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Talanta 65 (2005) 888–894

Talanta

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Modification of carbon ceramic electrode prepared with sol–gel technique by a thin film of chlorogenic acid: application to amperometric detection of NADH

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Received 16 March 2004; received in revised form 21 July 2004; accepted 12 August 2004
Available online 25 September 2004

Abstract

The carbon ceramic electrode prepared with sol–gel technique is modified by a thin film of chlorogenic acid (CGA). By immersing the carbon ceramic electrode in aqueous solution of chlorogenic acid at less than 2 s a thin film of chlorogenic acid adsorbed strongly and irreversibly on the surface of electrode. The cyclic voltammetry of the resulting modified CCE prepared at optimum conditions shows a well-defined stable reversible redox couple due to hydroquinone/quinone system in both acidic and basic solutions. The modified electrode showed excellent electrocatalytic activity toward NADH oxidation and it also showed a high analytical performance for amperometric detection of NADH. The catalytic rate constant of the modified carbon ceramic electrode for the oxidation of NADH is determined by cyclic voltammetry measurement. Under the optimised conditions the calibration curve is linear in the concentration range 1–120 μm. The detection limit (S/N = 3) and sensitivity are 0.2 μM and 25 nA μM^{-1}.The results of six successive measurement-regeneration cycles show relative standard deviations of 2.5% for electrolyte solution containing 1 mM NADH, indicating that the electrode renewal gives a good reproducible and antifouling surface. The advantages of this amperometric detector are: high sensitivity, excellent catalytic activity, short response time $t < 2$ s, remarkable long-term stability, simplicity of preparation at short time and good reproducibility.

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Keywords: Carbon ceramic electrode; Sol–gel; Chlorogenic acid; NADH; Amperometry

1. Introduction

Sol–gel technology has been used to prepare inorganic and organic–inorganic composite films through the hydrolysis and condensation of metal alkoxides, most notably the silicon alkoxides [1–3]. These materials are now being developed for use in chromatographic separations [4,5], full cells [6] and as supports for optical sensors [7,8]. Considerable research efforts are constantly being devoted to development of chemical sensors and biosensors based on sol–gel technology [9–13]. Due to the inert low temperature process, the sol–gel method provides an attractive way for the immobilization of heat-sensitive biological entitles (enzyme, protein and antibody). This class of sol–gel silicate matrix possesses chemical inertness, physical rigidity, negligible swelling in aqueous solutions, tenable porosity, high photochemical and thermal stability and optical transparency. These attractive features have led to an intensive research in the optical and electrochemical sensors and biosensors. Sol–gel electrochemistry has been rapidly developed over the last 5 years and has become an area of active research [11,14–16]. The sol–gel technique is a new, simple and renewable method for the preparation of the modified electrodes and solid electrolytes [11]. Since the pioneering work of Lev's group [17,18] on ceramic carbon composite electrodes (CCEs) much effort has been devoted to the fabrication of chemically modified CCEs and using them as sensor for amperometric and voltammetric detection of analytes. The chemical modifications of inert substrate electrodes with redox active thin films offer signif-

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doi:10.1016/j.talanta.2004.08.010

icant advantages in the design and development of electrochemical sensors. In operation, the redox active sites shuttle electrodes between solution analyte and the substrate electrodes often with significant reduction in overpotential. A further advantage of the chemically modified electrodes is their less prone to surface fouling and oxide formation compared to inert substrate electrodes. Although several organic, inorganic and organometalic compounds have been used to prepare modified electrodes most of these electrodes presented quasi-reversible electrochemical behaviour and even ill-defined cyclic voltammograms with large background current. So the preparation of modified carbon ceramic electrodes with various electron transfer reagents is receiving increasing interest in the filed of chemically modified electrodes and electroanalysis.

The electrochemical oxidation of NADH at low overpotential is of particular interest in biosensor development because of its ubiquitous use as cofactor for over 300 dehydrogenases. Significant overpotential 1.0 V is often required to oxidize NADH at bare electrode surfaces. In addition, the direct oxidation of NADH is often accompanied by electrode fouling and interference background currents in real samples and sometimes leds to the formation of enzymatically inactive forms of NAD^+ [19]. Oxidation of NADH at lower overpotentials can be achieved through a redox mediator and a number of systems capable of this have been identified. Electrode modification with transition metal complexes [20], transition metal hexacyanoferrates [21,22], tetrarutenated complex of cobalt porphyrin [23], and different electroactive dyes have been used for electrocatalytic oxidation of NADH [24–27]. One of the major difficulties attributed to approaches described above relates to the stability of the modified electrodes and in some cases the difficulty of modification. Recently most attention has been devoted to the GC electrodes modified with *O*-hydroquinone groups mediators, which are capable of effective mediation of NADH oxidation [28–33]. Although GC electrodes modified with *O*-hydroquinone films have been used successfully for the detection of NADH, to our knowledge the method of electrode preparation was carried out in two different steps (activation and electrodeposition) at least in 30 min and the experimental conditions for electrode preparation should be controlled carefully for optimum reproducibility. Hence, it is pertinent to explore and develop an electroless simple and reliable method to fabricate electrodes modified with catechol derivatives in short time. As described above, the carbon ceramic electrodes prepared by sol–gel techniques are interesting materials for electrode modification, especially, because they have high capacity for adsorption without any activation. Then they can be used as support for electroactive species either by adsorption or entrapment during formation [34,35].

In continuation of our study to prepare modified electrodes based on sol–gel technique [36–39] and also by application of catechol derivates [40] to modified electrode surfaces at the present study, we propose a simple electroless method to fabricate carbon ceramic electrode modified with a thin film of chlorogenic acid as a naturally antioxidant *O*-dipenolic compound that is found in many fruits such as apple, pear, peach, plum, cherry and apricot [41]. It is also known as an important factor in plant metabolism. The modified electrode is used for the electrocatalytic oxidation of NADH and finally the analytical performance of a chlorogenic acid modified carbon ceramic electrode is described as an amperometric sensor for NADH determination, a cofactor in several hundred enzymatic reactions.

2. Experimental

2.1. Reagents and solutions

Methyltrimethoxysilane (MTMOS) was purchased from Fluka and used without any further purification. Chlorogenic acid [1,3,4,5-tetrahydroxycyclohexane carboxylic acid 3-(3,4-dihydroxycinamate)] (CGA), NADH and other reagents were of analytical grade from Merck, Fluka and Aldrich and used as received. High Purity graphite powder was obtained from Merck. Buffer solution (0.1 M) was prepared from HCl, H_3PO_4, Na_2HPO_4, $NaH2PO_4$ and KOH for the pH range 3–9. Solutions were deaerated by bubbling high purity (99.999%) nitrogen gas through them prior to the experiments and the electrochemical cell was kept under nitrogen atmosphere throughout the experiments. The bare and modified CCEs were characterized by atomic force microscopy (AFM). The AFM was a digital Instruments Multimode SPM, operating in ex situ Tapping Model. A model J scanner was used, having a lateral range of 125 μm × 125 μm and a vertical range of 5 μm. Nanosensors NCH tapping mode tips having a spring constant of approximately $45\,nm^{-1}$ and a resonant frequency of approximately 380 kHz were used.

2.2. Apparatus

A computer controlled Auto Lab modular electrochemical system (ECO Chemie, Ultrecht, and The Netherlands) equipped with a PSTA 20 module and driven by GPES (ECO Chemie) was used for amperometric and voltammetric measurements. A three electrode cell, consisting of a carbon ceramic electrode modified with a thin film of chlorogenic acid as a working electrode, an Ag/AgCl (satd., KCl) $3\,M^{-1}$ KCl reference electrode and a platinum wire counter electrode were employed for the electrochemical studies. A Methrom drive shaft to rotate working electrodes was used in amperometric determination. The electrochemical measurements were carried out at the thermostated temperature of $25 + 0.1\,^{\circ}C$.

2.3. Preparation of bare and chlorogenic acid modified carbon ceramic electrodes

The bare carbon ceramic electrodes were prepared according to the procedure described by the Lev and co-workers

[17] by using 0.2 ml MTMOS, 0.6 ml methanol and 20 μl hydrochloric acid 11 M. This mixture was magnetically stirred for 2 min, after which 0.5 g graphite powder was added and the mixture was shaken for an additional 5 min. The mixture was added to Teflon tube (with 2 mm inner diameter and 5 cm length, and the length of composite material in the tube was about 0.5 cm) and dried for 48 h at room temperature. The electrode was polished with polishing paper to remove extra composite material. By immersing the bare carbon ceramic electrode in 1 mM aqueous solution of chlorogenic acid for (5–40 s), a stable film of chlorogenic acid adsorbed on the surface of the electrode, and after rinsing with water it can be used for electrochemical experiments immediately. Electric contacts were made with copper wire through the backs of the electrodes.

3. Results and discussion

3.1. Electrochemical properties of CCE modified with thin film of chlorogenic acid

Fig. 1 shows cyclic voltammograms of a chlorogenic acid modified carbon composite electrode modified with different surface concentration of chlorogenic acid, obtained by soaking the carbon ceramic electrode in 1 mM chlorogenic acid solution for different times at 20 mV s^{-1} in the blank solution of 0.1 M phosphate buffer (pH 6). As shown, by immersing the electrode for 2 s a stable thin layer of chlorogenic acid adsorbed at the surface of carbon composite electrode fabricated with sol–gel technique. By increasing the immersing time, the surface concentration of chlorogenic acid is increased and starts to level off after 50 s. We found a single and well-defined redox couple with formal potential 0.27 V versus SCE, low background current and small potential peak separation ΔE_p = 15 mV. The pH dependence, stability and reproducibility of the electrochemical behaviour of the modified chlorogenic acid carbon composite electrode have been reported in our previous report [42].

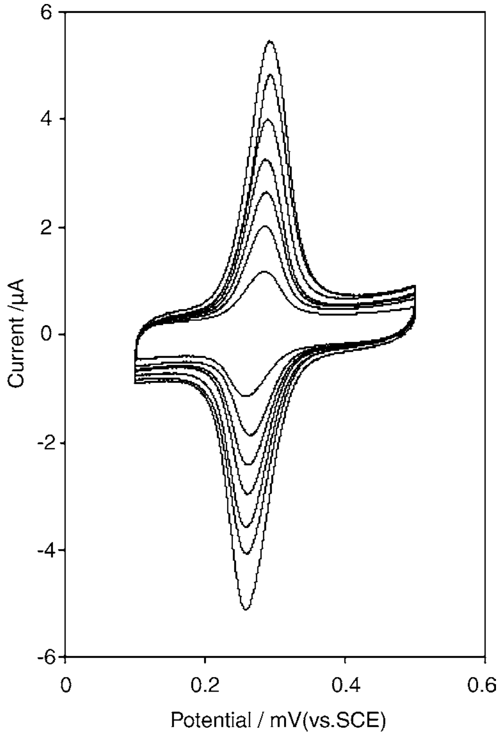

Fig. 1. Cyclic voltammetric responses of carbon ceramic electrode in 0.1 M buffer solution (pH 6) at scan rate 20 mV s^{-1} modified with immersion in 1 mM chlorogenic acid for different time (from inert to outer) 2, 5, 10, 15, 20, 30 and 40 s.

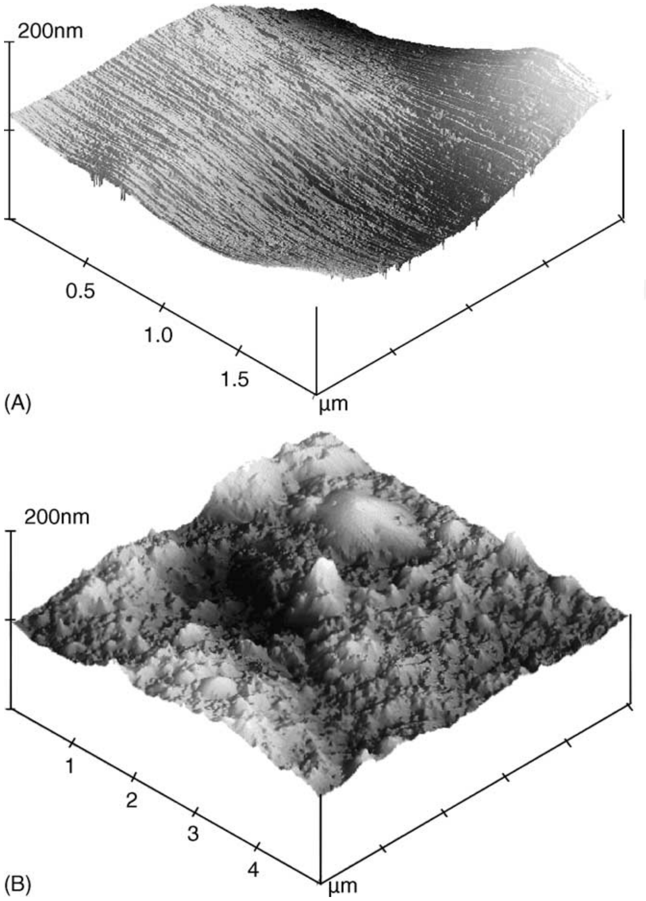

Fig. 2. The AFM image (A) is for bare electrode and (B) is for modified carbon ceramic electrode.

Fig. 2 shows the atomic force microscopy (AFM) images of bare carbon composite electrode and carbon composite electrode modified with chlorogenic acid. As it can be seen, a thin film of chlorogenic acid has been adsorbed on electrode surface.

3.2. Electrocatalytic properties of modified chlorogenic acid CCE for oxidation NADH

Due to high electrochemical reversibility and stability of carbon ceramic electrodes modified with thin film of chlorogenic acid, they are widely used in electrochemistry as elec-

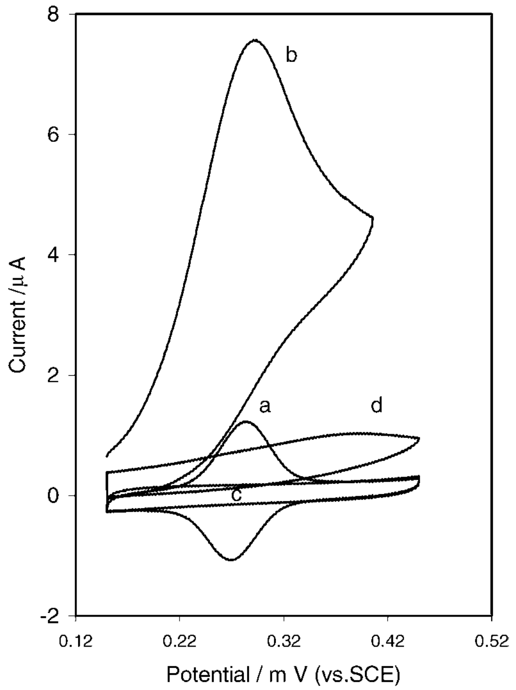

Fig. 3. Cyclic voltammograms of CGA modified carbon composite electrode in 0.1 M phosphate buffer solution (pH 6) at scan rate 20 mV s^{-1} in the absence (a) and 1 mM NADH solution (b). (c and d as a and b) for bare electrode at the same condition.

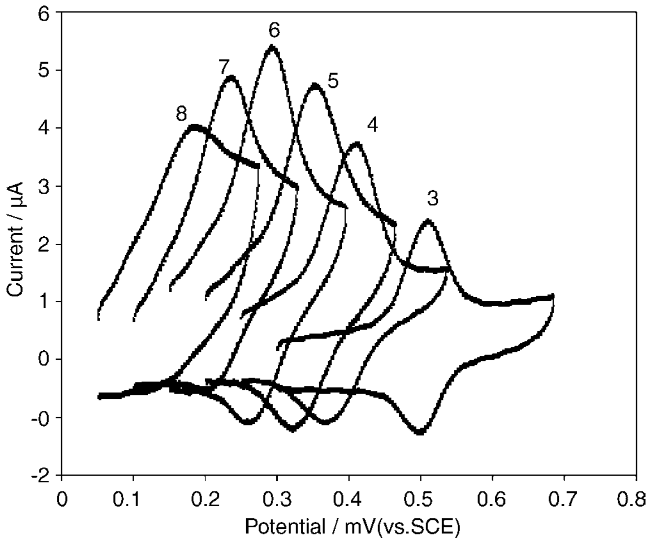

Fig. 4. Cyclic voltammetric response of modified carbon ceramic electrode in solutions containing 1 mM NADH at scan rate 20 mV s^{-1} at different pH.

tron transfer mediator to shuttle electrons between NADH and substrate electrodes. Fig. 3 shows the cyclic voltammograms of the bare and modified CCE in the presence and absence of NADH. As shown in Fig. 3c and d, no response is observed at the surface of bare CCE in the presence and absence of NADH in potential range 0.15–0.45 V. The CGA modified CCE in the blank solution (0.1 M phosphate buffer solution pH 6), exhibits a well-defined cyclic voltammogram at 0.27 V versus reference electrode (Fig. 3a). Upon the addition of 1 mM NADH, there is a dramatic enhancement of the anodic peak current at less positive potentials (before oxidation potential of chlorogenic acid) and the cathodic peak current disappears, indicating a strong electrocatalytic effect of chlorogenic acid for NADH oxidation (Fig. 3b). The electrocatalytic oxidation of NADH at low potentials is also very useful for practical applications since there is less risk for interfering electrochemical reactions to take place. As we reported previously [42], the electrochemical properties of CGA-modified CCE are strongly pH-dependent in buffered solutions. To optimise the electrocatalytic response of the modified CCE to NADH oxidation, we investigated the effect of pH on the catalytic oxidation. The cyclic voltammograms of modified CGA–CCE in 1 mM NADH solution at different pH values (pH 3–8) are presented in Fig. 4. As it can be seen, with increasing pH values, the peak potential shifts to less positive values and the peak current increase. At pH > 6 the peak current decreases, due to conversion of chlorogenic acid to the more soluble anionic form. The modified electrode can be used for NADH detection in pH range 4–8, but the most stable and sensitive signal was observed at pH 6.

The cyclic voltammogram responses for a series of NADH solution with various concentrations are shown in Fig. 5. With the addition of NADH (1–3 mM), there was an increase in the

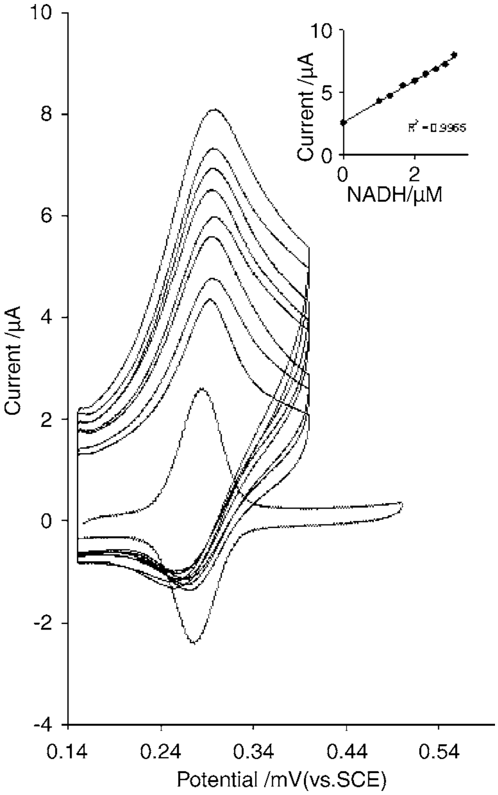

Fig. 5. Cyclic voltammograms of modified CCE in 0.1 M phosphate buffer solution (pH 6) at scan rate 20 mV s^{-1} with increasing NADH concentration (from inner to outer) 1–3 mM. Inset, plot of peak current vs. NADH concentration.

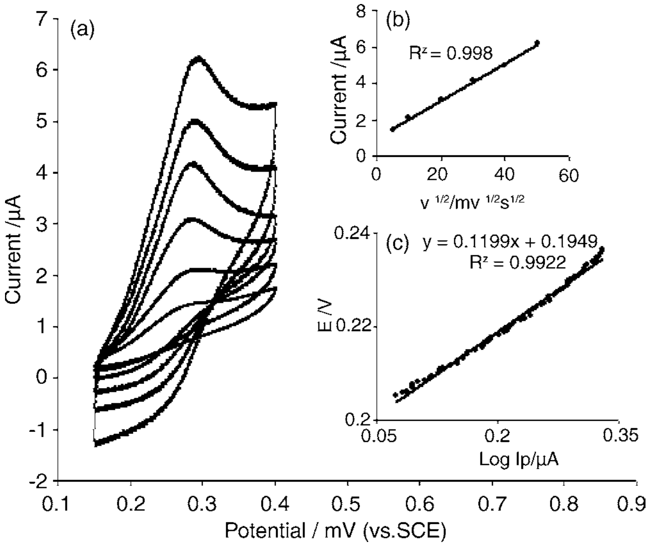

Fig. 6. (A) Cyclic voltammograms of modified CCE in 0.1 M phosphate buffer solution (pH 6) containing 1 mM NADH at different scan rates (from inner to outer) 5, 10, 20, 30, 40 and 60 mV s^{-1}. (B) Plot of I_p vs. (scan rate)$^{1/2}$. (C) Tafel plot derived from a current–potential curve at scan rate 20 mV s^{-1}.

anodic peak current. Plot of catalytic current versus NADH concentration was linear in the concentration range 1–10 mM. At higher concentration there appears to be a levelling of the response, probably due to the saturation of active sites of chlorogenic acid with NADH and its oxidation products.

By recording cyclic voltammograms of 1.5 mM NADH solution in different scan rates (Fig. 6), the peak currents for the anodic oxidation of NADH are proportional to the square root of scan rate (Fig. 6B). This result indicates that at sufficiently positive potential the reaction is controlled by NADH concentration, which is the ideal case for quantitative applications. It can also be seen that by increasing the sweep rate, the peak potential for the catalytic oxidation of NADH shifts to more positive values and the plot of peak current versus square root of scan rate deviates from linearity, suggesting a kinetic limitation in the reaction between the oxidized form of the chlorogenic acid and NADH. Based on these results, the following catalytic scheme (EC′) describes the reaction sequence in the oxidation of NADH by the chlorogenic acid

$$\text{CGA(reducedform)} \rightarrow \text{CGA(oxidizedform)} + 2e^{-} \tag{1}$$

$$\text{CGA (reduced form)} + \text{NADH} \rightarrow \text{CGA (reduced form)} + \text{NAD}^{+} \tag{2}$$

In order to obtain information about the rate-determining step, a Tafel plot was drawn using the data derived from the rising part of the current–voltage curve recorded at scan rate 10 mV s^{-1} (Fig. 6C). A slope of 120 mV dec^{-1} is obtained, indicating that a one electron transfer was involved in the rate limiting step, assuming a charge transfer coefficient (α) of 0.53.

Under the above conditions for EC′ mechanisms, the theoretical model [43] can be used to calculate the catalytic rate constant. Based on this theory, a relation exists between the peak current and the concentration of NADH for the case of slow scan rate and large catalytic rate constant

$$I_{\mathrm{P}} = 0.446 nFAD^{1/2}\left(\frac{\nu F}{RT}\right)^{1/2} C_{\mathrm{S}} \tag{3}$$

where D is the diffusion coefficient of NADH (2.1 × 10^{-6} cm^2 s) [44], C_{S} is the bulk concentration (mol cm^{-3}) of NADH and other symbols have their usual meanings. A low value of K_{cat} results in values of the coefficient lowers than 0.446. For low scan rates (5–20 mV s^{-1}), the average value of this coefficient was found to be 0.28 for a chlorogenic acid modified electrode with surface concentration 1.3 × 10^{-10} mol cm^{-2} and a geometric area (A) 0.0314 cm^2 in 0.2 mM NADH at pH 6. According to the approach of Andrieux and Saveant and using Fig. 1 in Ref. [43], the average value of K_{cat} was calculated to be 1.7 (±0.2) × 10^3 M^{-1} s^{-1}.

Repetitive scanning at scan rate 20 mV s^{-1} checked the stability of electrocatalytic activity of CGA/CCE toward oxidation NADH. In the first three scans, the electreocatalytic currents decreased with scan number, but the currents then remained at 90% of the initial value after 20 cycles. The reproducibility of CGA-modified CCE for catalytic oxidation of NADH is evaluated by six successive polishing and modification with chlorogenic acid and then the cyclic voltammograms are recorded in 1 mM NADH solution (Fig. 7). The R.S.D. for six measured anodic peak currents was 2.5%). Also the relative standard deviation (R.S.D.) of the peak currents of 2 mM NADH for eight replicate determinations was 1.5%. Then at the surface CGA-modified CCE not only overvoltage for NADH oxidation decreased but also the antifouling properties of ceramic composite improved reproducibility.

3.3. *Amperometric detection of NADH at CGA-modified carbon composite electrode*

As shown in the above section the modified carbon composite electrode has excellent and strong electrocatalytic properties and facilities at low potential amperometric measurements of NADH. Fig. 8 shows chronoamperograms which were recorded for a rotating modified CCE (rotation speed 2000 rpm), under conditions where the potential was kept at 0.30 V in phosphate buffer solution (pH 6). As shown, during successive addition 1 and 25 μM NADH a well-defined response is observed. For each addition of NADH within a response time of less than 1 s, a sharp rise in the current was observed. The plot of current versus NADH concentration is shown in inset of Fig. 8. The sensitivity of the electrode is 25 nA μM^{-1}, and the linear range spans the concentration of NADH from 1 to 120 μM with a correlation coefficient of 0.998. The electrode has a detection limit of 0.2 μM at signal-to-noise ratio of 3. These analytical parameters are comparable or better than results that reported for analyti-

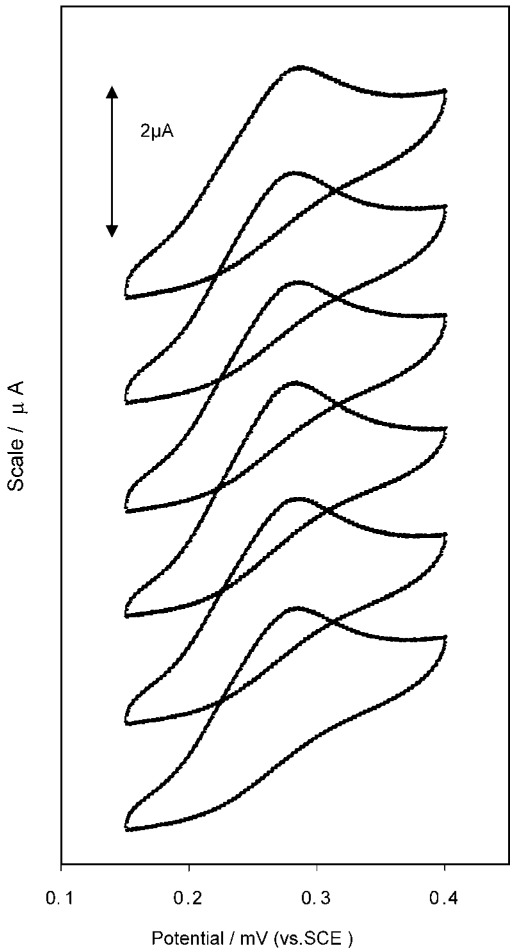

Fig. 7. Cyclic voltammograms (six successive) of CGA modified CCE after polishing and modification (5 s immersing time), in 0.1 M phosphate buffer solution (pH 6) containing 1.5 mM NADH at scan rate 20 mV s^{-1}.

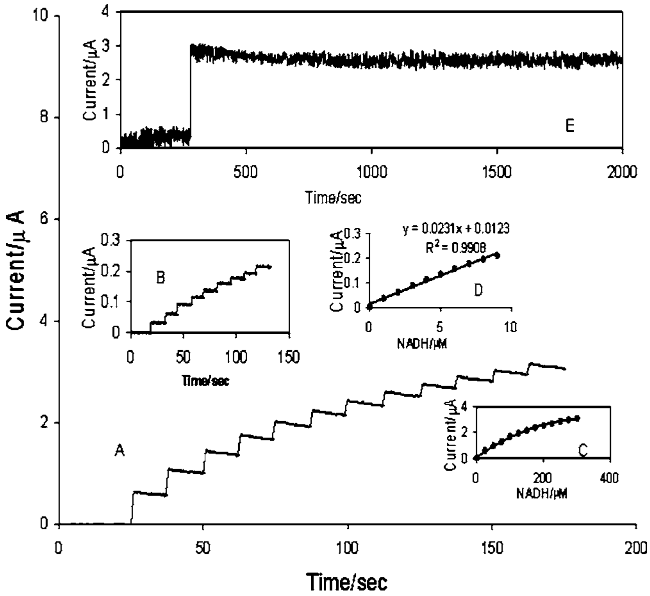

Fig. 8. Amperometric response at rotating modified carbon composite electrode (rotation speed 2000 rpm) held at 0.30 V in phosphate buffer solution (pH 6) for successive addition of (A) 25 µM and (B) 1 µM NADH addition. (C and D) Plots of chronoamperometric currents vs. NADH concentration. (E) Stability of the response to 0.1 mM NADH using modified CGA–CCE.

cal determination of NADH at the surface of different electrodes modified with transition metal complexes [20], transition metal hexacyanoferrates [21,22], tetrarutenated complex of cobalt porphyrin [23], different electroactive dyes [24–27], GC electrodes modified with *O*-hydroquinone groups mediators [28–33], carbon past electrode modified with diaphorase (DAP) and ferrocene [45], graphite electrode modified with dibenzo-dithia-diazopentacene [46], meldola blue immobilized on silica coated with niobium oxide [47], NAD$^+$ modified carbon paste [48], glassy carbon electrode modified with electropolymerized porphyrin film [49], poly(thionine) modified screen printed carbon electrode [50], meldola blue screen printed [51], carbon nanotube modified glassy carbon electrode [52] and self-assembled monolayer of Ni(II) macrocycle on gold electrode [53]. An extremely attractive feature of the CCE modified with chlorogenic acid is its highly stable amperometric response toward NADH. Fig. 8E shows the amperometric response of 100 µM NADH as recorded over a continuous 35 min period. The response of modified CGA–CCE remains stable throughout the experiment (only 5% decrease in current is observed after 35 min) indicating no inhibition effect of NADH and its oxidation products for modified electrode surface. Also the amperometric current of NADH remained unchanged after the modified electrode was stored for two weeks in air at room temperature, indicating that it could be used as stable sensor for NADH detection. Thus, the chlorogenic acid modified carbon ceramic electrode was found to exhibit very high sensitivity, stability, reproducibility and fast response time for NADH detection.

4. Conclusion

A modified electrode was successfully fabricated by electroless deposition of chlorogenic acid on the surface of carbon composite electrode prepared with sol–gel technique. A stable film of chlorogenic acid with long stability, electrochemical reversibility and excellent catalytic activity and reproducibility for NADH oxidation was achieved on the carbon composite surfaces. It offers a marked decrease in the overvoltage for the NADH oxidation and eliminates surface fouling effects. The electrode allowed for multiple measurements without significant decrease in signals that indicates no or little fouling of the electrode surface for NADH and its oxidation products. A carbon ceramic electrode modified with thin film of chlorogenic acid was used for fast amperometric detection of NADH at low potentials. The ability of CGA modified CCE to promote electron-transfer reactions of NADH suggests great promise for the design of amperometric biosensors in connection to the immobilization of suitable dehydrogenase enzymes.

Acknowledgements

This project has been supported by research office of Kurdistan University. The authors also gratefully acknowledge Michael E. Hyde (Oxford University) for taking the AFM images, Mr. Taher Sarhadi and Dr. Golam-Hosain Karimi-Dostan for their helpful discussions.

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