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DNA biosensor based on chitosan film doped with carbon nanotubes

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Abstract

A biosensor based on chitosan doped with carbon nanotube (CNT) was fabricated to detect salmon sperm DNA. Methylene blue (MB) was employed as a DNA indicator. It was found that CNTs can enhance the electroactive surface area threefold (0.28 ± 0.03 and $0.093 \pm 0.06\ cm^2$ for chitosan–CNT- and chitosan-modified electrodes, respectively) and can accelerate the rate of electron transfer between the redox-active MB and the electrode. A low detection limit of 0.252 nM fish sperm DNA was achieved, and no interference was found in the presence of 5 μg/ml human serum albumin. The differential pulse voltammetry signal of MB was linear over the fish sperm DNA concentration range of 0.5–20 nM.
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Keywords: Chitosan; Carbon nanotubes; Fish sperm DNA; Methylene blue; Differential pulse voltammetry

Carbon nanotubes (CNTs)[1] consisting of cylindrical graphite sheets with nanometer diameter are relatively novel materials showing many attractive properties, including improved electrochemical behavior [1,2]. There are two kinds of CNTs with different structures: single-wall carbon nanotubes (SWNTs) and multiwall carbon nanotubes (MWNTs). SWNTs have diameters of 1–2 nm and are made from a single graphite sheet rolled flawlessly, whereas MWNTs are composed of homocentric and closed graphite tubules with diameters varying from 2 to 50 nm and an interlay distance of approximately 0.34 nm. Since the discovery of CNTs in 1991 [3], considerable efforts have been made to study the application of this new material. Because of their special structural feature and its unique electronic and mechanical properties, CNTs are widely used in electrochemical investigations, for example, in direct electrochemistry of proteins [4], construction of electrochemical sensors and biosensors [5,6], electrocatalysis [7,8], and electrode materials in batteries [9].

DNA correlated with the genic diseases is highly important for human life. Various techniques, including quartz crystal microbalance [10], electrochemical [11], fluorescent [12], electrophoretic [13], electrochemiluminescence [14], enzymatic [15], surface plasmon resonance [16], and atomic force microscopy [17], have been developed to detect DNA hybridization. However, the electrochemical method has attracted more attention than other methods because it is simple, rapid, highly sensitive, and cheap. Thus, the immobilization of DNA to the surface of the working electrode is a significant step in the fabrication of the DNA biosensor. CNTs can be employed to bind with DNA through covalent bond or electrostatic attraction [18,19]. However, lack of stability and uniformity of CNTs directly immobilized onto the electrode surface without other reagents can be a problem. Chitosan, electrodeposited zirconia thin film, and cetyltrimethyl ammonium bromide have also been

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[1] *Abbreviations used:* CNT, carbon nanotube; SWNT, single-wall carbon nanotube; MWNT, multiwall carbon nanotube; MB, methylene blue; EDTA, ethylenediamine tetraacetic acid; CV, cyclic voltammetry; DPV, differential pulse voltammetry; UV–vis, UV–visible; HSA, human serum albumin.

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applied to immobilize DNA [20–22], although the conductivity of these films has been less than ideal, leading to the conclusion that the electron transfer may be retarded to some extent. To solve this problem, functionalized conductive polymers were electropolymerized on the electrode surface to detect DNA hybridization directly [23–25]. Nevertheless, the synthesis of the functionalized monomers has always been complicated, limiting its application. Therefore, films were doped with CNTs to improve conductivity. The sol-gel-derived ceramic CNT-modified electrode exhibits a tunable electrode dimension, depending on the amount of CNTs, and can catalyze the oxidation of ascorbic acid or glutathione in both reduced and oxidized forms [26]. Chitosan films mixed with CNTs have been used to develop a biosensor based on dehydrogenase enzyme [27]. However, whether chitosan–CNT film can be used to enlarge the electrochemical signal of the DNA indicator and increase sensitivity for DNA detection has not been investigated previously.

In this study, CNTs wrapped by chitosan film were immobilized on the surface of graphite electrodes. Because chitosan–CNTs can form a stable complex through noncovalent binding, the stability of CNTs in aqueous chitosan solution was greatly improved. Thus, CNTs could be uniformly distributed in the chitosan film. Using fish sperm DNA as the model and methylene blue (MB) as the indicator, the properties of the chitosan–CNT system were characterized by electrochemical methods. The interference of human serum albumin was also investigated.

Materials and methods

Chemicals and solutions

MWNTs were purchased from Tsinghua University. Chitosan was obtained from Shanghai Yuanju Biocompany (China). MB was purchased from Beijing Chemical Reagent (China). Fish sperm DNA was obtained from AMRESCO (Solon, OH, USA), and human serum albumin was purchased from Shanghai Biological (China). In the experiment, 40 mM Tris–HCl buffer (pH 8.0) and 10 mM Tris–HCl + 1.0 mM ethylenediamine tetraacetic acid (EDTA, TE buffer, pH 8.0) were used. Other chemicals were commercially available, and all were of analytical reagent grade. Double distilled water was used.

Instrumentation

A CHI 660B electrochemical analyzer (Chenhua, China) was employed to perform cyclic voltammetry (CV) and differential pulse voltammetry (DPV). A DU-800 spectrophotometer (Beckman, USA) was used to determine the DNA concentration. The three-electrode system, containing a working electrode made of graphite, an Ag/AgCl reference electrode, and a platinum foil as a counterelectrode, was employed.

Preparation of chitosan–CNT film on graphite electrode

A 2% chitosan solution was prepared in 1% acetic acid. MWNTs were refluxed in concentrated nitric acid for 5 h, were filtered and washed with double distilled water until the filtrate was neutral, and finally were dried under vacuum. Then, a different amount of CNTs (0.1–1.5 mg/ml) was dissolved in chitosan solution under 30 min sonication. Chitosan–CNT solution (10 μl) was spread uniformly to the freshly smoothed graphite electrode surface. After the solution was dried in air, the graphite electrode was immersed in 0.1 M NaOH for 30 min to make the film more stable. Then, the electrode was rinsed with double distilled water three times and air-dried. In this way, a robust chitosan film doped with CNTs was formed.

Fish sperm DNA immobilization

All fish sperm DNA solutions were prepared in TE buffer. The modified graphite electrode was immersed in the DNA solution under stirring for 2 h at room temperature (~20 °C). Then, the electrode was washed with TE buffer three times and immersed in 2.0×10^{-5} M MB solution containing 20 mM NaCl. After the accumulation of MB for 60 min, the electrode was rinsed with 40 mM Tris–HCl buffer three times to remove the nonspecifically adsorbed MB.

Electrochemical detection

CV experiments were carried out in 5 mM $K_3Fe(CN)_6$ + 5 mM $K_4Fe(CN)_6$ with 0.1 M KCl as supporting electrolyte or 40 mM Tris–HCl buffer. The effective area of the electrode was determined by CV in 20 mM $K_4Fe(CN)_6$ + 0.2 M KCl. DPV was performed in MB-free 40 mM Tris–HCl buffer between −0.6 and 0.2 V with the amplitude of 50 mV.

UV–visible spectrophotometric detection

UV–visible (UV–vis) spectrophotometric detection was carried out using a DU-800 spectrophotometer. To evaluate the stability of the film, the modified graphite electrodes were immersed in 2 ml of 0.1 M NaOH or 0.1 M HCl aqueous solution and incubated at room temperature for 60 min under shaking, and then the UV–vis spectra of the solutions were recorded. After the adsorption of DNA to the modified electrode, the graphite electrode was put in 2 ml of 0.1 M NaOH or 1% acetic acid aqueous solution. Then, the absorbance

between 220 and 400 nm was recorded. The double-stranded fish sperm DNA concentration was determined spectrophotometrically using the known molar absorption coefficient 6600 $(mol/ml)^{-1}$ cm^{-1} at 260 nm (per P or nucleotide unit) [28].

Results and discussion

Characterization of the chitosan–CNT film

MWNTs are not soluble in water and acid solution due to the aggregation caused by hydrophobic interactions and van der Waals attractive force between the nanotubes. However, CNTs would be hydrophilic after treatment with concentrated nitric acid because the –COOH and –OH groups are generated on the surface of CNTs. In these experiments, it was found that the aqueous chitosan–CNT solution was black and that the color remained for a long time. As a cationic biopolymer, chitosan can absorb anionic chemicals such as CNTs containing –COOH groups. Hence, CNTs could be dispersed uniformly in aqueous solution of chitosan and the stability of CNT solution was greatly improved.

Fig. 1 demonstrates the steady-state CVs for the bare, chitosan-modified, and chitosan–CNT-modified (concentration of CNTs in chitosan solution was 1.2 mg/ml) graphite electrodes in 20 mM $[Fe(CN)_6]^{4-}$ containing 0.2 M KCl at a scan rate of 20 mVs^{-}1 versus Ag/AgCl (saturated KCl) reference electrode. Well-defined reduction and oxidation peaks were found for the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox couples. However, the reversibility of the redox probes was not good at the freshly smoothed graphite electrode. Because chitosan can adsorb negatively charged $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ through electrostatic adsorption, the peak current of the chitosan-modified graphite electrode was greater than that of the bare graphite electrode. Although the positive charge of chitosan can be compensated to some extent in the presence of –COOH groups on the surface of CNTs, the peak current of $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ did not decrease, indicating that CNTs can accelerate the electron transfer between redox probes and the electrode. At the same time, the background current was also enlarged, showing that the electroactive surface can be enhanced by CNTs. According to the Randles–Sevcik equation [29], the electroactive surface area of the chitosan- and chitosan–CNT-modified graphite electrodes could be calculated as

$$I_p = 2.69 \times 10^5 A D^{1/2} n^{3/2} \gamma^{1/2} C, \quad (1)$$

where I_p is the cationic peak current (A), A is the electroactive area (cm^2), D is the diffusion coefficient of the molecule in solution ($cm^2\ s^{-1}$), n is the number of electrons transferred in the redox course, γ is the potential scan rate ($V\ s^{-1}$), and C is the concentration of the probe chemical in the bulk solution ($mol\ cm^{-3}$). For 20 mM $[Fe(CN)_6]^{4-}$, the diffusion coefficient (D) is $(6.70 \pm 0.02) \times 10^{-6}\ cm^2\ s^{-1}$. Consequently, the average electroactive areas of chitosan–CNT- and chitosan-modified graphite electrodes were estimated as (0.28 ± 0.03) and $(0.093 \pm 0.06)\ cm^2$, respectively, indicating that a close electrical contact existed between chitosan–CNTs and the graphite electrode. In contrast, bare graphite electrodes give less active surface area than do modified electrodes. After modification with CNTs, the property of the electrodes was greatly improved because CNTs were of large surface area and could also accelerate the electron transfer between the redox couple in bulk solution and the electrodes.

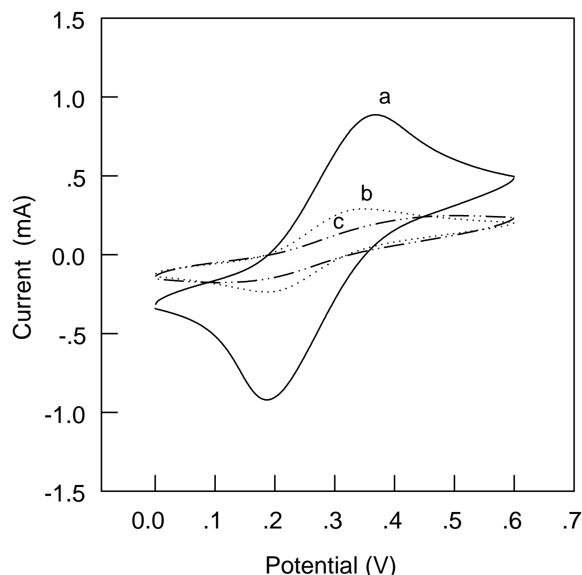

Fig. 1. Estimation of electroactive surface area by CV in 20 mM $K_4Fe(CN)_6$ and 0.2 M KCl at 20 mV s^{-1} versus Ag/AgCl reference electrode: (a) chitosan–CNT-modified graphite electrode, (b) chitosan-modified graphite electrode, and (c) bare graphite electrode.

Adsorption time for fish sperm DNA

When DNA was adsorbed on the chitosan–CNT film, the cathodic peak current of the $[Fe(CN)_6]^{3-}/[Fe(CN)_6]^{4-}$ redox probe decreased because DNA with a negative charge could retard the redox probes to the surface of graphite electrode. Thus, the decrease of the peak current of ferricyanide indicated the formation of the DNA/chitosan–CNT film modified at the graphite electrode surface. Fig. 2 shows the relationship between the adsorption time for DNA and the decrease of the anodic and cathodic peak currents of $[Fe(CN)_6]^{3-} + [Fe(CN)_6]^{4-}$. It is clear that the anodic and cathodic peak currents decreased drastically for immersion times in 20 nM DNA solution that were less than 2 h. When the adsorption times were more than 2 h, no further decrease of peak currents was observed,

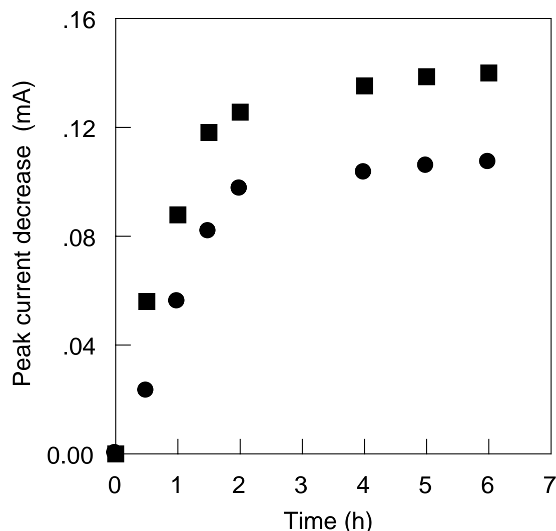

Fig. 2. Relationship between DNA adsorption time and peak current of CV in 5 mM $K_3Fe(CN)_6$ + 5 mM $K_4Fe(CN)_6$. ■, anodic peak current decrease; ●, cathodic peak current decrease. Supporting electrolyte: 0.1 M KCl; DNA concentration: 20 nM; scan rate: 50 mV s^{-1} versus Ag/AgCl reference electrode.

indicating that no more adsorption occurred. In this work, 2 h was selected to immobilize DNA to the chitosan–CNT film.

Effect of MB concentration

The concentration of MB used to label the immobilized DNA affected the quantity of MB accumulated on DNA. Therefore, the sensitivity of the sensor can be influenced by MB concentration. After the adsorption of DNA to the chitosan–CNT-modified graphite electrode, the electrodes were immersed in MB solutions with different MB concentrations. Fig. 3 demonstrates the DPV signals of MB with different MB concentrations for labeling fish sperm DNA. It is clear that the DPV signals do not increase significantly when the concentration of MB is greater than 20 μM. The accumulation of MB with diverse concentrations to DNA had an isotherm-like shape. From a fit of these data to the Langmuir model, a surface-binding constant of 3.50×10^5 M^{-1} was obtained:

$$\theta = ap/(1 + ap), \quad (2)$$

where θ is the surface coverage, a is the surface-binding constant, and p is the MB concentration. The fit curve is also presented in Fig. 3. It can be seen that the fit data are very similar to the experimental data, indicating that the Langmuir model is suited for describing the adsorptive behavior of MB to DNA.

Effect of accumulation time for MB binding to DNA

Accumulation time was another factor influencing the sensitivity of the sensor. The chitosan–CNT-modified graphite electrodes were dipped in 10 nM DNA solution for 2 h and then in 20 μM MB aqueous solution containing 20 mM NaCl for different time periods. The DPV signals of MB at approximately −0.2 V (vs. Ag/AgCl) in MB-free Tris–HCl buffer were recorded correspondingly. Fig. 4 shows the relation between the cathodic peak current of MB by DPV and the accumulation time. It can be seen that the signals did not increase when the accumulation time was longer than 60 min. At shorter times, the cathodic peak currents

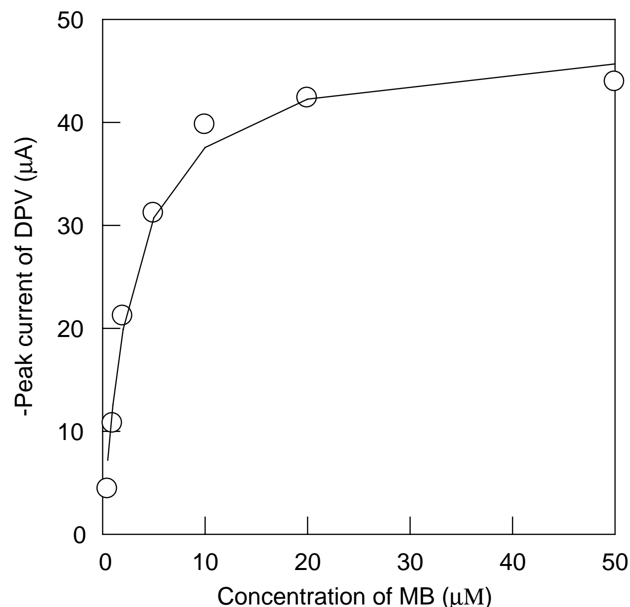

Fig. 3. Effect of MB concentration on DPV signal of MB. ○, experimental data; —, fitting curve. DNA concentration: 20 nM; incubation time for DNA/chitosan binding: 2 h; adsorption time of MB on DNA: 40 min.

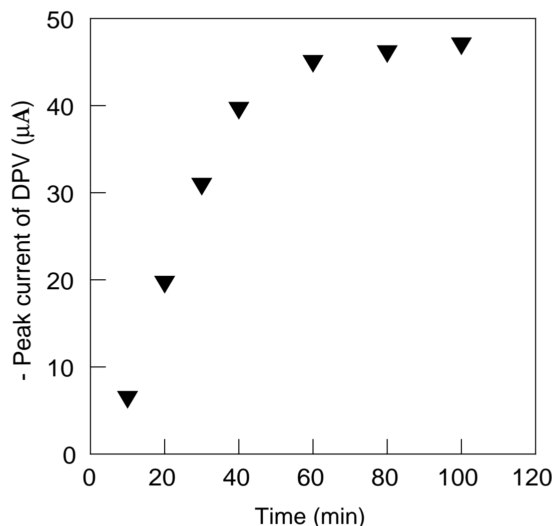

Fig. 4. Effect of adsorption time of MB to DNA/chitosan–CNT film. MB concentration: 20 μM.

changed dramatically because the adsorption of MB to the DNA was uncompleted and many adsorption sites for MB existed in double-stranded DNA. However, MB molecules intercalated to DNA cannot increase infinitely because binding sites in DNA are limited by DNA structure. Therefore, 60 min was selected as the optimal accumulation time.

Stability of chitosan–CNT film

The chitosan–CNT-modified graphite electrodes were immersed in 0.1 M NaOH and 0.1 M HCl under stirring for 60 min each. After the treatment, no visible changes were found in the solution or on the surface of graphite electrodes; in particular, no black precipitates were generated in aqueous solution, indicating that the CNTs were well immobilized in the chitosan film and could not flake off from the surface of modified graphite electrodes. It can also be concluded that the chitosan film was very stable in 0.1 M NaOH or HCl aqueous solution. However, the chitosan film can be destroyed by 1% acetic acid solution. If the chitosan–CNT-modified graphite electrode was incubated in 1% acetic acid solution for 60 min, the film was dissolved and black particles were suspended in the solution because chitosan is soluble in 1% acetic acid aqueous solution. These facts were verified by UV–vis spectrophotometry using CNT absorption at 230 nm as a criterion [30]. No CNT absorption at approximately 230 nm was observed in 0.1 M NaOH or HCl incubated with the chitosan–CNT-modified electrodes. However, a UV absorption peak at 230 nm appeared in 1% acetic acid (data not shown) treated with chitosan–CNT film, demonstrating that CNT flaked off from the electrode surface.

Stability of DNA/chitosan–CNT/graphite electrodes

DNA and other polyanions can be effectively immobilized on the surface of the polycationic polymer film of chitosan through electrostatic attraction [20]. The stability of the DNA/chitosan–CNT complex was also investigated by UV absorption of DNA after the electrodes were treated with 0.1 M NaOH, 0.1 M HCl, 40 mM Tris–HCl buffer (pH 8.0), and 1% acetic acid for 60 min each. Hardly any DNA absorption at 260 nm was observed using 0.1 M HCl solution or 40 mM Tris–HCl buffer as baseline correction, showing that the DNA/chitosan complex was stable in 0.1 M HCl or 40 mM Tris–HCl buffer and that CNTs could not affect the stability of the DNA/chitosan complex. However, the DNA/chitosan system was unstable in 0.1 M NaOH, demonstrating that electrostatic attraction between DNA and chitosan could be destroyed by a high pH. This phenomenon may suggest that the electrode can be refreshed by rinsing with alkali solution. Fig. 5 shows the UV spectra of CNTs and chitosan

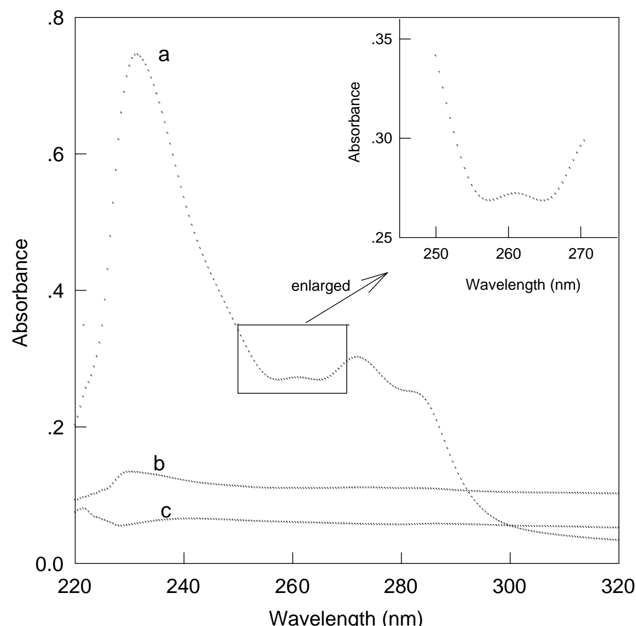

Fig. 5. UV absorption spectra recorded in 1% acetic acid solution incubated with DNA/chitosan–CNT-modified graphite electrode for 1 h (a), 1% acetic acid solution of CNTs (b), and 1% acetic acid solution of chitosan (c). Inset: UV absorption spectra of trace (a) between 250 and 270 nm.

in 1% acetic acid and the aqueous acetic acid after incubation with DNA/chitosan–CNT electrodes. A new absorption at approximately 270 nm was found if the DNA/chitosan–CNT-modified electrodes were incubated in 1% acetic acid for 60 min, whereas a peak at approximately 260 nm remained. This may be explained as follows. Most of the DNA can form a complex with chitosan in aqueous acetic acid solution, resulting in the DNA absorption shift from 260 to 270 nm. However, this DNA/chitosan complex can be dissociated partly in the presence of acetic acid; hence, the DNA absorption at approximately 260 nm remained. Because the absorbance at 260 nm was much lower than that at 270 nm, it can be concluded that the DNA/chitosan complex was rather stable. Nevertheless, no absorption at 250–280 nm was observed when the chitosan–CNT-modified electrodes were treated with 1% acetic acid for 60 min.

Electrocatalysis of CNT doped into chitosan film

MB can intercalate into double-stranded DNA as an indicator. To improve the DNA detection sensitivity, CNTs were mixed in the chitosan film. Fig. 6 illustrates the cyclic voltammograms of MB accumulated on chitosan, DNA/chitosan, and DNA/chitosan–CNT-modified graphite electrodes in 40 mM Tris–HCl buffer each. No redox peaks were observed when chitosan-modified electrodes were treated with MB, indicating that nonspecific adsorption of MB can be avoided using the given

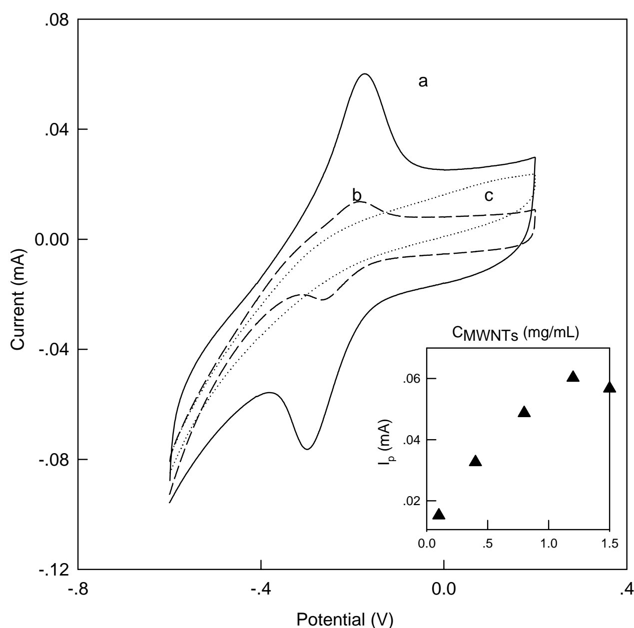

Fig. 6. Cyclic voltammograms of 40 mM Tris–HCl at a graphite electrode modified with MB/DNA/chitosan–CNT (a), MB/DNA/chitosan (b), and chitosan (c). Inset: Influence of CNT concentration in chitosan solution on anodic peak current of MB. CNT concentrations: 0.1, 0.4, 0.8, 1.2, and 1.5 mg/ml.

procedure. In contrast, MB can give redox peaks on the DNA/chitosan-modified electrodes as a result of the binding of MB with DNA. However, the chitosan–CNT-modified graphite electrodes can give more distinct signals than can the DNA/chitosan-modified graphite electrodes. Furthermore, the background current is also enlarged, showing that CNTs mingled with chitosan can increase the electroactive area of the modified electrodes. Because the CNTs contacted to graphite electrodes closely, the new channels for electron transfer between the electroactive substances and the electrodes can be formed; therefore, the rate of heterogeneous electron transfer can be increased. However, as can be seen in Fig. 6, the amount of CNTs can greatly affect the sensitivity of the sensors (the relative standard deviations were <8%, $n = 4$). The maximum anodic peak current (I_p) was found when the concentration of CNTs in chitosan solution was 1.2 mg/ml, whereas I_p began to drop at the CNT concentration of 1.5 mg/ml. Although CNTs can enlarge the signal of MB, positive charges of chitosan can also be compensated by –COOH groups on the surface of CNTs. Therefore, the amount of DNA immobilized to the film will be affected by the presence of CNTs, causing changes of electrochemical signals of MB. However, if the amount of CNTs immobilized on the film was too small, such as 0.1 mg/ml in chitosan solution, the electrocatalytical function of CNT was not obvious; the anodic peak current of MB was 0.0152 and 0.0137 mA for CNT–chitosan-modified graphite electrode and chitosan graphite electrode, respectively. It is reasonable that a small quantity of CNTs cannot offer enough electron transfer channels between MB and the graphite electrode. Therefore, it can be inferred that CNTs not only increase the electroactive surface area of the graphite electrode but also shorten the distance between the DNA and the graphite electrode.

Electrochemical detection of fish sperm DNA using chitosan–CNT film

To electrochemically detect DNA based on the chitosan–CNT film, fish sperm double-stranded DNA was used. Chitosan can effectively immobilize single- or double-stranded DNA through attachment of the phosphate group at the 5′ end of DNA with the $–NH_2$ group of chitosan. In this study, chitosan film similarly showed good affinity for double-stranded DNA as well as single-stranded DNA [20]. The voltammetric signal of MB at the electrode surface can indirectly reflect the quantity of DNA immobilized onto chitosan film, which was closely correlated with the DNA concentration. Fig. 7 shows the results of DNA detection. The

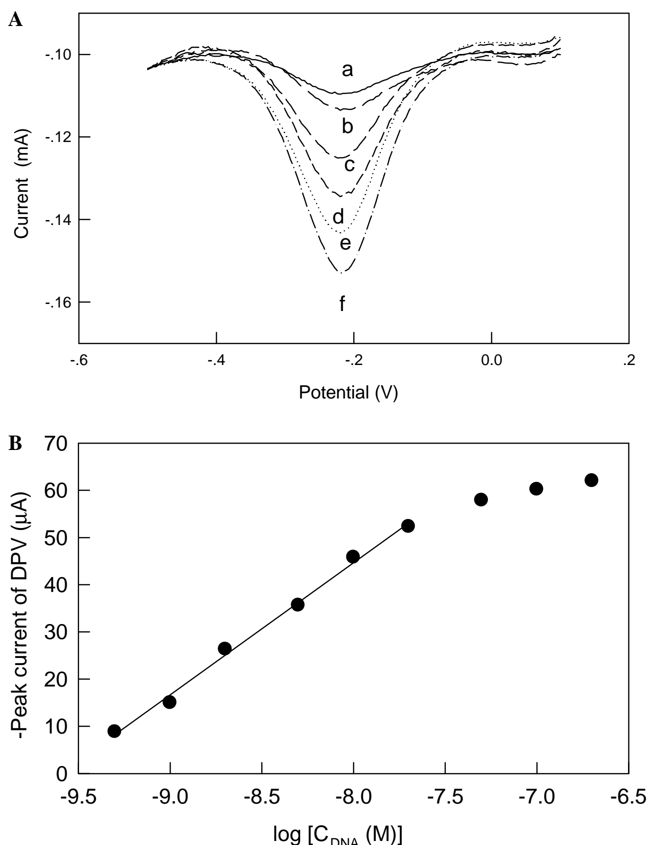

Fig. 7. (A) Differential pulse voltammograms using 20 μM MB as DNA indicator for different DNA concentrations: (a) 0.5 nM, (b) 1 nM, (c) 2 nM, (d) 5 nM, (e) 10 nM, and (f) 20 nM. (B) Logarithmic standard plot of DPV signal to fish sperm DNA concentration. DNA incubation time: 2 h; MB incubation time: 60 min.

logarithmic value of DNA concentration between 0.5 and 20 nM is linear with the DPV signal of MB. The regression equation was

$$-y = 27.976 \log x + 268.43, \quad (3)$$

where y is the DPV peak current of MB (μA) and x is the concentration of fish sperm double-stranded DNA (M). The regression coefficient (r) of the linear part was 0.9953. The detection limit is estimated as 0.252 nM for fish sperm DNA using 3 SD, where SD is the standard deviation of the blank solution ($n = 4$).

Recovery and interference of the sensor for fish sperm DNA

The recovery of this DNA sensor was also investigated. The chitosan–CNT-modified graphite electrodes were immersed in various standard DNA solutions under the above experimental conditions. After labeling with MB, voltammetric signals of MB were recorded and the concentration of DNA was calculated by Eq. (3). The results are listed in Table 1. The recovery was between 89 and 106% at different DNA concentrations examined, indicating that the chitosan–CNT film was suitable for the detection of DNA. We also studied the interference of human serum albumin (HSA) on this DNA sensor. To the fish sperm DNA solutions of different concentrations, 5 μg/ml HSA was added. Table 2 gives the results. The recovery was between 90 and 104%, showing that HSA cannot be specifically adsorbed onto the chitosan–CNT film and does not affect the performance of the sensor for DNA detection. Therefore, this sensor may be used to detect the trace DNA in serum.

Table 1
Recovery of DNA determination with biosensor based on chitosan–CNT system

C_{DNA} added (nM)	C_{DNA} found (nM)	Recovery (%)	Mean recovery (%)
0.60	0.6333	105.60	98.43
1.25	1.2625	101.00	
5.75	5.6417	98.11	
8.00	7.1200	89.00	
12.50	13.1970	105.58	

Note. MB concentration: 20 μM; accumulation time for MB: 60 min; DNA incubation time: 2 h.

Table 2
Study on interference of HSA on DNA sensor

C_{DNA} added (nM)	C_{DNA} found (nM)	Recovery (%)	Mean recovery (%)
0.60	0.5881	98.02	96.40
1.25	1.2415	99.32	
3.25	2.9268	90.06	
5.75	5.9360	103.23	
12.50	11.4230	91.38	

Note. HSA concentration: 5 μg/ml; MB concentration: 20 μM; accumulation time for MB: 60 min; DNA incubation time: 2 h.

Conclusion

In this study, chitosan–CNT film employed for DNA detection was prepared on the surface of graphite electrodes. The solubility of MWNTs was enhanced in the presence of chitosan, and the stability of CNTs was also improved. Under high ionic strength, black sediment was found in the chitosan–CNT solution, identifying the chitosan–CNT system as a colloidal suspension of charged particles. CNTs can be fixed tightly to the surface of the graphite electrode through chitosan. The chitosan–CNT film was nondestructive in 0.1 M HCl or NaOH. CNTs doped into the chitosan film not only can increase the electroactive surface of the electrode but also act as a bridge to accelerate the rate of heterogeneous electron transfer between the electrode and the redox-active MB. In this way, the sensitivity of this electrode was greatly increased with the detection limit of 0.252 nM for fish sperm double-stranded DNA, and the cathodic current of MB was linear with the logarithmic value of the DNA concentration between 0.5 and 20 nM. The recovery of DNA was between 89 and 110%. Compared with other DNA detection techniques, this study provides a simple and practical method that displays a satisfactory detection limit and a linear dynamic range, indicating that the chitosan–CNT system can be used as a stable and sensitive platform for DNA detection. The detection signals were enlarged immediately by the introduction of CNTs, indicating that CNTs have great potential to be used to improve sensor performance. At the same time, some disadvantages in the assay were observed, such as a relatively long manipulation time, and these may be overcome in future work.

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