Journal of Applied Electrochemistry **31**: 1095–1101, 2001.

Surface-modified electrodes for NADH oxidation in oxidoreductase-catalysed synthesis

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Received 16 October 2000; accepted in revised form 29 May 2001

Key words: modified electrodes, NADH oxidation, synthesis

Abstract

Three surface-modified electrodes were investigated: 3,4-dihydroxybenzaldehyde electropolymerized on glassy carbon, hexacyanoferrate modified nickel electrodes, Meldola Blue adsorbed on graphite, for application to NADH oxidation in oxidoreductase-catalysed synthesis. The high overpotential, which is generally required for NADH oxidation on clean electrodes, was perfectly overcome with the three electrodes and especially with the Meldola blue modified graphite electrode. This electrode allowed fast NADH oxidation at potentials as low as 0.00 V vs SCE. It also ensured good storage stability. It is stressed that the key problem in applying this electrode to synthesis is the decrease in its electrocatalytic properties under operating synthesis conditions. An operating procedure was established that allowed ageing of the electrode to be directly linked to the current measurement. Following this procedure, several methods were investigated for improving the half-life of the electrode: pulse electrolysis, replacing NADH by NAD^+ in the initial solution, lowering the NAD^+ concentration and using dextran-bound NAD^+. In the best conditions, the half-life reached 152 min instead of 34 min with the initial conditions, proving that fouling by anodically produced NAD^+ was first of all identified as a very important cause of electrode ageing. Modification of intrinsic surface properties of the electrode also led to the decrease of the interface efficiency.

1. Introduction

The combination of enzyme-catalysed reactions and electrochemistry has led to great successes in the field of electrochemical biosensors. As yet, it has been more difficult to apply this type of combination to synthesis technology. Nevertheless, a few studies have suggested opportunities for coupling enzymatic catalysis with electrochemical processes [1–3]. Combining NAD-dependent oxidoreductases with the electrochemical regeneration of NAD^+ should open a wealth of new synthesis routes [4]. Many domains are concerned, such as oxidation of alcohols for aldehyde production [5, 6] in the flavour and perfume industries, oxidation of diols for synthesis of lactones [7, 8], functionalization of some steroids [9, 10], resolution of racemic mixtures [11] etc.

The direct electrochemical oxidation of NADH into NAD^+ is easy to achieve:

$$\mathrm{NADH} \rightarrow \mathrm{NAD}^+ + \mathrm{H}^+ + 2\ \mathrm{e}^-$$

It is a fast reaction which selectively produces NAD^+ on carbon, gold and platinum electrodes [12,13]. However, the high overpotential required for the reaction to be sufficiently fast constitutes the main drawback of the technique.

For the moment, the electrochemical oxidation of NADH has essentially been studied for the design of electrochemical biosensors. Several studies have aimed at improving the electrocatalytic properties of the electrodes to reduce the overpotentials required for NADH oxidation. Various surface-modification techniques have been proposed [14–17]: adsorption, polymerization, electropolymerization, covalent linkage etc.

The basic electrocatalytic properties required for an electrode are similar for a biosensor and an electrochemical reactor since decreasing the overpotential is necessary in both cases to lower the risk of side-reactions. Nevertheless, the two applications have very different requirements in other aspects. The electrode area is very small in biosensors, and it is not a real hindrance to use sophisticated surface modification procedures. In contrast it is difficult, and costly, to scale-up cumbersome surface modification procedures to produce the large electrode surface areas required for industrial synthesis. The simplicity of the electrode fabrication procedure is then essential for electrosynthesis reactors. The problem of electrode ageing is also very different. Biosensors are used in a repetitive mode:

short measurement periods are followed by washing periods, and sometimes storage periods. The purpose is generally to measure very low concentrations. The electrode must be stable in these conditions. In contrast, syntheses are carried out in a continuous mode: the purpose is to reach high conversion rates with high product concentrations. Experience shows that the behaviour of the electrodes is often very different when they are only discontinuously used with low current densities, or when they are continuously exposed to high current densities and high product concentrations. Checking the stability of electrodes for analytical or synthesis applications is consequently very different. Unfortunately, although there is promising work in the literature dealing with electrode modification for biosensors, studies devoted to synthesis are scarce.

The purpose of the present work was to give a first insight into scaling up surface-modified electrodes from the field of biosensors to the field of synthesis. The efficiency of three surface-modified electrodes for NADH oxidation were compared: (i) 3,4-dihydroxybenzaldehyde electropolymerized on glassy carbon, (ii) hexacyanoferrate modified nickel electrodes, and (iii) Meldola Blue adsorbed on graphite. These surface-modified electrodes were chosen because they were technically easy to manufacture, and have given good results in biosensor applications. It is important to note that the work did not aimed at conceiving new surface-modified electrodes, but at checking the suitability to synthesis of surface-modified electrodes that have already been described, and at suggesting possible adaptation of these electrodes to long-time electrolysis. The second part of the work focused on the Meldola Blue modified electrodes, which gave the best results. Various techniques are proposed to delay ageing of the electrode under operating conditions suitable for synthesis.

2. Material and methods

2.1. *Chemicals*

The chemicals were purchased from Prolabo, Fluka, Sigma or Aldrich. D-sorbitol dehydrogenase (EC 1.1.1.14) from sheep liver was purchased from Sigma. Activity was measured according to the Sigma protocol. One unit (1 U) transforms one micromole of D-fructose into D-sorbitol in one minute.

Enzymatically active NAD^+ and NADH concentrations were measured by an enzymatic test followed spectrophotometrically at 340 nm.

2.2. *Electrochemical measurements*

Electrolysis at constant potential and current–potential curves were performed in a three-electrode cell with an EG&G Princeton Applied Research 362 potentiostat. A platinum grid was used as counter-electrode. All potentials were monitored and measured versus a saturated calomel electrode (SCE) connected to the cell by a Luggin capillary.

2.3. *Working electrode materials and their preparation*

Glassy carbon from Le Carbone Lorraine (France), was mechanically polished with 1 μm diamond paste and rinsed with distilled water. Samples were then inserted in a Teflon holder to define a 28.3 mm^2 disc area in contact with the solution (dia. 6 mm). Pretreatment was carried out as proposed by Pariente et al. [18]. The polished electrodes were activated in 1.00 M NaOH solution with a potential held at +1.20 V vs SCE for 5 min; this was followed by potential cycling at 100 mV s^{-1} from −0.20 to +1.00 V vs SCE in buffer solution (tris-$NaNO_3$, 0.10 M, pH 8.0) for 5 min.

The nickel used was a 1.7 mm thick foam (Resocell MN90) with a 95% porosity supplied by SORAPEC (France). The electrodes were strips of 5 mm width. The surface was delimited using Araldite glue to define an area of 50 mm^2. The pretreatment reported by Yon Hin and Lowe [19] that consisted in a reductive electrolysis at a fixed potential of –1.00 V vs Ag/AgCl for 15 min was not used here. Indeed with our porous nickel electrode this electrolysis step could result in a pH increase inside the pores of the electrode, which would explain the nickel hydroxide precipitation observed during the following oxidation scan. The electrode was only cycled, in a phosphate buffer (0.01 M, pH 7.5) containing 0.10 M perchlorate between −0.10 and +1.00 V vs SCE at 10 mV s^{-1}, until a constant background profile was observed.

The spectrographic graphite supplied by Le Carbone Lorraine was used inserted into a Teflon holder adapted to the rotating disk electrode (Tacussel, mod. EDI). The area in contact with the solution was a 6 mm diameter disc. No pretreatment was needed [20]. The rotation speed was 100 rad s^{-1}.

3. Results

3.1. *3,4-dihydroxybenzaldehyde electropolymerized on glassy carbon*

3,4-dihydroxybenzaldehyde was electropolymerized on glassy carbon. After electrochemical pretreatment of the electrode, electrolysis for 3 min was performed at +0.20 V vs SCE in 3,4-dihydroxybenzaldehyde (0.5 mM) gave rise to a stable electropolymerized film [18]. The initial clean carbon electrode, the same electrode after pre-treatment, and the surface-modified electrode were checked for NADH oxidation by recording current–potential curves in a 0.25 M pH 7.0 phosphate buffer containing 1.0 mM NADH (Figure 1). The current peak corresponding to NADH oxidation occurred at approximately 0.71 and 0.37 V vs SCE, for the clean (curve 1) and the pretreated electrode (curve 2), respectively. The

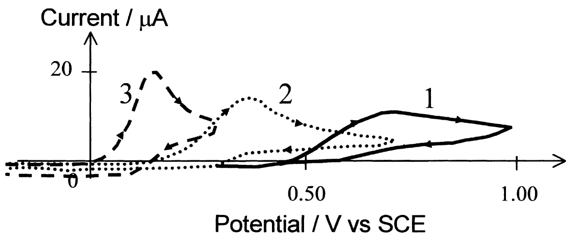

Fig. 1. Influence of the surface modifications of glassy carbon on NADH oxidation. Current–potential curves recorded at 10 mV s^{-1} in a 0.25 M, pH 7.0 phosphate buffer containing 1.0 mM NADH. Key: (1) — clean glassy carbon, (2) ···· glassy carbon after pretreatment, (3) - - - - glassy carbon with a 3,4-dihydroxybenzaldehyde electropolymerised film.

electrochemical pretreatment resulted in significant activation of the electrode. The surface-modified electrode exhibited a peak at around 0.14 V vs SCE (curve 3). The total decrease in the overpotential from the clean electrode to the surface-modified electrode was 0.57 V.

3.2. *Hexacyanoferrate modified nickel electrode*

The nickel electrode was modified by electrochemically synthesising a nickel-hexacyanoferrate complex ($Na_2NiFe(II)(CN)_6/NaNiFe(III)(CN)_6$) on its surface. The protocol proposed by Yon Hin and Lowe [19] was adapted to the characteristics of our nickel electrode. The electrocatalytic activity of the final electrode appeared to be closely dependent on the initial electrode material. After pretreatment of the electrode, the complex was formed by continuous potential scans between –0.10 and 1.00 V vs SCE in the solution containing 5 mM $Fe(CN)_6^{4-}$. To ensure complete coverage of the electrode surface with the catalytic complex, 30 cycles were necessary. Figure 2 compares the voltammograms recorded with the clean nickel electrode (curve 1) and the surface-modified electrode (curve 2) obtained in a 0.01 M, pH 7.5 phosphate buffer containing 0.10 M sodium perchlorate and 5 mM NADH. The oxidation of NADH was observed at 0.75 V vs SCE for the clean nickel electrode and at 0.53 V vs SCE for the modified one. This surface-modification allowed the overpotential for NADH oxidation to be decreased by approximately 0.22 V.

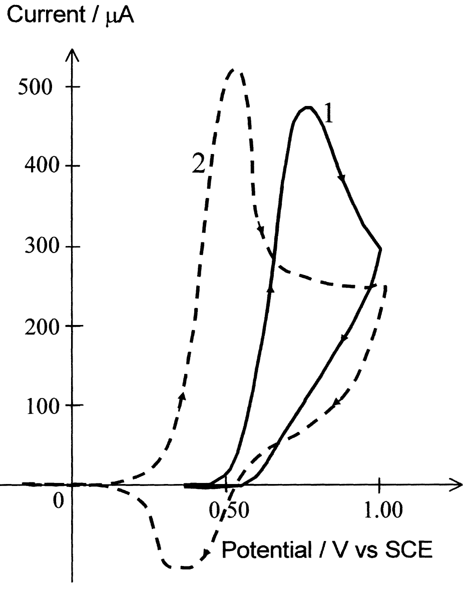

Fig. 2. Influence of the surface modifications of nickel on NADH oxidation. Current–potential curves recorded at 10 mV s^{-1} in a 0.01 M, pH 7.5 phosphate buffer containing 0.1 M sodium perchlorate and 5.0 mM NADH. Key: (1) — clean nickel foam electrode, (2) - - - - hexacyanoferrate modified nickel electrode with 30 cycles.

3.3. *Meldola Blue adsorbed on graphite*

The graphite electrode was modified by simple adsorption of Meldola Blue on its surface. The procedure described by Gorton et al. [20] was modified here; the adsorption time was increased from 1 to 15 min, and the Meldola Blue concentration was 10^{-3} M instead of 10^{-4} M. With the graphite material used here, dissolving Meldola Blue in 0.1 M HCl solution also gave better results than using a buffer solution. Voltammograms obtained with the clean electrode and the surface-modified electrode were recorded in a 0.25 M, pH 7.0 phosphate buffer with and without NADH (Figure 3). Without NADH, the voltammogram obtained with the modified electrode (curve 2′) exhibited an anodic and a cathodic peak corresponding to the reversible oxidation and reduction of the adsorbed Meldola Blue. When 3 mM NADH was added (curve 2), the current of the anodic peak increased, which corresponded to the catalytic oxidation of NADH by the oxidized form of Meldola Blue (MB^+). At this interface, Meldola Blue acts as a mediator in NADH oxidation following the scheme [20]:

$$\begin{array}{r}
\mathrm{MBH} \leftrightarrow \mathrm{MB}^+ + 2e^- + \mathrm{H}^+ \\
\mathrm{NADH} + \mathrm{MB}^+ \rightarrow \mathrm{NAD}^+ + \mathrm{MBH} \\
\hline
\mathrm{NADH} \rightarrow \mathrm{NAD}^+ + 2e^- + \mathrm{H}^+
\end{array}$$

The gain in potential between the clean graphite electrode ($E_{\mathrm{peak}} = +0.34$ V vs SCE, curve 1) and the surface-modified electrode ($E_{\mathrm{peak}} = -0.14$ V vs SCE curve 2) was 0.48 V.

3.4. *Comparison*

The results presented here are the best results obtained for each interface by checking several modifications of the preparation procedure. Comparing Figures 1, 2 and 3 showed that the Meldola Blue modified electrode obviously gave the best results, as far as diminishing the risk of side-reactions is concerned. Actually, the 3,4-dihydroxybenzaldehyde modified glassy carbon electrode resulted in the greatest potential gain between the clean material and the surface-modified material. Nevertheless, the Meldola Blue modified electrode allowed NADH to be oxidized at the lowest potential. Figure 3

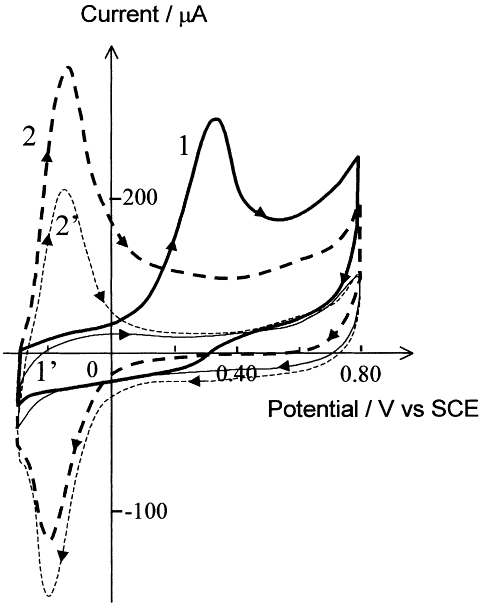

Fig. 3. Influence of the adsorption of Meldola Blue on graphite toward NADH oxidation. Current–potential curves recorded at 50 mV s^{-1} in a 0.25 M, pH 7.0 phosphate buffer without NADH (1′ and 2′) and with 3.0 mM NADH (1 and 2). Key: (1) and (1′) — clean graphite electrode, (2) and (2′) - - - - graphite with Meldola Blue adsorbed.

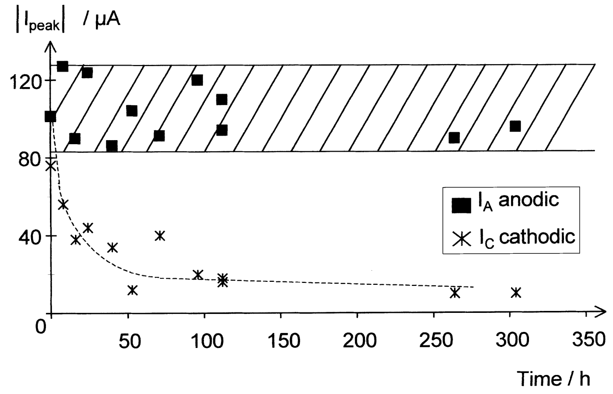

Fig. 4. Stability of the Meldola Blue modified electrode under storage. I_{peak} are taken from the current–potential curves recorded at 50 mV s^{-1} in a 0.25 M, pH 7.0 phosphate buffer containing 3.0 mM NADH. Meldola Blue modified electrodes stored at room temperature for various durations.

shows that NADH oxidation effectively started at about –0.25 V vs SCE, which is not far from the formal potential of the couple NAD$^+$/NADH: –0.35 – 0.03 pH = –0.56 V vs SCE at pH 7.0 [21].

This surface-modified electrode can, therefore, be considered as excellent, with respect to the well-known high overpotentials which are generally required to perform electrochemical NADH oxidation. There was a gain of around 0.65 V and 0.25 V when comparing the graphite–Meldola Blue interface with the Ni-modified electrode and the glassy carbon modified one, respectively. Moreover, the procedure for performing modification on graphite is very simple and could easily be scaled-up to large surface areas. It has also been claimed in the literature that this electrode has very high stability [14]. This is a keypoint for any application in synthesis.

3.5. *Stability of the Meldola Blue modified electrode under storage*

Meldola Blue modified electrodes were always prepared following the same protocol, and then stored at room temperature, without any special care. Tests were performed after different storage times. They consisted of voltammograms recorded at 50 mV s^{-1} between −0.30 and +0.80 V vs SCE in 0.25 M, pH 7.0 phosphate buffer, containing 3.0 mM NADH. The current–potential curves obtained were similar to those reported in Figure 3, that is with a high oxidation peak, corresponding to the catalytic oxidation of NADH, and a smaller cathodic peak due to the reduction of adsorbed Meldola Blue. The maximum values of the anodic and cathodic peaks versus storage time are reported in Figure 4. The parameter noted I_C is the current of the cathodic peak, minus the base line value. This parameter was chosen to approach the current due to the reduction of adsorbed Meldola Blue. The parameter noted I_A is an estimation of the peak current actually due to NADH oxidation. Its value was derived by subtracting from the raw anodic peak value: (a) the value of the anodic residual current (measured on a clean electrode), which was always about 10 μA, and (b) the value of the cathodic peak (I_C), which was an estimation of the current due to the oxidation of the adsorbed Meldola Blue.

Parameter I_C decreased exponentially as a function of the time storage, while the value of I_A remained approximately constant. A rather high dispersion of the values was observed in the range from 80 to 130 μA. The cathodic peak value stabilized at a minimum value around 20 μA after approximately 150 h storage, and this value remained constant for longer storage times. The decrease in the cathodic peak current did not result in a proportional decrease in the electrocatalytic activity of the electrode for NADH oxidation. Actually, the oxidation current measured with the electrodes which had been stored for the longest times seemed to be slightly lower than the average value, but they remained exactly in the same order of magnitude. The variation in the intrinsic features of the modified electrode during storage did not significantly alter its electrocatalytic properties. As a conclusion, storage periods around two weeks, without any particular care, lead to ageing of the electrode, but not to any real variation of its electrocatalytic effectiveness.

3.6. *Ageing of the Meldola Blue modified electrode under operating conditions*

The transformation of D-sorbitol ($C_6H_{14}O_6$) into fructose ($C_6H_{12}O_6$) catalysed by D-sorbitol dehydrogenase (SDH) was used to check ageing of the Meldola Blue modified electrode under synthesis conditions. The SDH-catalysed transformation of D-sorbitol occurred in the bulk solution:

$$C_6H_{14}O_6 + NAD^+ \xrightarrow{SDH} C_6H_{12}O_6 + NADH + H^+$$

and was coupled to the electrochemical oxidation of NADH.

It was essential to define a protocol which allows ageing of the electrode to be clearly distinguished from the variations in the composition of the solution during the synthesis. An electrode was used with a very small surface area with respect to the volume of solution to be processed. The solution also contained a large excess of substrate. In these conditions, the low consumption of D-sorbitol did not significantly modify the composition of the solution and did not limit the overall reaction rate. On the other hand, SDH was used in large excess in order to make sure the electrochemical step was the rate limiting step.

A 6 mm diameter rotating disc electrode was used in 4 ml of 0.25 M, pH 7.0 phosphate buffer initially containing 0.20 M D-sorbitol. Each experiment started with a potential scan from –0.30 to 0.00 V vs SCE (Figure 5, curve a) to verify the quality of the surface-modified electrode. NADH was not initially present in the solution. So the well-defined peak observed corresponded to oxidation of the adsorbed Meldola Blue only. The potential was then kept constant at 0.00 V vs SCE. 0.2 ml SDH solution was added to give a final activity of 2 U ml^{-1}. The residual current became stable at approximately 4 μA (it decreased by 1 or 2 μA after 15 h electrolysis without NADH). Finally, NADH was added to obtain a final concentration of 1.0 mM. The current rose rapidly to its maximum value and then decreased slightly and continuously (Figure 5, curve b).

From Figure 5, the quantity of electricity consumed during one-hour electrolysis was found to be 0.13 C. According to the two-electron oxidation of NADH, 6.7×10^{-7} mol of D-sorbitol were transformed, that is, less than 0.2 mM. The transformation ratio was less than 0.1% after one hour electrolysis. On the other hand, the SDH activity of 2 U ml^{-1}, should be able to transform $8 \times 10^{-6} \times 60 = 5 \times 10^{-4}$ mol of substrate per hour. Even if this value, which is derived from the maximum rate, represents a maximum value, it indicates a very large excess. Consequently, the enzymatic reaction was not rate limiting, and the current decrease was only linked to the alteration of the electrode electrocatalytic properties. Ageing of the electrode was characterized by its half-life time, that is, the time necessary for the current to fall to half its maximum value. In this case, the electrode had a half-life of 34 min only. Three hypotheses were raised and explored to explain this short time: (i) fouling of the electrode by NAD$^+$ electrochemically produced, (ii) spontaneous hydrolysis of NADH/NAD$^+$ in solution, and (iii) modification of the intrinsic properties of the electrode surface.

It has already been observed that the electrochemical oxidation of NADH produces NAD$^+$, or some intermediate species, which may remain adsorbed on the electrode surface and inactivate it [13, 22, 23]. In order to enhance NAD$^+$ desorption from the electrode surface, experiments were performed with alternative potential steps. The potential was repetitively switched between 0.00 V vs SCE and a cathodic value which was intended to result in NAD$^+$ desorption. Experiments performed with 1 min electrolysis at 0.00 V vs SCE, alterning with 1 min electrolysis at –0.60 V vs SCE, gave a half-life of 44 min (this duration only took into account the periods of the 0.00 V electrolysis). The result was significant enough to support the assumption that the adsorption of the anodically produced NAD$^+$ may be responsible of the electrode ageing. However, an optimization of the pulse conditions (potential and duration) may improve the results.

With the same objective, experiments were performed with lower NADH concentrations. Using concentra-

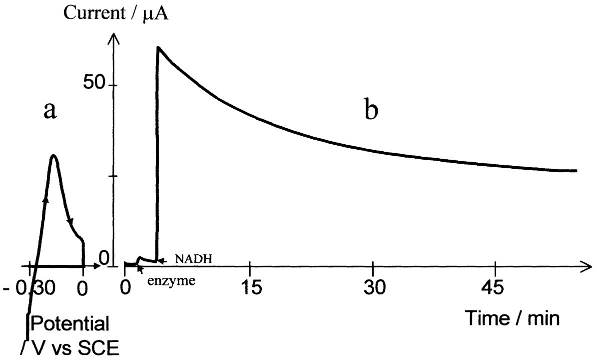

Fig. 5. Ageing of the Meldola Blue modified electrode under synthesis conditions. (a) Current–potential curve recorded at 50 mV s^{-1} in a 0.25 M, pH 7.0 phosphate buffer containing 0.2 M D-sorbitol. (b) Current–time curve recorded during electrolysis at 0.00 V vs SCE in a 0.25 M, pH 7.0 phosphate buffer containing 0.2 M D-sorbitol. Enzyme (SDH) is added to obtain a 2 U ml^{-1} as final activity and then NADH is added to obtain a 1 mM concentration.

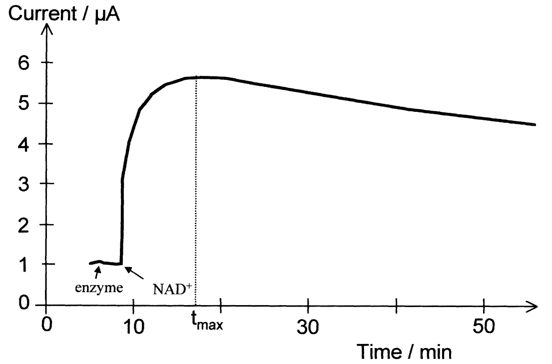

Fig. 6. Ageing of the Meldola Blue modified electrode under synthesis conditions using NAD$^+$ instead of NADH. Current–time curve recorded during an electrolysis at 0.00 V vs SCE in a 0.25 M, pH 7.0 phosphate buffer containing 0.2 M D-sorbitol. Enzyme (SDH) is added to obtain a 2 U ml^{-1} final activity and then NAD$^+$ is added to obtain a 0.1 mM concentration.

tions of 0.1 mM instead of 1.0 mM NADH, lengthened the half-life of the electrode to 57 min.

Figure 6 represents the current recorded versus time when 0.1 mM NAD$^+$ was added instead of NADH. In this case, NADH was only produced by D-sorbitol transformation. The electrode worked with very low NADH concentrations at the onset of the process. This is clear from Figure 6 where the current progressively increased for about ten min until it reached a maximum value. As a consequence, anodically formed NAD$^+$ was steadily produced in small quantities on the electrode surface. Comparison with Figure 5 shows that when NADH was added initially, the electrode worked at maximum current from the onset. In this case, the anodic production of NAD$^+$ was maximum at the onset, when the electrode was still clean. The history of the electrode differed greatly according to the protocol followed and the results obtained here confirm its importance, the electrode exhibited a half-life of 152 min when the NADH was replaced by NAD$^+$.

Dextran-bound NAD$^+$ was tested with the hope that the steric hindrance might modify the interfacial behaviour of NAD$^+$ and lower its ability to adsorb on the electrode surface. Experiments performed with 0.1 mM dextran-bound NAD$^+$ gave absolutely no current during the electrolysis at 0.00 V vs SCE. Nevertheless, spectrophotometric measurements of the solution proved that the enzymatic reaction occurred and resulted in complete dextran-bound NAD$^+$ reduction into NADH after a few minutes. The voltammogram obtained in this solution with the modified electrode showed direct oxidation of dextran-bound NADH at 0.63 mV vs SCE, but indicated no catalytic oxidation with adsorbed Meldola Blue at –0.16 V vs SCE (Figure 7, curve 2). Comparing this curve with the voltammogram obtained in the same conditions, but with a clean graphite electrode (Figure 7, curve 1) confirms that only the direct oxidation of dextran-bound NADH occurred on the Meldola Blue modified electrode. The modified electrode was not able to catalytically oxidize dextran-bound NADH. Further experiments would be necessary to explain this result, and could help in better understanding the properties of the Meldola Blue modified electrode.

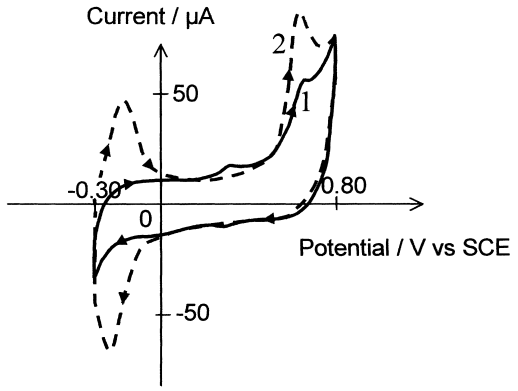

Fig. 7. Influence of the adsorption of Meldola Blue on graphite toward the oxidation of dextran-bound NADH. Current–potential curves recorded at 50 mV s^{-1} in a 0.25 M, pH 7.0 phosphate buffer containing 0.2 M D-sorbitol and 0.1 mM dextran-bound NADH enzymatically obtained. Key: (1) — clean graphite, (2) - - - - Meldola Blue modified electrode.

Independent experiments were performed to quantify the spontaneous NADH and NAD$^+$ denaturation (second hypothesis). Tests were carried out with 0.1 mM NADH in phosphate buffer on the one hand, and with 0.1 mM NAD$^+$ in phosphate buffer also containing 0.20 M sorbitol on the other hand. No enzyme and no electrochemical step were used. NADH and NAD$^+$ concentrations were measured as a function of time using an enzymatic test. After three hours no denaturation of NAD$^+$ was observed and the concentration of NADH decreased of approximately 12%. Consequently, even for 152 min, the low effect of NADH denaturation will not significantly modify the discussion of the results.

The third hypothesis concerned the possible modification of the intrinsic properties of the electrode. Electrolyses were performed with the Meldola Blue modified electrode immersed in solution without NADH. After three hours, 0.1 mM NADH was added. The maximum current obtained was around two or three times lower than the current obtained previously when 0.1 mM NADH was added just at the onset of the electrolysis, proving there was a decrease in the interface activity. Desorption of Meldola Blue can be suggested, but the quantity liberated in solution was too low to be detected spectrophotometrically.

This interface modification was consequently another key parameter explaining the decrease in electrode efficiency. This effect was simultaneous to the fouling by the electrochemically produced NAD$^+$. At this state of the study, it seemed that fouling by NAD$^+$ was minimized thanks to the new operating conditions (NAD$^+$ instead of NADH, low concentration). Effort must now be focused on the minimization of this modification perhaps by fighting against the Meldola Blue desorption.

4. Conclusion

This work was a first illustration of the difficulty of scale-up of surface-modified electrodes to enzyme-catalysed synthesis. Though several surface-modified electrodes give excellent results for bioanalytical applications, specific work is still required to adapt them to the operating synthesis conditions.

Among the three different surface-modified electrodes studied here, the Meldola Blue modified electrode gave the best results in terms of overpotential decrease for NADH oxidation. The stability under storage was also excellent. The stability of the electrode under operation was stressed here as the keypoint to be improved for practical applications. Adsorption of the anodically produced NAD$^+$ on the electrode surface was identified as one of the main causes of electrode ageing. A suitable procedure (initially NAD^+ instead of NADH, and low concentration) allowed this effect to be avoided. Work is in progress to decrease the alteration of the intrinsic properties particularly by adding low quantities of Meldola Blue in solution to continuously regenerate the modified electrode surface.

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