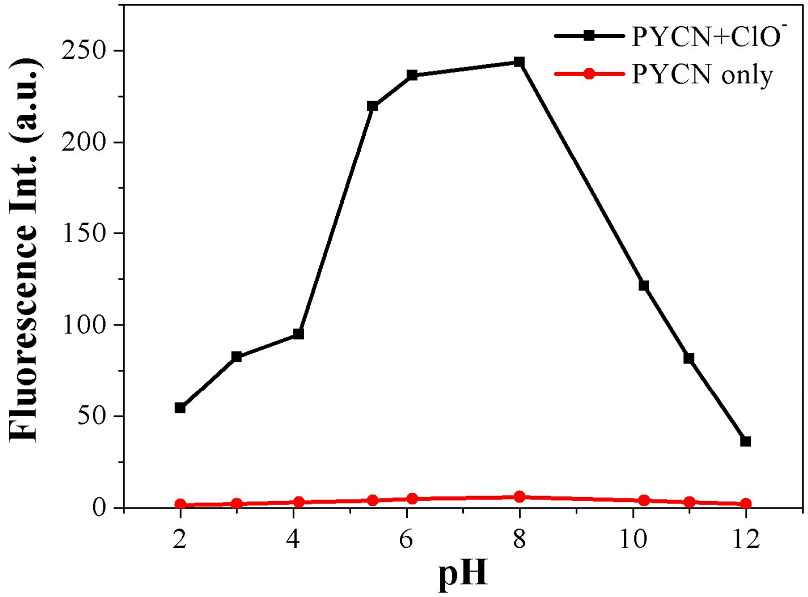


Fig. 5. The variation of the fluorescence at 420 nm for PYCN (10 μM) at different pH values in the absence and presence of ClO^- (10 Eq).

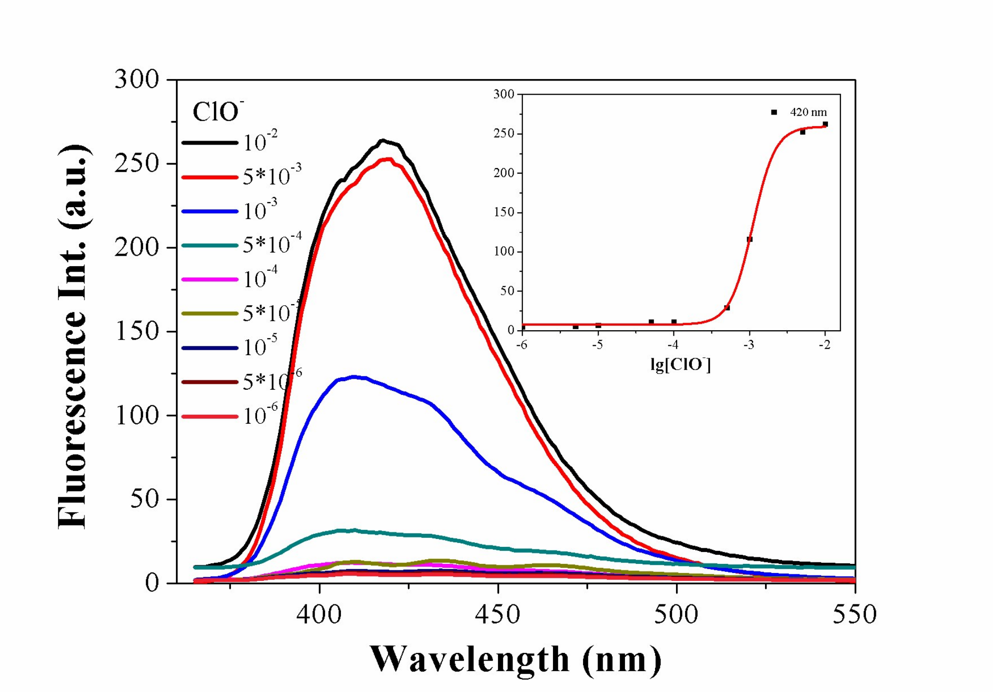
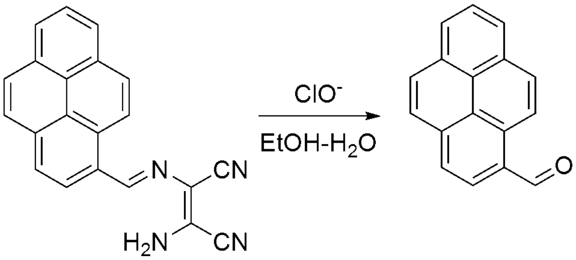


Fig. 6. Determination of ClO$^-$ in water samples with PYCN (10 μM).



Scheme 2. Proposed sensing mechanism of PYCN in the presence of ClO$^-$.

Graphical Abstract