Analytical Methods

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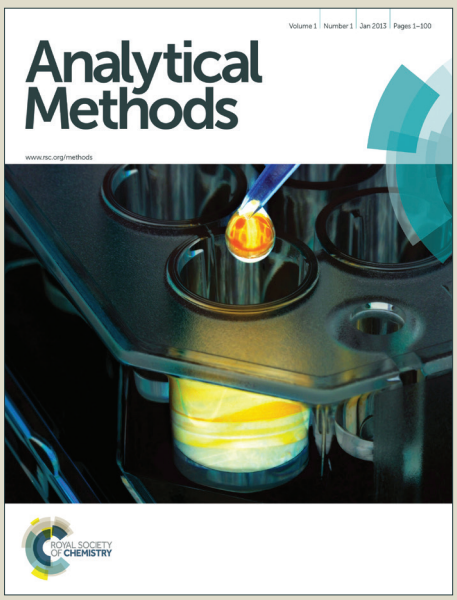

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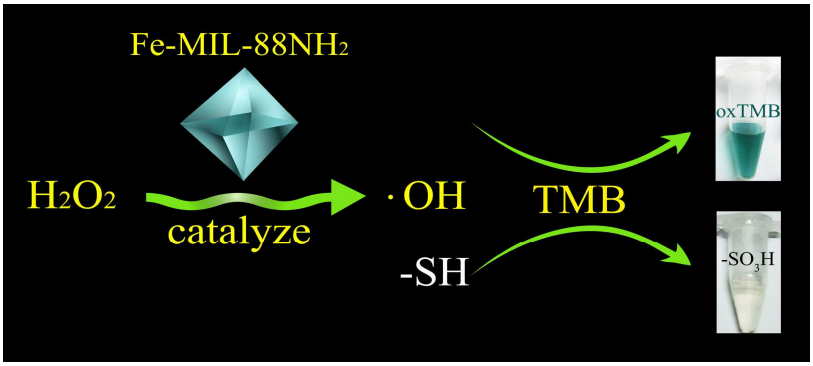
Colorimetric determination of thiol compounds in serum based on Fe-MIL-88NH$_2$ metal-organic framework as peroxidase mimetics

Zhongwei Jiang, Yali Liu, Xiaoli Hu, 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, China. E-mail: liyf@swu.edu.cn Tel: (+86) 23 68254659, Fax: (+86) 23 68367257.



The peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) could be quickly catalyzed oxidation by Fe-MIL-88NH$_2$ in the presence of H_2O_2 and produced a typical blue color reaction, but when thiol compounds existed in the solution, the color of the responsive solution gradually became shallow due to the competitive reaction between TMB and thiol compounds with H_2O_2.

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Colorimetric determination of thiol compounds in serum based on Fe-MIL-88NH$_2$ metal-organic framework as peroxidase mimetics

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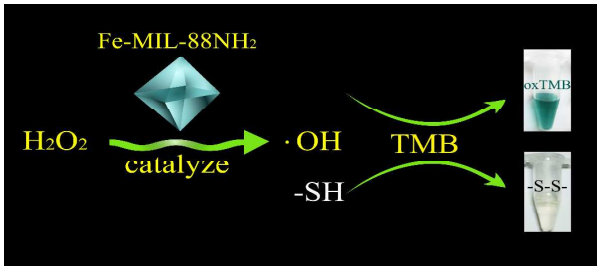
Herein, a novel and simple colorimetric method for the detection of thiol compounds was established based on iron contained metal-organic framework Fe-MIL-88NH$_2$ as peroxidase mimetics. Fe-MIL-88NH$_2$ could catalyze the oxidation of the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 to develop a blue color in aqueous solution. However, when thiol compounds, such as glutathione (GSH), cysteine (Cys) or homocysteine (Hcy), coexisted in the solution, the color of the responsive solution gradually became shallow due to the competitive reaction between TMB and thiol compounds with H_2O_2. Therefore, the content of thiol compounds could be calculated according to the change of absorbance in the system. Under the optimum experimental conditions, the linear response ranges for GSH, Hcy and Cys were 1.0 ~ 100.0 μM , 1.0 ~ 80.0 μM and 1.0 ~ 80.0 μM, and the detection limits were 0.45 μM, 0.40 μM and 0.39 μM, respectively. Furthermore, the colorimetric method was successfully applied to the detection of total thiol compounds in serum.

1. Introduction

Endogenous low molecular-mass thiol compounds, such as glutathione (GSH), cysteine (Cys) and homocysteine (Hcy), are an important part of amino acids and proteins and they play vital roles in many physiological and pathological processes.[1-4] For example, glutathione can maintain redox dynamic balance, oxidative stress and the growth of cells; furthermore, glutathione is also closely related to cancer and many other diseases.[5-7] Cysteine is often involved in many cellular functions, including protein synthesis, detoxification and metabolism. Lack of cysteine will lead to hair fade, edema, somnolence, liver cell damage, obesity and dermatosis.[8] The content of homocysteine in serum is associated with Alzheimer's disease, cardiovascular disease and atherosclerosis.[9] Hence, the content of low molecular-mass thiol compounds in biological systems is of great importance to cell functions and healthy.

Up to now, many analysis methods, such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), have been used for analyzing thiol compounds in biological samples.[10-15] Otherwise, resonance light scattering and near infrared fluorescent methods were established by Yan and co-workers for high sensitive and selective detecting biothiols in biological fluids.[16, 17] Owing to the potential for direct visual readout, colorimetric biosensing has drawn much attention in biological science and analytical chemistry. It provides the advantages of simplicity, rapidity, sensitivity, and there is no requirement for any sophisticated instrumentations. In the past few years, biosensors based on enzyme-mimetic inorganic nano materials have emerged as a new class of ideal and important colorimetric detection approaches, including Fe_3O_4 magnetic nanoparticles,[18] carbon nanodots,[19] functionalized graphene,[20] gold nanoparticles,[21, 22] Co_3O_4[23]and nanostructured FeS[24]. For instance, Ma and his co-workers have reported that Fe_3O_4 MNPs could be used for detecting GSH in cells.[25]

Metal-organic frameworks (MOFs) as a class of functional materials, and their unusual properties such as porosity, high specific surface areas and catalytic efficiency, good thermal stability and outer-surface modification are attractive for diverse analytical applications. For example, MOFs have widely used in chromatographic separation and sensing.[26-32] Recently, our group first discovered that a metal-organic framework Fe-MIL-88NH$_2$ possesses intrinsic peroxidase-like activity，and it was employed for the detection of glucose.[33] In comparison with horseradish peroxidase (HRP), Fe-MIL-88NH$_2$ has higher catalytic activity and stability,[33] so Fe-MIL-88NH$_2$ has a great superiority to be used in biological assay as peroxidase mimetics.



Scheme1 Schematic illustration of the detection of thiols in the system of TMB+H_2O_2+Fe-MIL-88NH$_2$.

In this work, we find Fe-MIL-88NH_2 also can be used for detecting thiol compounds. As shown in Scheme1, TMB could be oxidized by ·OH which produced from the catalytic decomposition of H_2O_2, and simultaneously yielding a blue colored oxidized TMB (oxTMB). The existence of thiols could consume ·OH and cause color change of the system, which could be observed by naked eye and quantitatively analyzed by UV-vis spectrophotometer.

2. Experimental

2.1 Materials

Glutathione (GSH), cysteine (Cys), homocysteine (Hcy) and other amino acids were obtained from Beijing Dingguo Changsheng Biotech Co.,Ltd. 3,3′,5,5′-tetramethylbenzidine (TMB) was purchased from Sigma-Aldrich (St. Louis, MO). H_2O_2 (30wt %), acetic acid and sodium acetate were purchased from Chongqing Pharmaceutical Co., Ltd. Keyi Assay Glass Branch (Chongqing, China). Serum samples were obtained from Southwest University Hospital of Chongqing. All reagents were analytically pure and without further purification. The ultra-pure water (18.2 MΩ) was used in the whole experiment. Fe-MIL-88NH_2 was synthesized according to the previous work of our group[33]. The SEM (Fig. S1) and XRD patterns (Fig. S2) agree with the reported Fe-MIL-88NH_2. [33, 34]

2.2 Apparatus

An S-4800 scanning electron microscope (SEM) (Hitachi, Japan) was used for imaging the size and shape of the Fe-MIL-88NH_2. Powder X-ray diffraction (PXRD) patterns were collected on an XD-3 X-ray diffractometer with Cu Kα radiation (λ = 1.5406 Å) in the range of 5−25θ at a scan rate of 2.0° min^{-1} (Purkinje, China). A constant-temperature water-base boiler (Jiangsu, China) was employed to control the reaction temperature. A U-3010 spectrophotometer (Hitachi, Tokyo, Japan) was used to record absorption spectra and measure absorbance.

2.3 Preparation of serum samples

The serum samples of three healthy adults were treated by spin dialysis at 12000 rpm for 30 min, and then the eluents were diluted to double with phosphate buffered saline (PBS, 10 mM, pH 7.0) before determination.

2.4 Method for thiol compounds detection in aqueous medium and serum samples

The detection process carried out as follows: 50 μL of 0.2 M HAc-NaAc buffer (pH = 4.0), 75 μL of 10 mM TMB, 75 μL of 1.0 mM H_2O_2 and 100 μL of 0.2 mg·mL^{-1} Fe-MIL-88NH_2 nanocrystals solution were added into 1.5 mL EP vial. Then a certain amount of thiol compounds or serum were added into the mixture, further diluting the mixture to 500 μL with ultra-pure water (18.2 MΩ). The ultimate mixture was incubated at 40°C for 25 min. For the control experiments, the deionized water was used instead of thiol compounds or serum eluent. Finally, U-3010 was applied to determine the absorption spectra of the solution.

3. Results and discussion

3.1 Spectral characteristic

The peroxidase-like activity of Fe-MIL-88NH_2 is showed in Fig. 1. The TMB-H_2O_2 mixed solution was nearly colorless without Fe-MIL-88NH_2 after treatment for 25min, indicating that TMB was slowly oxidized by H_2O_2. On addition of Fe-MIL-88NH_2 to the TMB-H_2O_2 system, TMB was catalyzed oxidation to produce the typical blue color reaction. The system presented a much higher absorbance than before. The maximum absorbance of the reaction mixture was at 650nm, which came from the oxidation of TMB. But the existence of GSH made the color shallow by consuming ·OH and the difference could be observed from the color change obviously by naked eye (Fig. 1, inset). Moreover, the existence of other biothiols such as Cys and Hcy led to the same phenomenon, suggesting that this reaction system could be employed for the determination of GSH, Cys or Hcy.

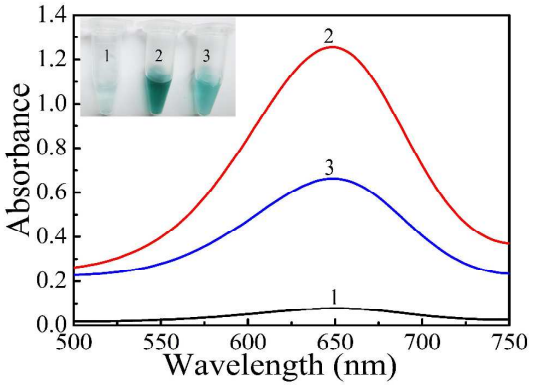


Fig. 1 Typical absorption spectra of TMB-H_2O_2 mixed solution in the absence and presence of GSH. (1) TMB+H_2O_2; (2) TMB+H_2O_2+Fe-MIL-88NH_2; and (3) TMB+H_2O_2+Fe-MIL-88NH_2+GSH. The inset: a photograph of the solutions. Concentrations: TMB, 1.5 mM; H_2O_2, 150 μM; Fe-MIL-88NH_2, 0.04 mg·mL^{-1}; GSH, 50 μM.

3.2 Optimization of experimental conditions

Similar to peroxidase, the catalytic activity of Fe-MIL-88NH_2 was found to be closely dependent on pH, temperature, and H_2O_2 concentration. The peroxidase-like activity of Fe-MIL-88NH_2 was measured by varying the pH from 3.0 to 5.0, and the temperature from 25°C to 60°C. After optimization, pH 4.0 and 40°C were set as the optimal acidity and temperature, respectively (Fig. 2A and B). These conditions were similar to the previously reported for nanostructure-based peroxidase mimetics and horseradish peroxidase (HRP)[18]. Through the optimization of H_2O_2 concentration, as shown in Fig. 2C, the catalytic activity was relatively stable with the concentration from 100 μM to 200 μM. Like the catalytic activity of nature enzyme, the catalytic activity of Fe-MIL-88NH_2 would be inhibited at high H_2O_2 concentration. As a result, 150 μM was selected as the optimal concentration of H_2O_2. Meanwhile, the optimum Fe-MIL-88NH_2 concentration has been investigated to be 0.04 mg·mL^{-1} (Fig. 2D).

3.3 Method validation

Under the optimal conditions, we explored the relationship between the absorbance at 650nm in the presence of different concentrations of GSH. The UV-vis spectra and the typical photographs are showed in Fig. 3. It obviously suggested that the color of the solution became more and more pale with the GSH concentration increasing.

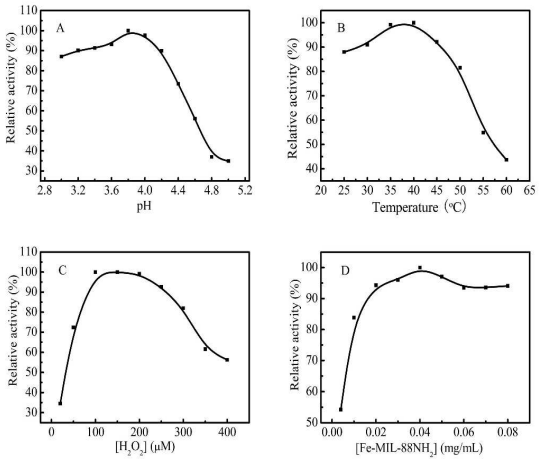


Fig. 2 Dependency of the Fe-MIL-88NH_2 peroxidase-like activity on (A) pH; (B) temperature; (C) H_2O_2 concentration and (D) Fe-MIL-88NH_2 concentration for 50 μM GSH detection, in 0.2 M HAc-NaAc buffer solutions incubated for 25 min.

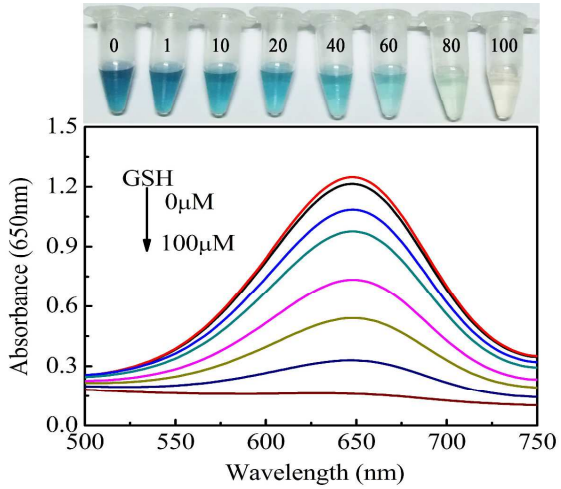


Fig. 3 UV-vis spectra and the typical photographs of mixture solutions with the concentrations of 0, 1.0, 10.0, 20.0, 40.0, 60.0, 80.0, and 100.0 μM GSH. Concentrations: TMB, 1.5 mM; H_2O_2, 150 μM; Fe-MIL-88NH_2, 0.04 mg·mL^{-1}; incubation 25 min in 0.2 M HAc-NaAc buffer (pH 4.0) at 40ºC.

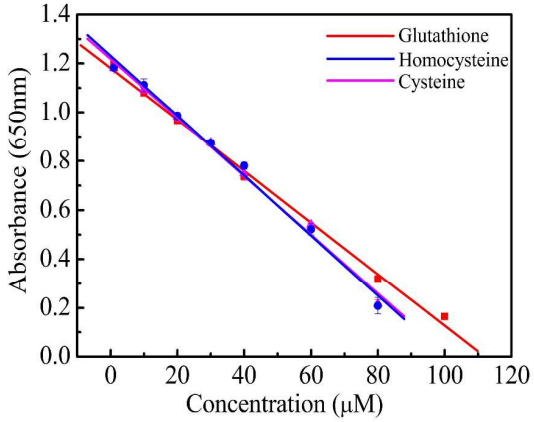


Fig. 4 The linear calibration plots for different concentrations of GSH, Cys and Hcy standard solutions. Concentrations: TMB, 1.5 mM; H_2O_2, 150 μM; Fe-MIL-88NH_2, 0.04 mg·mL^{-1}.

To examine the feasibility of this detection strategy for biothiols, the calibration curves and performance characteristics of the method for detecting GSH, Hcy and Cys were investigated under the optimized conditions. As shown in Fig. 4 and Table 1, good linear relationships and low detection limits were obtained for GSH, Hcy and Cys. The results indicated that this method could be used to detect the content of thiol compounds.

Table 1 Quantitative analyses of GSH, Hcy and Cys through this colorimetric method

Analyte	Linear range(μM)	Regression equation	Correlation coefficient(R)	Detection limits(μM)
GSH	1.0-100.0	*A*=1.18-0.0105c	0.998	0.45
Hcy	1.0-80.0	*A*=1.21-0.0120c	0.996	0.40
Cys	1.0-80.0	*A*=1.23-0.0122c	0.996	0.39

3.4 The effect of coexisting substances in serum on determination of biothiols

We chose GSH as target analyte to investigate the effect of coexisting substances of this detection platform for GSH, Cys and Hcy over common metal ions, glucose, serum albumin and other amino acids. As shown in Fig. 5, although the concentration of coexisting substances were 10-fold higher than that of GSH, the obtained signals for GSH had not changed much. It illustrated that the coexisting substances in serum did not interfere with the determination of biothiols. Therefore, this reaction system can specificly detect biothiols in serum.

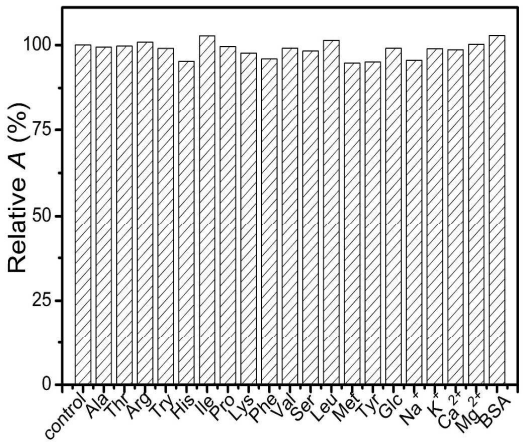


Fig. 5 The effect of coexisting substances on determination of biothiols. Concentrations: TMB, 1.5 mM; H_2O_2, 150 μM; Fe-MIL-88NH_2, 0.04 mg·mL^{-1}; coexisting substances, 500 μM; GSH, 50 μM.

3.5 Analytical applications

In order to explore the practicality of the proposed method, we detected the thiol compounds in serum through the standard addition method. The determination results were shown in Table 2. The mean recoveries for GSH are from 97% to 100%, suggesting that the colorimetric method was largely free from the sample matrix effect of the serum. The experimental results were in agreement with the concentration of thiol compounds (4.87×10^{-4} ~ 6.64×10^{-4} mol/L) in human blood.[35, 36] Comparing to capillary electrophoresis and fluorometry[11, 37, 38] (Table S1),

this method is smiple, sensitive, and it realized the visual detection of thiol compounds in serum.

Table 2 The determination of thiol compounds in three healthy human serums

Sample	Amount found(μM)	Added GSH (μM)	Total found (μM)	Recovery (%)	RSD (%) (n=3)
Sample1	32.90	35	67.96	100	2.7
Sample2	32.93	35	66.74	97	2.1
Sample3	32.36	35	66.52	98	2.9

4. Conclusion

In summary, a new colorimetric method for the determination of biothiols has been developed based on Fe-MIL-88NH_2 as newly peroxidase-like. In this method, Fe-MIL-88NH_2 was used as a catalyst to catalyse the reaction of H_2O_2 and TMB, which is similar to HRP. The presence of thiol compounds led to the consumption of ·OH and caused color change, which could be observed by naked eye. On this basis, we provide a simple, rapid, and sensitive method for colorimetric detection of biothiols. The analytical platform for the detection of biothiols developed here displayed great potential applications of utilizing MOFs as enzymatic mimics in biological samples analysis and medical diagnostics.

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Notes and references

Key Laboratory of Luminescent and Real-Time Analytical Chemistry (Southwest University), Ministry of Education, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, China. E-mail: liyf@swu.edu.cn, Tel: (+86) 23 68254659, Fax: (+86) 23 68367257.

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