Full Research Paper

Electrochemical Sensor for Tryptophan Determination Based on Copper-cobalt Hexacyanoferrate Film Modified Graphite Electrode

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Abstract: In this work, the development of a tryptophan sensor and its application to milk are described. The mixed metal (copper and cobalt) hexacynoferrates are electrodeposited on the graphite electrode, and this film exhibits an electrocatalytic activity towards for the oxidation of tryptophan. The experimental conditions, including the scan cycles, the ratio of copper(II) and cobalt(II), pH value, applied potential, are investigated in detail. At the optimal conditions, the eletctrocatalytic response is a linear relationship with the concentration of tryptophan in the range of 10 μM and 900 μM, with a detection limit of about 6 μM. This modified electrode was also successfully used to detect the tryptophan concentration in milk.

Keywords: tryptophan, copper-cobalt hexacyanoferrate, electrocatalysis, sensor

1. Introduction

Determination of amino acids is important in various fields of research, particularly in food, soil, biotechnology and pharmaceutical industries [1-3]. For example, tryptophan is an important amino acid owing to its crucial roles in biological systems. It is a vital constituent of proteins and indispensable in human nutrition for establishing and maintaining a positive nitrogen balance [4]. It is also an essential amino acid for brain functions and neuronal regulatory mechanisms. Therefore, the detection of tryptophan is very important. Many methods have been applied for the measurement of

tryptophan, including liquid chromatography [5], capillary electrophoresis [6] or spectroscopic detection [7] is usually based on the indirect detection or the corresponding derivations.

Recently, electrochemical methods have found many applications in the determination of electroactive amino acids due to their inherent electroactivity of thiol or aromatic groups, including cysteine, tyrosine and tryptophan [8]. For these applications, various electrode materials such as carbon, platinum and gold are used [9]. Unfortunately, the electrochemical behaviors are usually poor. Therefore, many groups employed the chemically modified electrodes to improve the anodic oxidation of amino acids.

Nowadays, it still continues to be of interest in the developments of new materials capable to form the electrode surface with better analytical properties, including conductive polymers, nanoparticles, and inorganic polymeric films [10-13]. Among them, the immobilization of polynuclear transition metal hexacyanoferrates on an electrode surface is widely employed. According to earlier studies, this kind of films are usually formed by electrodeposition from the solutions containing hexacyanoferrate(III), a metal cation and a potassium electrolyte [14]. It has been considered for many purposes including such applications as chemical sensors, charge storage, electroanalysis, ion exchange, electron mediator and electrocatalysis. Especially, its use in electrocatalysis has been widely found in the determination of dopamine [15], thiosulfate [16], sulfite [17], nitrite [18], and so on. Furthermore, its application in DNA field has also been reported by Abbaspour in the determination of guanine [19] and DNA [20]. However, in another life science field, amino acid and protein, the reports are very few. Since some amino acids can be oxidized at certain potential of about 0.7 V(vs. SCE) [10], if suitable metal hexacyanoferrates were selected to make one pair of peaks at this site, it is possible that amino acids can be electrocatalyzed by these compounds.

The most employed transition metal hexacynoferrate was single transition metal atoms, such as copper(II) [18], cobalt(II) [19], nickel(II) [21], ferric(III) [17] and zinc(II) [22]. Our earlier work has concentrated on the iron-cobalt hexacyanoferrate film, and its higher peak is at about 0.8 V [23]. The components of the mixed metal hexacyanoferrate film can be easily adjusted by the ratio of metals, so it is easy to select working voltages according to the detected materials as an electrocatalyst. In this work, mixed metal (copper and cobalt) hexacynoferrate (CuCoHCF) film is electrodeposited on the graphite electrode for the electrocatalysis of amino acids, and the optimal experimental conditions, including the ratio of copper(II) and cobalt(II), pH value, applied potential. This method is also used in the actual application of milk.

2. Experimental

2.1 Reagents and apparatus

All the chemicals were of analytical grade unless stated. $CuCl_2$, $CoCl_2$, and $K_3Fe(CN)_6$ were from Tianjin Chemical Reagents (China). Tryptophan was biological grade and purchased from Shanghai (China). Phosphate buffer solution (PBS, pH 7.4) was prepared from 1/15 M NaH_2PO_4 and 1/15 M Na_2HPO_4 (2:8 V/V). Tryotophan solution was prepared in PBS. Double-distilled water was used throughout.

Electrochemical measurements were performed at a CHI 660C electrochemical workstation (Shanghai Chenhua Instruments, China) with a conventional three-electrode cell. The graphite

electrode (0.12 cm^2) was used as working electrode, with an Ag/AgCl electrode as reference electrode and a platinum electrode as auxiliary electrode. The elemental composition of CuCoHCF film was detected by EPMA-1600 (Shimadzu) in South China University of Technology.

2.2 Preparation of CuCoHCF film

Prior to the preparation of CuCoHCF film, the graphite electrode was scrubbed with fine sand paper, washed with water, and then scanned for 40 cycles in 0.1 M H$_2$SO$_4$ in the range of 0 and 1.6 V to remove the impurity from the electrode surface. Then the CuCoHCF films was electrodeposited by cyclic voltammetry from 0 to 1.0 V at 50 mV s^{-1} in a fresh solution containing 0.5 M KCl, 0.33 mM CuCl$_2$, 0.33 mM CoCl$_2$, and 0.66 mM K$_3$Fe(CN)$_6$. After 30 cycles, the electrode was taken out and rinsed thoroughly with water. The detection of tryptophan was performed at 0.5 M KCl solution by adding of tryptophan.

3. Results and Discussions

3.1 Electrochemical characterization of CuCoHCF modified graphite electrode
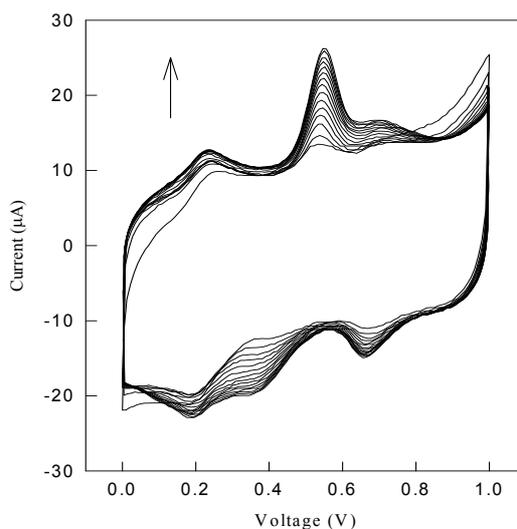

Figure 1. Cyclic voltammograms in the eletrodeposition of copper-cobalt hexacyanoferrate films from a solution of 0.5 M KCl, 0.33 mM CuCl$_2$, 0.33 mM CoCl$_2$, and 0.66 mM K$_3$Fe(CN)$_6$ from 0 to 1.0 V at 50 mV s^{-1}.

Figure 1 illustrates the cyclic voltammogram during the electrodeposition process. The consecutively increasing currents for both anodic and cathodic peaks demonstrate the CuCoHCF films are deposited continuously on the electrode surface. Three pairs of redox peaks of this film appeared near 0.20, 0.50 and 0.65 V. The peaks at 0.50 V and 0.65 V are associated with the redox process between [Fe(CN)$_6$]$^{2-}$ and [Fe(CN)$_6$]$^{3-}$ in the CuCoHCF films [24]. Unlike the report [24], a pairs of peaks at about 0.20 V was found, which may be due to the redox process of free Fe(CN)$_6^{4-/3-}$ in the solution, since these two peaks disappeared if the modified electrode was transformed to KCl solution without K$_3$Fe(CN)$_6$. Figure 2 was the EDS spectra of the CuCoHCF modified graphite electrode. It

shows that C, Co, Fe, Cu, K, and Cl are the major elements, which confirm that the hybrid HCFs of iron, cobalt and copper are deposited on the gold electrode surface.
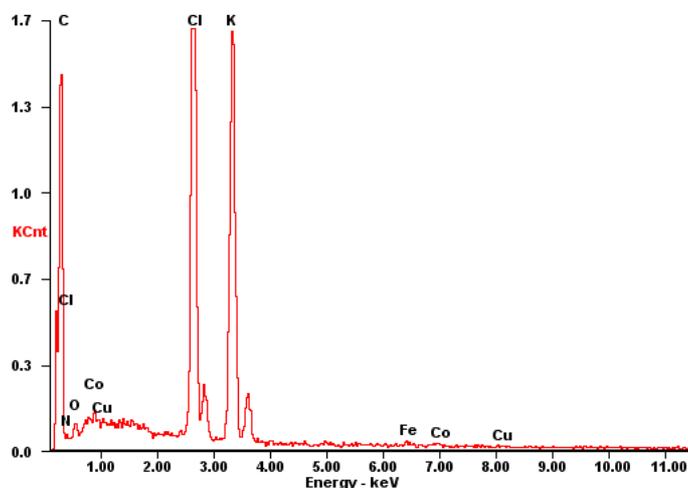

Figure 2. The EDS spectra of CuCoHCF-modified electrode.

Then, the modified electrode was transformed to 0.5 M KCl solution and the cyclic voltammograms at various scan rates were recorded as in Figure 3A. With the increase in scan rates, the cathodic and anodic current both increased. As the peaks at 0.5 V an example (Figure 3B), the peak currents were linearly proportional to the scan rates in the range between 20 and 300 mV s^{-1}. The i_{pa}/i_{pc} was almost unity, as expected for a surface-type behavior. This deviation of redox process, from the ideal surface redox behavior appeared at even low scan rates, may be attributed to the limitations associated with charge propagation in the film, chemical interaction between the ions and the modified film, polarizability of the cation influencing its penetration in or out of the CuCoHCF film [15].
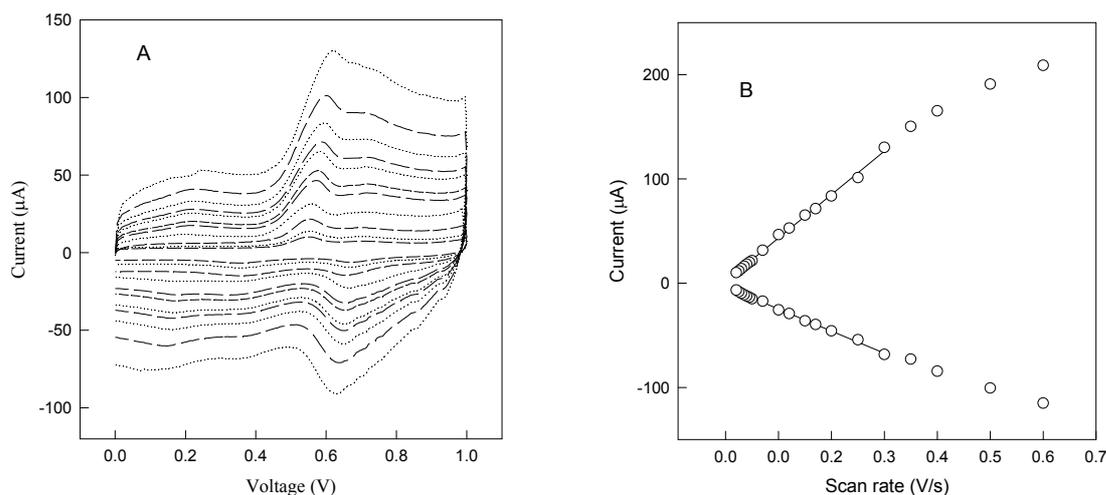

Figure 3. The cyclic voltmmograms of CuCoHCF-modified electrode at various scan rates in 0.5 M KCl solution (A): 20, 30, 50, 70, 100, 120, 150, 170, 200, 250, 300 mV/s. The plot of cathodic and anodic currents at 0.5 V with scan rates (B).

The formal potential changes with the scan rates in the high scan range. Figure 4 shows the relationship between the peak potential (E_p) and the natural logarithm of scan rate (lnv) for the modified electrode in 0.5 M KCl. As can be seen, in the range from 170 to 600 mV/s, the cathodic peak potential (E_{pc}) changed linearly versus lnv with a linear regression equation of $y = 0.285 - 0.044x$(r = 0.993). According to the following equation [25]:

$$E_p = E^{0'} + \frac{RT}{\alpha nF} - \frac{RT}{\alpha nF} \ln v \qquad (1)$$

where α is the cathodic electron transfer coefficient, n, the number of electrons, *R*, *T* and *F* are the normal meanings, so αn is calculated to be 0.58. Given $0.3 < \alpha < 0.7$ in general [26], it could be concluded that $n = 1$ and $\alpha = 0.58$. So the redox reaction between the film and the electrode is a single electron transfer process.

In order to calculate the value of apparent heterogeneous electron transfer rate constant (k_s), the following equation [27] was used:

$$\log k_s = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/nFv) - \alpha(1-\alpha)\frac{nF\Delta E_p}{2.3RT} \qquad (2)$$

According to Figure 4, in a range from 170 to 600 mV/s, the anodic peak potential is also linear to lnv with a linear regression equation as $y=0.692+0.058x$($r=0.991$). It also indicates that at cross point of these two lines, $\ln v_c = \ln v_a = -3.99$, and k_s was calculated to be 0.35 s^{-1}.
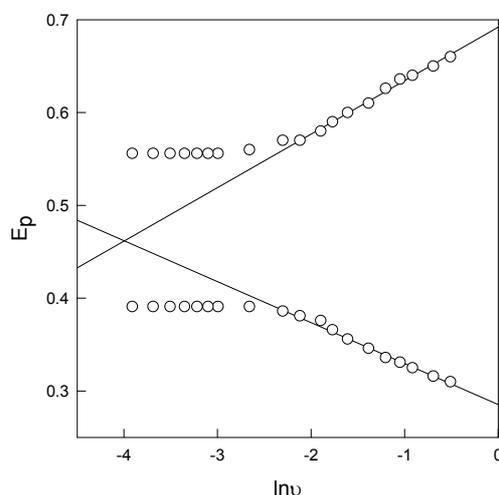

Figure 4. Relationship between the peak potential (E_p) and the natural logarithm of scan rate (ln v) for CuCoHCF-modified electrode in 0.5 M KCl solution and the linear fitting at scan rates from 170 mV/s to 600 mV/s.

The surface coverage can be evaluated from the equation $\Gamma=Q/nFA$, where Q is the charge obtained by integrating the anodic peak at a low scan rate and other symbols have their usual meanings. Therefore, the value of Γ can be calculated as 2.27×10^{-8} mol cm^{-2} by the involvement of one electron in the process. The film thickness (*l*) was also estimated by means of [28]:

$$l = \frac{Q}{nFA} \frac{d^3 N_A}{4} = \frac{\Gamma d^3 N_A}{4} \tag{3}$$

Where *d* is the length of the unit cell (10.17Å) and N_A is Avogadro's number. Thus, the film thickness can be calculated as about 36 nm, which is similar to earlier report on other metal-based hexacyanoferrate film [28, 29].

3.2 Electrocatalytic oxidation of tryptophan at the surface of CuCoHCF-modified graphite electrode

The electrochemical reduction and oxidation of amino acids are usually irreversible and occur at relatively higher negative and positive potentials, respectively. In earlier reports, tryptophan show well-defined oxidation peaks at about 0.7 V [30], which is very close to the third oxidation peak potential of CuCoHCF film that appeared in 0.5 M KCl medium. Therefore, it can be expected an electrocatalytic mechanism initiated by electrochemical oxidation of the reduced form of the complex exist at the surface of the electrode and then completed by chemical oxidation of tryptophan. To reveal the electrocatalytic activity of CuCoHCF film toward the oxidation of tryptophan, the voltammetric behavior of tryptophan was investigated at the surface of bare and CuCoHCF film-modified graphite electrode.

Figure 5 shows the cyclic voltammograms of CuCoHCF film-modified graphite electrode in 0.5 M KCl solution (a) and in the presence of 300 μM tryptophan (b) and also the cyclic voltammogram of tryptophan (c) at the surface of the bare electrode. At a bare electrode, the direct oxidation of tryptophan is not significant, and just a small anodic current due to the oxidation of tryptophan is observed. However, if the electrode is modified with CuCoHCF film, and then placed in the electrochemical cell containing tryptophan, a large anodic peak is observed with cathodic peak current decreasing accordingly. Therefore, it can be indicated an electrocatalytic process has occurred.
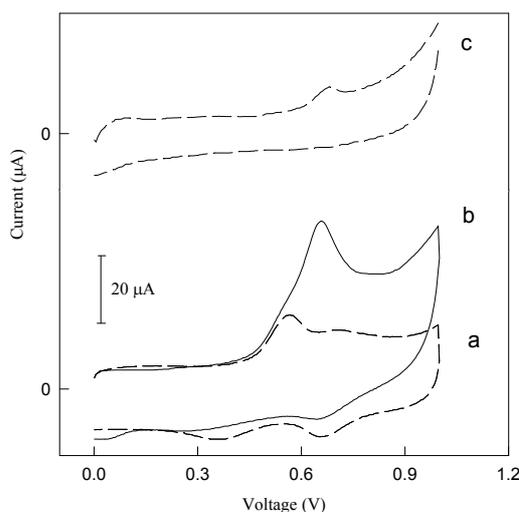

Figure 5. The cyclic voltammograms of CuCoHCF-modified graphite electrode in 0.5 M KCl solution (a) and in the presence of 300 μM tryptophan (b), and tryptophan (c) on the bare graphite electrode.

3.3 Optimization of experimental variables for electrocatalytic ability on tryptophan

3.3.1 Effect of scan cycles in electrodeposition

By altering the scan cycles in the electrodeposition process, CuCoHCF films of different thickness were obtained. In our experiments, with the increase in scan cycles, the response current increased, while the scan cycles were too larger, the response current may decrease due to a greater barrier for electron transfer in the thick films. Therefore, a moderate cycles, 30, was chose as the optimal scan cycles.

3.3.2 Effect of the ratio of Cu(II) and Co(II) in the solution

Cyclic voltammograms of the CuCoHCF film deposited from the solution with different χ_{Cu} values to tryptophan in 0.5 M KCl solution are shown in Figure 6. During the deposition, the concentration of KCl and $K_3Fe(CN)_6$ were kept constant as 0.5 M 0.66 mM, respectively. From the inset of Figure 5, it can be seen that the response current gradually increased with increase in χ_{Cu} from 0.3 to 0.5, while clearly decreased higher than 0.5. The oxidation voltages at higher potential (about 0.65 V) showed a little difference at different ratio, therefore, at the ratio of Cu(II) and Co(II) with 0.5, the CuCoHCF exhibit the best electrocatalytical response to tryptophan, and in the following work, the ratio was selected as 0.5.
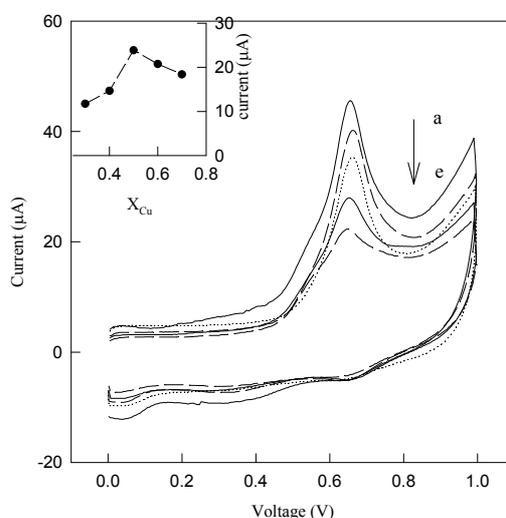

Figure. 6 Effect of the ratio of Cu(II) and Co(II) in the electrodeposited solution on the catalytic ability of tryptophan. KCl: 0.5 M; $K_3Fe(CN)_6$: 0.66 mM. From *a* to *e*: 5:5, 6:4, 7:3, 3:7, 4:6.

3.3.3 Effect of pH value

In order to optimize the solution pH for tryptophan detection, the effect of pH on the response current was studied in buffered solution (1/15 M PBS) containing 0.5 M KCl. The pH is adjusted by different ratio of KH_2PO_4 and Na_2HPO_4. The relationship between steady-state current and pH value is shown in Figure 7. There was a little increase of the response in the catalytic current at lower than 7, however, a decrease in the catalytic current above pH 7 was observed, which may be due to the hydroxylation of CuCoHCF film in the alkaline conditions. Furthermore, the activity of tryptophan is

much higher at its isoelectric point, so the higher current response may occur at this pH value. Thus, for the consistence of the film preparing solution and the detection solution, pH value of 7 is selected.
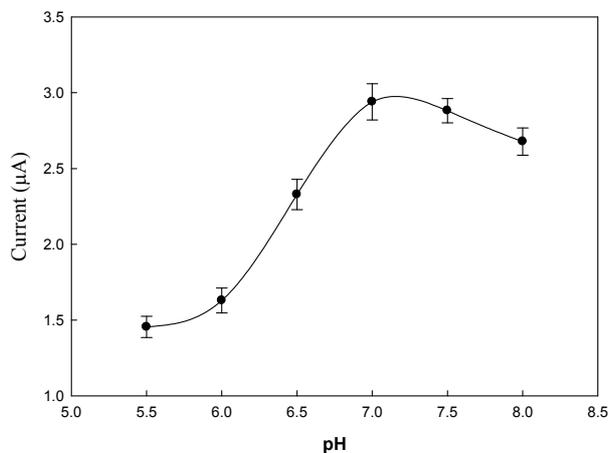

Figure 7. The pH influence of the solution on the detection of
50 μM tryptophan. Applied potential: 0.65 V.

3.3.4 Effect of applied voltage

To optimize the applied potential for the tryptophan determination, the effect of applied potential on the response current was investigated. As shown in Figure 8, the response current gradually increases with the applied potential from 0.4 V to 0.65 V, while decrease if the potential is higher than 0.7 V.

Then, other amino acids which can be oxidized at the electrode surface in earlier reports, i.e., cysteamine and tyrosine, were selected to carry out the interference study. The oxidation of tyrosine on bare graphite electrode appeared at 0.85 V, and our follow work is to select a suitable metal hexacynoferrate film for its electrocatalysis. The oxidation of cysteamine on the bare graphite electrode can hardly be found. The response of CuCoHCF film to these amino acids was also investigated and the electrocatalytic effect for cysteamine and tyrosine is small. Therefore, considering the possible interference, the optimal potential was chose as 0.65 V.
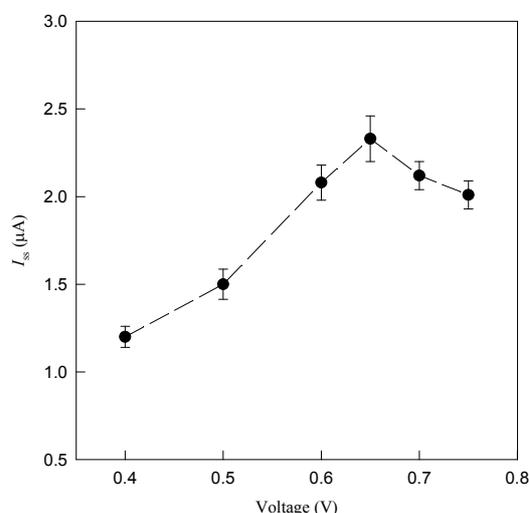

Figure 8. Effect of applied potential on the detection of 50 μM tryptophan in 0.5 M KCl solution.

3.4 Analytical properties

The current response of the bare graphite electrode and CuCoHCF-film modified electrode to tryptophan has been investigated at the optimized conditions, and the results are shown in Figure 9. Standard tryptophan solution was added stepwise after the background current became stable. It can be observed that CuCoHCF-film modified electrode shows a plateau with a large response current to tryptophan. From the inset of Figure 9, the response current is linear with the tryptophan concentration in the range of 10 μM – 900 μM. The regression equation is i_{ss} (μA)=0.67+0.046$C_{\mathrm{Tryptopahn}}$ (μM) with a correlation coefficient of 0.994, indicating that the regression line is fitted very well with the experimental data and it can be applied in the unknown sample determination. The detection limit, based on a signal to nose ratio of 3, was 6 μM.
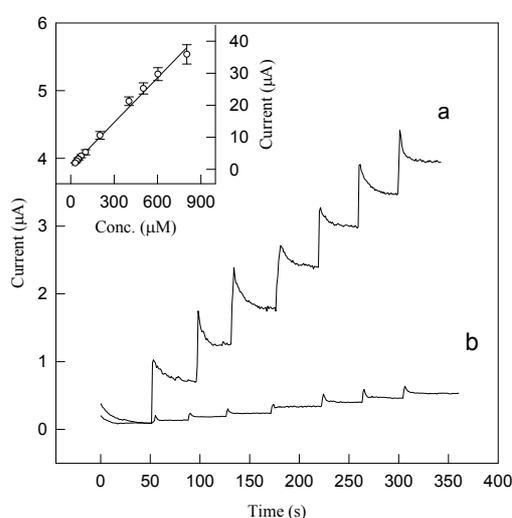

Figure 9. The current response to successive addition of tryptophan in 0.5 M KCl solution: (a) bare graphite electrode and (b) CuCoHCF-film modified electrode. The inset is the relationship of the current response of (b) with the tryptophan concentration. Applied potential: 0.65 V.

The stability of the CuCoHCF-film modified electrode was studied using the same KCl solution containing 50 μM tryptophan. During the repeating amperometric detection process, a little decrease of response current can be observed and 2% of the response current will be lost after 10 repeated detections. The long-term storage stability of this electrode was also investigated, and only 6% current loss after a week and 20% current loss after 1 month are observed.

3.5 Determination of tryptophan in milk

As a practical application, the CuCoHCF-film modified electrode was also used to detect tryptophan content in milk (a product of a milk factory in South China Agriculture University). At first, 1-mL of milk was diluted to 10-ml, and then a series of 0.01 M tryptophan solution was added. The concentration of tryptophan was calculated by standard addition, and results are shown in Table 1. Thus, the average content of tryptophan in milk is about 36 mg/100g, suggesting that the CuCoHCF-film modified electrode is very reliable and sensitive in the determination of tryptophan.

Table 1. Determination of tryptophan in milk by standard addition.

	Added tryptophan (10^{-4} M)	Detected tryptophan[a] (10^{-4} M)	Tryptophan in milk (10^{-4} M)	Average (10^{-4} M)	SD
1	0.5	2.4±0.2	1.9±0.2		
2	1.0	2.7±0.3	1.7±0.4		
3	1.5	3.3±0.3	1.8±0.3	1.8	0.15
4	2.0	3.8±0.2	1.8±0.2		
5	2.5	4.5±0.1	2.0±0.1		

a: Mean values ± standard deviations (*n*=3)

4. Conclusion

We have proposed here a new method for the electrocatalytic determination of tryptophan based on copper-cobalt hexacynoferrate film modified graphite electrode. The experimental conditions, including scan cycles in the electrodeposition, the ratio of copper(II) and cobalt(II), pH value, applied potential are investigated in detail. The eletctrocatalytic response shows a linear relationship on the concentration of tryptophan in the range of 10 μM and 900 μM, with a detection limit of about 6 μM. This electrode was also successfully used to detect the tryptophan concentration in milk.

Acknowledgment

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