

H.R. Zare · S.M. Golabi

Caffeic acid modified glassy carbon electrode for electrocatalytic oxidation of reduced nicotinamide adenine dinucleotide (NADH)

Received: 6 January 1999 / Accepted: 11 May 1999

Abstract A new modified electrode was prepared by electrodeposition of caffeic acid (CFA) at the surface of an activated glassy carbon electrode. Cyclic voltammetry was used to investigate the redox properties of this electrode at various solution pH values and at various scan rates. The pH dependence of the electrode response was found to be 58.5 mV/pH, which is very close to the expected Nernstian value. The electrode was also employed to study electrocatalytic oxidation of reduced nicotinamide adenine dinucleotide (NADH), using cyclic voltammetry, chronoamperometry and rotating disk voltammetry as diagnostic techniques. It was found that the modified electrode exhibits potent and persistent electrocatalytic properties toward NADH oxidation in phosphate buffer solution (pH 7.0) with a diminution of the overpotential of about 450 mV compared to the process at an unmodified electrode. The electrocatalytic current increases linearly with NADH concentration in the range tested from 0.05 to 1.0 mM. The apparent charge transfer rate constant and transfer coefficient for electron transfer between the electrode surface and immobilized CFA were calculated as 11.2 s^{-1} and 0.43, respectively. The heterogeneous rate constant for oxidation of NADH at the CFA-modified electrode surface was also determined and found to be about $3 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. Finally, the diffusion coefficient of NADH was calculated as $3.24 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for the experimental conditions, using chronoamperometric results.

Key words Caffeic acid · NADH · Modified electrodes · Catalytic methods · Electrocatalytic oxidation

Introduction

Electrocatalysis at chemically modified electrodes (CMEs) is widely utilized for the determination of many inorganic and organic substrates, including bio-substrates. Various inorganic and organic materials such as polynuclear transition metal hexacyanometallates [1–3], polyoxometallates [4], a poly(vinylimidazole) complex of osmium [5], thionine derivatives [6, 7], *o*- and *p*-quinones [8–13], methylene blue [14], 3,4-dihydroxybenzaldehyde and related analogues [15–17], phenazines [18–20], phenoxazines [21–24], phenothiazines [25–28], pyrroloquinoline quinone [29], dopamine [30, 31], 2,6-dichlorophenol-indophenol salts [32] and chlorogenic acid [33] have been used to fabricate CMEs which can enhance the electron transfer rate and reduce the overpotential for the oxidation of reduced nicotinamide adenine dinucleotide (NADH). Studies of the oxidation of the reduced form of the nicotinamide coenzymes, NADH and NADPH, to the corresponding oxidized form, NAD^+ and NADP^+ , in aqueous solution have received considerable attention, since the largest group of redox enzymes known today is the one where the enzymatic process depends on a soluble nicotinamide coenzyme. These enzymes are usually termed dehydrogenases. Some 300 dehydrogenases depend on the NAD^+/NADH couple and 150 on $\text{NADP}^+/\text{NADPH}$ [34–36]. Also, the electrochemical oxidation of NADH and NADPH is of increasing interest in developing amperometric enzyme electrodes for substrates which are enzymatically coupled to NAD^+/NADH or $\text{NADP}^+/\text{NADPH}$ [34, 36]. The amperometric electrodes combine the specificity and selectivity of biological molecules with the direct transduction of reaction rate into current response and represent powerful tools in the field of analysis and biotechnology. However, the oxidation of NADH at the bare electrode surface is highly irreversible [37], and the reaction takes place at considerable overpotential [38, 39]. It is assumed that the oxidation of NADH at a solid electrode proceeds via two

H.R. Zare
Department of Chemistry, Faculty of Science, University of
Tehran, Tehran, Iran

S.M. Golabi (✉)
Electroanalytical Chemistry Laboratory, Faculty of Chemistry,
University of Tabriz, Tabriz, Iran

successive one-electron transfer steps involving radical intermediates, which often cause side reactions and electrode fouling. These processes lead to a loss in selectivity which appears as co-oxidation of other electroactive species present in the complex analytes [40–43]. To avoid these problems, many attempts have been made to reduce the high overvoltage and to suppress radical intermediate formation by favoring a simultaneous two-electron transfer mechanism. For this purpose, the electrode surface is modified with mediators, which can undergo fairly fast redox reactions with NADH. Some of these mediators were mentioned above.

Recently we have reported a new modified electrode for the electrocatalytic oxidation of NADH, based on the grafting of chlorogenic acid onto an activated glassy carbon (GC) electrode [33]. Owing to the importance of naturally occurring *o*-quinones in the processing of mediated electron transfer reactions and in order to clarify the effect of *o*-hydroquinone ring substituents on the reactivity of the mediator, we describe in this paper the characteristics of another modified electrode, prepared from the electrochemical bonding of caffeic acid on the surface of an preactivated GC electrode. The reactivity of this electrode was also examined toward the electrocatalytic oxidation of NADH with the aim of finding its capabilities as an electron transfer mediator.

Experimental

NADH- Na_2 (92% purity) and caffeic acid (CFA) or 3,4-dihydroxycinnamic acid (97% purity) were obtained from Merck and Riedel-de Haen, respectively, and used as received. All other chemicals were of analytical grade. All solutions were prepared with doubly distilled water. The buffer solutions (0.15 M) were made up from Na_2HPO_4 , NaH_2PO_4 and H_3PO_4 . Caffeic acid and NADH solutions were prepared just prior to use and all experiments were carried out at ambient temperature.

All electrochemical experiments were carried out using a 746 VA Trace Analyzer electrochemical system (Metrohm) in a cell equipped with a modified GC disk (2 mm diameter) as the working electrode, a platinum wire as an auxiliary electrode and with Ag/AgCl (sat)-KCl (3 M) as a reference electrode. All potentials in the text are quoted versus this reference electrode. A personal computer was used for data storage and processing.

The GC electrode was first polished mechanically with 0.05 μm alumina in a water slurry using a polishing cloth and then rinsed with doubly distilled water and acetone. The electrochemical activation of the electrode was performed by a continuous potential cycling from -1.1 to 1.6 V at a sweep rate of 100 mV s^{-1} in sodium bicarbonate (0.1 M) solution until a stable voltammogram was obtained. After rinsing with doubly distilled water, the activated electrode was modified by cycling the potential between 0.1 and 0.9 V at 20 mV s^{-1} for 12 min in a 1.0 mM solution of CFA in 0.15 M phosphate buffer (pH 4.0). After the CFA film had formed on the electrode surface, the electrode was rinsed thoroughly with water and dipped into the buffer solution to test its electrochemical behavior. The reproducibility of the modification stage was examined by measuring the coverage (Γ) of the modified electrode from the cyclic voltammograms recorded after 35 cycles of potential. A coefficient of variation of 5.0% was calculated for Γ ($n=5$). All solutions tested were deaerated by passing highly pure nitrogen (99.999%) before the electrochemical experiments and a continu-

ous flow of nitrogen was maintained over the sample solution during the experiments. The surface coverage of such modified electrodes was determined from cyclic voltammograms recorded at a low scan rate (25 mV s^{-1}) and by integration of anodic and cathodic peaks, assuming an n value of 2.

Results and discussion

Voltammetric properties of the CFA-modified GC electrode

The mechanism of bonding of substituted *o*-hydroquinones as modifiers on the surface of an activated GC electrode has already been discussed [16, 33]. Indeed, electrochemical treatment of the electrode surface leads to the formation of some reactive functional groups which can react as nucleophiles with the *o*-quinone produced during the electrooxidation of the modifier. The stability and reactivity of the deposited layer depends mainly on the appropriate conditions in which the modification is processed. For a CFA-modified electrode, the pH of the modifier solution is a critical factor affecting the surface coverage and is discussed in some detail below.

Cyclic voltammograms of a CFA-modified GC electrode prepared in optimal condition (see below) were taken in 0.15 M phosphate buffer solution (pH 7) at several different potential scan rates ranging from 25 to 1000 mV s^{-1} (Fig. 1). The anodic and cathodic currents of the redox waves increased in proportion to the potential sweep rates below 1000 mV s^{-1} , as shown in the inset of Fig. 1, indicating that the immobilized CFA exhibits electrochemical responses which are characteristic of the redox species confined on the electrode. In addition, the formal potential, taken as the average of the anodic and cathodic peak potentials, $E^{\circ'} = (E_{p,a} + E_{p,c})/2$, is about 200 mV and is almost independent of the potential scan rate for sweep rates ranging from 25 to 5000 mV s^{-1} . This value is lower than that reported for a chlorogenic acid (CGA) modified electrode [33], since the substituent on the *o*-quinone ring in CFA has a less electron-withdrawing effect than in CGA. The surface charge transfer rate constant, k_s , and the transfer coefficient, α , for the electron transfer between the electrode surface and the immobilized CFA can be calculated from cyclic voltammetric experiments using the variation of the oxidation and reduction peak potentials with the scan rate following the treatment developed by Laviron [44]. This peak separation will be close to zero when the electron transfer rate is fast relative to the scan rate and will increase when such a condition is fulfilled. Figure 2A shows the magnitudes of peak potentials (E_p) as a function of the potential sweep rate. We found that for scan rates above 2 V s^{-1} the values of E_p were proportional to the logarithm of the scan rate (Fig. 2, inset B). Although in the present case the values of k_s and α were pH dependent, using this plot at pH 7.0 the values of 11.2 s^{-1} and 0.43 were obtained

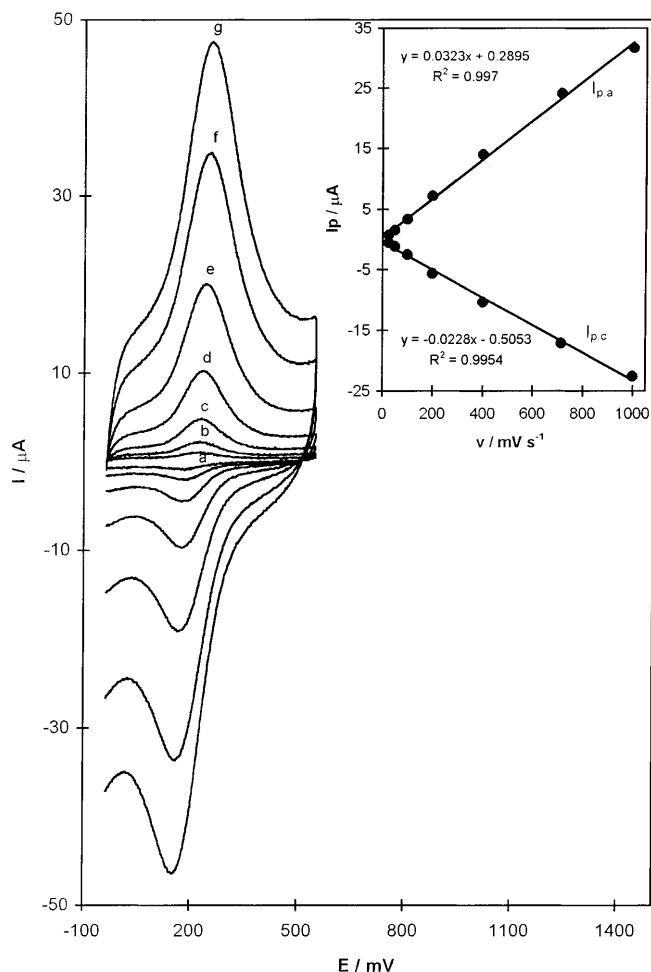


Fig. 1 Cyclic voltammograms of caffeic acid (CFA)-modified glassy carbon (GC) electrode in 0.15 M phosphate buffer (pH 7.0) at various scan rates: *a* 25, *b* 50, *c* 100, *d* 200, *e* 400, *f* 714 and *g* 1000 mV s⁻¹. Inset: variation of peak currents with sweep rate

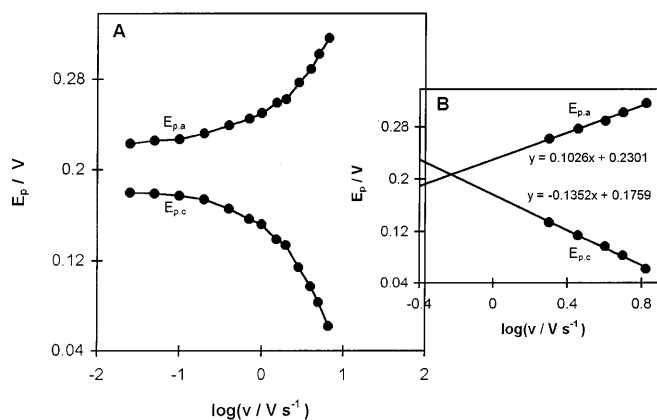


Fig. 2 A Experimental variation of *E*_p versus the logarithm of the scan rate for a CFA-modified GC electrode in 0.15 M phosphate buffer (pH 7.0). Inset B: magnification of the same plot for high scan rates

for these kinetic parameters. This value of *k*_s is almost in agreement with those reported for other deposited quinones at the same pH [45–47].

The effect of different pH values on the immobilization of CFA was investigated to optimize the testing performance. Figure 3A shows the affect of modifier solution pH on the surface coverage of the CFA-modified GC electrode. The best coverage for the CFA/GC electrode is obtained when the modification is carried out in a CFA solution at pH 4.0 (phosphate buffer 0.15 M); therefore this was selected in the subsequent modification process. For pH values above 4.0, a slow decrease in surface coverage is observed. It seems that at higher pH the bond formation between CFA *o*-quinone and active groups at the electrode surface begin to decrease because of a competing effect of interfering molecules reacting as nucleophiles with CFA *o*-quinone [48, 49].

The surface coverage (Γ) of the electrode modified at the optimum condition decreases rapidly at first by potential recycling between -50 and 550 mV and then remains almost constant (Fig. 3B, curve a). Such behavior is also observed during the time when the freshly modified electrode is kept in phosphate buffer (pH 7) (Fig. 3B, curve b). In all cases the surface coverage was evaluated from the cyclic voltammograms recorded at low scan rate and using the equation $\Gamma = Q/nFA$, where

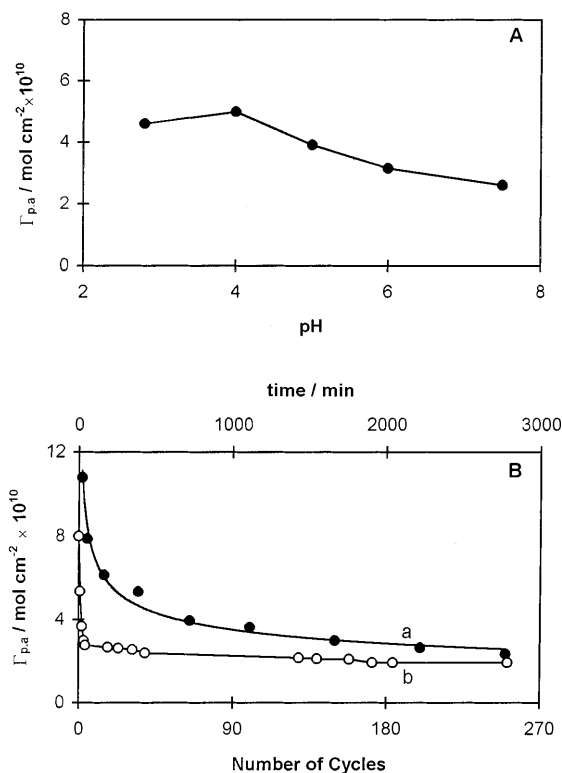


Fig. 3 A Variation in anodic peak surface coverage ($\Gamma_{p,a}$) for a CFA-modified GC electrode as a function of modifier solution pH during the preparation of the modified electrode. B Variation of $\Gamma_{p,a}$ during the repetitive recycling of the potential between -50 and 550 mV, *b* during the storage time of the modified electrode in phosphate buffer (pH 7)

Q is the charge obtained by integrating the anodic peak under the background correction and the other symbols have their usual meanings. The calculated value of $\Gamma = 5 \times 10^{-10} \text{ mol cm}^{-2}$ corresponds to the coverage of the voltammogram recorded after 35 cycles of potential. Figure 3B shows also the maximum and the minimum values of electrode coverage, which correspond to the electrodes newly modified and long stored in phosphate buffer (pH 7), respectively. Note that, despite the diminution of the electrode coverage during the time, its efficiency (defined as I_p/Γ) increases gradually until reaching a constant value. Such a feature has already been reported for a CGA-modified electrode [50].

Peak potential dependence on pH

The effect of pH on the CFA-modified GC electrode signal was investigated by cyclic voltammetry using a 0.15 M phosphate buffer at various pH values ranging from 2.0 to 11.2. As can be seen in Fig. 4, the formal

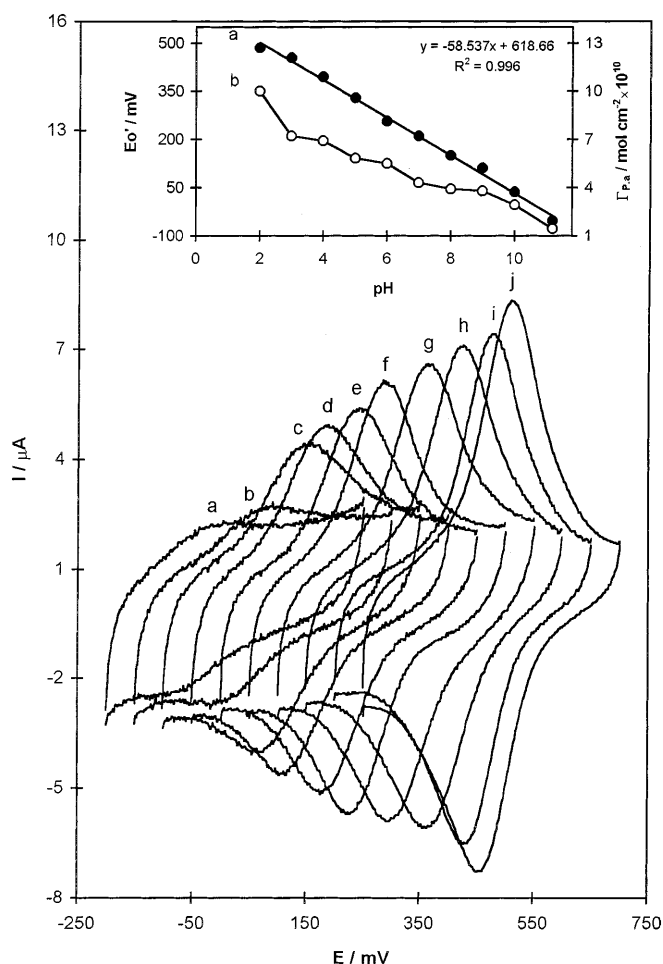
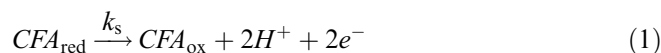


Fig. 4 pH dependence of the cyclic voltammetric response (at 100 mV s^{-1}) of a CFA-modified GC electrode. pH values are: a 11.2, b 10, c 9, d 8, e 7, f 6, g 5, h 4, i 3 and j 2. Inset: curve a, plot of formal potential versus pH; curve b, variations in surface coverage as a function of pH

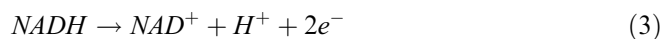
potential ($E^{\circ'}$) of the surface redox couple and anodic peak surface coverage were pH dependent. Curve a, (Fig. 4 inset) shows $E^{\circ'}$ as a function of pH. The slope was found to be 58.5 mV/pH unit over a pH range from 2.0 to 11.2, which is very close to the anticipated Nernstian value of 59 mV for a two-electron, two-proton process. In fact, such a process can be considered as a simple reaction with two successive one-electron exchanges as indicated by Laviron [51] for the conditions at which the transfer coefficients of the electrochemical reactions are about 0.5 and the protonations are at equilibrium. On the other hand, the reported value for pK_{a2} of soluble CFA is 8.55 [52], so that one would anticipate a change in the slope of $E^{\circ'}$ versus pH plot around this value. Since such behavior was not observed, it has to be assumed that no deprotonation of the electrodeposited CFA is taking place at the expected pH. Changes in pK_a of deposited species have also previously been reported [12, 33, 47, 53]. Another point that should also be mentioned is that there was a change in the surface coverage with pH. As can be seen in curve b of Fig. 4 inset, there was a decrease in surface coverage with an increase in pH values. The loss of coverage could be due to the displacement of surface-confined CFA by solvent molecules [54]. The deprotonation of surface-attached material is also proposed as the origin of the coverage decrease by some authors [12, 15]. However, this statement cannot be valid in our case since the surface-bound CFA remains in its protonated form even at higher pH (see inset of Fig. 4, curve a).

Catalytic oxidation of NADH at a CFA-modified GC electrode

Figure 5 curve b shows a cyclic voltammogram obtained with a CFA-modified GC electrode in a solution which was 1.0 mM in NADH. It can be seen that there is a great increase in the anodic current compared to a scan recorded in a buffer without NADH (curve a). This increasing oxidation current was due to the fact that the NADH in solution diffuses toward the electrode surface and reduces the electrochemically produced CFA_{ox} to CFA_{red} . As CFA_{red} is regenerated by NADH during the potential sweep, there will be an increase in the anodic current, while for the same reason the cathodic current is smaller in the presence of NADH. The process could be expressed as follows:



The overall oxidation of NADH by modified electrode is given as:



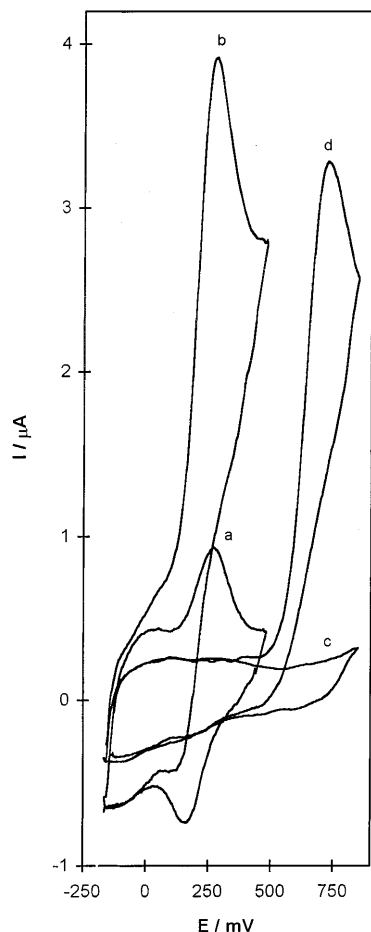


Fig. 5 Current potential curves for *a* a CFA-modified GC electrode in 0.15 M phosphate buffer (pH 7.0) solution and *b* the same electrode in a 1.0 mM NADH, pH 7.0 solution; *c* as *a* and *d* as *b* for an unmodified GC electrode. Scan rate = 25 mV s^{-1}

The rate determining step is given in Eq. 2 with a heterogeneous rate constant, k_h , which is representative of the catalytic reaction rate between the electrode surface-deposited CFA and solution-diffused NADH. The catalytic effect can be seen directly when curve *b* is compared with curve *d* in Fig. 5. The latter shows the cyclic voltammogram of NADH at an unmodified glassy carbon electrode under identical conditions with curve *b*. The catalytic peak potential is found to be about 245 mV, whereas that of the uncatalyzed peak is about 695 mV. Thus, a decrease in the overvoltage of approximately 450 mV and an enhancement of peak current also is achieved with the modified electrode.

The repeatability of the modified electrode response toward a 0.5 mM solution of NADH in buffer solution (pH 7) was examined by repetitive recording of the cyclic voltammogram of the test solution and measuring the peak current. A coefficient of variation of about 4.5% was obtained for I_p ($n=7$).

Figure 6 shows the cyclic voltammograms of the CFA-modified GC electrode at various scan rates obtained in 0.15 M phosphate buffer solution (pH 7.0)

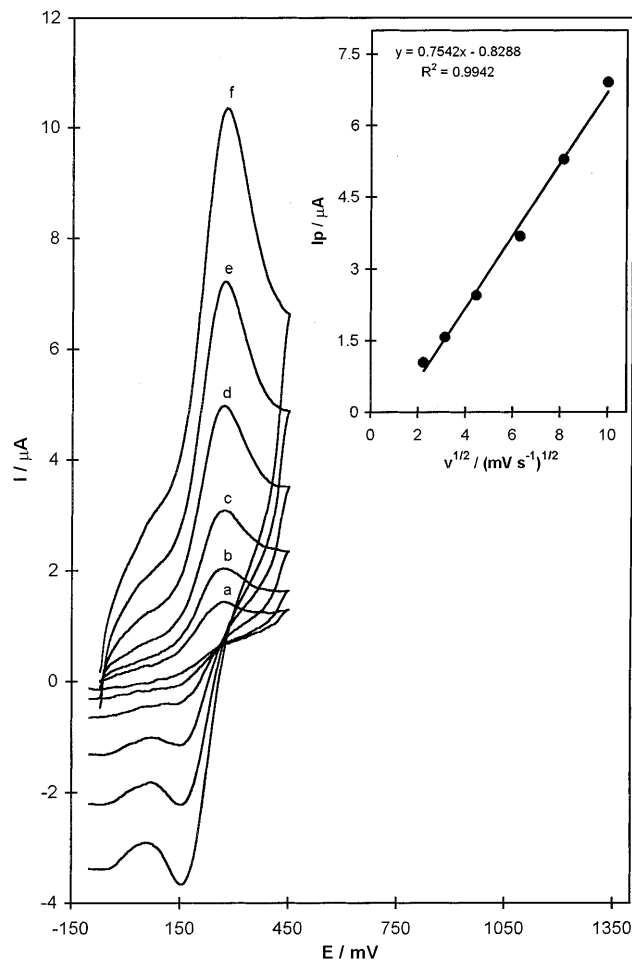


Fig. 6 Cyclic voltammograms of a CFA-modified GC electrode in 0.15 M phosphate buffer solution (pH 7.0) containing 0.8 mM NADH at various scan rates: *a* 5, *b* 10, *c* 20, *d* 40, *e* 66.7 and *f* 100 mV s^{-1} . Inset: variations of the electrocatalytic current with the square root of the sweep rate

containing 0.8 mM NADH. As indicated by Fig. 6, the catalytic oxidation peak potential gradually shifts slightly toward positive potentials with increasing scan rate, and the oxidation currents increase linearly with the square root of the scan rate. These results show that a kinetic limitation exists in the reaction between the NADH and the redox sites of the CFA film and indicates that, at sufficient overpotential, the reaction is controlled by the diffusion of NADH in solution. For the case of slow scan rate, v , and large catalytic rate constant, k_h , Andrieux and Saveant [55] developed a theoretical model for a heterogeneous catalytic reaction:

$$I_{\text{cat}} = 0.496 nFAD^{1/2}v^{1/2}C_b(nF/RT)^{1/2} \quad (4)$$

where D and C_b are respectively the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the bulk concentration (mol cm^{-3}) of NADH, and the other symbols have their usual meanings. Low values of k_h result in values of the coefficient lower than 0.496. For low scan rates (2.0–10.0 mV s^{-1}), we find the average value of this constant to be 0.31 for a

CFA-modified GC electrode with a coverage of $\Gamma = 5 \times 10^{-10} \text{ mol cm}^{-2}$, a net area, A , of 0.031 cm^2 and $D = 2.4 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ [56] in the presence 0.8 mM NADH. According to the approach of Andrieux and Saveant and using Fig. 1 in their theoretical paper [55], we calculated an average value of $k_{\text{h}} = 2.61 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. This value is approximately in accordance with that obtained below using rotating disk electrode (RDE) voltammetry.

The effect of increasing concentration of NADH on the voltammetric response of the CFA-modified GC electrode was also investigated. Figure 7 shows the cyclic voltammograms for different concentrations of NADH. Upon the addition of NADH, there was a dramatic enhancement in the anodic current. The peak current was proportional to the NADH concentration in the range from 0.05 to 1.0 mM , as is shown in the inset of Fig. 7. For solutions with higher concentrations of NADH there appears to be a leveling off the peak current, indicating the involvement of a rate decreasing

parameter. It seems likely that the electrocatalytic process could be inhibited by NAD^+ produced at elevated concentrations of substrate, as earlier reported by others [45].

Chronoamperometric experiment

The catalytic oxidation of NADH by a CFA-modified GC electrode was also studied by chronoamperometry. Chronoamperograms obtained at a potential step of 320 mV are depicted in Fig. 8A. In chronoamperometric studies, we have determined the diffusion coefficient of NADH for a glassy carbon electrode modified with CFA. The current for the electrochemical reaction (at a mass transport limited rate) of an electroactive material (NADH in this case) with a diffusion coefficient, D , is described by the Cottrell equation [57]:

$$I = nFAD^{1/2}C_b/\pi^{1/2}t^{1/2} \quad (5)$$

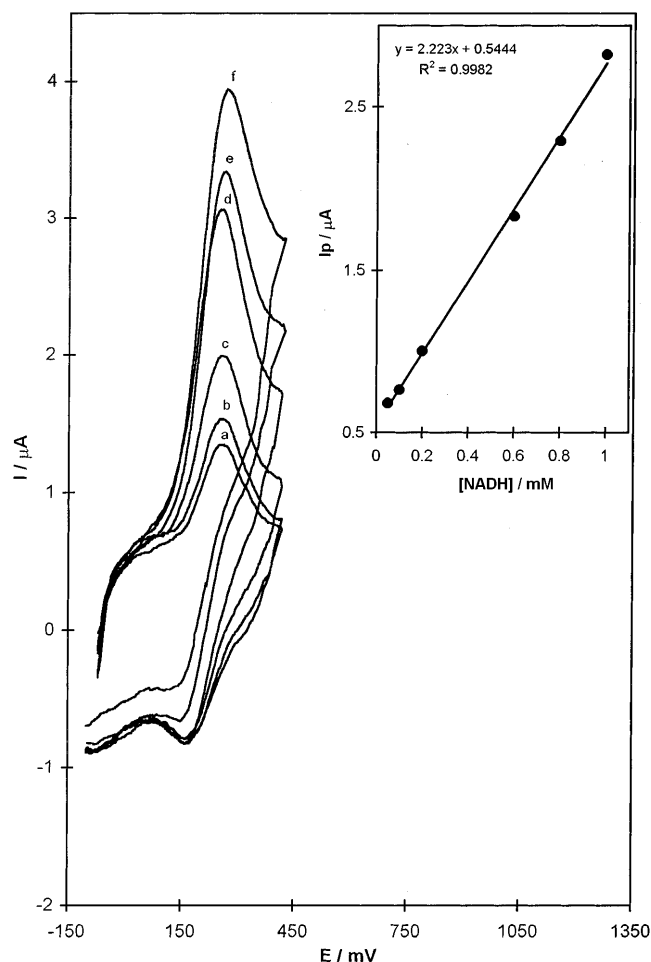


Fig. 7 Dependence of cyclic voltammetric response on NADH concentration for a CFA-modified GC electrode in 0.15 M phosphate buffer (pH 7.0) at 25 mV s^{-1} . NADH concentrations are a 0.05 , b 0.1 , c 0.2 , d 0.6 , e 0.8 and f 1.0 mM . Inset: variation of catalytic current versus NADH concentration

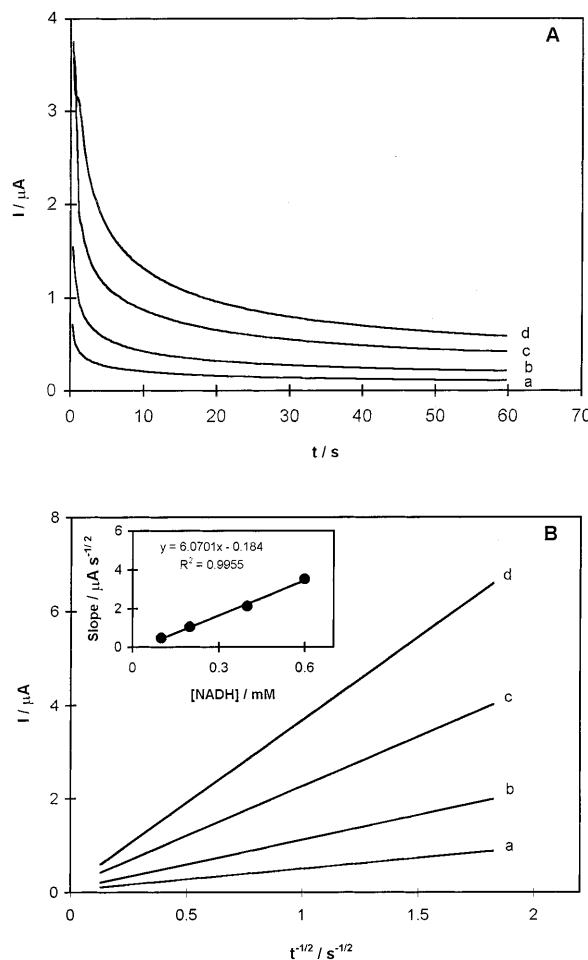


Fig. 8 **A** Chronoamperometric response of a CFA-modified GC electrode in 0.15 M phosphate buffer solution (pH 7.0) at a potential step of 320 mV for different concentrations of NADH: a 0.1 , b 0.2 , c 0.4 and d 0.6 mM . **B** Plots of I versus $t^{-1/2}$ obtained from chronoamperograms shown in A. Inset: plot of the slopes of the straight lines against the NADH concentration

where D is the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and C_b is the bulk concentration (mol cm^{-3}) of substrate. Under diffusion (mass transport) control, a plot of I versus $t^{-1/2}$ will be linear, and from the slope the value of D can be obtained. We have carried out such studies at various NADH concentrations, for a CFA-modified GC electrode. Figure 8B shows the experimental plots with the best fits for different concentrations of NADH employed. The slopes of the resulting straight lines were then plotted versus the NADH concentration (Fig. 8B, inset), from whose slope we calculated a diffusion coefficient of $3.24 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ for NADH. We designate the obtained value as an apparent diffusion coefficient, since we believe that in the experimental conditions the diffusion of NADH from solution bulk to electrode surface can be affected to some extent by the rate of electron transfer between substrate and modifier. However, as cited above, the calculated value of the diffusion coefficient is very close to the value reported elsewhere [56].

RDE measurements

With RDE voltammetry, the catalytic rate constant, k_h , of the reaction involved between CFA film and NADH was also measured. The RDE voltammograms were recorded for various concentrations of NADH in 0.15 M phosphate buffer (pH 7.0) and at various rotation rates using a CFA-modified GC RDE with a coverage of $2.8 \times 10^{-10} \text{ mol cm}^{-2}$. The results for a 0.2 mM solution of NADH are shown in Fig. 9A. The plot of catalytic currents measured at 280 mV versus $\omega^{1/2}$ (Levich plot) are shown in Fig. 9B. It can be seen that the clear lack of linearity immediately suggests that the reaction is limited by kinetics and not by mass transport. In addition, slow electron transfer between the electrodeposited CFA and the electrode can also be discarded as rate limiting. Thus, we conclude that the reaction between NADH and mediator CFA is the rate-determining step. Under these conditions, the Koutecky-Levich equation can be used to determine the rate constant for the process. The Koutecky-Levich equation can be formulated as follows [57]:

$$\frac{1}{i_{\text{lim}}} = \frac{1}{nFAk_h\Gamma C_b} + \frac{1}{0.62nFAv^{-1/6}D^{2/3}\omega^{1/2}C_b} \quad (6)$$

where C_b is the bulk concentration of the NADH (mol cm^{-3}), ω is the angular frequency of rotation (rad s^{-1}), D is the diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$), Γ is the surface coverage of CFA (mol cm^{-2}), ν is the kinematic viscosity ($\text{cm}^2 \text{ s}^{-1}$), k_h is the reaction rate constant ($\text{M}^{-1} \text{ s}^{-1}$) between surface-deposited CFA and solution-diffused NADH, and all other parameters have their conventional meanings. The Koutecky-Levich plots, obtained from the data in Fig. 9B, are shown in Fig. 9C. These plots show the anticipated linear dependence between $1/i_{\text{lim}}$ and $1/\omega^{1/2}$. The rate constant, k_h , can be calculated from the intercepts of the Koutecky-Levich plots. It was

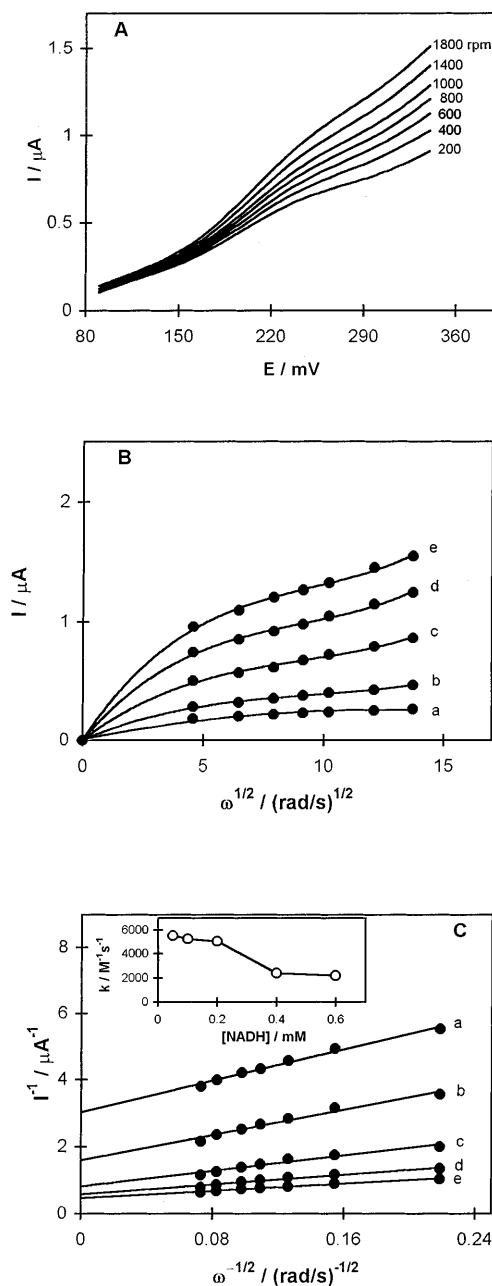


Fig. 9 A Voltammograms of a CFA-modified GC rotating disk electrode in 0.15 M phosphate buffer (pH 7.0) containing 0.2 mM NADH at the various rotation rates indicated for each voltammogram. B Levich plots for NADH oxidation at 280 mV at a CFA-modified GC electrode. The NADH concentrations were a 0.05, b 0.1, c 0.2, d 0.4 and e 0.6 mM. C Koutecky-Levich plots obtained from the Levich plots shown in B. The experimentally obtained curves correspond to an electrode with $\Gamma = 2.8 \times 10^{-10} \text{ mol cm}^{-2}$, and in parts B and C the points are the data and the lines are the least-squares fits

found that k_h decreases significantly with increasing the bulk concentration of NADH (Fig. 9C inset). A similar observation has been previously reported for the electrooxidation of NADH at electrodes modified with different mediators [1, 2, 17, 23, 30]. This observation also provides additional evidence against the restricted access

of the substrate, NADH in this case, as the rate limiting step. From the values of the intercepts, an average value of k_h was found to be $3.18 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$, which is approximately similar to that determined from cyclic voltammetric measurements. Also, this value is comparable with those previously reported for the electrocatalytic oxidation of NADH at electrodes modified with other mediators such as 1,2-benzophenoxazin-7-one [23, 24], 5-methylphenazinium [30], chlorogenic acid [33], 4-methylcatechol [58], poly(thionine) [59] and 3,4-dihydroxybenzaldehyde [60].

Conclusions

CFA can be oxidatively electrodeposited onto GC electrodes previously activated by potential recycling in alkaline solution. The redox response of the film is that anticipated for a surface-immobilized redox couple. The electrodeposition process and the electrochemical behavior of the modified electrodes are strongly dependent on solution pH. This modified electrode shows a satisfactory stability (see Fig. 3B) toward potential recycling and is capable of catalyzing the electrochemical oxidation of NADH. A quantitative analysis of the electrocatalytic reaction based on cyclic voltammetry and rotating disk voltammetry gives an electrocatalytic reaction rate constant, k_h , on the order of about $3 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$.

Acknowledgements This work was supported by Yazd University. Mr. M. Hamzaloo is gratefully acknowledged for technical assistance.

References

- Cai CX, Ju HX, Chen HY (1995) *J Electroanal Chem* 397: 185
- Cai CX, Chen HY, Ju HX (1995) *Anal Chim Acta* 310: 145
- Somasundrun M, Hall J, Bannister JV (1994) *Anal Chim Acta* 295: 47
- Essaadi K, Keita B, Nadjo L (1994) *J Electroanal Chem* 367: 275
- Ju H, Leech D (1997) *Anal Chim Acta* 345: 51
- Hajizadeh K, Tang HT, Halsall HB, Heinemann WR (1991) *Anal Lett* 24: 1453
- Chen HY, Zhou DM, Xu JJ, Fang HQ (1997) *J Electroanal Chem* 422: 21
- Ueda C, Tse DCS, Kuwana T (1982) *Anal Chem* 54: 850
- Huck H, Schmidt HL (1981) *Angew Chem* 93: 421
- Ravichandran K, Baldwin RP (1981) *J Electroanal Chem* 126: 293
- Fukui M, Kitani C, Degrand C, Miller LL (1982) *J Am Chem Soc* 104: 28
- Lorenzo E, Sanchez L, Pariente F, Tirado J, Abruna HD (1995) *Anal Chim Acta* 309: 79
- Murthy ASN, Sharma J (1998) *Talanta* 45: 951
- Chi Q, Dong S (1994) *Analyst* 119: 1063
- Pariente F, Lorenzo E, Abruna HD (1994) *Anal Chem* 66: 4337
- Pariente F, Tobalina F, Darder M, Lorenzo E, Abruna HD (1996) *Anal Chem* 68: 3135
- Pariente F, Tobalina F, Moreno G, Hernandez L, Lorenzo E, Abruna HD (1997) *Anal Chem* 69: 4065
- Torstensson A, Gorton L (1981) *J Electroanal Chem* 130: 199
- Kulys JJ (1981) *Anal Lett* 14: 377
- Grundig B, Wittstock G, Rudel U, Strehlitz B (1995) *J Electroanal Chem* 395: 143
- Huck H (1982) *Fresenius J Anal Chem* 313: 548
- Ni F, Feng H, Gorton L, Cotton TM (1990) *Langmuir* 6: 66
- Gorton L, Torstenson A, Jaegfeldt H, Johansson G (1984) *J Electroanal Chem* 161: 103
- Persson B, Gorton L (1990) *J Electroanal Chem* 292: 115
- Persson B (1990) *J Electroanal Chem* 287: 61
- Kulys J, Gleixner G, Schumann W, Schmidt HL (1993) *Electroanalysis* 5: 201
- Ohtani M, Kuwabata S, Yoneyama H (1997) *J Electroanal Chem* 422: 45
- Cai CX, Xue KH (1997) *J Electroanal Chem* 427: 147
- Katz E, Lotzbeyer T, Schlereth DD, Schuhmann W, Schmidt HL (1994) *J Electroanal Chem* 373: 189
- Albery WJ, Bartlett PN (1984) *J Chem Soc Chem Commun* 234
- Deinhammer RS, Ho M, Anderegg JW, Porter MD (1994) *Langmuir* 10: 1306
- Ageliki BF, Mamas IP, Miltiades IK, Stella MTK (1998) *Electroanalysis* 10: 1261
- Zare HR, Golabi SM (1999) *J Electroanal Chem* 464: 14
- Bergel JS, Comtat M (1989) *Anal Biochem* 179: 382
- Lee LG, Whitesides GM (1985) *J Am Chem Soc* 107: 6999
- Gorton L, Csoregi E, Dominguez E, Emneus J, Jonsson-Petersson G, Marko-Varga G, Persson B (1991) *Anal Chim Acta* 250: 203
- Elving PJ, Schmamel CO, Santhanam KSV (1976) *CRC Crit Rev Anal Chem* 6: 1
- Moiroux J, Elving PJ (1978) *Anal Chem* 50: 1056
- Jaegfeldt H (1980) *J Electroanal Chem* 110: 295
- Elving PJ, Bresenahan WT, Moiroux J, Samec Z (1982) *Bioelectrochem Bioenerg* 9: 365
- Moiroux J, Elving PJ (1979) *J Electroanal Chem* 102: 93
- Schmamel CO, Santhanam KSV, Elving PJ (1975) *J Am Chem Soc* 97: 5085
- Coughlin RW, Aizawa M, Alexander BF, Charles M (1975) *Biotechnol Bioeng* 17: 515
- Laviron E (1979) *J Electroanal Chem* 101: 19
- Tse DCS, Kuwana T (1978) *Anal Chem* 50: 1315
- Jaegfeldt H, Torstenson A, Gorton L, Johansson G (1981) *Anal Chem* 53: 1979
- Jaegfeldt H, Kuwana T, Johansson G (1983) *J Am Chem Soc* 105: 1805
- Nematollahi D, Golabi SM (1996) *J Electroanal Chem* 405: 133
- Golabi SM, Nematollahi D (1997) *J Electroanal Chem* 420: 127
- Golabi SM, Zare HR (1999) *J Electroanal Chem* 465: 168
- Laviron E (1983) *J Electroanal Chem* 146: 15
- Kiss T, Nagy G, Pecci M, Kozlowski H, Micera G, Strinna Erre L (1989) *Polyhedron* 8: 2345
- Gorton L, Johansson G (1980) *J Electroanal Chem* 113: 151
- Horspool WM, Smith PI, Tedder JM (1972) *J Chem Soc Perkin Trans I* 1024
- Andrieux CP, Saveant JM (1978) *J Electroanal Chem* 93: 163
- Moiroux J, Elving PJ (1980) *J Am Chem Soc* 102: 6533
- Electrochemical methods, fundamentals and applications. Wiley, New York
- Degrand C, Miller LL (1980) *J Am Chem Soc* 102: 5728
- Ohsaka T, Tanaka K, Tokuda K (1993) *J Chem Soc Chem Commun* 222
- Pariente F, Lorenzo E, Tobalina F, Abruna HD (1995) *Anal Chem* 67: 3936