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Highly selective and reproducible electroanalysis for histidine in blood with turn-on responses at a potential approaching zero using tetrahedral copper metal organic frameworks†

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A highly selective and reproducible electroanalysis strategy was developed for probing histidine using tetrahedral copper metal organic frameworks. It can facilitate His detection through the stable “turn-on” signal output of solid-state CuCl electrochemistry at a potential approaching zero, with the level down to about 25 nM in blood.

Histidine (His), an essential amino acid in humans and other mammals, plays crucial roles in many life activities such as the growth and repair of tissues, the transmission of metal elements like copper, and the control of the central nervous system.^{1–3} Recent studies have shown that a deficiency of His in the human body may cause an impaired nutritional state in patients with chronic kidney disease.^{1,4,5} To date, some classic technologies have been reported for the analysis of His, typically including capillary electrophoresis, fluorimetry, spectrophotometry, and electroanalysis.^{2,6–12} Among these techniques, the electroanalysis method has been preferably applied because of the outstanding advantages such as high detection sensitivity, easy operation, and portable devices suitable for on-site applications.^{9–12} For example, Zhang *et al.* described the electrochemical determination of His using functionalized indium tin oxide.⁹ Sutradhar and co-workers developed an electrochemical detection strategy for sensing His by utilizing a homocysteine-functionalized fullerene-C₆₀-gold nanocomposite.¹⁰ Radecka's group proposed an electroanalysis method with a Fe(III)-porphyrin complex for the evaluation of His.¹¹ However, most of the current electroanalysis methods for His have been conducted either at high electrochemical potentials or by way of a His-induced decrease of the signal outputs, indicating the meaningfully low detection selectivities and reproducibilities. Therefore, exploration of highly selective and sensitive detection candidates for sensing His in complicated biological media like blood especially co-existing structure-similar amino acids like lysine (Lys), is a challenging but attractive target to pursue.

Nowadays, metal-organic frameworks (MOFs), a new prominent class of porous crystalline materials, have gained great interest in the fields of adsorption, separation, storage, catalysis, and biological and chemical sensing.^{13–17} As the most common representatives, copper MOFs (Cu MOFs) have received special attention for applications in the biomedical field typically as carriers of enzymes, sensing probes, and electrocatalysts, due to their well-defined configuration, high electric conductivity, superior catalytic activity, large surface area, and ultrahigh porosity.^{18–20} For instance, Wu and co-workers utilized Cu MOFs to develop an electrochemical detection strategy for sensing sunset yellow and tartrazine.¹⁸ Xu *et al.* reported the exploitation of a Cu MOF as an electrocatalyst for H₂O₂ oxidation.²⁰ Yet, there has been no work reported on the applications of Cu MOFs for the analysis of His by way of a His-induced increase of signal outputs to date.

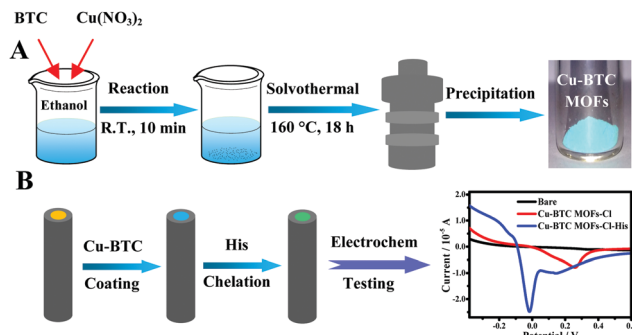
In the present work, a highly selective and reproducible electroanalysis strategy has been developed for sensing His in blood using Cu MOFs prepared by the one-pot solvothermal reaction route using the ligand precursor of benzenetricarboxylic acid (BTC). The main preparation procedure is schematically illustrated in Scheme 1A. The Cu-BTC MOFs as blue precipitates could be obtained with tetrahedral metal corners connected by BTC linkers, in which two Cu atoms could be coupled with the oxygen atoms of four BTC linkers to form 3D four-connected square-planar vertexes.¹⁶ The obtained Cu-BTC MOFs were further modified onto the electrodes for His electroanalysis through solid-state CuCl electrochemistry. Scheme 1B depicts the step-by-step setup procedure of sensing His using a Cu-BTC MOF modified electrode. The electrochemical procedures of solid-state Cu⁰/CuCl should be involved in the buffer containing Cl[−] ions:

- (1) Cu²⁺ from the MOF (solution) + 2Cl[−] (solution) + e[−] → CuCl (solid) + Cl[−] (solution)
- (2) CuCl (solid) + e[−] → Cu⁰ (solid) + Cl[−] (solution)
- (3) Cu⁰ (solid) + Cl[−] (solution) → CuCl (solid) + e[−]
- (4) CuCl (solid) + Cl[−] (solution) → CuCl₂ (solution) + e[−]

Herein, in the Cu reduction process, Cu²⁺ of the Cu-BTC MOF is reduced to yield CuCl and then Cu⁰. Moreover, in the forward Cu oxidation process, Cu⁰ is oxidized to CuCl with a

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Scheme 1 Schematic illustration of (A) the preparation procedure of Cu-BTC MOFs by a solvothermal reaction in alcohol and (B) the step-by-step setup of the electrodes modified with the Cu-BTC MOFs for sensing His showing comparably changing electrochemical responses.

peak potential of about -0.020 V, which is further oxidized to Cu^{2+} at a potential of about 0.20 V. Notably, during the reversible electrochemical process in cyclic voltammetry (CV), the currents of the two Cu oxidation peaks would gradually decrease till “turn-off” after several electrochemical scanning cycles. Furthermore, once His is added, it would convert CuCl into a Cu-His complex due to the higher Cu-chelating capability of His. An electrochemical oxidation was thus induced to regenerate the original passive layer of non-conductive CuCl. As a result, a sharp and extremely stable oxidation peak of solid-state CuCl electrochemistry was turned on at a potential of about -0.020 V, of which the oxidative currents should be employed to electrochemically evaluate the concentrations of His. Therefore, Cl^- ions can play a vital role in the electrochemical process of solid-state Cu^0/CuCl ,²¹ which enables the selective electroanalysis of His.

SEM imaging was firstly performed for the Cu-BTC MOFs before and after adding His (Fig. 1). As expected, the as prepared Cu-BTC MOFs display a well-defined tetrahedron structure, as clearly disclosed in the amplified view (Fig. 1A). After addition of His, the structure of the MOFs could collapse (Fig. 1B), which might be ascribed to the strong coordination between Cu^{2+} of the Cu-BTC MOFs and the nitrogen-containing groups of His. Moreover, Fig. S1A (ESI[†]) shows the UV-vis spectra of the resultant Cu-BTC MOFs, showing the characteristic absorbance peaks of the Cu^{2+} ions (*i.e.*, around 300 nm). Furthermore, the crystallinity and phase purity of the prepared MOFs were examined using X-ray diffraction (XRD) (Fig. S1B, ESI[†]). Apart from some deviations in the relative intensities, the XRD pattern can be

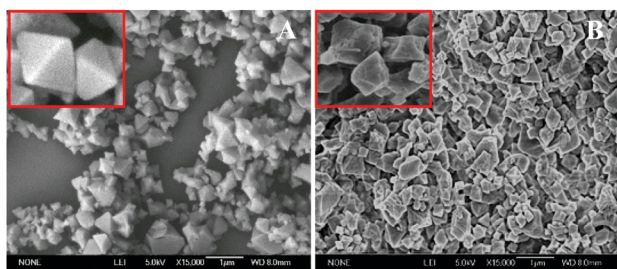


Fig. 1 Characteristic SEM images of the Cu-BTC MOFs (A) before and (B) after addition of His (inset: the amplified view).

fitted well with the simulated one, indicating the formation of Cu-BTC MOFs.

The characteristic sensing properties of the Cu-BTC MOF modified electrodes were investigated in phosphate buffers with and without Cl^- ions for comparison (Fig. S2, ESI[†]). It was discovered that the as prepared electrodes display a reversible CV process showing Cu redox peaks in the buffer without Cl^- ions (Fig. S2A, ESI[†]). Notably, a large Cu reduction peak could be well sustained at a potential of about -0.25 V even after electrochemically scanning for several times. Yet, when Cl^- ions (*i.e.*, 100 mM) were introduced into the buffer at high levels, the Cu-BTC MOF modified electrode showed two Cu oxidation peaks separately at about -0.020 V and 0.20 V, and the currents gradually decreased till “turn-off” after electrochemical scanning several times (*i.e.*, four cycles) (Fig. S2B, ESI[†]), presumably due to the increasing accumulation of non-conductive CuCl precipitates on the electrode surface. More interestingly, once His was added, the Cu-BTC MOF modified electrode showed different electrochemical behaviors in the buffers without and with Cl^- ions, showing a decreased Cu reduction peak at about -0.25 V (Fig. S2C, ESI[†]) and an increased Cu oxidation peak at about -0.020 V (Fig. S2D, ESI[†]), respectively. In comparison, obviously, the introduction of His could turn on a sharp and highly stable Cu oxidation peak triggered by the specific Cu-His binding therein. Such a phenomenon of solid-state CuCl electrochemistry was thought to result from the unique oxidation behavior of the exposed Cu active sites inside the Cu-MOFs by following the above electrochemical process $\text{Cu}(0) \rightarrow \text{Cu}(I) + e^-$ and $\text{Cu}(I) \leftrightarrow \text{Cu}(II) + e^-$.^{15,22,23} Besides, a comparison of the characteristic CV responses was conducted among His (Fig. S2D, ESI[†]), Lys (Fig. S2E, ESI[†]), and Cys (Fig. S2F, ESI[†]) in the Cl^- -containing buffer. One can note that the nitrogen-rich Lys can show a much lower current response than His, due to which they can possess largely different abilities of Cu^{2+} binding, with $\log K_{\text{His-Cu}} = 18.1$ ²⁴ versus $\log K_{\text{Lys-Cu}} = 7.23$.²⁵ Furthermore, the Cys containing $-\text{SH}$ groups, which may also exhibit strong interaction with Cu^{2+} and form CuS, could interestingly manifest a decreased current response at the two Cu oxidation peaks, in contrast to His showing a sharply enhanced one, which enables selective electroanalysis.

The characteristic electrochemical behavior of the Cu-BTC MOF modified electrodes was explored during the step-by-step procedure for sensing His (Fig. 2). Fig. 2A shows the linear

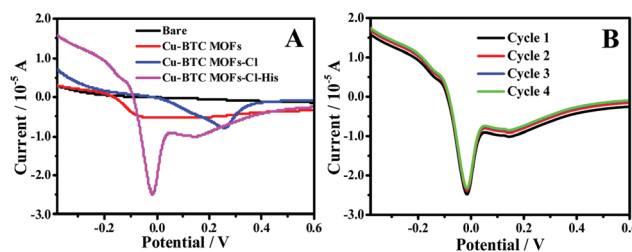


Fig. 2 (A) Characteristic electrochemical LSVs for the Cu-BTC MOF modified electrodes before and after adding His, taking the bare ones as the control. (B) Electrochemical LSV responses of the Cu-BTC MOF modified electrodes to His upon scanning four times in the Cl^- -containing phosphate buffer at a scanning rate of 100 mV s^{-1} .

sweep voltammograms (LSVs) for the modified electrode before and after adding Cl^- ions, followed by addition of His, in comparison with those of the bare electrode. One can note that the Cu-BTC MOF modified electrodes display a Cu oxidation peak at about 0.20 V in the presence of Cl^- ions, in contrast in the absence of Cl^- ions an insignificant oxidation peak is observed. Importantly, once His is added, the specific Cu-His binding could trigger a sharp and very stable “turn-on” oxidation peak of solid-state CuCl electrochemistry at the potential approaching zero (*i.e.*, -0.020 V). More interestingly, this peak current induced by His is extremely stable against scanning several times (Fig. 2B), which could be defined as the characteristic His responses of solid-state Cu^0/CuCl ,²⁷ promising a high sensing reproducibility of His electroanalysis afterwards.

Moreover, the main detection conditions of the Cu-BTC MOF-modified electrodes for the electroanalysis of His were investigated (Fig. S3, ESI[†]). Obviously, an optimal probe dosage of 2.0 mg mL^{-1} Cu-BTC MOF nanocomposites, pH 7.0, and 100 mM NaCl should be chosen (Fig. S3A–C, ESI[†]). Besides, Fig. S3D (ESI[†]) shows that the response of the Cu-BTC MOF modified electrodes to His can be completed within 30 s.

The electroanalysis selectivity of the Cu-BTC MOF modified electrodes was explored for His in comparison to some common ions and small molecules especially amino acids possibly co-existing in blood (Fig. 3). In comparison, all of the other tested analytes except for Lys could present negligibly low responses. Interestingly, the nitrogen-rich amino acid like Lys also shows an increased response due to the coordination between the Cu^{2+} ions and Lys, which however is much lower than that of His at the same concentration. Accordingly, the developed electroanalysis method can show high selectivity for probing in some complicated samples like blood.

Moreover, the detection reproducibility of the Cu-BTC MOF modified electrodes was evaluated in sensing His, showing a statistic standard deviation of 3.8% for the results obtained from the six electrodes tested (Fig. S4A, ESI[†]). Besides, electrochemical investigations were carried out on the storage stability of the Cu-BTC MOFs used for His electroanalysis (Fig. S4B, ESI[†]). As expected, no significant change of the His response

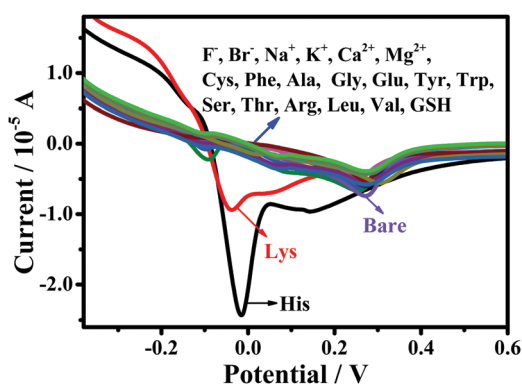


Fig. 3 Electrochemical LSV responses of the Cu-BTC MOF modified electrodes to His and different interferences indicated at the same concentration ($200 \mu\text{M}$) at a scanning rate of 100 mV s^{-1} .

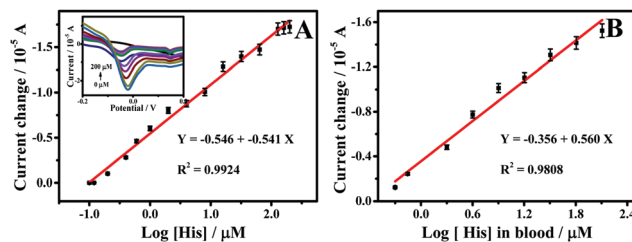


Fig. 4 Calibration curves of the relationship between the current responses and different concentrations of His in (A) buffer (inset: LSV responses to His) and (B) in blood. The error bars represent the relative standard deviations of the quantitative results.

signal was observed for the Cu-BTC MOF modified electrodes that were stored even up to 6 months. The data indicate that the developed electrochemical His sensor could present pretty high detection selectivity and reproducibility, in addition to the long-term stability of the Cu-BTC MOF probes.

Under the optimized conditions, the developed Cu-BTC MOF modified electrodes were applied for the electroanalysis of different concentrations of His separately spiked in buffer and blood samples (Fig. 4). It was discovered that the current responses of the developed electrodes would increase with increasing His concentrations (Fig. 4A, inset). A linear relationship was thus obtained for the electrochemical responses *versus* the $\log[\text{His}]$ concentrations ranging from $0.10 \mu\text{M}$ to $200 \mu\text{M}$ (Fig. 4A), with a limit of detection (LOD) of about $0.025 \mu\text{M}$, estimated by the 3σ rule. Furthermore, the practical application of the developed electroanalysis method was investigated by probing different concentrations of His in blood samples (Fig. 4B). The results indicate that His in blood can be detected at concentrations ranging from $0.50 \mu\text{M}$ to $128 \mu\text{M}$, with a LOD of about $0.125 \mu\text{M}$. In addition, the His detection performances of the developed electroanalysis strategy were compared with those of the current detection methods reported previously, and the results are summarized in Table S1 (ESI[†]). One can note that the developed method can present better or comparable detection performances in terms of detection ranges and LODs. Therefore, the developed electroanalysis strategy may have promise in clinical applications for monitoring His in low levels in various samples like blood. Herein, the current changes refer to the differences of the current responses between the Cu-BTC MOF modified electrodes before and after adding His.

In summary, a highly selective and reproducible electroanalysis strategy has been initially developed for probing His in blood by using tetrahedral Cu MOFs fabricated simply by the one-step solvothermal reaction route. It was discovered that the Cu-BTC MOF modified electrodes exhibit reversible Cu redox peaks in buffer. After Cl^- ions were introduced, unexpectedly, the currents of the Cu oxidation peaks could gradually decrease till “turn-off” after electrochemically scanning several times. More importantly, once His was added, the specific Cu-His binding ($\log K = 18.1$) triggered a sharp and extremely stable “turn on” oxidation peak of solid-state Cu^0/CuCl electrochemistry at a potential of about -0.020 V. The as-developed electroanalysis strategy possesses some outstanding advantages compared to the current analysis

methods for sensing His especially those by way of “turn-off” signal outputs at high potentials. First, the Cu–BTC MOF based electroanalysis method with the output of a His triggered “turn-on” signal can enable the highly selective detection of His in complicated biological media especially those (*i.e.*, blood) containing nitrogen or sulfur-rich amino acids (*i.e.*, Lys and Cys) that may bind with Cu²⁺. Second, it can display sharp and large current responses to His at a considerably low potential approaching zero so as to avoid the possible interference from electroactive substances co-existing in the sample backgrounds. Third, with the extremely stable His responses together with the long-term stability of the MOF probes, the as prepared electrodes can ensure high reproducibility for His detection. Finally, the selective and reproducible electroanalysis strategy with Cu–BTC MOFs can allow the evaluation of His in complicated blood samples with high sensitivity, which can display better or comparable His analysis performances with those of the detection methods previously reported. Therefore, the developed electroanalysis method shows promise for practical applications in the evaluation of His at trace levels in complicated media like blood for the clinical early diagnosis of kidney diseases. Yet, it should be pointed out that the detailed sensing mechanism should be further investigated in the future.

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Conflicts of interest

There are no conflicts to declare.

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