

## Electrochemical behaviour of $\text{NAD}^+$ -modified carbon paste electrodes

S B Saidman

Instituto de Ingeniería Electroquímica y Corrosión (INIEC), Departamento de Ingeniería Química,  
Universidad Nacional del Sur, Av. Alem 1253.8000-Bahía Blanca, Argentina  
Email: ssaidman@criba.edu.ar

Received 14 November 2002

The effects of electrochemical pretreatment time on the electrochemical response of  $\text{NAD}^+$ -modified carbon paste electrodes have been analysed. Prolonged pretreatment results in an improvement in the catalysis of NADH oxidation. First, pretreatment produces a removal of pasting liquid that increases the number of surface sites for NADH oxidation. But this surface activation reaches a limiting value and, at longer pretreatment times, activation is produced by the contribution of reduced surface functionalities.  $\text{NAD}^+$ -modified carbon paste electrodes have also been fabricated by incorporating appropriate quantities of  $\text{NAD}^+$  directly into the paste mixture. Analogous to solution-phase  $\text{NAD}^+$ , the electrodes constructed in this manner present true surface active groups and are able to catalyse NADH oxidation. Pretreatment does not result in a loss of their electrocatalytic properties.

### Introduction

It is well known that the reduced form of nicotinamide adenine dinucleotide (NADH), the main coenzyme of dehydrogenases, is oxidised electrochemically only with high overvoltages<sup>1</sup>. Carbon paste electrodes modified with  $\text{NAD}^+$  allow to decrease this overpotential<sup>2-4</sup>. The electrode was prepared by anodic polarisation at potentials ca. 1.25 V (SCE) in neutral and mild alkaline solutions containing  $\text{NAD}^+$ . The reversible system responsible for this catalysis was identified as the adenine moiety of  $\text{NAD}^+$ .

The performance of carbon paste electrodes can be improved by electrochemical pretreatments<sup>5,6</sup>. Previous work indicates that potentiodynamic cycling for activation results in higher catalytic response for NADH oxidation even with the paraffin-richest electrode composition<sup>4</sup>, which is the best configuration from a practical viewpoint. Cathodic polarisation also produces a catalytic current improvement that is independent of the carbon paste composition<sup>4</sup>.

In the present paper an attempt is made to improve the pretreatment procedure in order to enhance sensitivity in the development of amperometric biosensors. It has also been demonstrated that carbon paste can be used to co-immobilise enzyme and  $\text{NAD}^+$  for the design of biosensors. The  $\text{NAD}^+$ -modified carbon paste electrode has been used as amperometric transducer in determinations of glycerol<sup>1</sup> and sorbitol<sup>7</sup>. Then, the electrode response

of  $\text{NAD}^+$  incorporated directly into the paste mixture was characterised with cyclic voltammetry and this behaviour was compared to that obtained with  $\text{NAD}^+$  in solution.

### Materials and Methods

The experimental set-up has been described previously<sup>7</sup>. The working electrode was a piston-like carbon paste electrode with a Teflon body and stainless steel contact. The diameter of the electrode surface was 3 mm. Carbon pastes were prepared by hand mixing the graphite (Graphite extrapure, Merck) and the paraffin-oil (Uvasol, Merck). Paste formulation is expressed in terms of the weight ratio of paraffin/graphite. All potentials were measured against a saturated calomel electrode (SCE) and a platinum foil was used as counter electrode.

Unless stated otherwise, measurements were performed in 0.1 M sodium phosphate, pH 10 solution in a purified nitrogen gas saturated atmosphere. All chemicals were reagent grade and solutions were freshly made in twice distilled water.  $\text{NAD}^+$  and NADH were purchased from Sigma Chemical Company.

### Results and Discussion

#### *Electrochemical pretreatment*

It was demonstrated that the catalytic current for NADH oxidation at  $\text{NAD}^+$ -modified electrodes is enhanced if a previous pretreatment is applied<sup>1</sup>. This pretreatment procedure consists of potential cycling

between  $-0.5$  and  $2$  V at  $5$  V s $^{-1}$  for  $5$  min in a  $0.1$  M phosphate buffer pH 10. Immediately after, NAD $^{+}$  was added to a final concentration of  $10^{-3}$  M and the electrode was cycled between  $-0.25$  and  $1.4$  V at  $0.020$  V s $^{-1}$  in order to obtain the NAD $^{+}$ -modified carbon paste electrode. Then the electrode was washed and transferred to a phosphate solution without NAD $^{+}$ . When the background current was constant at  $0.0$  V, NADH was added to a final concentration of  $10^{-4}$  M while stirring, and the catalytic current was measured. For pretreatment times longer than  $5$  min, the amperometric response to NADH increased when the graphite-rich electrodes were used. But in these cases an increased background current was obtained at the same time, and the electrodes did not give reproducible responses. However, as the percentage of pasting liquid increased an improvement in the catalytic signal was obtained without any loss of reproducibility. The voltammogram obtained for a pasting liquid/graphite ratio of  $0.44$  after a pretreatment of  $15$  min is presented in Fig. 1. It can be observed that the initial potentiodynamic run in the anodic direction shows a broad anodic peak at  $-0.1$  V and, at more positive potentials, the peak associated with the oxidation of NAD $^{+}$ . In the subsequent scans, the voltammetric response exhibited redox peak with a formal potential of about  $-0.010$  V, indicating the presence of the adsorbed redox products. The anodic peak that appears at  $-0.1$  V in the first cycle did not appear if the pretreatment was applied for only  $5$  min. Furthermore, its peak current, corrected for the background current, increased with increasing pretreatment time. The currents measured for NADH oxidation at  $0.0$  V ( $i_{cat,1}$ ) for different pretreatment times are shown in Table 1. Each of these values is based on three independent measurements. When the carbon paste electrodes were pretreated without the posterior cycling in NAD $^{+}$ -solution, the surface was activated and the oxidation current of NADH was also determined at  $0.0$  V ( $i_{cat,2}$ ). It can be observed that with increasing pretreatment time, the electrocatalytic activity for NADH ( $i_{cat,2}$ ) does not present the important increase obtained when after this procedure, the electrode was modified with the NAD $^{+}$ -oxidation products ( $i_{cat,1}$ ).

Previously, it was found that after a pretreatment of  $5$  min, the relative increase in the catalytic current is higher for the rich-paraffin electrodes $^3$ . On the contrary, after longer pretreatment times, the relative

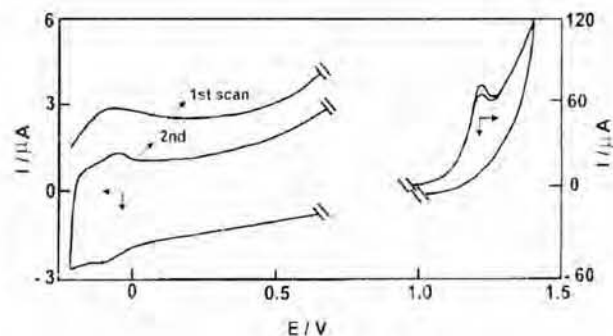


Fig. 1—Cyclic voltammograms obtained with a carbon paste electrode in a  $0.1$  M phosphate solution pH 10 containing  $10^{-3}$  M NAD $^{+}$ . Only the first scan and the second anodic scan are depicted. The carbon paste electrode was first cycled between  $-0.5$  and  $2.0$  V at  $5$  V s $^{-1}$  for  $15$  min. Paraffin to graphite ratio:  $0.44$ . Scan rate:  $0.020$  V s $^{-1}$ .

Table 1—Catalytic current for NADH oxidation as a function of pretreatment time. The  $i_{cat}$  values refer to the catalytic current measured at  $0.0$  V in a  $0.1$  M phosphate solution pH 10 for a  $10^{-4}$  M NADH, using: pretreated carbon paste electrodes (potentiodynamic cycling between  $-0.5$  and  $2.0$  V at  $5$  V s $^{-1}$ ) modified with NAD $^{+}$  oxidation products ( $i_{cat,1}$ ) and carbon paste electrodes pretreated ( $i_{cat,2}$ ).

Pretreatment time (min)	$i_{cat,1}$ ( $\mu$ A)	$i_{cat,2}$ ( $\mu$ A)
5	0.075	0.475
10	0.084	0.555
15	0.094	0.610
30	0.096	0.775

increment was practically independent of the pasting liquid content.

On the other hand, the relative increase of the conductor surface area obtained employing chronoamperometric measurements of the oxidation  $K_4Fe(CN)_6 \cdot 3H_2O$ , was  $3.14$  when the pretreatment was applied $^4$  during  $5$  min (ref. 4). This value was not substantially modified when the activation time was increased.

The dependence between the amperometric response to NADH examination using a pretreated NAD $^{+}$ -modified carbon paste electrode and the solution pH used in the pretreatment was not altered with the pretreatment time. Thus, there were no significant changes over pH value from  $8$  to  $10$ . For pH values above  $10$ , a decrease in the catalytic current was observed for a given pretreatment time. Carbon paste electrodes did not sustain prolonged pretreatment at pH higher than  $12$  due to the vigorous generation of oxygen bubbles. Therefore, a solution of pH  $10$  seems to be the best.

To gain insight into the process that occurs during pretreatment, the carbon paste electrode was first polarised at  $-0.5$  V for 5 min and then was treated in order to oxidise the adenine moiety in  $\text{NAD}^+$ . The first cycle exhibits a broad anodic peak such as that in Fig. 1. In the subsequent scan, the peaks due to the catalytic system can be observed. The peak at  $-0.1$  V is not associated with the presence of  $\text{NAD}^+$  because it was also observed after cathodisation in a solution without  $\text{NAD}^+$ . The anodic peak is considered therefore to originate from a surface bound species formed at the negative potential.

One may postulate that when the potentiodynamic cycling between  $-0.5$  and  $2$  V at  $5$  V  $\text{s}^{-1}$  was applied during prolonged times, an important contribution to activation was caused by the surface reduced functionalities. This explanation is based in the following observations: (a) Cathodic polarisation yielded a product whose electrooxidation is mainly associated with the peak at  $-0.1$  V. This peak also appeared after pretreatment, although it was observable only after activation periods longer than 5 min. Moreover, the height of this peak increased when pretreatment time increased. (b) After prolonged pretreatment, the relative increment in the catalytic current for NADH was practically independent of the amount of pasting liquid in the paste (only paraffin-rich electrodes were analysed). This behaviour was also found when cathodic polarisation was applied. Conversely, a dependence between this increment and the percentage of the pasting liquid in the paste was observed after 5 min. (c) Extending the duration of pretreatment did not lead to a substantially increase in the area of the conductor surface as occurred when a negative potential was applied.

Takeuchi and Murray<sup>8</sup> have suggested that a large fraction of pasting liquid is presented as droplets thickly interspersed on the graphite surface. According to this model, it was postulated that active surface sites for NADH oxidation exist at the three-phase boundary of carbon with aqueous media and pasting liquid<sup>1</sup>. The pretreatment produces a remotion of organic molecules and, in the case of pasting liquid-rich pastes, this results in more paraffin exposed to the aqueous solution. Then, the number of surface sites available increases with pasting liquid content. On the contrary, it was postulated that cathodic polarisation does not produce a significant desorption of organic molecules and replacement by

water. Thus, there seems to be a limiting value for surface activation by the above qualitative model, where further activation time does not improve the catalytic current for NADH oxidation. As the pretreatment time is increased, the activation of the carbon surface must be due primarily to the contribution of reduced surface functionalities.

#### *Electrochemical behaviour of electrodes with $\text{NAD}^+$ dispersed into the carbon paste*

Carbon paste containing  $\text{NAD}^+$  lead to relative higher background currents, but this situation was

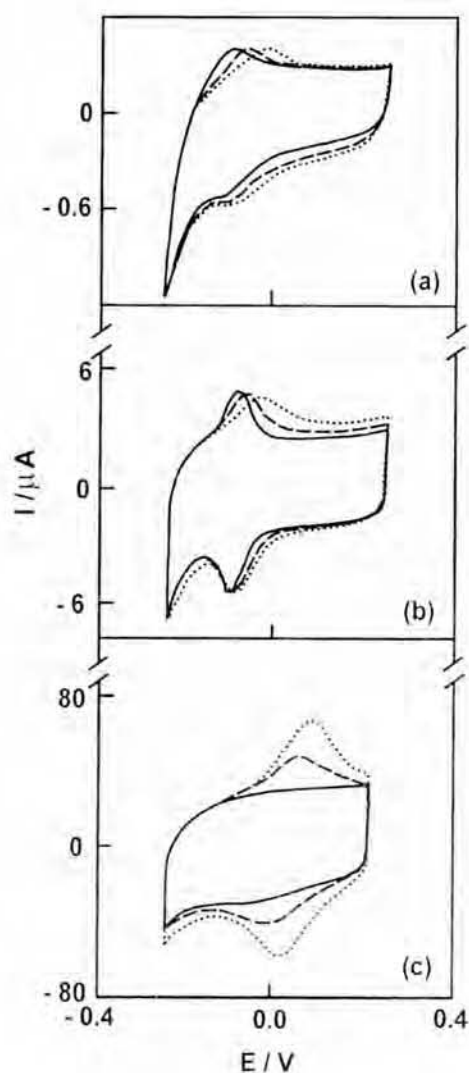


Fig. 2—Cyclic voltammograms obtained with carbon paste electrodes containing  $x$  %  $\text{NAD}^+$  incorporated into the paste in  $0.1$  M phosphate solution pH 10 at  $0.020$  V  $\text{s}^{-1}$ . The initial anodic switching potential was  $1.4$  V. Paraffin to graphite ratio:  $0.44$ . Scan rate:  $0.020$  V  $\text{s}^{-1}$ ,  $x$  = (a) 2; (b) 15 and (c) 20. (—) 1st; (---) 2nd and (---) 10th scan.

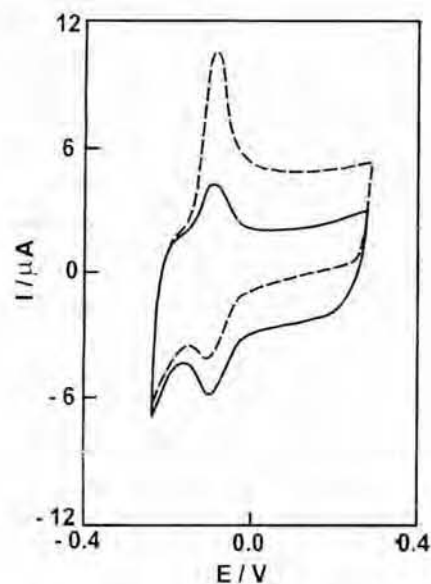


Fig. 3—Cyclic voltammograms obtained with a 15%  $\text{NAD}^+$  carbon paste electrode. The electrolyte solution was 0.1  $M$  phosphate solution  $\text{pH}$  10 (—) without and (---) with  $10^{-4}$   $M$   $\text{NADH}$ . Paraffin to graphite ratio: 0.44. Scan rate:  $0.020 \text{ V s}^{-1}$ .

changed when the voltammetric behaviour was examined over a narrower potential range. In Fig. 2 the cyclic voltammograms for carbon paste electrodes containing different amounts of  $\text{NAD}^+$  are depicted. All the pastes have a high paraffin/graphite ratio in order to increase their stability. A 2 %  $\text{NAD}^+$  electrode resulted in redox peaks obscured by the high signal of the base (Fig. 2a). In the case of 15 %  $\text{NAD}^+$  well-defined peaks were obtained (Fig. 2b), and in spite of the solubility of  $\text{NAD}^+$  in aqueous media continuous cycling did not result in a noticeable decrease of electroactivity. Conversely, the configuration of 20 %  $\text{NAD}^+$  was unstable and the voltammetric peaks are completely lost during cycling (Fig. 2c). It can be observed that the formal potential shifts to more positive potentials compared to that for lower amount of  $\text{NAD}^+$  in the paste, as occurred with increasing  $\text{NAD}^+$  concentration in solution.

When cyclic voltammograms for the 15%  $\text{NAD}^+$  electrode were obtained in the presence of  $\text{NADH}$ , an enhancement in the oxidation current and a decrease in the cathodic wave were obtained, denoting a very strong electrocatalytic effect (Fig. 3). The magnitude of the catalytic current was proportional to the  $\text{NADH}$  in solution. The catalytic behaviour toward  $\text{NADH}$  was then similar to that of the solution-phase  $\text{NAD}^+$ .

The ratio of peak currents, corrected for the background current for cyclic voltammograms at

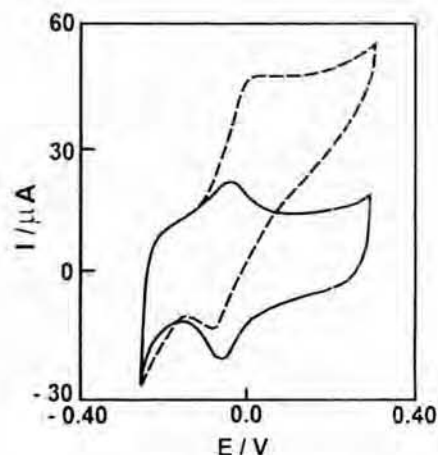


Fig. 4—Cyclic voltammograms of a 15%  $\text{NAD}^+$  carbon paste electrode in 0.1  $M$  phosphate solution  $\text{pH}$  10 (—) without and (---) with  $10^{-4}$   $M$   $\text{NADH}$ . The electrode was first cycled between  $-0.5$  and  $2.0 \text{ V}$  at  $5 \text{ V s}^{-1}$  for 15 min. Paraffin to graphite ratio: 0.44. Scan rate:  $0.020 \text{ V s}^{-1}$ .

different scan rates, was close to unity and only a small separation between the peaks was observed at the highest scan rates, indicating a reversible redox switching of surface bound species. Thus, the electroactive sites on the carbon paste behave as true surface active groups influenced by the properties of the electrolyte.

From the peak separation ( $\Delta E_p$ ) was possible to calculate the heterogeneous rate constant ( $k_s$ ), using the method proposed by Laviron<sup>9</sup>. A master curve of  $m$  vs  $\Delta E_p$ , where  $m = (RT/F)(k_s/nv)$ , yielded the value of  $m$  corresponding to different measured values of  $\Delta E_p$ , and  $k_s$  was obtained from the value of  $m$ . The transfer coefficient was taken as 0.5. The main value of  $k_s$  is  $8.5 \text{ s}^{-1}$ , which compares favourably with the values of others modified electrodes that were used to catalyse the oxidation of  $\text{NADH}$ <sup>10,11</sup>.

The influence of pretreatment on the electrochemical response of carbon paste electrodes with  $\text{NAD}^+$  incorporated into the paste was also explored. In this case, the oxidation of the adenine group in  $\text{NAD}^+$  occurred during the pretreatment. The oxidation products were strongly adsorbed on the electrode surface as shown in Fig. 4, which presents the voltammogram obtained when the same electrode was subsequently potentiodynamically polarised between  $-0.25$  and  $0.3 \text{ V}$ . As expected, an increased background current was measured. The value of  $k_s$  slightly increased after the pretreatment procedure. The cyclic voltammogram obtained in a 0.1  $M$

phosphate solution containing  $10^{-4}$  M NADH is also shown in Fig. 4. It is evident that pretreatment applied to this type of electrode did not inhibit the catalysis of NADH oxidation.

#### Acknowledgement

Fundación Antorchas and Consejo Nacional de Investigaciones Científicas y Técnicas are gratefully acknowledged for financial support.

#### References

- 1 Lobo M J, Miranda A J & Tuñón P, *Electroanalysis*, 9 (1997) 191.
- 2 Alvarez González S L, Saidman S B, Lobo Castañón M J, Miranda Ordieres A J & Tuñón Blanco P, *Anal Chem*, 72 (2000) 520.
- 3 Saidman S B & JBBessone, *Electrochim Acta*, 45 (2000) 3151.
- 4 Saidman S B, *Electroanalysis*, 14 (2002) 449.
- 5 Rice M E, Galus Z & Adams R N, *J electroanal Chem*, 143 (1983) 89.
- 6 Svancara I, Kalcher K, Diewald W & Vytras K, *Electroanalysis*, 8 (1996) 336.
- 7 Saidman S B, Lobo Castañón M J, Miranda Ordieres A J & Tuñón Blanco P, *Anal Chim Acta*, 424 (2000) 45.
- 8 Takeuchi E S & Murray R W, *J electroanal Chem*, 188 (1985) 49.
- 9 Laviron E, *J electroanal Chem*, 101 (1979) 19.
- 10 Gorton L, Johansson G & Torstensson A, *J electroanal Chem*, 196 (1985) 81.
- 11 Yo Hin B F & Lowe C R, *Anal Chem*, 59 (1988) 2111.