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Colorimetric and Fluorometric Assays for Dopamine with a Wide Concentration Range Based on Fe-MIL-88NH$_2$ Metal-organic Framework

Chao ZHAO, Yali LIU, and Yuanfang LI†

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China

Metal-organic framework Fe-MIL-88NH$_2$ as a dual colorimetric and fluorometric sensor has been designed for a wide range of dopamine quantitative detection. It is easy to implement the assay for visual detection of dopamine based on restraining the color change of the 3,3′,5,5′-tetramethylbenzidine-H_2O_2 system that is catalyzed by Fe-MIL-88NH$_2$ with intrinsic peroxidase-like catalytic activity. The linear range is from 50 nM to 30 μM. In addition, the Fe-MIL-88NH$_2$ can exhibit a dramatic decrease of its fluorescent intensity when exposed to dopamine, which may be attributed to the electron transfer from the Fe-MIL-88NH$_2$ to the oxidized dopamine-quinone. The linear response range is from 30 μM to 4 mM. Meanwhile, both colorimetric and fluorometric methods exhibit higher selectivity for DA over a number of possible interfering substances. Furthermore, the proposed method has been successfully applied to detect DA in human serum samples, which suggests its great potential for analytical applications.

Keywords Fe-MIL-88NH$_2$ metal-organic framework, colorimetric detection, fluorescence quenching, dopamine

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Introduction

Dopamine (DA), as an important catecholamine neurotransmitter, is closely associated with motor and cognitive functions, as well as the reward system; deficits in brain dopamine cause Parkinson's disease in humans.[1-4] Hence, numerous techniques for accurate detection of DA have been developed during the past decades, such as electrochemical assay,[5-7] chemiluminescence,[8,9] capillary electrophoresis[10] and high-performance liquid chromatography.[11] Recently, more attention has been paid to spectrometric methods due to their simple procedures and low detection limits.[12-15] For example, Qu *et al.* reported a dual fluorometric and colorimetric sensor for DA based on BSA-stabilized Au nanoclusters.[16] Nevertheless, it is still a challenge to achieve low detection limits coupled with wide linear range, as well as high sensitivity and selectivity. Therefore, in order to meet the increasing demand for detecting trace dopamine in biological fluids, substantial efforts are still needed for the development of low-cost, facile and efficient methods.

Metal-organic frameworks (MOFs), self-assembled from metal ions or clusters and multidentate organic ligands, have stimulated extensive enthusiasm for research owing to their fascinating structural topologies and intriguing properties, such as large surface areas, tunable surface properties, and high chemical and thermal stability.[17,18] Up to now, MOFs and hybrid materials incorporating MOFs[19-21] have generated many practical applications including those associated with gas storage,[22,23] separations,[24,25] catalysis,[26] sensing,[27,28] and imaging.[29] Notably, colorimetric or luminescent properties of MOFs offer unlimited potential for sensing and detection applications.[30-32] For instance, recently, our group has reported that Fe-MIL-88NH$_2$ metal-organic framework possesses intrinsic peroxidase-like activity, and further used it to establish sensitive colorimetric assays.[33,34]

In this context, we were surprised to find that Fe-MIL-88NH$_2$ as a novel peroxidase mimic could selectively assay DA. As shown in Fig. 1A, Fe-MIL-88NH$_2$ was employed to quickly catalyze the oxidation of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) to generate a blue colored oxidized TMB (oxTMB) by decomposing H_2O_2 to produce ·OH radicals. However, DA induced an inhibitory effect on the oxidation by competitively consuming ·OH, thus resulting in the shallow color. Therefore, the content of DA could be calculated according to colorimetrics. In addition, Fe-MIL-88NH$_2$ has strong fluorescence attributable to the organic ligand 2-aminoterephthalic acid, and DA could efficiently quench the fluorescence of the Fe-MIL-88NH$_2$ through electron transfer (Fig. 1B). Herein, a fluorescence method for DA detection with excellent selectivity was also developed. As stated above, a colorimetric and fluorometric dual channel probe has been developed for DA detection based on the metal-organic framework Fe-MIL-88NH$_2$.

† To whom correspondence should be addressed.
E-mail: liyf@swu.edu.cn

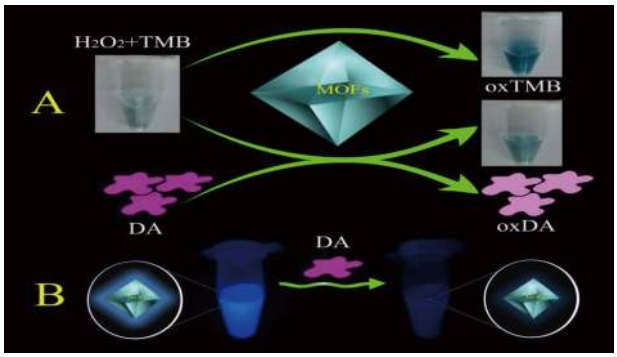

Fig. 1 Schematic diagram of the experimental system. (A) Schematic representation of peroxidase-like catalytic color reaction for sensitive sensing of DA (dopamine), (B) Schematic illustration of the fluorescence response of the Fe-MIL-88NH$_2$ to DA.

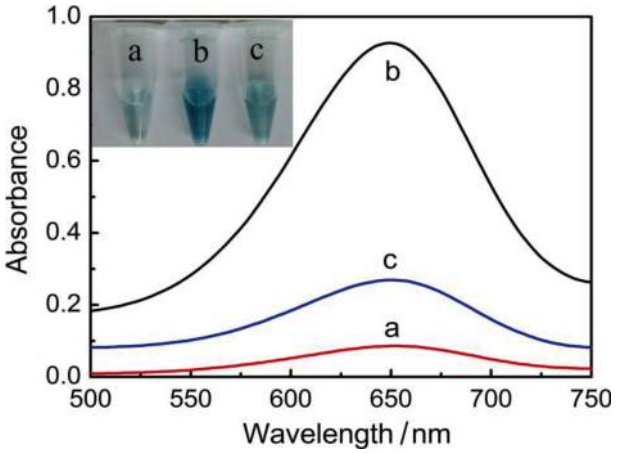

Fig. 2 Typical absorption spectra of the TMB-H_2O_2 mixed solution in the absence and presence of 30 μM DA. (a) TMB + H_2O_2. (b) TMB + H_2O_2 + Fe-MIL-88NH$_2$. (c) TMB + H_2O_2 + Fe-MIL-88NH$_2$ + DA. Inset: a photograph of the solutions.

Experimental

Reagents and chemicals

Supplies of 3,3′,5,5′-tetramethylbenzidine (TMB), dopamine hydrochloride (DA), and uric acid (UA) were purchased from Sigma-Aldrich (St. Louis, MO). Ascorbic acid (AA), cysteine (Cys), tyrosine (Tyr), phenylalanine (Phe) and other amino acids were obtained from Beijing Dingguo Changsheng Biotech Co., Ltd. We obtained H_2O_2 (30 wt%), acetic acid, sodium acetate, potassium dihydrogen phosphate, disodium hydrogen phosphate, glucose and lactose from Chongqing Pharmaceutical Co., Ltd., Keyi Assay Glass Branch (Chongqing, China). Serum samples were obtained from the Southwest University Hospital of Chongqing. All reagents were of analytical reagent grade, and used as received. Ultra-pure water (18.2 MΩ) was used throughout the experiment. Fe-MIL-88NH$_2$ was synthesized in previous studies performed by our group.[33] In addition, SEM and XRD patterns are shown in Figs. S1 and S2 (Supporting Information).

Apparatus

A constant-temperature water-base boiler (Jiangsu, China) was used to control the reaction temperature. The UV-vis absorption spectra were recorded with a U-3010 spectrophotometer (Hitachi Co., Tokyo, Japan). Fluorescence measurements were carried out on an F-2500 fluorescence spectrophotometer (Hitachi Co.). Fourier transform infrared (FTIR) spectra were recorded on an FITI-8400 (Shimadzu, Japan) in the range of 4000 – 500 cm^{-1} using the KBr disk method. The fluorescence lifetimes were measured with a FL-TCSPC fluorescence spectrophotometer (Horiba Jobin Yvon Inc., France). An S-4800 scanning electron microscope (SEM, Hitachi, Japan) was used to scan SEM images of Fe-MIL-88NH$_2$. Powder X-ray diffraction (PXRD) patterns were obtained on an XD-3 X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) at a scan rate of 2.0° min^{-1} (Purkinje, China).

Preparation of serum samples

Two human serum samples from healthy adults were treated by spin dialysis at 12000 rpm for 30 min. All samples were subjected to a 20-fold dilution before measurement, and no other pretreatment process was necessary.

Measurement procedures of colorimetric method

A typical test was carried out as follows: 50 μL of 0.2 M HAc-NaAc buffer (pH 4.0), 35 μL of 10 mM TMB, 50 μL of 0.8 mM H_2O_2, 70 μL of 0.2 mg mL^{-1} Fe-MIL-88NH$_2$ nanocrystal solution and certain amounts of DA were added into a 1.5 mL EP vial. Then the mixture was diluted to 500 μL with ultra-pure water (18.2 MΩ). Finally, the mixture was incubated at 45°C for 30 min, and the peroxidase activities were carried out by monitoring the absorbance changes at 650 nm on a U-3010 spectrophotometer.

Measurement procedures of fluorimetric method

In a typical test, 100 μL of 0.2 mg mL^{-1} Fe-MIL-88NH$_2$ nanocrystal solution and 50 μL of 0.067 M Na_2HPO_4-KH_2PO_4 buffer (pH 8.0) were mixed with certain amounts of DA. Then the mixture was diluted to 500 μL with ultra-pure water (18.2 MΩ). The ultima mixture was incubated at 47°C for 45 min. Finally, fluorescence spectra were recorded under excitation at 330 nm.

Results and Discussion

Spectral characteristic

To demonstrate the feasibility of the methods, we evaluated the spectral changes from the interaction between Fe-MIL-88NH$_2$ and DA. Firstly, colorimetric assay for DA was tested. As shown in Fig. 2, the TMB-H_2O_2 mixed solution without Fe-MIL-88NH$_2$ exhibited negligible color change (curve a, Fig. 2). In contrast, when Fe-MIL-88NH$_2$ was introduced into the TMB-H_2O_2 system, TMB was catalyzed to oxidation to produce the typical blue colored oxTMB with maximum absorbance at 650 nm (curve b, Fig. 2). However, the presence of DA resulted in the shallow color (curve c, Fig. 2). Moreover, all color changes could be observed obviously by the naked eye (Fig. 2 inset). As shown in Fig. S3A (Supporting Information), we have investigated the effect of a mixed solution of $FeCl_3$ and 2-aminoterephthalic acid without forming MOF structures. In spite of similar phenomenon, the results were less effective than using the Fe-MIL-88NH$_2$ metal-organic framework. This may be because the forming of MOF structures provide the advantage of enhancing surface area and stability, which may be helpful to improve the efficiency of reaction. Therefore, we deemed the Fe-MIL-88NH$_2$-TMB-H_2O_2 system could be used as the

colorimetric indicator for DA detection.

The fluorescence method was also investigated. The typical emission spectrum of the Fe-MIL-88NH_2 displayed an emission peak at around 430 nm upon excitation at 330 nm (Fig. 3, curve a). When DA was added into the solution, the fluorescence of the Fe-MIL-88NH_2 was quenched greatly without an obvious shift of the emission peak (Fig. 3, curve b), and weaker fluorescence was observed under 365 nm UV light (Fig. 3 inset). We also investigated the effect of a mixed solution of $FeCl_3$ and 2-aminoterephthalic acid without forming MOF structures, and the results indicated that the Fe-MIL-88NH_2 as a fluorescent probe to detect DA could be more efficient (Fig. S3B, Supporting Information).

Optimization of conditions

As is already known, the practical applicability of an analytical method is often strongly influenced by the experimental conditions such as reaction temperature, time, and pH. Therefore, the as-developed dopamine detection parameters were optimized in our study, while keeping the other parameters constant. First, we investigated the dependability of the Fe-MIL-88NH_2 peroxidase-like activity. When Fe-MIL-88NH_2 was used as a colorimetric probe, we found the optimal acidity, temperature, reaction time, and concentrations of H_2O_2, Fe-MIL-88NH_2 and TMB were 4.0, 45°C, 30 min, 80 μM, 0.028 mg mL^{-1} and 0.7 mM, respectively (Fig. S4, Supporting Information). When Fe-MIL-88NH_2 was introduced as a fluorescent probe, pH 8.0, 47°C, 45 min and 0.040 mg mL^{-1} were set as the optimal pH, temperature, time and Fe-MIL-88NH_2 concentration, respectively (Fig. S5, Supporting Information).

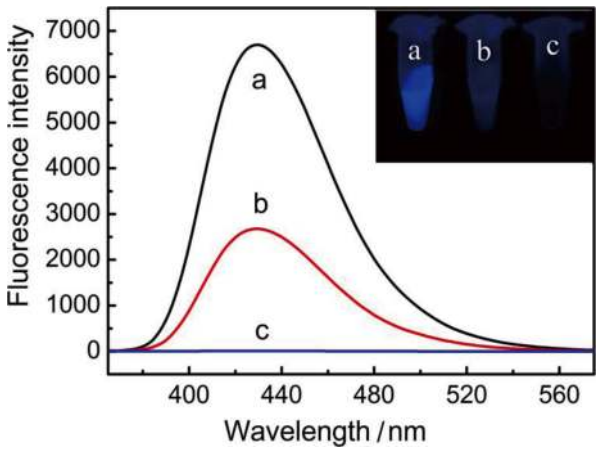

Fig. 3 The typical emission fluorescence spectra of Fe-MIL-88NH_2 in the absence and presence of 0.3 mM DA. (a) Fe-MIL-88NH_2, (b) Fe-MIL-88NH_2 + DA, (c) DA. Inset: a photograph of the solutions upon excitation (365 nm) under a UV lamp.

Sensitivity and selectivity

Under optimized experimental conditions, we further examined the sensitivity of both colorimetric and fluorometric sensing systems. In the colorimetric sensing system, more DA introduced weaker absorption peak intensity at 650 nm, and the linear detection range was estimated to be from 50 nM to 30 μM (Fig. 4). The linear equation is $A = -0.02c$ (μM) + 0.89, $R = -0.998$. The direct detection limit for analyzing DA could be as low as 50 nM. In the same way, the fluorescence emission of Fe-MIL-88NH_2 at 430 nm in the presence of different concentrations of DA was measured. As shown in Fig. 5, the fluorescent intensity of Fe-MIL-88NH_2 decreased gradually with increasing concentrations of DA from 1 μM to 6 mM, and linear regression analysis with the concentration ranges from 30 μM to 4 mM were obtained (inset figure). The linear equation is lg $F = -0.25$lg c (μM) + 4.05, $R = -0.998$, with the direct detection limit of 30 μM.

For an excellent detection system, high selectivity is crucial especially in real sample detections. In order to verify the specificity of the proposed methods, possible foreign species were examined, such as UA, AA, amino acids, glucide, human serum albumin (HSA), γ-globulin (γ-G) and metal ions. First, the absorption values were determined. As shown in Fig. 6A, the solution containing DA was almost colorless, while the solutions containing other interferences revealed a deep blue

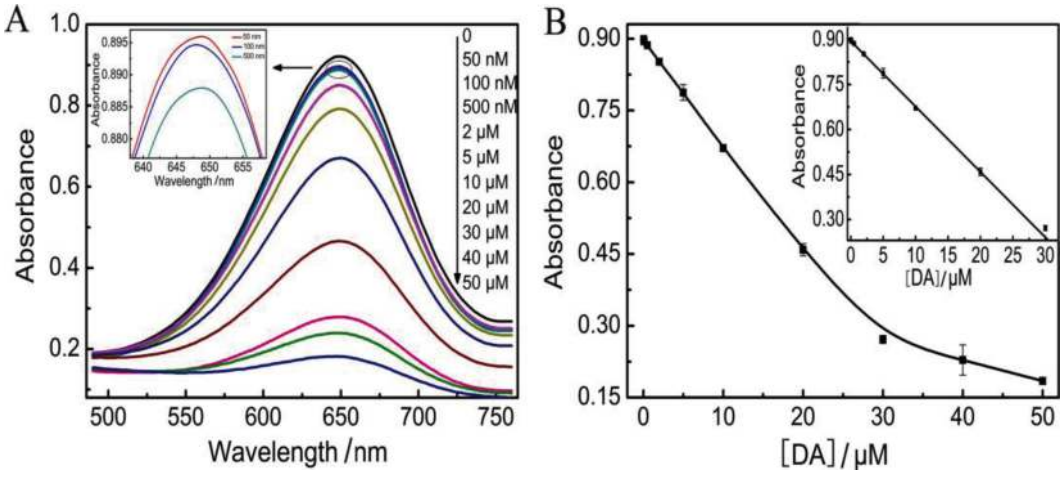

Fig. 4 (A) Typical absorption spectra of the TMB solution in the presence of DA at various concentrations using Fe-MIL-88NH_2 as an artificial enzyme. Inset: absorption spectra of the TMB solution in the presence of DA from 50 to 500 nM. (B) Plots of the absorbance of TMB at 650 nm as a function of concentration of DA. The error bars represent the standard deviation of three measurements. Inset: linear calibration plot for DA.

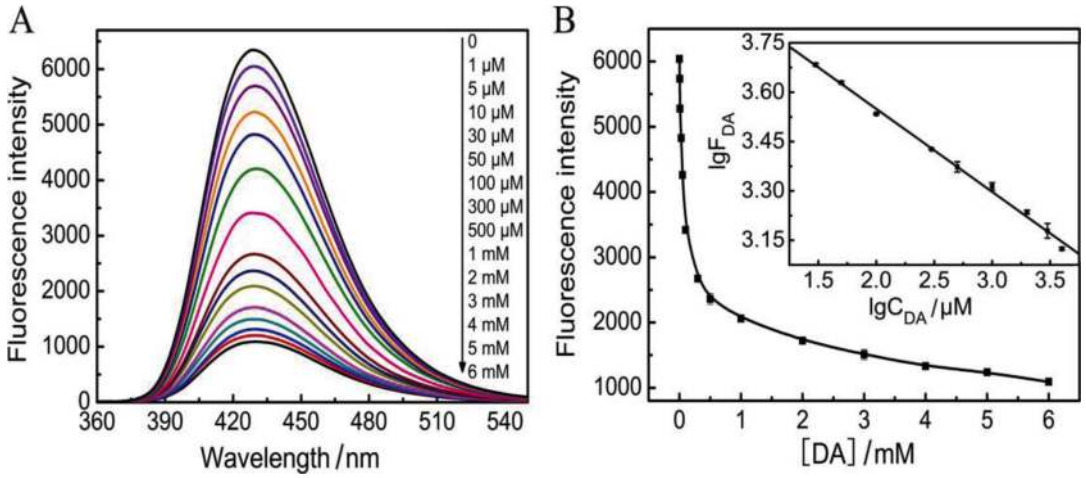

Fig. 5 (A) Fluorescence emission spectra of the Fe-MIL-88NH$_2$ in the presence of increasing DA concentrations (0 – 6 mM). (B) Plots of the fluorescence intensity at 430 nm as a function of the DA concentration (0 – 6 mM). The error bars represent the standard deviation of three measurements. Inset: the linear plot.

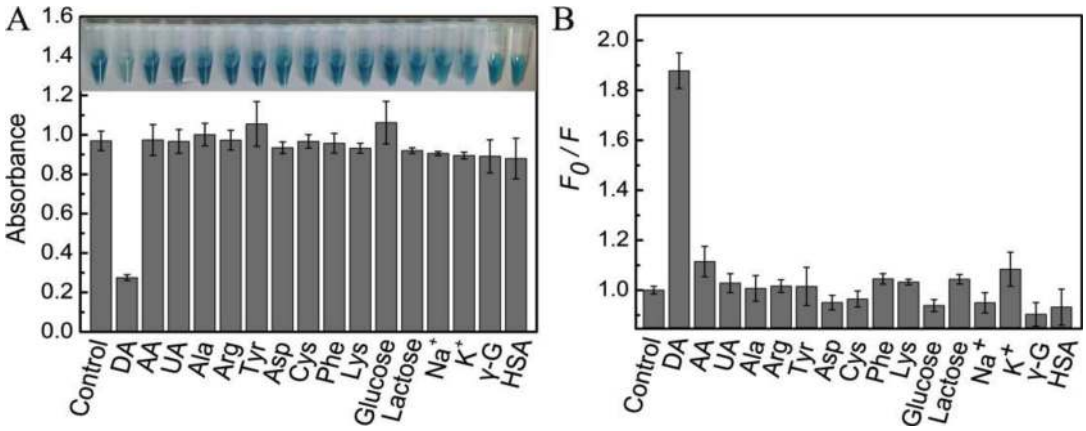

Fig. 6 (A) Absorbance changes of the TMB solution in the presence of 30 μM DA, 30 μM Cys, 60 μM AA, HSA (10 mg mL^{-1}), γ-G (10 mg mL^{-1}) or 300 μM other analytes. Inset: a photograph of the solutions. (B) Fluorescence responses of the Fe-MIL-88NH$_2$ in the presence of 0.1 mM DA, HSA (40 mg mL^{-1}), γ-G (40 mg mL^{-1}) or 2 mM other analytes. F_0 and F are the fluorescence intensity of Fe-MIL-88NH$_2$ in the absence and presence of DA and the other analytes, respectively.

color, indicating that this visual method could effectively identify DA under experimental concentrations, even with the naked eye. So the tolerable concentrations of Cys, AA, HSA, γ-G and other foreign substances were determined to be 30 μM, 60 μM, 10 mg mL^{-1}, 10 mg mL^{-1} and 300 μM, respectively. Then, selectivity of the fluorescence method was investigated. As shown in Fig. 6B, while the concentrations of HSA and γ-G were 40 mg mL^{-1} and other foreign substances were 20-fold higher than that of DA, there were still no obvious effects on the fluorescence emission intensity of Fe-MIL-88NH$_2$ except DA. Although higher concentrations of AA and Cys have some interferences in the colorimetric sensing system, Fe-MIL-88NH$_2$ as a colorimetric and fluorometric sensor still exhibits higher selectivity to DA over other interferences under experimental concentrations, especially as a fluorometric sensor.

Determination of DA in human serum samples

In order to verify the practicality of the proposed system, it was employed for selective detection of DA in human blood serum through the standard addition method. As the analytical results show in Table 1, the mean recoveries for DA were from 98 to 107%, suggesting that the methods were largely free from the matrix effect of the serum samples. Moreover, compared with some other methods, the present method yielded a wider liner range, better detection limit and higher sensitivity for DA (Table S1, Supporting Information). Therefore, it proved that the methods have potential application for the clinical analysis of DA.

Conclusions

In this work, a very sensitive and selective sensor using Fe-MIL-88NH$_2$ was developed for colorimetric and fluorometric assay of dopamine. The as-prepared methods take advantage of fluorescence quenching through an electron transfer process and the inhibition of the intrinsic peroxidase-like activity of Fe-MIL-88NH$_2$ upon addition of DA to detect a notably wide range

Table 1 Results for the determination of DA in human serum samples (n = 3)

	Colorimetric method				Fluorimetric method			
	Added/μM	Measured/μM	Recovery, %	RSD, %	Added/μM	Measured/μM	Recovery, %	RSD, %
Sample 1	1.00	1.07	107	2.7	30.00	29.50	98	3.0
	2.00	2.05	103	2.4	40.00	39.91	100	4.1
Sample 2	1.00	1.05	105	4.8	30.00	29.59	99	1.8
	2.00	1.97	98	1.5	40.00	39.43	99	2.7

of dopamine from the millimole level to the nanomole level. Therefore, this study provided an example for facile and efficient sensing of DA using the Fe-MIL-88NH_2 metal-organic framework, which may offer a new approach for detecting small biomolecules based on the intrinsic peroxidase-like activity and fluorescent property of metal-organic frameworks at the same time.

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Supporting Information

Synthesis and characterization of Fe-MIL-88NH_2, figures of optimization of conditions, and study on the quenching mechanism of the fluorimetric method. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

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