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An amperometric penicillin biosensor with enhanced sensitivity based on co-immobilization of carbon nanotubes, hematein, and β-lactamase on glassy carbon electrode

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ABSTRACT

An amperometric penicillin biosensor with enhanced sensitivity was successfully developed by co-immobilization of multi-walled carbon nanotubes (MWCNTs), hematein, and β-lactamase on glassy carbon electrode using a layer-by-layer assembly technique. Under catalysis of the immobilized enzyme, penicillin was hydrolyzed, decreasing the local pH. The pH change was monitored amperometrically with hematein as a pH-sensitive redox probe. MWCNTs were used as an electron transfer enhancer as well as an efficient immobilization matrix for the sensitivity enhancement. The effects of immobilization procedure, working potential, enzyme quantity, buffer concentration, and sample matrix were investigated. The biosensor offered a minimum detection limit of 50 nM (19 $\mu g\,L^{-1}$) for penicillin V, lower than those of the conventional pH change-based biosensors by more than two orders of magnitude. The electrode-to-electrode variation of the response sensitivity was 7.0% RSD.

1. Introduction

The presence of antibiotic residues in food products, drinking water, and environment constitutes a worldwide concern because of its serious health consequences, including the emergence of antibiotic-resistant bacterial strains, disturbance or disruption of the normal ecological equilibrium, and increasing instances of allergic reactions. Penicillins and other β-lactam antibiotics are the most frequently used antibiotics and have long been widely used for treating various bacterial infections in human, livestock, poultry, and aquaculture. Consequently, detection of the residues of these antibiotics in food and environment is vital for protecting the public health.

HPLC/MS methods are the current gold standard for the detection of these drugs [1,2]. These methods are reliable and have adequate sensitivity for enforcement of the maximum residue limits or tolerance levels of antibiotic residues in different samples, e.g., 4–6 $\mu g\,kg^{-1}$ (ppb) for penicillin and 30–100 ppb for other β-lactam antibiotics in milk, 50–1000 ppb for different β-lactam antibiotics in bovine muscle [3–5]. However, HPLC/MS methods require expensive instruments and extensive sample pretreatment and are thus mostly used in centralized laboratories. A variety of commercial test kits and strips are available for rapid screening of β-lactam antibiotics in certain samples [3,6,7]. Some examples are Delvotest based on inhibition of bacterial growth, Penzyme based on inhibition of D-carboxypeptidase, and those tests based on the receptor protein-antibiotic binding such as Charm SL Beta Lactams, Charm II, DelvoX-Press βL, New SNAP and Beta Star. These commercial tests are convenient to use but also have some limitations: the microbial inhibitor tests need a lengthy incubation step and are thus very time consuming, most of the tests can only provide semi-quantitative results (i.e., above or below a certain level), and some of them lack adequate sensitivity. Therefore, there is a need for the development of new alternative methods for rapid and sensitive detection of β-lactam antibiotics.

β-Lactamase can catalyze the hydrolysis of penicillin to penicillinic acid, resulting in a decrease of solution pH. This forms the basis of many reported penicillin biosensors, in which the pH change is usually monitored by potentiometry [8–10], conductometry [11], colorimetry [12], or fluorescence [13]. The potentiometric modes have been employed most frequently; they are simple in configurations but have drawbacks of slow response speeds and high detection limits (0.08–1.5 mM). The conductometric and optical modes are advantageous to the potentiometric ones in response speeds, but they still have relatively high detection limits (0.05–0.25 mM). Most recently, several new penicillin biosensors were developed based on sensing the pH change with a capacitive field-effect [14,15] or charge transfer [16] technique. The detection

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limit of these biosensors is as low as 5 μM; however, the fabrication of these biosensors is complicated.

The huge commercial success of electrochemical glucose biosensors has exemplified advantages of the amperometric transduction over others in terms of sensitivity, response time, simplicity, cost, etc. Nevertheless, there have been relatively scarce reports on pH change-based amperometric biosensors. Stred'anský et al. [17] pioneered the work on amperometric biosensors for detection of penicillin and other analytes using hematein, lauryl gallate, or methylene blue dissolved in solution as a pH-sensitive redox probe. Using a penicillinase electrode and dissolved hematein, a detection limit of 4 μM (~1.4 ppm) was obtained for amperometric detection of penicillin G in phosphate buffer solution (PBS); the detection limit is considerably lower than those of the conventional potentiometric, conductometric, and optical ones. This has stimulated several other studies on this type of biosensors [18–20].

On another hand, carbon nanotubes (CNTs) including multi-walled CNTs (MWCNTs) and single-walled CNTs (SWCNTs) have found increasing applications in developing electrochemical chemo/biosensors with improved performance and features, particularly with enhanced sensitivity [14,15,21–24]. This is benefited from the unique electronic, mechanical, and chemical properties of CNTs such as excellent electron mobility and electrical conductivity, high surface area to volume ratio, high sorption capability and capacity, and good chemical stability. Compared to SWCNTs, MWCNTs exhibit higher electron transfer rates and possess a higher sorption capacity [24], which are favorable for the sensitivity enhancement of electrochemical biosensors.

Inspired by the preceding studies, we reasoned that MWCNTs could facilitate the construction of an amperometric biosensor with enhanced sensitivity for penicillin detection. The primary objective of this study was to prove this hypothesis with penicillin V, a monobasic penicillin commonly used in human and veterinary practice, as a model β-lactam antibiotic. An amperometric biosensor with enhanced sensitivity for penicillin V was successfully developed by co-immobilization MWCNTs, hematein, and β-lactamase on glassy carbon electrode (GCE) using a layer-by-layer assembly (LbL) technique. This work represents the first attempt to take advantages of CNTs and LbL technique to develop a pH-sensitive amperometric biosensor with improved sensitivity for β-lactam antibiotics.

2. Experimental

2.1. Reagents and materials

MWCNTs of 20–30 nm outer diameter, 5–10 nm inside diameter, 10–30 μm length, >100 S cm^{-1} electrical conductivity, and >95 wt% purity used in this work were provided by Cheap Tubes Inc. (Brattleboro, VT, USA) as free samples at one conference. Chromatography-purified β-lactamase (penicillinase, EC 3.5.2.6, type I, >90% purity) was supplied by Lion Biotechnology Co. (Hangzhou, Zhejiang, China). Penicillin V (phenoxymethyl penicillin) potassium, hematein, and chitosan (≥90% deacetylated) were obtained from Jiehui Bioreagent Co. (Changsha, Hunan, China), Sigma–Aldrich Co. (St. Louis, MO, USA), and Sinopharm Chemical Reagent Co. (Shanghai, China), respectively. Doubly-distilled water prepared by an SZ-93 system of Yarong Biochemical Instrument Factory (Shanghai, China) was used throughout.

The MWCNTs were carboxylated to improve their dispersion in water basically following the procedure described by Kim and Sigmund [25]. In a typical experiment, 15 mg of MWCNTs was added into 20 mL of concentrated H_2SO_4–HNO_3 (3:1, v/v) and sonicated in a water bath for 8 h. After the sonication, the mixture was vacuum-filtrated through a PVDF microporous membrane (pore size 0.22 μm) and washed with water until the filtrate was neutral. The resulted carboxylated MWCNTs were dispersed in water under sonication for further use. Penicillinase solutions were prepared in 1 mM pH 6.8 PBS (containing 0.1 M NaCl, the same below). A 1% chitosan solution was prepared by dissolving a calculated quantity of chitosan in 2% HAc and adding 5 M NaOH to adjust the pH to 4.7.

2.2. Biosensor fabrication and surface characterization

The amperometric biosensor was fabricated on 3.0 mm-diameter GCE (Aida Hengcheng Technology Co., Tianjin, China) using an LbL technique. First, 100 μL of 1.6 mg mL^{-1} carboxylated MWCNTs and 100 μL of 10 mM hematein were mixed, and an aliquot of 10 μL of the mixture was deposited on the GCE surface; after drying, the electrode was rinsed with 1 mM pH 6.8 PBS to remove unimmobilized MWCNTs and unadsorbed hematein molecules. Then, 10 μL of 1% chitosan was deposited on the layer of immobilized MWCNTs preadsorbed with hematein; after drying, the electrode was rinsed with 1 mM pH 6.8 PBS. Finally, 10 μL of 60,000 U mL^{-1} penicillinase solution was deposited on the layer of chitosan; after being stored at 4 °C overnight, the electrode was rinsed with 1 mM pH 6.8 PBS to remove unbound enzyme molecules. The resulted enzyme electrode was kept at 4 °C until use.

The surface morphology of the electrode was characterized by scanning electron microscopy (SEM) using JSM-6701F (JEOL Ltd., Tokyo, Japan) after each step of modification. The biosensor surface polarity was characterized by contact angle measurement using JC2000C (Zhongchen Digital Equipment Ltd., Shanghai, China).

2.3. Sample preparation and biosensor measurements

The biosensor was evaluated for the detection of penicillin V spiked in PBS or milk samples. The spiked PBS samples were directly analyzed with the biosensor. Milk samples, containing protein ≥2.9% (w/w) and fat ≥3.3% (w/w), were purchased from a local supermarket. The spiked milk samples were pretreated by salting-out with ammonium sulphate (6 g per 20 mL of milk) followed by centrifugation to remove protein and fat; the supernatants were collected for the biosensor assay.

Amperometric measurements were performed on CHI 660C electrochemical workstation (CH Instruments Inc., Austin, TX, USA) using a three-electrode system. The working, reference, and counter electrodes were the MWCNT–hematein–penicillinase electrode (i.e., the MWCNT–hematein/chitosan/penicillinase electrode), type 218 Ag/AgCl electrode (Precision & Scientific Instrument Co., Shanghai, China), and type 213 platinum electrode (Hengyu Water Analysis Instrument Co., Shanghai, China), respectively. In biosensor measurements, the amperometric response of the penicillinase electrode was monitored at −200 mV in a 5 mL-static electrochemical cell containing 2 mL of 1 mM pH 6.8 PBS–0.1 M NaCl as the supporting electrolyte solution. Aliquots of 5–10 μL of penicillin V spiked PBS or milk sample solutions were successively injected into the electrochemical cell under magnetic stirring. The resulted current changes were correlated to the final concentrations of penicillin V in the tested solution.

3. Results and discussion

3.1. Assembly of the biosensor

The present biosensor is based on co-immobilization of MWCNTs, hematein, and penicillinase on GCE. The immobilized penicillinase catalyzes the hydrolysis of penicillin, causing a

decrease in local pH on the electrode surface. Hematein acts as a pH-sensitive redox probe for amperometric detection of the pH change, which is proportional to the concentration of penicillin present in the sample. MWCNTs are used as an electron transfer enhancer as well as an immobilization matrix for the sensitivity enhancement.

Initially, the amperometric biosensor was fabricated by co-immobilization of hematein-adsorbed MWCNTs and penicillinase on GCE via bovine serum albumin (BSA)–glutaraldehyde crosslinking. This approach turned out to be tricky, exhibiting a poor repeatability. Moreover, in terms of the slopes of calibration graphs, the enzyme electrode prepared by the crosslinking method was apparently inferior to that prepared by the LbL approach later investigated (4.1 nA/100 μM vs. 10 nA/100 μM in the range of 200–1000 μM of penicillin V). LbL has proven to be a simple and versatile technique for assembling biomolecules onto CNT-coated electrode surfaces with fine control over the film thickness and architecture [15,26]. In this work, chitosan was chosen for interfacing between MWCNTs and penicillinase in the LbL because it is a natural cationic polyelectrolyte, has good biocompatibility and excellent film-forming ability, and has been used in LbL to construct a variety of CNT-based electrochemical biosensors [26–31]. First, a negatively charged layer of carboxylated MWCNTs preadsorbed with hematein is casted on GCE through solvent evaporation. Chitosan has a pK_a of 6.3 and is thus positively charged in the acidic solution (pH 4.7) due to the protonation of its amino groups [32]. The penicillinase, with an isoelectric point of 5.4, is negatively charged in pH 6.8 PBS. Therefore, the layers of chitosan and penicillinase are successively assembled onto the MWCNT coated GCE via electrostatic attractions.

3.2. Characterization of the biosensor

The morphological changes of the electrode caused by the LbL process were investigated by SEM. As shown in Fig. 1, the GCE was first covered by a porous film of single MWCNTs and small bundles that were randomly but homogenously packed (a). Then, the deposition of chitosan resulted in a roughly uniform and more smooth surface (b). Upon adsorption of penicillinase, the surface became more rough and showed numerous nodular protrusions (c). These notable morphological changes suggest that the LbL films were successfully fabricated on the GCE. The water contact angle of the LbL-assembled enzyme electrode measured was 38–42°, indicating a porous and hydrophilic surface, which is favorable for the penetration of the generated enzymatic product and hence the response speed.

The pH-dependence of the MWCNT–hematein–penicillinase electrode was demonstrated by cyclic voltammetry (CV) as well as by potentiostatic amperometry. As shown in Fig. 2a, both the anodic and cathodic peaks shift to more positive potentials when the solution pH decreases; this is consistent with the CV behavior of soluble hematein [18] and therefore confirms successful adsorption of hematein into MWCNT. The shifts of the anodic and cathodic peaks between pH 7 and 4, which corresponds to the range of the pH change caused by enzymatic hydrolysis of penicillin, are 123 and 96 mV, respectively, and both larger than those between pH 10 and 7.

From a practical viewpoint, monitoring the current change with potentiostatic amperometry will be more convenient than measuring the shift of anodic or cathodic peak with cyclic voltammetry. Fig. 2b demonstrates that, at a working potential of 200 mV, the amperometric response of the described penicillinase electrode displays a strong pH-dependence. This amperometric pH-dependence was employed to monitor the enzymatic hydrolysis of penicillin V and then detect the target analyte.

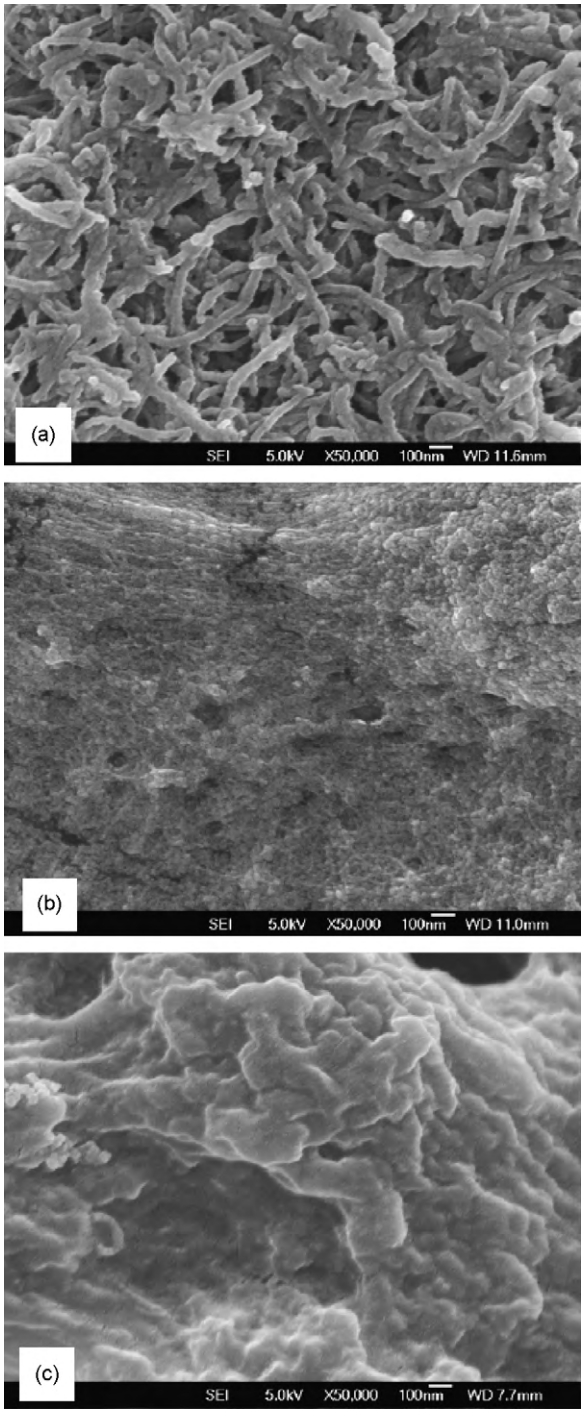

Fig. 1. SEM images of MWCNT–hematein (a), MWCNT–hematein/chitosan (b), and MWCNT–hematein/chitosan/penicillinase (c) modified-GCEs.

3.3. Selection of working potential

Several key factors were optimized in order to obtain an enhanced sensitivity for the biosensor assay. First, the effect of working potential on the amperometric pH response was investigated. Hematein, as a staining dye, is usually prepared by the process called ripening, which involves partial oxidation of hematoxylin to hematein [33]. Under appropriate conditions, hematein can be either reduced (to hematoxylin) or oxidized (e.g., to trioxyhematein). In addition, hematoxylin contained in the dye can be oxidized to hematein. In this work, it was found that at −100

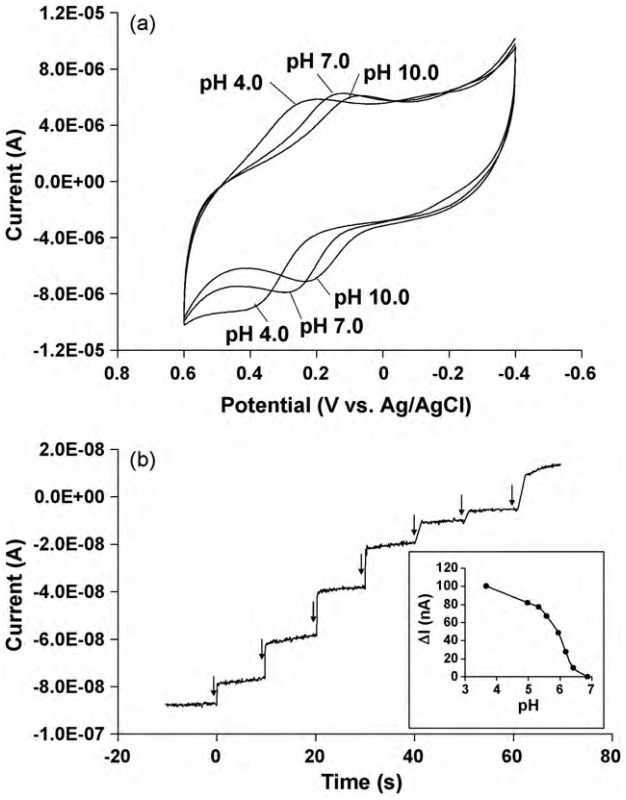

Fig. 2. Cyclic voltammograms (a) of the MWCNT–hematein–penicillinase electrode in 0.1 M PBS of different pH values and the amperometric response (b) at 200 mV to solution pH starting at pH 6.8 (1 mM PBS containing 0.1 M NaCl). The arrows indicate successive additions of 0.1 M HCl.

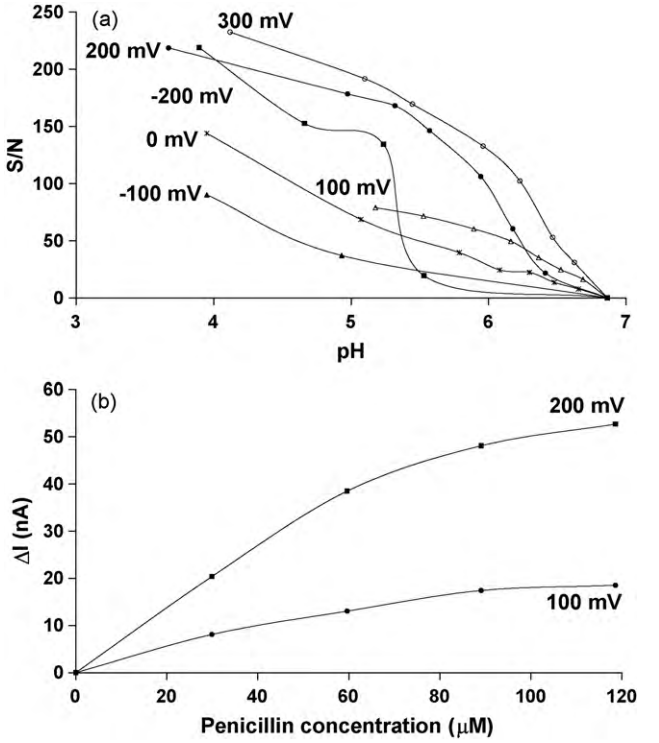

Fig. 3. Signal-to-noise ratios of the amperometric pH responses (a) and dependence of the current change on penicillin V concentration (b) of the MWCNT–hematein–penicillinase electrode at different working potentials.

and −200 mV, the reduction current increased (became more positively) with decreasing pH. Contrastively, at 0–300 mV, the oxidation current decreased (became less negatively) with decreasing pH.

Similar pH dependences of oxidation and reduction current changes were also observed by other researchers on soluble hematein [18] and hematein-modified platinum electrode [19], respectively. However, these previous studies had not compared the measurements of reduction and oxidation current changes. As indicated in Fig. 3a, measuring the change of oxidation current at 0–300 mV is more favorable in terms of signal-to-noise (S/N) ratios. The slope of the current change–pH plot basically increases with the increase of working potential in the range of 0–300 mV; nevertheless, the slope obtained at 200 mV is only slightly smaller than that obtained at 300 mV. In detection of penicillin V, the slope of the calibration graph obtained at 200 mV is approximately 3 times that obtained at 100 mV (Fig. 3b).

Accordingly, 200 mV was chosen as the working potential to ensure a high sensitivity while avoiding potential interference occurring at higher applied potentials. It is worth noting that when hematoxylin was used alone instead of the dye hematein as the pH-sensitive probe, the resulted electrode displayed a poor stability and failed to give a concentration-dependent response to penicillin V, probably due to the easy autooxidation of hematoxylin [34].

3.4. Selection of enzyme quantity

The effect of enzyme quantity used for the electrode modification is illustrated in Fig. 4. In the investigated range (60–1500 U/10 μL of penicillinase), the amperometric responses at the same analyte concentrations all increased with increasing enzyme quantity. It was also shown that the detection limit and dynamic detection range were significantly affected by the enzyme quantity. With an enzyme quantity of 60 or 300 U/10 μL, the resulted enzyme electrode could hardly discriminate among analyte concentrations of 50 nM to 50 μM and the former gave a relatively poor detection limit (50 μM). When an enzyme quantity of 600 or 1500 U/10 μL was used, both the dynamic detection range and detection limit were markedly improved. Finally, the enzyme quantity of 600 U/10 μL was selected as the detection limit and dynamic detection range obtained with this quantity were evidently superior to those obtained with 60 and 300 U/10 μL and comparable to those obtained with 1500 U/10 μL.

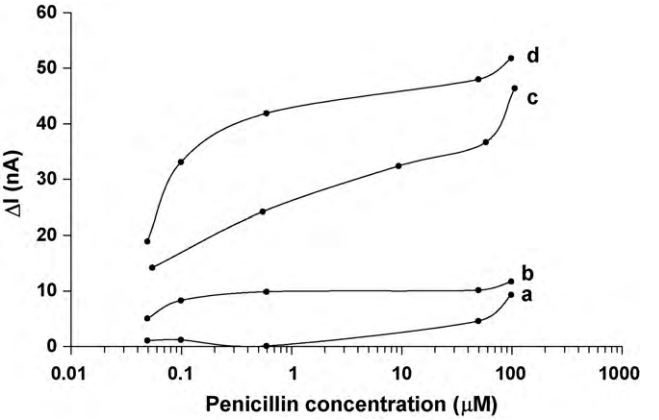

Fig. 4. Dependence of the amperometric responses on penicillin V concentration of the MWCNT–hematein–penicillinase electrodes prepared with different enzyme quantities: (a) 60 U/10 μL; (b) 300 U/10 μL; (c) 600 U/10 μL; (d) 1500 U/10 μL.

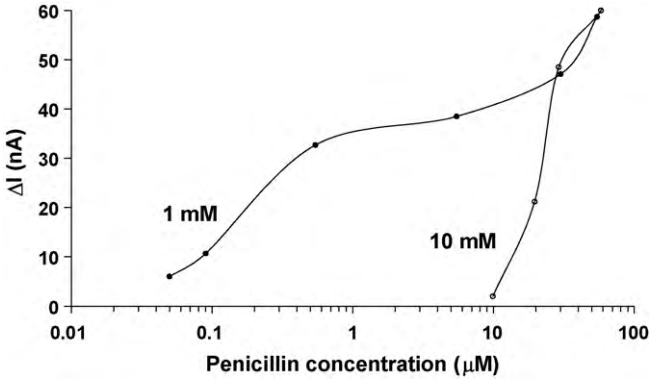

Fig. 5. Dependence of the amperometric response on penicillin V concentration of the MWCNT–hematein–penicillinase electrode in 1 and 10 mM PBS containing 0.1 M NaCl (pH 6.8).

3.5. Selection of buffer pH and concentration

It is well known that the buffer pH and concentration are important operating parameters for pH change-based biosensors. The pH for optimum activity of the immobilized penicillinase is in the range of 6.5–7.0 [8]. Consequently, a pH of 6.8 was chosen for the supporting buffer solution. The effect of PBS concentration was investigated in the range of 0.1–10 mM. As displayed in Fig. 5, 1 mM PBS is better in both detection limit and dynamic detection range when compared to 10 mM PBS. At a given analyte concentration, the amperometric response generally decreased with increasing PBS concentration. When the PBS concentration was decreased from 1 to 0.1 mM, the noise level increased severely and the signals became unstable. As a result, 1 mM pH 6.8 PBS containing 0.1 M NaCl was used as the supporting solution for the biosensor measurements.

3.6. Calibration characteristics and effect of the matrix of milk

Three different calibration curves are presented in Fig. 6a to demonstrate the analytical characteristics of the proposed biosensor and a control one for the detection of penicillin V spiked in PBS or milk samples. Curve 1 was obtained with the proposed MWCNT–hematein–penicillinase electrode for detection of 5.0 nM to 2.7 mM penicillin V spiked in PBS under the optimized conditions mentioned above. For clarity, the low concentration portion of the curve 1 is also plotted in a semi-log format with an inset showing the temporal response curve (Fig. 6b), from which the response time of the proposed electrode was determined to be <10 s. The calibration curve is nonlinear in the entire range investigated; the response increases with the increase in the analyte concentration in the range of 5.0 nM to 2.6 mM (1.9 ppb to 1.0×10^3 ppm) and reaches a plateau thereafter. In the range of 5.0 nM to 9.3 μM (1.9 ppb to 3.6 ppm), the response is linearly correlated to the logarithmic concentration of penicillin V. This is followed by two linear regions in the ranges of 9.3 μM to 1.0 mM (3.6 ppm to 3.9×10^2 ppm) and 1.0–2.6 mM (3.9×10^2 to 1.0×10^3 ppm), respectively. The response sensitivity, i.e., the slope of calibration graph, becomes smaller at higher analyte concentrations. This could be explained by the fact that the activity of the immobilized penicillinase can be inhibited by the acidic products of the enzymatic hydrolysis [8] when the penicillinase electrode is tested continuously in a static cell and the acidic products are accumulated to a certain high level. Within a certain detection range, the influence of the inhibition effect is negligible. With the criterion of $S/N \geq 3$, the lowest limit of detection was measured to be 50 nM (19 ppb, $S/N = 6$) from Fig. 6b.

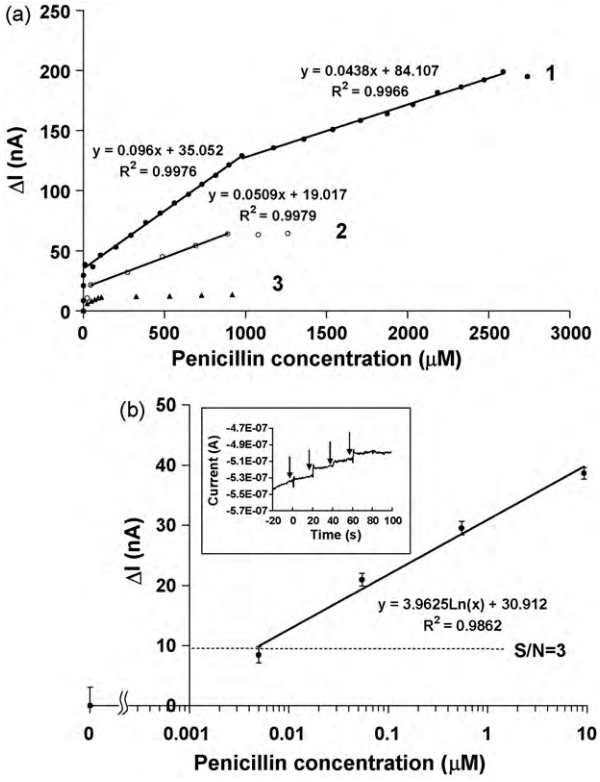

Fig. 6. (a) Calibration curves of the MWCNT–hematein–penicillinase electrode for detection of penicillin V in PBS (1) or milk (2) and of the hematein–penicillinase electrode for detection of penicillin V in PBS (3). (b) The low concentration portion of the calibration curve a1 shown in a semi-log format; the inset shows the temporal response curve, and the arrows indicate successive additions of penicillin V sample solutions. Working potential, 200 mV; supporting solution, 1 mM pH 6.8 PBS containing 0.1 M NaCl. Error bars represent standard deviations calculated from the temporal response curve.

Curve 2 was obtained with the MWCNT–hematein–penicillinase electrode for detection of penicillin V spiked in milk. In initial tests, severe interference was observed from the matrix of raw milk samples. This was most likely caused by the adsorption of milk proteins (e.g., casein) and fat onto the electrode surface, which could form a barrier between the immobilized enzyme and analyte molecules. Even with salting-out and centrifugation pretreatments, the interference from the matrix of milk was still obvious: the detection limit (24 μM), detection range (24 μM to 0.89 mM), and response sensitivity obtained were all notably inferior to those obtained in PBS. Such matrix effects of milk samples have been frequently observed on electrochemical biosensors [19,20,35]. The sample matrix effect observed in this study likely stemmed from the interference from residual milk proteins and other components. With the use of appropriate chemometric methods, e.g., the state-of-the-art multidimensional data projection techniques [36], the influence from the matrix may be able to be accounted for.

Curve 3 was obtained with the hematein–penicillinase electrode, which was prepared the same way as the MWCNT-based one except that no MWCNT was used, for detection of penicillin V spiked in PBS. The detection limit was 24 μM, more than two orders of magnitude higher than that of the MWCNT-based penicillinase electrode. Moreover, both the detection range (24 μM to 0.11 mM) and response sensitivity were apparently poorer than those of the MWCNT–hematein–penicillinase electrode. These results clearly demonstrate that MWCNTs play a critical role for the sensitivity enhancement: as an electron transfer enhancer and an efficient immobilization matrix, they can provide an excellent microenvironment for hematein molecules to sense a subtle change of the

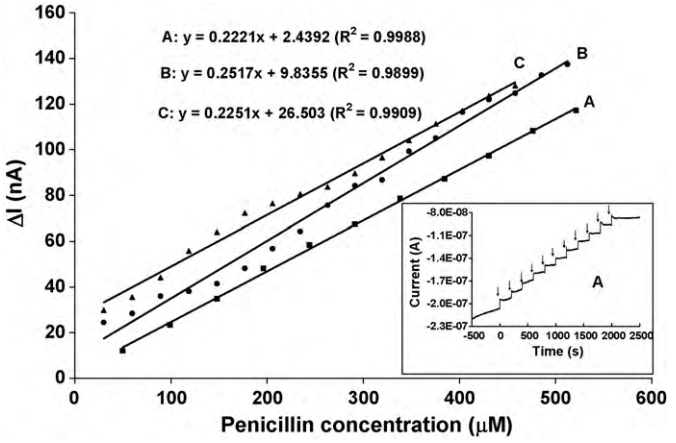

Fig. 7. Calibration graphs for amperometric detection of penicillin V in 1 mM, pH 6.8 PBS containing 0.1 M NaCl at 200 mV with three MWCNT–hematein–penicillinase electrodes (denoted as A, B, and C). The inset shows the temporal response curve of electrode A; the arrows indicate successive additions of penicillin V sample solutions.

local pH. In addition, as demonstrated in recent studies [37,38], CNTs have an important effect on the morphology of the LbL films through the formation of highly interconnected nano-networks, which can substantially improve the matrix connectivity and hence the sensitivity and response speed.

3.7. Reproducibility and selectivity

The electrode-to-electrode reproducibility is demonstrated in Fig. 7. Although the amperometric responses obtained at the same analyte concentrations varied somehow from electrode-to-electrode, the slopes of calibration graphs of individual electrodes, which were prepared using the same LbL procedure, were close to one another with a relative standard deviation (RSD) of 7.0%. The sample-to-sample variation of the response sensitivity of the same electrode was as small as 3.0% RSD ($n = 11$, Fig. 7-electrode A). The good inter- and intra-electrode reproducibility can be attributed to the excellent controllability of the LbL approach, as well as the robustness of the electrode coatings.

The selectivity of the present biosensor was investigated by comparing its responses to penicillin V, another β-lactam antibiotic (ampicillin), and four non-β-lactam antibiotics (namely roxithromycin, levofloxacin, clindamycin, and fluconazol) in PBS. At the same concentration of 50 μM, ampicillin and the four non-β-lactam antibiotics gave a relative response of 1.2, 0.03, 0.11, 0.13, and 0.19, respectively, when compared to the response of penicillin V. All the four non-β-lactam antibiotics, giving an S/N of <3 at 50 μM, did not interfere with the detection of penicillin V when its concentration was ≥50 nM. Hence, the biosensor has a good selectivity to β-lactam antibiotics. Like other amperometric biosensors, the present biosensor might be subject to the interference from certain electroactive species that can also be oxidized under the operating potential (200 mV), e.g., uric acid and ascorbic acid present in milk [39]. This could also be a part of the reasons why the performance of the biosensor in milk was worse than that in PBS.

4. Conclusions

This study has proven our hypothesis that MWCNT can facilitate the development of an amperometric biosensor with enhanced sensitivity for penicillin detection. A detection limit of 50 nM (19 ppb) was achieved in PBS with the proposed MWCNT–hematein–penicillinase electrode under the roughly optimized operating conditions. This detection limit is lower than those of the control electrode without MWCNT and most of the reported pH change-based biosensors by more than two orders of magnitude. Moreover, the MWCNT-based biosensor is obviously advantageous over the control one in both detection range and response sensitivity. The main limitation of the present biosensor is that like other pH change-based ones, it is vulnerable to the interference from the matrix of milk. Nevertheless, the detection limit obtained in pretreated milk samples with the present biosensor is still superior or at least comparable to those of the conventional potentiometric, conductometric, and optical ones, which were reported primarily for detection of penicillins in relatively simple aqueous samples. The present work suggests that the present biosensor could be a cost-effective alternative for sensitive detection of β-lactam antibiotics in water samples, but more studies are required to overcome the interference of sample matrices so as to extend the applications to milk and more complicated samples.

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