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 nanofibers. The modified electrode showed excellent selectivity and acceptable reproducibility.

The selective and sensitive detection of hydrogen peroxide is becoming increasingly important because of its wide and various applications in several fields such as food, pharmaceutical, and environmental analysis. Hydrogen peroxide 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 titrimation, spectrometry and chemiluminescence, electrochemistry has been proved to be a sensitive, inexpensive and effective way to examine hydrogen peroxide. 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 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 first treated with 30% HNO3 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 HNO3. The concentration of 30% of 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 deoxygenated 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...
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, $[[\text{Fe(CN)}_6]^3/4^-]$. The bare GCE shows an $R_{ct}$ value of 278 $\Omega$, while the value at the CNF-film-modified GCE is only 48 $\Omega$, 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$_2$O$_2$ 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$_2$O$_2$ (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$_2$O$_2$, 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$_2$O$_2$ and the facilitation of electron transfer kinetics of the electroactive H$_2$O$_2$ by the edge sites on the outer wall of CNFs. Thus, the CNFs offer a significant decrease in the overpotential for H$_2$O$_2$ reduction and allow convenient low-potential amperometric detection, similar to the observation at other CNT-modified electrodes.

The amperometric experiments at various operation potentials indicate that the modified electrode has a good amperometric response to H$_2$O$_2$ 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$_2$O$_2$ aliquots into a stirred buffer solution. The trace illustrates that the modified electrode responds very rapidly to these changes in the H$_2$O$_2$ concentration, producing steady-state signals within 5 s. The response displays a linear range from 1.0 to 220 m$\mu$M with a correlation coefficient of 0.999 and a slope of 19 nA m$^{-1}$. The limit of detection is 0.15 m$\mu$M at the signal-to-noise ratio of 3, which is much lower than those of 150 and 1.5 m$\mu$M for GC/CNT- and GC/Pt nanoparticle-modified electrodes, and even lower than that of 1.8 m$\mu$M based on a europium-ion-based luminescent sensing probe. 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$_2$O$_2$ 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$_2$O$_2$ 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 m$\mu$M H$_2$O$_2$ are 3.3 and 2.9%, respectively. When the modified electrode is immersed in continuously stirred pH 7.0 PBS containing 20 m$\mu$M H$_2$O$_2$ 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 m$\mu$M H$_2$O$_2$ in the presence of UA and AA (Fig. 4B). A well-defined H$_2$O$_2$ response is observed after its injection. However, the subsequent injections of 2.0 m$\mu$M of UA, AA do not show an obvious additional response. It is obvious that the low overpotential for H$_2$O$_2$ 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$_2$O$_2$, which is very important for the

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Fig. 2 Cyclic voltammograms of 5.0 mM H$_2$O$_2$ 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$_2$O$_2$ in pH 7.0 PBS at $-0.3$ V. The H$_2$O$_2$ addition each time is from 1.0 to 20 m$\mu$M as indicated. Upper inset: amplified response curve; 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 H₂O₂ increases linearly. The linear range is from 1.0 to 200 μM (R = 0.9999), showing better analytical performance than that for continuous monitoring of H₂O₂ with aid of an enzyme or an enzyme mimic.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 H₂O₂ 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 H₂O₂, which leads to high sensitivity and good selectivity and can be used for continuous monitoring of H₂O₂ 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 H₂O₂ makes the CNFs extremely attractive for the development of pertinent oxidase-based amperometric biosensors.

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Notes and references