A microelectrochemical switch responsive to NADH

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A carbon dual-microband electrode coated with a poly(aniline)–poly(vinylsulfonate) composite film exhibits an off-to-on switching response when exposed to NADH at pH 7; the device takes a few minutes to fully switch conductivity and has a linear response for NADH concentration up to *ca.* 4 mmol dm^{-3}.

Work in this laboratory has shown that a microelectrochemical enzyme switch responsive to glucose can be constructed by coating a carbon dual-microband electrode with a poly(aniline) film upon which the enzyme glucose oxidase (GOx) is entrapped in an insulating film of poly(1,2-diaminobenzene).[1–3] The device works in pH 5 buffered solution and shows an optimised switching time of about 10 s from the fully insulating to the fully conducting state. This conductivity change is brought about by reduction of the poly(aniline) by the reduced form of the enzyme mediated by tetrathiafulvalenenium present in the solution. Here we report a microelectrochemical switch responsive to NADH. An enzyme switch sensitive to NADH has previously been described by Masue *et al.*[4] Their device was based on a microarray electrode coated with a pyrrole (*N*-methylpyrrole) copolymer containing the enzyme diaphorase and used anthraquinone-2-sulfonate present in the solution as a mediator. With this system they demonstrated switching between 'on' and 'off' states albeit at a rather slow rate (typically about 15–20 min for 1 mmol dm^{-3} NADH). In contrast, the device described here is simply prepared by electrodepositing a poly(aniline)–poly(vinylsulfonate) (PANI–PVS) composite film on to a carbon dual-microband electrode and requires no additional mediator because the PANI–PVS film itself acts as an effective catalyst for the oxidation of NADH.[5] Reduction of the oxidised (pernigraniline) form of poly(aniline) in the composite film occurs in pH 7 buffered solution by reaction with NADH leading to the formation of the conducting (emeraldine) form of poly(aniline) and bringing about a switch in the conductivity of the device. The switching time is relatively fast in buffered solution at pH 7 with only a few minutes required to switch from the fully insulating 'off' to the fully conducting 'on' state. The precise time required to switch the device depends on the thickness of the polymer film and the concentration of NADH present in the solution.

Full fabrication details for the carbon microband electrodes have been given elsewhere.[1] The two carbon microband electrodes are *ca.* 10 μm wide and *ca.* 4.5 mm long and are separated by a *ca.* 20 μm gap. Use of poly(vinylsulfonate) as the counter ion in the poly(aniline) is necessary in order to retain the electroactivity of the polymer at neutral pH.[6] The PANI–PVS film was deposited potentiostatically at +0.9 V *vs.* SCE from a solution containing 21.5% poly(vinylsulfonate) [made from a 25% poly(vinylsulfonate) solution supplied by Aldrich], 1.88 mol dm^{-3} H_2SO_4 and 0.44 mol dm^{-3} freshly distilled aniline. The polymerisation process was monitored by integrating the current passed during deposition and the process was stopped when the required total charge (Q_t) had been passed. Investigation of the effect of film thickness (as determined by Q_t) showed that the switching rate was maximised when Q_t was *ca.* 4 mC. Therefore all data reported in this paper refer to films with a total deposition charge of 4 mC. After polymerisation the devices were thoroughly rinsed with deionised water and kept in 0.1 mol dm^{-3} citrate–phosphate buffer at pH 5 until required. The same pH 5 buffer was used to set the devices to their 'off' state by oxidising at +0.7 V until the residual current was *ca.* 350 nA. The devices were then transferred to a 0.1 mol dm^{-3} phosphate buffered solution containing 0.5 mol dm^{-3} Na_2SO_4 for the switching experiments. Before addition of NADH, a small bias potential (V_d) of 30 mV was applied between the two microband electrodes. All switching experiments were carried out under thermostatic conditions at 25 ± 0.5 °C in stirred solution.

A typical response of a device to NADH is illustrated in Fig. 1. The drain current ($i_{\rm drain}$) through the PANI film remains close to zero during the first minute after addition of NADH (indicated by the arrow). After approximately one minute, $i_{\rm drain}$ begins to rise slowly and then more quickly for the next two minutes, finally reaching a plateau after about 5.5 min. The corresponding dashed curve in Fig. 1 shows the variation of the potential of PANI–PVS film ($E_{\rm PANI-PVS}$) during the switching process. This simultaneous measurement clearly shows that the 'off' to 'on' transition of the device is the result of reduction of the PANI–PVS film rather than any local pH variation. Comparing the two curves, we can see that the reaction between NADH and PANI–PVS film starts immediately the NADH is added to the solution but that whilst the potential of the film ($E_{\rm PANI-PVS}$) drops continuously, no significant current is observed until the potential reaches approximately 0.10 V *vs.* SCE. This is consistent with separate measurements of the resistance of PANI–PVS films as a function of the applied potential. Films of poly(aniline) prepared with counter ions of AsF_6^{-1} or HSO_4^{-1} show a U-shaped dependence of film resistance on the applied potential with the minimum resistance observed for the emeraldine form of the polymer at intermediate

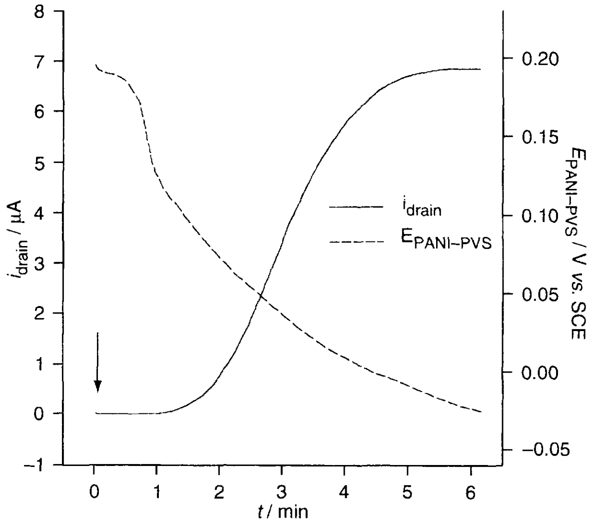

Fig. 1 Plots of the drain current, $i_{\rm drain}$, and film potential, $E_{\rm PANI-PVS}$, as a function of time after exposure to a solution of 3.38 mmol dm^{-3} NADH in 0.1 mol dm^{-3} phosphate buffer solution (pH 7) containing 0.5 mol dm^{-3} Na_2SO_4 at 25 ± 0.5 °C. The polymer film was deposited with a total charge of 4 mC and the applied drain voltage was 30 mV.

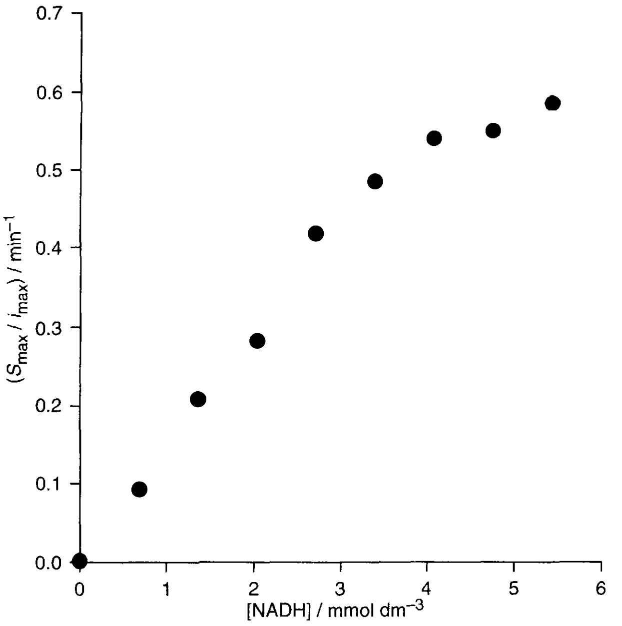

Fig. 2 Plot of the switching rate for a set of PANI–PVS based devices as a function of the concentration of NADH used. Experimental conditions are the same as in Fig. 1.

potentials.[7,8] Steady-state resistance measurements for our PANI–PVS films at pH 7 shows a similar potential dependence but with the low resistance region shifted to more negative potentials. The increase in the drain current in Fig. 1 occurs at potentials which correspond to those for which the film resistance falls rapidly in our steady-state measurements. The occurrence of the plateau in the drain current at longer times is consistent with the observed broad minimum in the film resistance as a function of potential.

Fig. 2 shows the relationship between switching rate and NADH concentration. The switching rate is defined as a ratio of the maximum slope of i_{drain} transient to the maximum i_{drain} (S_{max}/i_{max}) and normalises the effects of film thickness and electrode variations.[6] (S_{max}/i_{max}) increases linearly with NADH concentration up to about 4 mmol dm^{-3}, indicating that devices of this type can be applied in quantitative analysis. It should be noted that the nine data points in the calibration curve in Fig. 2 were obtained using nine different devices, indicating that the reproducibility of the fabrication and response of these devices are good. Each device is also reusable since the PANI–PVS film can be electrochemically reoxidised to its insulating 'off' state.

Our results show that microelectrochemical switches responsive to NADH in neutral aqueous solution can be constructed using PANI–PVS films. By coupling such devices to different NADH dependent dehydrogenase enzymes it should be possible to develop a range of microelectrochemical enzyme switches responsive to a range of substrates.

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