

Electrocatalysis and Determination of Uracil on Polythionine/Multiwall Carbon Nanotubes Modified Electrode

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ABSTRACT: A new type of poly (thionine)/multiwall carbon nanotube/glassy carbon (PTH/MWNTs/GC) electrode was fabricated by electropolymerization thionine onto the surface of MWNTs modified GC electrode. The properties and behaviors of the modified electrode were characterized by scanning electron microscopy (SEM) and cyclic voltammetry (CV). The results show that the high sensitivity and selectivity are mainly caused by the unique carbon surface of the carbon nanotubes and the catalytic activity of thionine. The modified electrode exhibited excellent electrocatalytic behavior to the oxidation of uracil, and was firstly applied to determinate the concentration of uracil for the differential pulse voltammograms.

Under the optimum conditions, linear calibration equation was obtained over the uracil concentration range from 1.0×10^{-5} to $5.5 \times 10^{-2} M$ with a correlation coefficient of 0.9978 and a detection limit $2.0 \times 10^{-7} M$ (based on $S/N = 3$) was also gained. The good electrocatalytic response of uracil at PTH/MWNTs/GC electrode suggests that the PTH/MWNTs are an excellent platform for electrochemical biosensing. The modified electrode displays excellent repeatability, stability, and high sensitivity. © 2007 Wiley Periodicals, Inc. *J Appl Polym Sci* 107: 3173–3178, 2008

Key words: multiwall carbon nanotube; thionine; uracil; modified electrode; electrocatalytic oxidation

INTRODUCTION

Since their initial discovery by Iijima¹ in 1991, carbon nanotubes (CNTs), as one of the most interesting carbon materials have attracted considerable attention of researchers over the past years.^{2,3} Owing to their unique advantages such as high conductivity, high chemical stability, extremely high mechanical strength, and modulus, CNTs are widely used in many fields including catalyst, nanoscale electronic parts, electron filed emission sources, and hydrogen storage material.^{4–7} In the electroanalytical field, as they can promote electron-transfer reactions, they are widely used as electrode materials and to prepare sensors.^{8,9} Recently, the fabrication of CNTs/conducting polymer (CNTs/CP) electrode has gained great interest as the CNTs can improve the

electrical and mechanical properties of polymers,^{10,11} and it has been demonstrated that the obtained CNTs/CP possess properties of the individual components with a synergistic effect.^{12–17} Different CP has been used, such as polypyrrole (PPy),^{12,13} polythiophene (PTH)^{14,15} and its derivatives,^{16,17} polyaniline (PAN) composites,¹⁸ and so on. At present, the combination of CP and CNTs composite with high surface-to-volume ratio are mainly focused on polypyrrole (PPy),¹⁹ Pan,^{20,21} and PTh.²² For example, PPy/CNTs were prepared by template-directed electropolymerization of PPy in the presence of a CNTs dopant,¹⁹ Pan/CNTs were prepared by self-assembly²⁰ or in situ polymerization,²¹ PTh/CNTs were fabricated through van der Waals and electrostatic interactions between PTh and CNTs.²² Moreover, to functionalize CNTs modified electrode with biomolecules or catalysts, a number of intriguing physicochemical approaches have recently been devised for modification of CNT.^{23–25} As we known that thionine is a small planar molecule and has two $-NH_2$ groups symmetrically distributed on each side. And thionine is a kind of purple dye, which can be easily dissolved in water and ethanol. Thionine molecules have been demonstrated to be easily adsorbed onto the surfaces of CNTs through strong π - π stacking force to introduce other molecules onto tube surfaces and enriching the chemistry of CNT.^{26,27} However,

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to the best of our knowledge, there are no reports on the fabrication of the polythionine/multiwall carbon nanotube/glassy carbon (PTH/MWNTs/GC) electrode and its response on uracil.

On the other hand, uracil is well known as a kind of pyrimidine bases in RNA. It plays an important role in biochemical processes which relate to several diseases and metabolic disorders.²⁸ The electrochemical behavior and determination of uracil is significance in life science and clinical iatrology. Thus, uracil is currently the object of intense research for pharmacologists and chemists, and it is essential to develop rapid and simple methods for the determination of the concentration of uracil. Generally, the determination of uracil is carried out by liquid-chromatography-tandem mass spectrometry,²⁹ high-performance liquid chromatography,³⁰ capillary electrophoresis,³¹ gas chromatography-mass spectrometry,³² spectrophotometric analysis.³³ Unfortunately, these methods mentioned above have some disadvantages such as limits of analysts, low sensitivity, long analysis time, environment unfriendly solvents, expensive instrumentation, and the tedious procedures. As we know, electrochemical analysis methods are more sensitive, convenient, rapid, and are low cost. So it is essential to develop a new electrochemical sensor to determine the concentration of uracil. And from the molecular formula of thionine and uracil, we have gained the result that it is possible to develop an electrochemical sensor of uracil, may be the thionine, which was modified on the surface of electrode, can attract the uracil by strong π - π stacking force between them.

In the present work, we describe a new method to determine the concentration of uracil through fabricating a PTH/MWNTs/GC electrode by simple electropolymerization of thionine onto the surface of MWNTs modified GC electrode. The PTH/MWNTs/GC electrode, possessing a maximum surface area and adsorbed abundant thionine molecules, exhibits a wonderful electrochemical response to the trace amount of uracil. Electrocatalytic property to the oxidation of uracil on the PTH/MWNTs/GC electrode has been investigated utilizing cyclic voltammetry. And we have also determined the concentration of trace uracil using differential pulse voltammetry. This modified electrode, which possesses maximized surface area and adsorbed abundant thionine molecules, exhibits a wonderful electrochemical response to uracil.

EXPERIMENTAL PART

Apparatus and reagents

Electrochemical experiments were performed using a CHI660B electrochemical workstation (CH Instruments, Chenhua Corp, Shanghai, China). A conventional three-electrode system was employed with a

bare or a modified electrode (3.0 mm in diameter) as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the auxiliary electrode. All the potentials quoted in this article were referred to the SCE. The field emission scanning electron microscopy images were taken using a JEOL JSM-67700F SEM.

The MWNTs were obtained from Chengdu Institute of Organic Chemistry of Academy of Sciences with the purity is more than 95%. Thionine and uracil were purchased from the Shanghai Institute of Biochemistry of the Academy of Sciences. Other chemicals were of analytical grade and were used without further purification. The phosphate buffer solution (PBS, 0.1M) was prepared by 0.1M KH_2PO_4 and 0.1M K_2HPO_4 , and adjusted the pH with 0.1M H_3PO_4 and 0.1M KOH solutions. All the solutions were prepared with double distilled water and all electrochemical experiments were carried out at room temperature ($20^\circ\text{C} \pm 5^\circ\text{C}$).

The electrochemical experiments were performed in 0.1M KCl solutions containing a certain concentration of uracil deaerated by purging with high-purity nitrogen. Under the various conditions, the CVs and DPV were recorded in a suitable potential range. All experiments were carried out at ambient temperature (about 20°C) under a nitrogen atmosphere.

Preparation of the PTH/MWNTs/GC electrode

MWNTs (1 mg) were dispersed in 10 mL DMF by ultrasonic agitation for about 60 s to give a 0.1 mg/mL black suspension. Prior to modification, the bare glassy carbon electrode (GCE, 3 mm in diameter) was polished successively with 0.3 and 0.05 μm Al_2O_3 slurry on emery paper. Then it was rinsed with double distilled water and sonicated in nitric acid (1:1), acetone and double distilled water for 5 min, respectively. After cleaning, the electrode was activated by 20 cycles sweeping from -1.0 to 1.2 V in pH 7.0 PBS. The MWNTs modified electrode was prepared by casting 10 μL of the dispersion on the surface of a GC electrode, which was air-dried at room temperature. When the DMF was volatilized, a MWNTs film was formed. The PTH/MWNTs/GC electrode was obtained by electropolymerize TH on the MWNTs modified electrode by cyclic voltammetry (CVs) between -0.4 and $+0.4$ V at 50 mV/s in 0.1M PBS (pH 7.0) containing 5 mM thionine for 40 cycles and rinsed with double distilled water.

RESULTS AND DISCUSSION

Characterization of the PTH/MWNTs/GC electrode

Figure 1 displays a typical morphology of the MWNTs/GC electrode (a) and PTH/MWNTs/GC

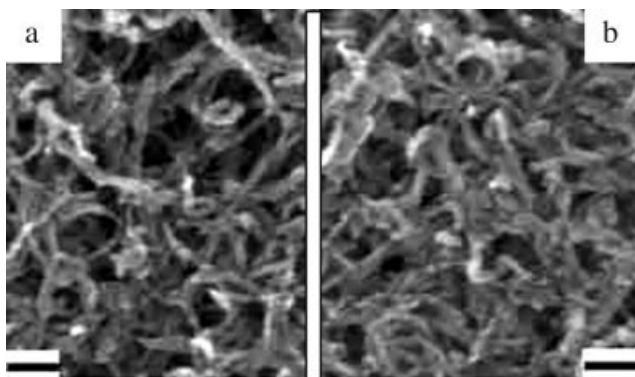


Figure 1 SEM images of: (a) MWNTs and (b) PTH/MWNTs on GCE surface. Scale bar = 100 nm.

electrode (b) characterized by SEM. From Figure 1, it can be seen that the PTH/MWNTs were in small bundles and single nanotubes and distributed almost homogeneously on the surface of GCE exhibiting a special three-dimensional structure. The electropolymerized TH on the surface of CNTs did not change the morphology of CNTs, but made it more compact. While the diameters of PTH/CNTs were slightly thicker than that of CNTs, which means the PTH layer is very thin. This shows that the thionine has been modified on MWNTs and distributed on GC electrode by electropolymerize.

Electrochemical behavior of the PTH/MWNTs/GC electrode

Figure 2 represents the cyclic voltammograms (CVs) of a bare GC electrode (curve a), MWNTs/GC electrode (curve b), and PTH/MWNTs/GC electrode (c),

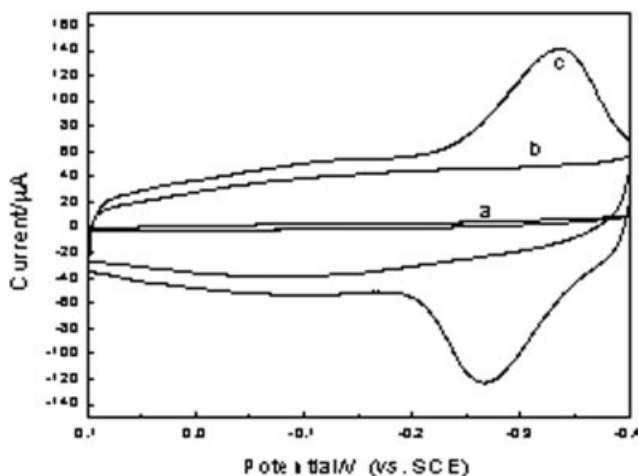


Figure 2 Cyclic voltammograms of bare GC electrode (a), MWNTs/GC electrode (b), and PTH/MWNTs/GC electrode (c) in 0.1M PBS (pH 7.0). Scan rate: 100 mV/s; Sensitivity: 10^{-4} A/V.

which were recorded in 0.1M PBS (pH 7.0). As it shows, the PTH/MWNTs/GC electrode has a quasi-reversible redox couple (curve c), where the anodic and cathodic peak potentials are -0.269 and -0.335 V, respectively. The oxidative and reductive peak currents were nearly equal, and the shapes of the redox peaks were symmetric, suggesting that all electroactive thionine in the MWNTs/GC electrode was reduced on the forward negative scan and the reduced thionine was oxidated fully again on the reverse positive scan.

The effect of scan rate on the peak current was investigated, and the results are shown in Figure 3. As can be seen, oxidative peak currents increased linearly with scan rates in the range of 20 to 180 mV/s, while the peak potentials only slightly changed. This demonstrates that the redox process of the PTH on the surface of MWNTs is controlled by adsorption scan rate.

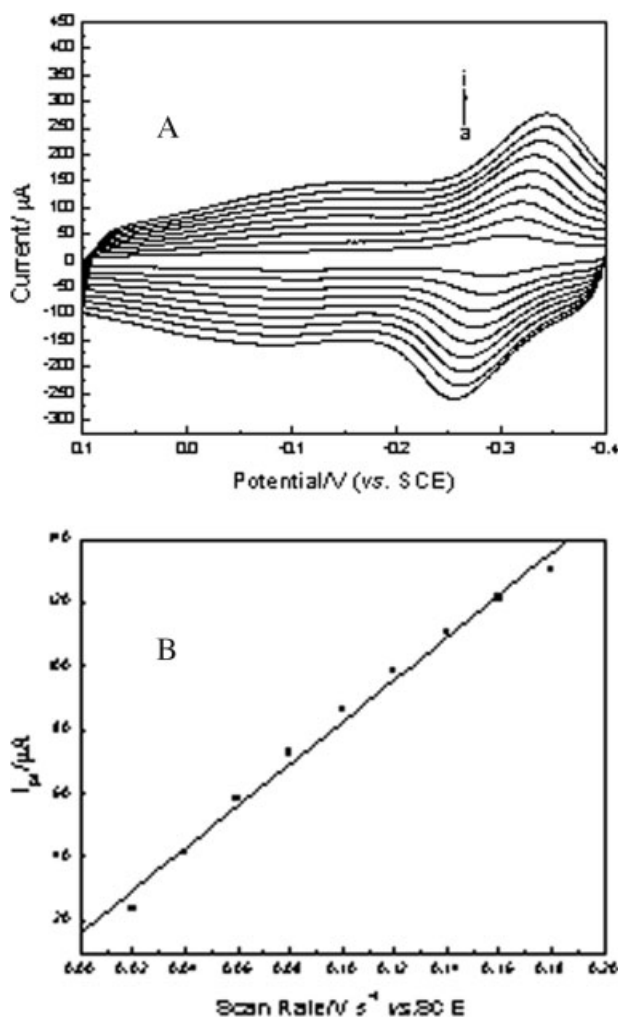


Figure 3 (A) Cyclic voltammograms of PTH/MWNTs/GC electrode in 0.1M PBS (pH 7.0) at scan rate 20, 40, 60, 80, 100, 120, 140, 160, 180 mV/s from a to i. (B) Plot of anodic peak current versus scan rate.

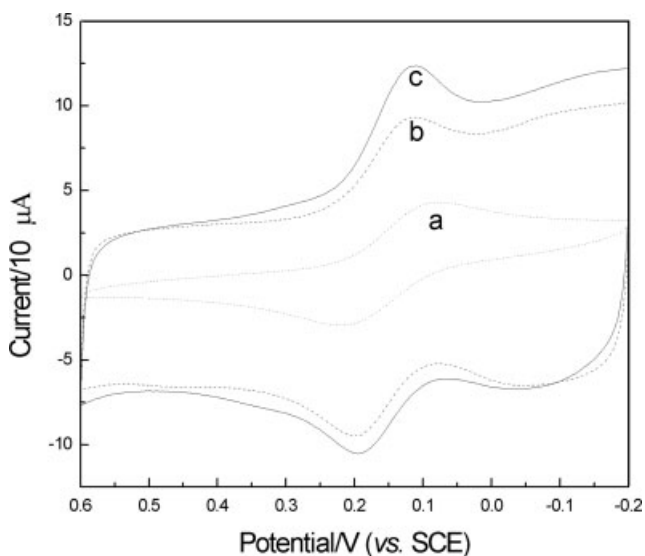


Figure 4 Cyclic Voltammograms for: (a) bare GC electrode, (b) MWNTs/GC electrode and (c) PTH/MWNTs/GC electrode in 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution containing 0.1M KCl. Scan rate: 100 mV/s; Sensitivity: 10^{-5} A/V.

Electrochemical characterization of modified electrode

According to Hrapovic and Yang's view,^{34,35} higher electroactive surface area of the CNTs-based nanomaterials always means higher electrocatalytic activity resulting higher sensitivity. So, we select CVs to estimate the electroactive surface of the modified electrodes. Figure 4 represents the CVs of the bare GC electrode (curve a), the MWNTs/GC electrode (curve b), and the PTH/MWNTs/GC electrode (curve c), which were recorded in 0.1M KCl containing 5 mM $[\text{Fe}(\text{CN})_6]^{3-/4-}$ solution at 100 mV/s. As shown, well-defined oxidation and reduction peaks due to the $\text{Fe}^{3+}/\text{Fe}^{2+}$ redox couple were observed. It could be seen from the Figure 4 that the peak-to-peak potentials separation of the bare GC electrode, MWNTs/GC electrode, and PTH/MWNTs/GC electrode decreased in sequence. This may be due to the higher electrochemical activity of MWNTs and PTH. Compared with the bare GC electrode, the peak currents at the MWNTs/GC electrode, the PTH/MWNTs/GC electrode were all increased and the PTH/MWNTs/GC electrode exhibited the highest peak currents. The highest peak current illustrates the PTH/MWNTs/GC electrode possesses the highest electroactive surface area.^{34,35} The highest electroactive surface area means the PTH/MWNTs/GC electrode possesses the highest electrocatalytic activity.^{34,35} On the other hand, the separation of peak potential ΔE_p is about 143 mV and the ratio of peak current i_{p_a}/i_{p_c} is about 1. When it is modified with MWNTs, the ΔE_p reduces to about 87 mV and the i_{p_a}/i_{p_c} is also about 1, suggesting the electrode pro-

cess is more reversible. When it is further modified by thionine, the ΔE_p also reduced to about 77 mV. This should be attributed to thionine, which is one of the redox indicators.

Electrochemical behavior of uracil

Figure 5 depicts the Cyclic Voltammograms of uracil at the bare GC electrode (curve a), the MWNTs/GC electrode (curve b), and the PTH/MWNTs/GC electrode (curve c) in 0.1M KCl solutions. At the bare GC electrode, there was no peak in 0.1M KCl solutions (curve a). From curve b, it is found that uracil exhibited poor electrochemical response at the MWNTs/GC electrode, which has an anodic peak potential at 0.95 V. However, at the PTH/MWNTs/GC electrode (curve c), the anodic peak potential shifted negatively to 0.89 V and the peak current obviously enhanced higher than that at the MWNTs/GC electrode. This may contribute to three facts: first, the MWNTs could increase the assembled TH amounts; second, the PTH/MWNTs nanomaterials are in the form of nanotubes, which could provide a huge catalytic surface; third, the MWNTs could facility the electron translation.^{24,25} As a result, the response current of uracil increased greatly. The modified electrode provides a wonderful biomembrane-like microenvironment.

Effect of electrolyte

The effect of electrolyte on the response of uracil was investigated among 0.1M PBS, KNO_3 , NaOH, KCl, and Tris-HCl solutions. The results show that the uracil peak was more stable and higher in KCl

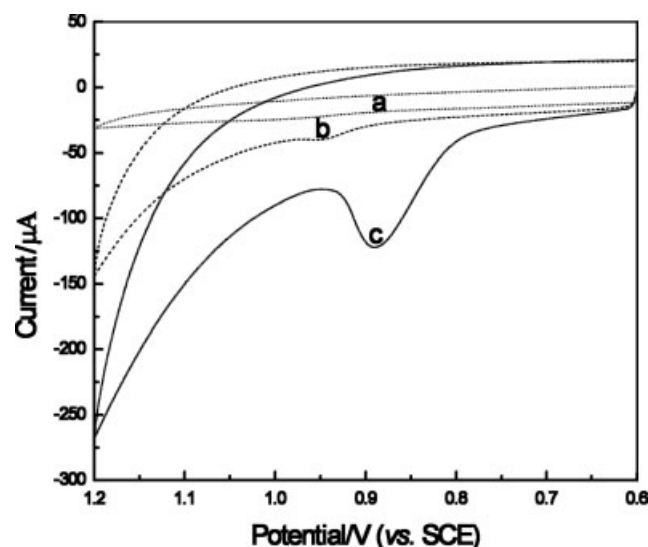


Figure 5 Cyclic Voltammograms of 5×10^{-3} M uracil in 0.1M KCl solutions at: (a) bare GC electrode; (b) MWNTs/GC electrode; and (c) PTH/MWNTs/GC electrode. Scan rate: 100 mV/s; Sensitivity: 10^{-5} A/V.

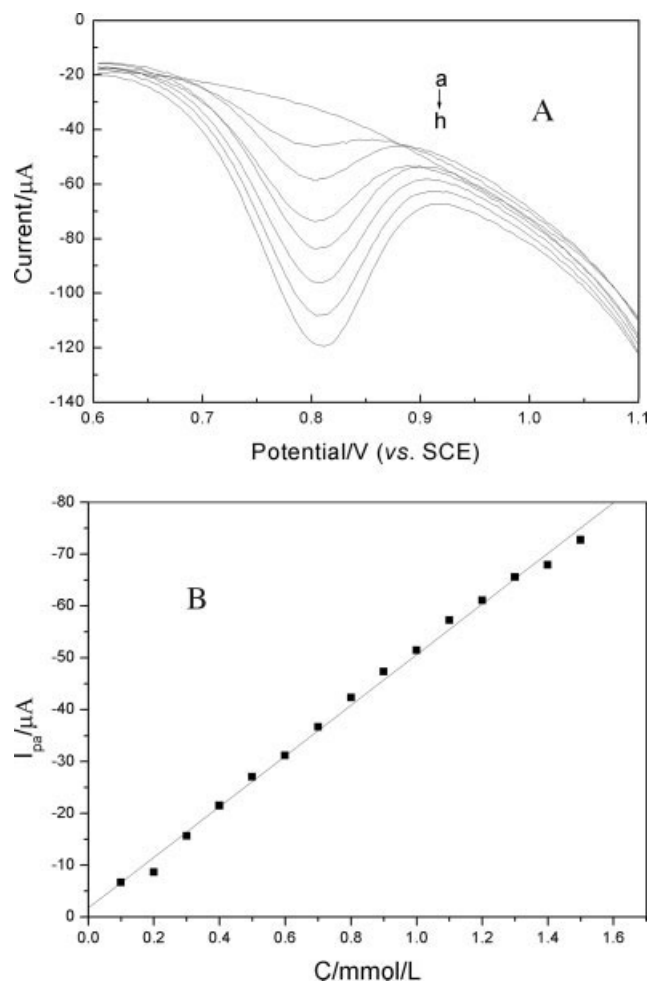


Figure 6 (A) Differential pulse voltammograms obtained for the determination of uracil at the modified electrode in 0.1M KCl. Concentration of uracil (M): (a–h) 0,1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0×10^{-4} M. (B) Plots of the peak currents as a function of concentration of uracil. Amplitude: 0.05 V; Pulse width: 0.06 s; Pulse period: 0.2 s.

solutions. In addition, there was no obvious change observed in peak current and peak potential when the concentration of KCl solutions changed from 0.05 to 0.2M. So the 0.1M KCl solutions were chosen as the supporting electrolyte in the electrochemical detection of uracil.

Determination of uracil

Under the optimum conditions described above, the DPV peak currents of uracil increased linearly with the corresponding uracil concentration (Fig. 6). The experimental results show that the DPV peak current is linear with the concentration of uracil in the range of 1.0×10^{-5} to 5.5×10^{-2} M. The corresponding linear equation can be described as follows: I_{pa} (μ A) = $1.377 + 49.24C$ (mM), with a correlation coefficient of 0.9978 and the detection limit was 2.0×10^{-7} M ($S/N = 3$).

The effect of coexistence material

Under the chosen conditions, the following compounds were investigated: hippuric acid (200), citric acid (50), cysteine (50), glucose (200), NaCl (400), KCl (400), CaCl_2 (200), adenine (50), and cytosine (100), where the numbers in parentheses represent the concentration ratios to 1.0×10^{-4} M uracil. No shift in the oxidation peak potential of uracil was observed in the presence of all the interferences. The current response of uracil was nearly not affected under the condition of the above interference.

Reproducibility and stability

Evaluation of the maintenance, lifetime, and storage of the PTH/MWNTs/GC electrode have been studied by measuring its voltammetric response on storage for a longer duration. The modified electrode is very stable and the capacitance of this electrode is unchanged while cycling the electrode potential in the above potential window. It was observed that no apparent decrease in response for nearly 7 days. The modified electrode retained 96 and 91% of its initial response up to 14 and 30 days, respectively. Such a good stability is acceptable for most practical applications. To ascertain the reproducibility of the results, eight different modified electrodes were modified with thionine and MWNTs and their response towards the oxidation of uracil were tested by 10 repeated measurements of the same solution. The peak potential and the peak current obtained in the 10 repeated measurements of eight independent electrodes showed a relative standard deviation of 1.0%, confirming that the results are reproducible.

CONCLUSION

In summary, a new type of PTH/MWNTs/GC electrode was prepared by electropolymerization of thionine onto the surface of MWNTs modified GCE. Significantly electrochemical response to trace the amount of uracil on thionine modified MWNTs electrode, has been observed in this article. This modified electrode provides a sensitive method and was firstly applied to determine the concentration of uracil. The present novel strategy for construction of PTH/MWNTs/GC electrode proposed an excellent platform for electrochemical biosensing towards trace amount of uracil.

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