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Enzymatically induced formation of neodymium hexacyanoferrate nanoparticles on the glucose oxidase/chitosan modified glass carbon electrode for the detection of glucose

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ABSTRACT

The formation of neodymium hexacyanoferrate (NdHCF) nanoparticles (NPs) on the surface of glucose oxidase/chitosan (GOx/CHIT) modified glass carbon electrode induced by enzymatic reaction was described and characterized. CHIT can be used not only as enzyme immobilizer, but also to provide active sites for NPs growth. Results showed that the optimized conditions of the GOx/CHIT film induced NdHCF NPs for the biosensing of glucose were 1.0 mM Nd^{3+} and 20.0 mM $Fe(CN)_6^{3-}$. The biocatalyzed generation of NdHCF NPs enabled the development of an electrochemical biosensor for glucose. The calculated apparent Michaelis–Menten constant was 7.5 mM. The linear range for glucose detection was 0.01–10.0 mM with the correlation coefficient of 0.9946, and the detection limit was 5 μM (S/N = 3). Furthermore, this system avoids the interferences of other species during the biosensing process and can be used for the determination of glucose in human plasma samples.

1. Introduction

In recent years, the application of biocatalysts is becoming more and more attractive for the synthesis and the enlargement of metallic NPs, especially the integration of NPs into biocatalytic reaction which opened a new way for designing simple and sensitive electrochemical and optical sensors (Katz and Willner, 2004; Medintz et al., 2005; Möller et al., 2005). Such new field that conjugates biomolecules to metal nanoparticles (MNPs) has recently attracted substantial interest in the rapidly developing area of nanobiotechnology (Katz et al., 2004; Niemeyer, 2001).

The unique catalytic properties of MNPs have been exploited by Willner and co-workers for amplified optical enzymatic assays in connection with biocatalytically induced particles-growth processes (Shlyahovsky et al., 2005; Xiao et al., 2004; Zayats et al., 2005a; Baron et al., 2005; Xiao et al., 2005a,b). Hwang et al. (2005) have reported the metal deposition by the enzymatically produced reducing agent for the amplified electrochemical detection of DNA. Zhou et al. (2006) also reported the design of a self-assembly monolayer modified electrode with the biological catalytic reaction for the biosensing of cholesterol. Such technique involves the reduction and deposition of metal onto the NPs by an enzymatically generates reducing agent, has been exploited for the quantitative optical monitoring of several enzymatic reactions. Further application studies, based on the enzyme-stimulated catalytic deposition of metals on MNP seeds, have been extended to other biocatalysts that yield products capable of reducing metal salts. For example, the NADH-stimulated growth of Au NPs under special conditions led to shaped NPs consisting of dipods, tripods, and tetrapods (Xiao et al., 2005a,b). The alkaline phosphatase hydrolysis of *p*-aminophenol phosphate yields *p*-aminophenol that catalyzes the reduction of Ag^+ on Au NP seeds (Basnar et al., 2006). Most recently, a kind of metal–NP–enzyme hybrid system have been employed as biocatalytic templates (or "biocatalytic inks") for the generation of enlarged metal NPs or metallic nanowires (Willner et al., 2006; Xiao et al., 2003; Zayats et al., 2005b). It is expected that the metal–NP–enzyme conjugates may act as biocatalytic labels for amplifying a variety of biorecognition events, such as antigen–antibody or nucleic acid–DNA, and enabling the optical, electrochemical, or microgravimetric readout of the recognition processes (Möller et al., 2005).

However, the present study has emphasized the biocatalyzed synthesis of metallic NPs. In fact, the biocatalyzed synthesis of other materials such as semiconductor NPs (e.g., CdS, PbS) or magnetic NPs is a very attractive field (Willner et al., 2006). It is expected that once such biocatalytic transformations are materialized, the development of new optical or electrochemical biosensing systems can be accomplished. Generally, the biocatalytic precipitation of an insoluble product on electronic transducers was used as a general amplification route for sensing and recognition events by layered

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biomaterial-sensing interface (Patolsky et al., 1999; Abad et al., 1998), such as enzyme-based electrodes, immunosensors and DNA sensors. Recently, Wang and Arribas (2006) represented the first example of deliberately exploiting enzymatic reactions for generating cupric ferrocyanide (CuHCF) NPs and exploiting their formation for monitoring biocatalytic reactions. However, since such protocol was performed in solution, only a small part of $Fe(CN)_6^{4-}$ formed by enzymatic reaction will react with Cu^{2+} and deposited on the carbon-paste electrode surface, while a large portion diffuse into bulk solution. Obviously, this limited the application of the protocol, especially for real sample analysis. So, it is desirable to develop novel method to solve the problem. Entrapment or encapsulation within a biocompatible material by simple procedures has been widely used for enzyme immobilization. Among various biocompatible materials, chitosan (CHIT) was widely used as an immobilization matrix for biosensors and biocatalysis (Huang et al., 2002; Bindhu and Abraham, 2003).

Here, we wish to develop a novel method for the improvement of biosensor design and the application of metal hexacyanoferrate (MHCF) NPs from biocatalyzed synthesis. A kind of real-earth MHCF (neodymium ferrocyanide, NdHCF) was chosen as an example to illustrate the protocol. The use of rare-earth metal ion (Nd^{3+}) avoided the formation of unpredicted precipitate, which extended the application of enzymatic reactions for generating MHCFs. The amine function of CHIT have the ability to chelate metal ions, which can provide better nucleation sites for the growth of NdHCF NPs. After optimized, the proposed biosensor was used for the determination of glucose. The combination of cooperative performance of polymer, highly efficient enzyme catalysis, and attractive electrochemical properties of MHCFs provides a general platform for the synthesis of nanowires and nanocircuits, the construction of bioelectronic devices, and the design of novel biosensors.

2. Experimental

2.1. Reagents

Glucose oxidase (GOx, E.C. 1.1.3.4, 182 U/mg, Type X–S from *Aspergillus niger*), CHIT (MW 1×10^6, >90% deacetylation) was purchased from Shanghai reagent company (China). β-D(+)-glucose, potassium hexacyanoferrate, potassium hexacyaniferrate, neodymium chloride hexahydrate (99.9%), glutaraldehyde (GA), and KCl were of analytical grades and used without further purification. All solutions were prepared with ultrapure water (>18 MΩ cm) obtained from a Millipore Milli-Q water purification system. All electrochemical experiments were conducted in 0.5 M KCl solution unless otherwise specified.

2.2. Apparatus

A Model CHI660A Electrochemistry Workstation (Chenhua Instruments in Shanghai, China) was employed for all the electrochemical techniques. A conventional three-electrode system was used. The prepared GOx/CHIT film modified electrode was used as the working electrode. A standard saturated calomel electrode (SCE) and a platinum wire electrode were served as the reference electrode and the auxiliary electrode, respectively. All the electrochemical experiments were conducted at room temperature.

Scanning electron microscopic (SEM) measurements were carried out on a scanning electron microscope (JEOL JSM-6700F) at 20 kV. Samples were placed in aluminum stubs and were then coated with a gold layer by sputtering in order to enhance their conductivity. Energy-dispersive X-ray (EDX) analysis of the GOx/CHIT film before and after the generation of NdHCF NPs was also performed during the SEM measurements.

2.3. Preparation of the CHIT and GOx/CHIT modified film electrodes

A 1.0 wt% CHIT solution was prepared by dissolving certain amount of CHIT into a 0.5 wt% acetic acid (HAc) and diluted with ultrapure water. Prior to use, a GCE was polished on a polishing cloth with alumina of successively smaller particles (1.0 and 0.05 μm diameter, respectively), rinsed with water, ultrasonicated in ethanol and ultrapure water, respectively. The concentration of GOx and CHIT were optimized in control experiments to obtain good current responses of GOx/CHIT modified GCE. Typically, 5 μL of the mixture containing 2.0 mg mL^{-1} GOx and 3.0 mg mL^{-1} CHIT was spreaded evenly on the pretreated GCE surface. Then, 2 μL of 0.5% GA solution was used to cross-link GOx with CHIT. The CHIT modified GCE was prepared the same as described above, except the addition of GOx. The resulting electrodes were dried at room temperature. Without use, the electrodes were stored at 4 °C in a refrigerator.

2.4. Procedures and electrochemical measurements

For NdHCF particles accumulation assays without enzymatic reaction, the prepared CHIT modified GCE was placed in a stirred 0.5 M KCl solution, containing 1.0 mM Nd^{3+}, 0.5 mM $Fe(CN)_6^{4-}$ and 5.0 mM $Fe(CN)_6^{3-}$, for the selected time. As for the investigation of the relationship between the formation of NdHCF particles and the $Fe(CN)_6^{4-}$ concentration, the CHIT modified GCE was placed in a stirred 0.5 M KCl solution, containing 1.0 mM Nd^{3+} and different concentrations of $Fe(CN)_6^{4-}$ for certain times.

For assays involving enzymatically induced formation of NdHCF NPs, the GOx/CHIT modified GCE was placed in a stirred 0.5 M KCl solution, containing 1.0 mM Nd^{3+}, 20.0 mM $Fe(CN)_6^{3-}$ and different concentrations of glucose for a given time. Before the incubation, the reaction solution was bubbled with N_2 for 15 min to eliminate interferences from dissolved O_2. Subsequently, the working electrode was removed from the solution, washed with ultrapure water and transferred into a 0.5 M KCl solution. Then, square-wave voltammetric (SWV) voltammograms were recorded.

3. Results and discussion

3.1. Preparation and characterization of the biocatalytically induced NdHCF NPs

To illustrate the general design strategy for biosensing of glucose based on the biocatalytically induced NdHCF NPs, we chose a well-characterized picture reported previously (Wang and Arribas, 2006) and made appropriate modifications as shown in Fig. 1S in Supplementary information. As can be seen, ferricyanide, severed as the electron acceptor, is reduced to ferrocyanide by the enzymatic reaction (Dzyadevich et al., 1998; Chaubey and Malhotra, 2002), which subsequently reacts with neodymium ion to produce NdHCF NPs on the surface of GOx/CHIT modified GCE. It has been reported that the amine function of CHIT has the ability to chelate metal ions (Domard, 1987; Couphlin et al., 1990; Koshijima et al., 1973). It is necessary to mention that a competitive adsorption and desorption exist under the experimental condition when the solution pH is 7.

$$\text{CHIT} + H_3O^+ \leftrightarrow \text{CHIT–}H^+ + H_2O \quad (1)$$

$$\text{CHIT} + M^{n+} \rightarrow (\text{Complex})^{n+} \quad (2)$$

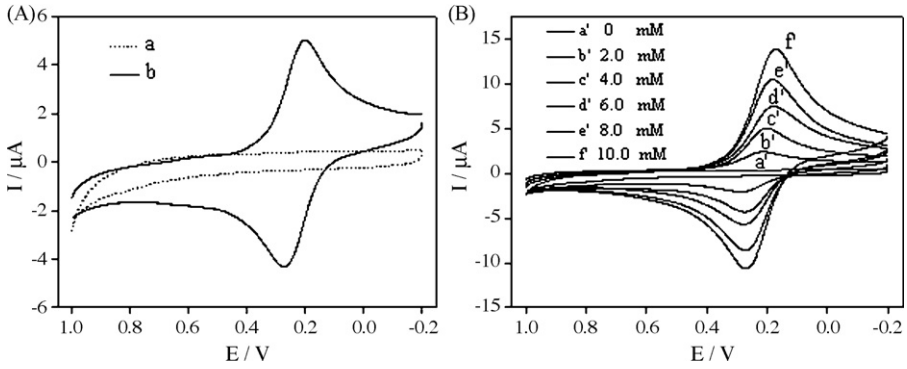

Fig. 1. (A) Cyclic voltammograms of the (a) CHIT modified electrode and (b) GOx/CHIT modified electrode in 0.5 M KCl at a scan rate of 50 mV s^{-1}. Incubation was performed in a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+}, 20.0 mM $Fe(CN)_6^{3-}$ and 4.0 mM glucose for 10 min. (B) Cyclic voltammograms of GOx/CHIT modified GCE in 0.5 M KCl solution at a scan rate of 50 mV s^{-1}. Incubation was performed in a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+}, 20.0 mM $Fe(CN)_6^{3-}$ and different concentrations (a′–f′) of glucose for 10 min (pH 7, 25 °C).

When the competitive adsorption process is superior (pH > 6), the adsorption of ions by CHIT can reach more than 50%. Contrarily, when the solution pH is less than 6, the amino group of CHIT will combine H^+ to form $-NH^{3+}$, leading to the decrease of chance for M^{3+} complex with CHIT (Muzzarelli, 1977; Rhazi et al., 2002). Moreover, if the solution pH > 7, precipitation of metal hydroxide will be formed. So, it can be drawn the conclusion that the adsorption plays a main role when the solution pH is 7. Such properties of CHIT could provide better nucleation sites for the growth of NdHCF NPs.

At first, we examined the enzymatic reaction conditions by immersing the CHIT film modified electrodes (a) with or (b) without GOx into the incubate solution for certain time. No current response was observed on (b) electrode, while (a) electrode showed a well-defined redox couple with peak potential of 0.23 V (Fig. 1A). This result suggested that the presence of GOx plays the critical role for the formation of NdHCF NPs. Fig. 1B showed the cyclic voltammograms of GOx/CHIT modified GCE after incubation in a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+}, 20.0 mM $Fe(CN)_6^{3-}$ and different concentrations (a′–f′) of glucose for 10 min. As shown in Fig. 1B, well-defined redox peaks were obtained and the peak current increased with the increase of glucose concentrations. This suggested that the amount of NdHCF NPs formed on electrode surface was related to the glucose concentration under enzymatic reaction. This relationship provides the possibility for new glucose biosensor design.

SEM experiments were also carried out in order to have an intuitionistic understand about the conformation of NdHCF NPs on the GOx/CHIT film modified GCE surface. As can be seen from Fig. 2a, a uniform GOx/CHIT film was obtained. After immersing the GOx/CHIT modified GCE in a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+}, 20.0 mM $Fe(CN)_6^{3-}$ and 5.0 mM glucose for 10 min, the surface of the electrode was covered with NdHCF NPs (average size of 80 nm) under the enzymatic reaction (inset of Fig. 2b). Meanwhile, the generated NdHCF NPs clustered on the electrode surface and the particle size was about 500 nm. Furthermore, the EDX analysis was accompanied with SEM to verify the composition of the resulting NdHCF NPs. As shown in Fig. 2c and d, the EDX analysis confirmed that the NdHCF NPs consisted of Nd and Fe in a ratio of 1:1. The Au peaks originated from gold layer sputtered for SEM in order to enhance their conductivity of samples. This confirmed that our attempt to utilize the enzymatic reaction to generate NdHCF NPs by using GOx/CHIT film modified electrode was fairly feasible. It is necessary to mention that, in our system there was no need to use special buffer solution to prevent undesired precipitation, compared with the formation of CuHCF reported by Wang and Arribas (2006). The reason was that only one stoichiometric compound formed after the reaction between Nd^{3+} and $Fe(CN)_6^{4-}$ (Sheng et al., 2007).

Fig. 3 showed the cyclic voltammograms of the resulting electrode obtained in 0.5 M KCl at various scan rates. The electrochemical response of NdHCF NPs exhibited a pair of well-defined redox peaks with the formal potential of 0.236 V in 0.5 M KCl solution. This was in good agreement with our earlier studies that the NdHCF particles were mechanically attached to the surface of carbon ceramic electrode (Sheng et al., 2007). Inset graph of Fig. 3 showed that the cathodic and anodic peak currents increase linearly with the increase of square root rates, suggesting the electrochemical reaction of NdHCF NPs formed on electrode surface was not a surface-controlled process but a diffusion-controlled one at the time of reaching the peak potential (Ayers and Piggs, 1971). This phenomenon has been confirmed by Moon et al. (1993) and described as electrolytic conduction. Here, it is indeed only the diffusion of K^+ in the NdHCF lattice, and not in the solution, which can be rate determining when the solution concentration is 0.5 M, and the bulk diffusion is not rate determined. Also, the peak current increased with the increasing of K^+ concentration, suggesting that the concentration of K^+ in NdHCF crystal is not high enough and the diffusion of K^+ in NdHCF crystal cannot be neglected. Quantificationally, from the relationship between the peak current and the concentration of K^+, the value of peak current is related to the concentration of K^+. Here, the partition coefficient of K^+ at the crystal/solution interface will not be influenced by the changes of K^+ concentration, which guarantee the concentration of K^+ in NdHCF crystal is proportional to the concentration of K^+ in solution.

3.2. *Effect of experimental conditions during the formation of NdHCF NPs*

Because of the superior sensitivity, SWV, rather than cyclic voltammetry, was used in quantification experiments. At first, to compare with enzymatic reaction, we examined the formation of NdHCF particles through the direct reaction of Nd^{3+} with $Fe(CN)_6^{4-}$ in the presence of $Fe(CN)_6^{3-}$. The CHIT film modified GCE was immersed in a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+}, 0.5 mM $Fe(CN)_6^{4-}$ and 5.0 mM $Fe(CN)_6^{3-}$. Well-defined SWV signals with peak potentials of about 0.21 V involved the conversion of $Fe(CN)_6^{3-/4-}$ were obtained. The current response increased rapidly during the first 8 min and became steady afterwards. Further work was to examine the relationship between the peak current of NdHCF particles (direct reaction of Nd^{3+} with $Fe(CN)_6^{4-}$) and the concentration of $Fe(CN)_6^{4-}$. Results showed that the peak

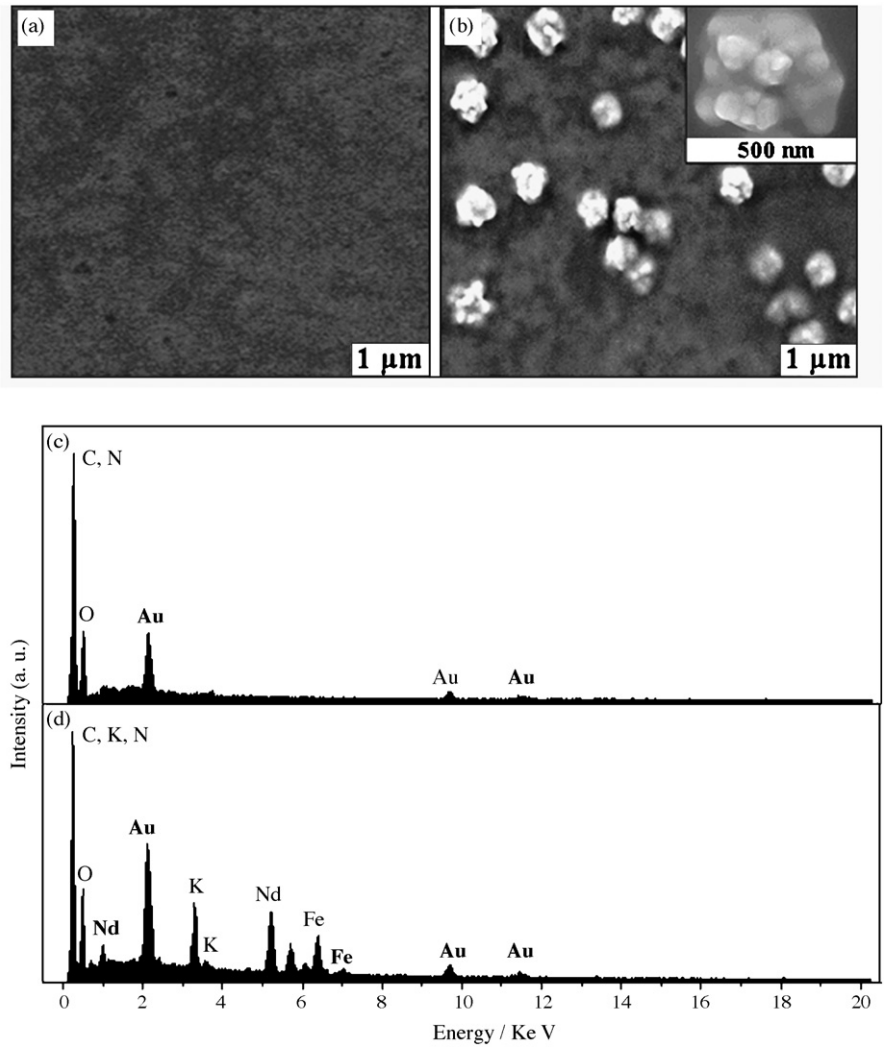

Fig. 2. SEM images of the electrode surface covered with (a) GOx/CHIT film and (b) NdHCF NPs formed on GOx/CHIT film after enzymatic reaction. Inset of (b) represents high magnification of the NdHCF NPs. EDX analysis of the GOx/CHIT film before (c) and (d) after the enzymatic reaction.

current increased linearly for a serious of $Fe(CN)_6^{4-}$ solution with various concentrations and exhibited good linearity over the 0–0.8 mM range (Fig. 2S in Supplementary information). Evidently, these results further indicated that the formation of NdHCF NPs on the GOx/CHIT film modified electrode was feasible.

Subsequent experiments examined the effects of accumulation time, the GOx, Nd^{3+} and $Fe(CN)_6^{3-}$ concentrations under the bio-catalytic accumulation of the NdHCF NPs on electrode surface. The dramatic biocatalytic accumulation of the NdHCF NPs was indicated in Fig. 4A, which displayed the dependence of the SWV peak upon the accumulation time. The peak current at first increased rapidly

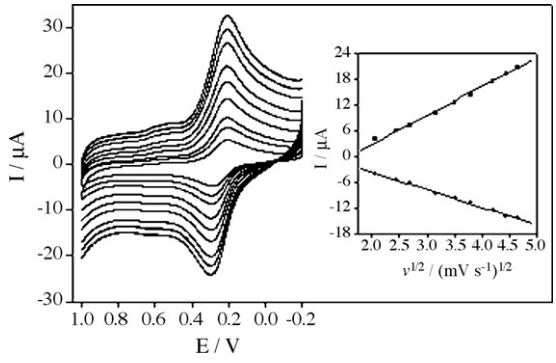

Fig. 3. Cyclic voltammograms of the modified electrode in 0.5 M KCl at various scan rates (from inner to outer curve): 10, 20, 30, 50, 70, 90, 120, 140 and 160 mV s^{-1}. Inset graph: plots of peak currents *vs*. square root of scan rates.

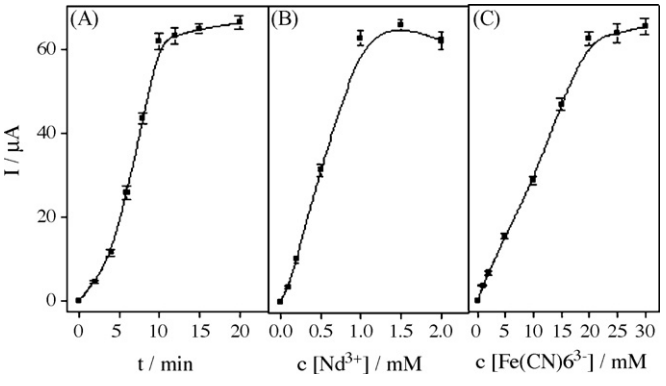

Fig. 4. Plots of peak current *vs*. (A) accumulation time, (B) Nd^{3+} concentration, and (C) $Fe(CN)_6^{3-}$ concentration. Reaction conditions: (A) a stirred 0.5 M KCl solution containing 20.0 mM $Fe(CN)_6^{3-}$; (B) a stirred 0.5 M KCl solution containing 20.0 mM $Fe(CN)_6^{3-}$ and different concentrations of Nd^{3+} for 10 min; and (C) a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+} and different concentrations of $Fe(CN)_6^{3-}$ for 10 min (pH 7, 25 °C).

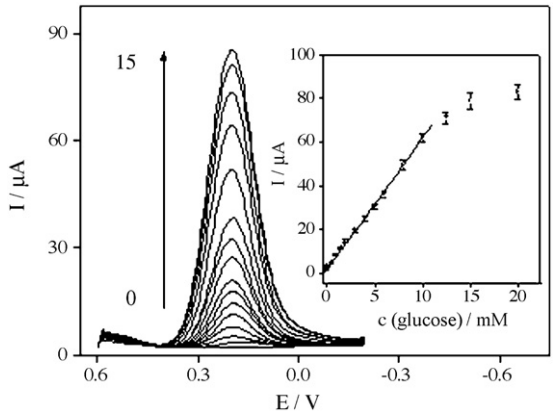

Fig. 5. Square-wave voltammograms of the GOx induced formation of NdHCF NPs on GOx/CHIT film modified GCE for an increasing concentration of glucose. Glucose concentration ranges: (0) 0, (1) 0.01, (2) 0.1, (3) 0.5, (4) 1.0, (5) 1.5, (6) 2.0, (7) 3.0, (8) 4.0, (9) 5.0, (10) 6.0, (11) 8.0, (12) 10.0, (13) 12.5, (14) 15.0 and (15) 20.0 mM. Inset graph: calibration plot of peak current *vs.* glucose concentration. Reaction solution: a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+}, 20.0 mM $Fe(CN)_6^{3-}$ and different concentrations of glucose; reaction time: 10 min (pH 7, 25 °C).

and nearly linearly with time, then more slowly above 10 min. This profile was similar to the behavior observed by the direct reaction between Nd^{3+} and $Fe(CN)_6^{4-}$. Thus, indicating that the enzymatic reaction was fast enough under these conditions.

We next accessed the effect of the concentration of different components of the enzymatic reaction solution relative to a 10 min reaction time. The current response increases rapidly at first with the concentration of Nd^{3+} and $Fe(CN)_6^{3-}$ ions up to 1.0 and 20.0 mM (Fig. 4B and C), respectively, reaching steady signals thereafter. The effect of GOx concentration upon the NdHCF NPs response was thus examined in the presence of 1.0 mM Nd^{3+} and 20.0 mM $Fe(CN)_6^{3-}$. The response increased with the levels of GOx at first, then more slowly, and levels off above 2.0 mg mL^{-1}.

3.3. Biosensing of glucose

Since the current response of the generated NdHCF NPs on electrode surface was related to glucose concentration, the proposed biosensor can be used for glucose detection. Fig. 5 showed SWVs for increasing concentration of glucose over the 0–20.0 mM range. As was shown, the peak current increased rapidly upon increasing the glucose concentration up to 10.0 mM and then more slowly. Furthermore, the change of voltammetric current showed a linear relationship with the glucose concentration in the range of 0.01–10.0 mM with the correlation coefficient of 0.9946. The detection limit was 5 μM with the signal-to-noise ratio of S/N = 3. It can be concluded that the proposed strategy holds great promise for biosensing of glucose.

The use of the Michaelis–Menten equation is quite efficient in the kinetic analysis of the enzymatic reaction. For biosensors the reaction rates are substituted with steady-state current, based on the equation (Kamin and Wilson, 1980):

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} - \frac{K_m}{(I_{max}\, C_s)} \quad (3)$$

Here I_{ss} is the steady-state current, C_s is the concentration of substrate, K_m is the apparent Michaelis–Menten constant, and I_{max} is the intercept on the current axis. The corresponding plot yielded an "apparent" K_m of 7.5 mM. The value was smaller than those reported for other enzymatic electrodes (Wang et al., 1998; Vidal et al., 1998; Chen et al., 2003). Wang and Arribas have explained that the reason may be due to different reaction kinetics when using ferricyanide as an electron acceptor, compared with oxygen as an electron acceptor (Wang and Arribas, 2006).

Previous studies have confirmed that competition between O_2 and $Fe(CN)_6^{3-}$ (threshold-type electron acceptors) as oxidizing substrates for GOx exists during the enzymatic reaction (Schläpfer et al., 1974; Wilson and Turner, 1992). This phenomenon was also observed in the present study. Previously, the GOx/CHIT film modified electrodes were incubated in the incubation solution with different concentrations of glucose, saturated with air, over a period of 10 min. Then, SWV signals were recorded. Interestingly, a linear dependence between the peak current and glucose concentration was observed in the range of 2.0–10.0 mM. At lower concentrations of glucose below 2.0 mM, the curve is not linear (Fig. 3S, curve ● in Supplementary information). The lower of glucose concentration, the less of NdHCF NPs generated from the reaction of Nd^{3+} with $Fe(CN)_6^{4-}$ during the enzymatic reaction. The reason can be explained by the fact that, under the condition of low glucose concentration, the rate of the enzymatic-oxidation of glucose by immobilized GOx is mainly determined by O_2 (Shul'ga et al., 1994; Schläpfer et al., 1974). At higher glucose concentration, "O_2 deficit" occurred, which made the reaction rate for glucose oxidation was determined by $Fe(CN)_6^{3-}$. When the incubation was carried out in a N_2 saturated solution, a good linear dependence was found between peak current and glucose concentration in the range of 0.01–10.0 mM (Fig. 3S, curve ■ in Supplementary information). The above results showed that the formation of NdHCF NPs induced by enzymatic reaction should be carried out under unaerobic conditions.

3.4. Interference study on the determination of glucose

Interferences were evaluated by adding external matters into the reaction solution and SWV signals were recorded. Our experiments results showed that even when the concentration of foreign species, such as uric acid, ascorbic acid, and L-cysteine, were 20 times larger than glucose, the current responses for the biosensing of glucose are almost the same as the original one (Table 1 in Supplementary information). The relative response deviation ($n = 10$) was less than 5.0%. The interferences of metal ions, such as Fe^{3+}, Cu^{2+}, Co^{2+} and Ni^{2+} were also investigated. It was found from a lot of experiments that those metal ions can react with $Fe(CN)_6^{3-}$ and precipitated immediately once added into the incubate solution. However, the concentrations of metal ions should be limited to 1.0 mM exist together with glucose to limit the interferences effects within a relative error of 5.0%. The reason can be ascribed to the decrease of $Fe(CN)_6^{3-}$ concentration for enzyme reaction which further interferes the detection of glucose.

3.5. System stability and reproducibility

In order to examine the system stability and reproducibility of the enzymatically induced NdHCF NPs, six GOx/CHIT film modified electrodes were immersed in a stirred 0.5 M KCl solution (1.0 mM $NdCl_3$, 20.0 mM $K_3Fe(CN)_6$ and 10.0 mM glucose) for a given time at room temperature. Then voltammetric signals were recorded. Results showed that the current responses of the resulting electrode were not changed when cycled in blank 0.5 M KCl solution within 20 cycles. This was sufficient for the measurements. Moreover, the current responses of six electrodes after incubation under the same conditions were almost the same and the R.S.D. for six measured anodic currents was 8.5%.

3.6. Application for the glucose determination

The determination of glucose in human plasma was performed by the proposed biosensor. The complete blood was prepared by immediate centrifuged for 10 min at 4 °C to obtain the plasma sample. After that, the sample solutions were prepared by diluting the corresponding plasma sample with buffer solution to the original volume and used for bioassay. At first, the GOx/CHIT film modified GCE was immersed in a stirred 0.5 M KCl solution containing 1.0 mM Nd^{3+} and 20.0 mM $Fe(CN)_6{}^{3-}$. Then, 1.0 mL of sample solution was added. After incubation in the above solution for 10 min, the electrode was washed with water and transferred into 0.5 M KCl solution, then SWV signals was recorded. The glucose level in human plasma was found to be 5.4 mM as an average value of three measurements. The recoveries for the determination of glucose were between 97.6% and 104.2% for three times determination.

4. Conclusion

In conclusion, the growth of NdHCF NPs induced by enzymatical reaction on the GOx/CHIT film is achieved and subsequently applied to glucose determination. Furthermore, this system avoids the interferences from other species, which holds great promise for developing novel glucose biosensors for practical detection. Such enzymatically controlled formation of ferrocyanide-based NPs exhibit favorable electrochemical properties that offer new prospects for electrochemical transduction of biocatalytic events and hold great promise for the construction of bioelectronic devices and the design of novel electrochemical biosensors with other bio-systems. Further works derived by enzymatically induced formation of NPs are on our schedule.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2008.04.024.

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