Highly sensitive flow injection detection of hydrogen peroxide with high throughput using a carbon nanofiber-modified electrode

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A highly sensitive method for the rapid flow injection amperometric detection of hydrogen peroxide is reported, which is based on the excellent catalytic activity of carbon nanofiber coated simply on an electrode towards the reduction of H_2O_2 and has high throughput and interesting potential for the development of rapid and highly sensitive biosensors.

The rapid and highly sensitive determination of biomoleclues is of great importance in clinic diagnoses, environment, food industries and defense areas. Over the past decades enormous progress for such needs has been made towards the development of the highly sensitive electrochemical biosensors by using efficient catalytic processes. Carbon nanotubes (CNTs) as the catalytic materials have attracted considerable attention in electrochemical sensing because of their remarkable electronic and mechanical properties.[1–3] However, the insolubility of CNTs in most solvents is a major obstacle in implementing their widespread use.[4] Compared with CNTs, the oxidation treatment of carbon nanofibers (CNFs) can produce a range of oxygen-containing groups without degradation of the structural integrity of its backbone,[5] leading to better dispersion and wettability.

CNFs have diameters varying from a few to hundreds of nanometers and lengths ranging from less than a micron to millimeters,[6] and possesses excellent mechanical characteristics such as high tensile strength and elastic modulus, and high thermal and electric conductivity.[7] Thus, well-dispersed CNFs can be conveniently coated onto a support surface to form a firm film as scaffold for protein immobilization.[8,9] The main distinguishing characteristic of CNFs from nanotubes is the stacking of graphene sheets of varying shapes, producing more edge sites on the outer wall of CNFs than CNTs,[10] which can facilitate the electron transfer of electroactive analytes. Based on this characteristic this work used soluble CNFs obtained by a simple oxidation treatment with acid to modify a glassy carbon electrode (GCE). The resulting modified electrode showed excellent catalytic activity towards the reduction of H_2O_2, thus a highly sensitive method for flow injection amperometric detection at a relatively low overpotential was proposed.

The selective and sensitive detection of H_2O_2 is becoming increasingly important because of its wide and various applications in several fields such as food, pharmaceutical, and environmental analysis.[11,12] H_2O_2 is also a product of the enzymatic reactions between most oxidases and their substrates, thus its detection is very interesting for the development of biosensors for oxidase substrates and monitoring the activity of oxidases. Compared with titrimetry,[13] spectrometry[14] and chemiluminescence,[15] electrochemistry has been proved to be a sensitive, inexpensive and effective way to examine H_2O_2.[16] However, high overpotentials are generally required on many electrode materials, placing the voltammetric wave in the same potential region as such common interferences as paracetamol, uric acid (UA) and ascorbic acid (AA). The proposed modified electrode has excellent stability and offered a significant decrease in the overpotential for the H_2O_2 reaction; thus, this method showed good selectivity and acceptable repeatability.

The CNFs were a gift from WPI (Sarasota, FL, USA). All reagents were of analytical reagent grade. All solutions were prepared with doubly distilled water. The CNFs were firstly treated with 30% HNO_3 and refluxed for 24 h at 140 °C. The solubility of the resulting CNFs and the carboxylic groups formed on their surface or their electrocatalytical activity depended on the concentration of HNO_3. At the concentration of 30% the obtained CNFs showed good solubility without any degradation of the structural integrity. The saturated concentration of the obtained CNFs was about 5 mg mL^{-1}. After a GCE was successively polished to a mirror finish using 0.3 and 0.05 μm alumina slurry (Beuhler) followed by rinsing thoroughly with doubly distilled water, 3.0 μL of CNF solution was dropped onto the GCE to form a uniform CNF film. Electrochemical measurements were performed in deoxygened solution on a CHI 660 electrochemical analyzer (CHI Co., USA) with a conventional three-electrode system comprised of platinum wire as the auxiliary, a saturated calomel electrode as reference and the modified GCE as working electrodes. The morphology of the CNFs was analyzed with a transmission electron microgram (TEM, JEOL-JEM-1005) and a scanning electron microscope (SEM, LEO 1530 VP).

The TEM image shows that the diameter for the soluble CNF is in the range of 30–50 nm (Fig. 1A). The nanofiber surface is

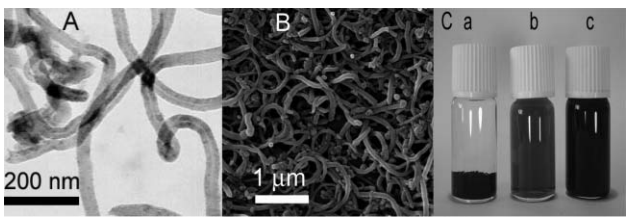

Fig. 1 TEM of CNFs (A), SEM of CNF film on an electrode surface (B), and photographs of vials containing untreated CNF (a), 0.1 (b) and 5 mg mL^{-1} (c) nitric acid-treated CNFs in water (C).

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smooth. After direct casting of the CNF solution onto the electrode surface a homogeneous and porous membrane can be obtained (Fig. 1B). The obtained porous membrane of CNFs possesses good stability and preparation reproducibility. Fig. 1C displays photographs of vials containing untreated CNFs (a), and nitric acid-treated CNFs in water (b, c). The untreated CNFs are insoluble in water, while the nitric acid-treated CNFs can completely dissolve in water – the obtained CNF solution is stable for a long time.

The charge transfer resistance, R_{ct}, of the formed film on the electrode surface greatly influences the electron transfer kinetics of the electroactive compounds. The value of R_{ct} can be measured with a redox probe, $[Fe(CN)_6]^{3-/4-}$. The bare GCE shows an R_{ct} value of 278 Ω, while the value at the CNF-film-modified GCE is only 48 Ω, implying that the CNF film accelerates the electron transfer of the electrochemical probe. This is ascribed to the good conductivity of the CNF film.

In the potential window from +0.8 to −0.6 V the CNF/GCE exhibits a pair of small redox peaks at −0.12 and −0.092 V at 0.01 V s^{-1}(curve c, Fig. 2), while no response is observed at the naked electrode (curve a). These peaks are ascribed to the reduction and oxidation of the oxygen-containing groups on the CNF surface. Upon addition of H_2O_2 the reduction current dramatically increases, which begins at −0.05 V and reaches a maximum current at the potential of −0.357 V, and the oxidation peak current of the oxygen-containing groups on the CNF surface decreases, while the naked electrode does not show any response to H_2O_2 (curves b and a, respectively, Fig. 2). From the changes in both the reduction and oxidation peak currents and the reduction potential upon addition of H_2O_2, it can be concluded that the increased oxidation response resulted from both the electrocatalytic action of the oxygen-containing groups to the reduction of H_2O_2 and the facilitation of electron transfer kinetics of the electroactive H_2O_2 by the edge sites on the outer wall of CNFs.[17] Thus, the CNFs offer a significant decrease in the overpotential for H_2O_2 reduction and allow convenient low-potential amperometric detection, similar to the observation at other CNT-modified electrodes.[18]

The amperometric experiments at various operation potentials indicate that the modified electrode has a good amperometric response to H_2O_2 at −0.3 V, which is used as the applied potential for the following determination. Fig. 3 displays the amperometric trace of the CNF film recorded at −0.3 V during the spiking of H_2O_2 aliquots into a stirred buffer solution. The trace illustrates that the modified electrode responds very rapidly to these changes in the H_2O_2 concentration, producing steady-state signals within 5 s. The response displays a linear range from 1.0 to 220 μM with a correlation coefficient of 0.999 and a slope of 19 nA μM^{-1}. The limit of detection is 0.15 μM at the signal-to-noise ratio of 3, which is much lower than those of 150 and 1.5 μM for GC/CNT- and GC/Pt nanoparticle-modified electrodes,[19] and even lower than that of 1.8 μM based on a europium-ion-based luminescent sensing probe.[20] The high sensitivity comes from the excellent catalytic activity of the carbon nanofibers, produced by both the oxygen-containing groups and the edge sites.

The sensor shows acceptable preparation reproducibility with a relative standard deviation of 5.2% for the current determined at 0.1 mM H_2O_2 at six different electrodes, which is ascribed to the good dispersion of the CNFs in the casting solution. No obvious decrease in the response to H_2O_2 is observed after six months of storage, indicating good storage stability. Repeated use of the electrodes does not affect the long-term stability. The coefficients of variation of the current signals for eight repeated injections of 9.0 and 101 μM H_2O_2 are 3.3 and 2.9%, respectively. When the modified electrode is immersed in continuously stirred pH 7.0 PBS containing 20 μM H_2O_2 it can remain highly stable for the amperometric response for at least 3 h (Fig. 4A). The good stability is ascribed to the formation of the firm film.

The biosensors are often interfered with by electroactive compounds which exist within samples. The interference test of the modified electrode is carried in pH 7.0 PBS containing 2.0 μM H_2O_2 in the presence of UA and AA (Fig. 4B). A well-defined H_2O_2 response is observed after its injection. However, the subsequent injections of 2.0 μM of UA, AA do not show an obvious additional response. It is obvious that the low overpotential for H_2O_2 detection at this electrode excludes the effects from interferents, indicating good selectivity.

The proposed modified electrode can be further used for flow injection detection of H_2O_2, which is very important for the

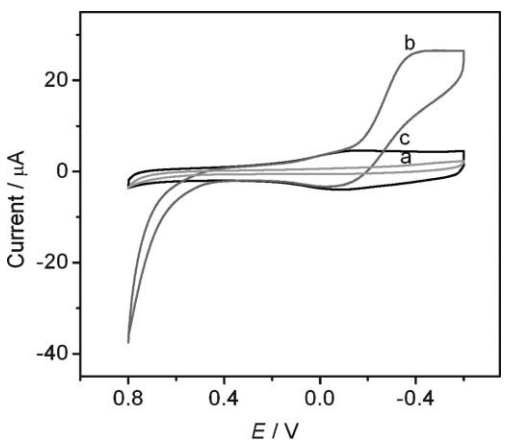

Fig. 2 Cyclic voltammograms of 5.0 mM H_2O_2 at bare (a) and CNF-modified GCE (b) in pH 7.0 PBS, and the CNF-modified GCE in pH 7.0 PBS (c). Scan rate: 0.01 V s^{-1}.

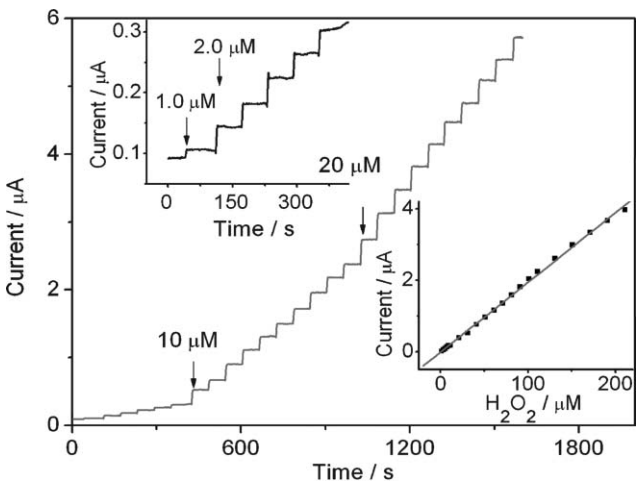

Fig. 3 Successive amperometric response of the CNF-modified GCE to H_2O_2 in pH 7.0 PBS at −0.3 V. The H_2O_2 addition each time is from 1.0 to 20 μM as indicated. Upper inset: amplified response curve; lower inset: linear calibration curve.

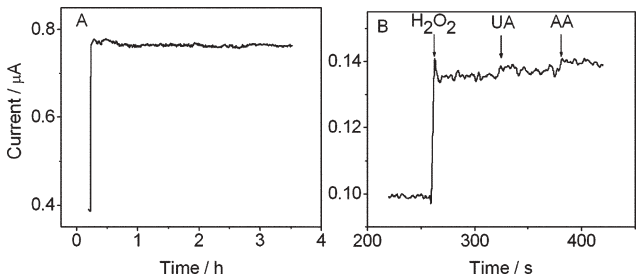

Fig. 4 Amperometric responses of CNF-modified GCE at −0.3 V to (A) 20 μM H_2O_2 in continuously stirred 0.2 M pH 7.0 PBS, and (B) additions of 2.0 μM H_2O_2, UA and AA in stirred pH 7.0 PBS.

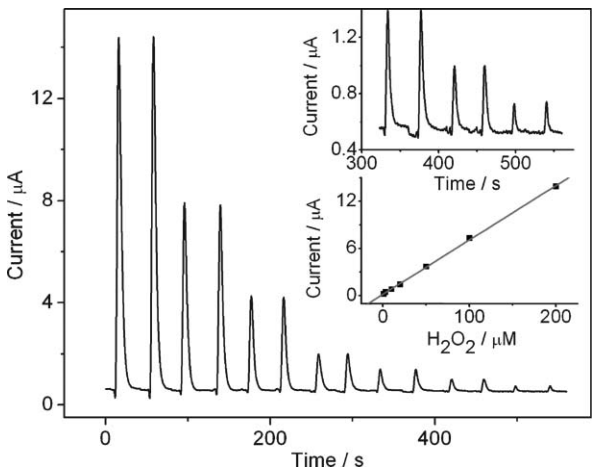

Fig. 5 Flow injection analysis with successive injections of 200, 100, 50, 20, 10, 3.0 and 1.0 μM H_2O_2 (from left to right) into pH 7.0 PBS at flow rate of 2.0 mL min^{-1} at −0.3 V. Upper inset: amplified response curve for injections of 10, 3.0 and 1.0 μM H_2O_2; lower inset: linear calibration curve.

development of an automated detection device. The flow injection analysis using 0.2 M pH 7.0 PBS as a carrier buffer is carried out at an applied potential of −0.3 V. The flow rate is optimized to be 2.0 mL min^{-1} based on the peak height and response time. As shown in Fig. 5, the amperometric response for flow injection detection of H_2O_2 increases linearly. The linear range is from 1.0 to 200 μM (R = 0.999), showing better analytical performance than that for continuous monitoring of H_2O_2 with aid of an enzyme or an enzyme mimetic.[21] From the linear slope a detection limit of 0.5 μM, corresponding to the signal-to-noise ratio of 3, can be obtained, indicating a high sensitivity for the flow injection detection. The amperometric responses of H_2O_2 show a half-width of 5 s with a base-width of 17 s (Fig. 5). Thus the sample throughput is more than 210 samples per hour.

In conclusion, soluble CNFs can be conveniently used for the preparation of a CNF-modified electrode, which shows good preparation reproducibility and stability. The modified electrode has been proved to have excellent catalytic activity towards the reduction of H_2O_2, which leads to high sensitivity and good selectivity and can be used for continuous monitoring of H_2O_2 without the need for an enzyme (such as peroxidase) or enzyme mimic. The proposed method for rapid flow injection amperometric detection of hydrogen peroxide is low-cost and high-throughput. The greatly enhanced reduction activity of H_2O_2 makes the CNFs extremely attractive for the development of pertinent oxidase-based amperometric biosensors.

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