ORIGINAL PAPER

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Electrochemical study of glassy carbon electrodes modified with zirconium phosphate and some azine-type redox dyes

Abstract Glassy carbon (GC) electrodes were modified with a layer of zirconium phosphate (ZrP), using either direct chemical synthesis onto the surface or a ZrP gel droplet evaporation procedure. The azine-type dyes nile blue A (NB) and toluidine blue O (TB) were immobilized onto the ZrP-modified GC electrodes either by adsorption onto just the formed layer of ZrP or by inclusion into the ZrP matrix during its chemical synthesis. The electrochemical behavior of the GC·ZrP·dye composite electrodes was studied. For GC·ZrP-modified electrodes prepared by chemical synthesis on the surface, coverage by NB or TB of one or a few monolayers was found, with E_m values for these redox couples slightly shifting by ca. 0.05 V to the negative direction. For the GC·ZrP electrodes prepared by gel droplet evaporation, the E_m values for NB and TB appear initially shifted by ca. 0.2 V to the positive direction; however, both cathodic and anodic peaks return to their usual positions on the potential scale during soaking these electrodes in a buffer solution.

Key words Modified electrode · Glassy carbon · Zirconium phosphate · Redox mediator

Introduction

Chemically modified electrodes (CMEs) are attracting a great deal of attention, particularly in the study of the electrocatalysis of some biologically important redox systems. Nicotinamide coenzymes present one of the most important biological redox systems that have been

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the subject of intense electrochemical study during the past two decades. The high degree of attention paid to the electrocatalysis of the $NAD^+/NADH$ redox couple is fueled essentially by its expected use, provided in combination with the corresponding enzymes, in the electroanalysis of metabolites and other biologically active substances.

A number of methods for the preparation of CMEs is known, and many of these techniques have been applied to study the electrooxidation of NADH [1, 2]. One group of these methods relates to the irreversible adsorption of some azine-type mediators onto carbon-based electrode materials, preferably graphite. A variety of these mediators, including the phenoxazine dyes nile blue [3, 4], meldola blue [3, 5, 6, 7], brilliant cresyl blue [3], coelestin blue [8, 9, 10], 1,2-benzophenoxazin-7-one [5, 11], 3-β-naphthoyl nile blue [3, 8, 9, 10], and the phenothiazine dyes toluidine blue [3, 12, 13] and its 3-β-naphthoyl derivative [14], were irreversibly adsorbed or immobilized onto the carbon-based electrodes, and the resulting CMEs were shown to be able to catalyze the anodic oxidation of the coenzyme NADH.

Glassy carbon (GC) is often considered to be one of the most suitable electrode materials in obtaining electrocatalytically active CMEs [15]. However, in contrast to graphite, weak adsorption of azine-type electron transfer mediators is usually observed on GC electrodes. Thus, some additional methods for fixing or immobilization of these mediators onto the GC surface should be applied in preparing CMEs, particularly those suitable for stable and reproducible electrocatalytic oxidation of NADH. Among others, the inclusion of redox-active species into inorganic microporous matrices is known as a useful procedure in obtaining CMEs [16, 17, 18]. Previously, it was shown that the adsorption of some azine-type dyes onto layered phosphates or oxides like zirconium or titanium phosphate or titanium oxide yield, upon inclusion of the resulting composite materials into carbon paste, modified electrodes suitable for electrocatalytic oxidation of NADH [19, 20, 21]. Moreover, a high shift of the redox potential for the

immobilized mediator redox couple towards positive potential values, reaching ca. 0.3 V at pH 7, was found upon adsorption of the mediator on these materials [19, 20, 21]. A similar shift of the redox potential was found recently also for the mediators admixed mechanically with the zirconium phosphate and graphite composite [22, 23]. Next to carbon paste, some attempt has been made to modify carbon fibers with a layer of microporous materials like, for example, titanium dioxide [24], followed by adsorption of redox mediators [25].

In the present work, the modification of a GC electrode with zirconium phosphate, and its composite with some azine-type redox mediators, was investigated.

Experimental

Materials

NB and TB, both from Aldrich, zirconium tetrachloride (pract. grade) from Fluka, and potassium dihydrogen phosphate (analytical grade) from Merck were used as received. In electrochemical experiments, a 0.1 M Tris (analytical grade, Merck) buffer (pH 7.0), containing 0.1 M KCl (analytical grade, Merck), was used.

Rods of glassy carbon Sigradur G, 3 mm in diameter, were obtained from HTW Hochtemperatur-Werkstoffe (Thierhaupten, Germany).

Modification of GC electrodes

Five different methods to obtain GC electrodes, modified with a NB or TB complex with zirconium phosphate (ZrP) were used. Before each preparation, the GC electrodes were polished sequentially with 1 μm and 0.25 μm particle size alumina paste (Struers), and washed thoroughly with distilled water.

Method A

A polished GC electrode was immersed into a solution of 0.2 M $ZrCl_4$ in ethanol for 10 min, then dried in air for 2 min, and immersed into a 1 mM solution of either NB or TB in 0.2 M KH_2PO_4 for 10 min. After that, the modified electrode was dried in a desiccator under reduced pressure, and mounted in an electrochemical cell.

Method B

A polished GC electrode was kept in $ZrCl_4$ solution as above, and then immersed into a solution of 0.2 M KH_2PO_4 for 10 min. After that, NB or TB was adsorbed onto the electrode by immersing it into a solution of 1 mM of the corresponding dye either in 0.2 M KH_2PO_4 or in ethanol. Then, the modified electrode was dried and operated as above.

Method C

A droplet of ca. 5 μL of 0.2 M $ZrCl_4$ solution in ethanol was placed onto the flat surface of a polished GC electrode, and the solvent was evaporated under reduced pressure. Then, the electrode was immersed either consecutively into a solution of 0.2 M KH_2PO_4 for 10 min and after that into the same solution containing additionally 1 mM of the dye, for 10 min, or directly into 0.2 M KH_2PO_4 solution containing 1 mM of dye.

Method D

ZrP was prepared by mixing of 0.2 mL of 0.2 M $ZrCl_4$ solution in ethanol with 0.4 mL 0.2 M KH_2PO_4 solution. A droplet of this gel of ca. 5 μL was placed onto the flat surface of a GC electrode, and dried in a desiccator under reduced pressure. Then, the electrode was kept in a solution of 1 mM of the dye either in 0.2 M KH_2PO_4 or in ethanol for varying times, and dried under reduced pressure.

Method E

ZrP gel, containing the redox dye, was prepared by mixing of 0.2 mL of a 0.2 M $ZrCl_4$ solution in ethanol with 0.4 mL of a 0.2 M KH_2PO_4 solution containing 1 mM of the dye. A droplet of 5 μL of this gel was placed onto the electrode, and dried in the usual manner.

Electrochemical experiments

Electrochemical experiments were performed in a 2 mL three-electrode cell, arranged with a platinum wire counter electrode, a saturated calomel electrode (SCE) as reference, and the modified GC working electrode. The GC working electrode was press-fitted into a Teflon holder so that a flat circular surface contacted the solution. A BAS model 100 W (Bioanalytical Systems, West Lafayette, Ind., USA) potentiostat was used throughout the work. All potential values given below relate to the SCE.

Results and discussion

Figure 1 shows a cyclic voltammogram, obtained for a polished (GC) electrode onto which NB was adsorbed by immersing the electrode into 1 mM solution of the dye in ethanol. The cathodic current maximum, observed at around –0.4 V, and the anodic current maximum, at ca. –0.37 V in the back potential scan, indicate some amount of NB adsorbed onto the GC electrode. In
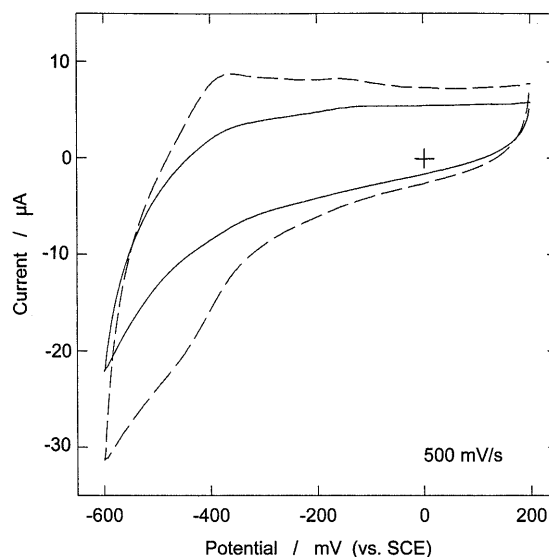
Fig. 1 Multicycle voltammograms, obtained at $v = 500$ mV/s for a polished glassy carbon (GC) electrode (*solid trace*), and for the same electrode kept initially in a solution of 1 mM NB in ethanol (*dashed trace*)

contrast, a ca. 10- or 20-fold higher cathodic and anodic current as well as a correspondingly higher electric charge, consumed during electrochemical redox processes, was obtained for NB adsorbed onto a graphite electrode [26]. For a graphite electrode, well-defined current peaks are usually obtained for adsorbed NB, characterized by a midpoint potential E_m of –0.414 V at pH 7, and thus shifted by 0.05 V to the negative potential direction compared to a solution of NB [27]. A similar picture as for NB was obtained for TB, also adsorbed onto a GC electrode. The results obtained show that, compared to graphite electrodes, only a small amount of redox dye adsorbs onto the GC electrode, showing poor electrochemical behavior.

A reasonable alternative to a simple adsorption onto the GC electrode is provided by the adsorption of the redox dyes onto layered microporous materials like ZrP with the subsequent inclusion of the resulting composite into an electrode material, e.g. into carbon paste [19, 20, 21, 22, 23]. Thus, attempts were made in the present work to modify the GC surface with a composite of ZrP and redox dye.

Two groups of methods were used for this purpose. One of them relates to the direct synthesis of a ZrP host matrix onto the surface of the GC electrode. In method A (for details, see Experimental section), the surface of the electrode was enriched by $ZrCl_4$, and after that the electrode was immersed into a solution of KH_2PO_4 containing the dissolved redox dye. An immediate reaction between $ZrCl_4$ and KH_2PO_4 proceeds on the surface of electrode, and a layer of ZrP is deposited on it. A visible blue appearance of the resulting surface layer indicates some amount of a redox dye incorporated either by adsorption or by mechanical inclusion into the surface layer of ZrP. In a modification of this method (method B), the deposition of a surface layer of ZrP was separated from the adsorption of the redox dye onto it. The adsorption was performed by immersion of a GC electrode, freshly modified with ZrP, into a dye-containing solution. Again, visible changes of the surface color to blue upon adsorption indicate some amount of the dye adsorbed onto the ZrP host matrix. Closely similar, a small droplet of $ZrCl_4$ solution can be evaporated on the surface of a GC electrode, followed by deposition of the dye-containing ZrP surface layer by immersion of the electrode into a solution containing both KH_2PO_4 and the dye (method C).

Another group of methods used consists of evaporation of a droplet of a separately prepared ZrP gel. The ZrP gel, prepared by mixing solutions of $ZrCl_4$ with KH_2PO_4, was evaporated onto the surface of the GC electrode, followed by adsorption of the dye from a separate solution (method D). Also, a ZrP gel has been prepared by the use of a dye-containing KH_2PO_4 solution, and a droplet of this dye-containing gel was evaporated onto the electrode surface (method E).

Figure 2 shows cyclic voltammograms (CVs) obtained for GC CMEs prepared by the direct synthesis of the ZrP matrix onto the surface of the electrode, more

specifically by the use of method A (top) and method B (bottom). In the first 10 potential scans, performed immediately after immersion of the ZrP·NB-modified electrode, prepared by method A, into the supporting electrolyte solution, values of E_{pc} of –0.435 V and E_{pa} of –0.266 V are observed, yielding an E_m of –0.351 V. By keeping the electrode for some time in the buffer solution, the E_{pc} of the CVs obtained slightly shifts to the negative direction, while the negative shift for the E_{pa} appears more pronounced. After 20 min, both the E_{pc} and E_{pa} stabilize, yielding an E_m of (-0.411 ± 0.002) V within the potential scan rate range 200–10,000 mV/s. The value obtained coincides within the error limits with the E_m obtained for NB adsorbed on graphite electrodes [26], and thus appears shifted by ca. 0.05 V to the negative direction compared to soluble NB [27]. Closely related to that, the ZrP·NB electrode, modified using method B (Fig. 2, bottom), shows initially an E_m of –0.382 V, shifting to –0.421 V in 30 min.
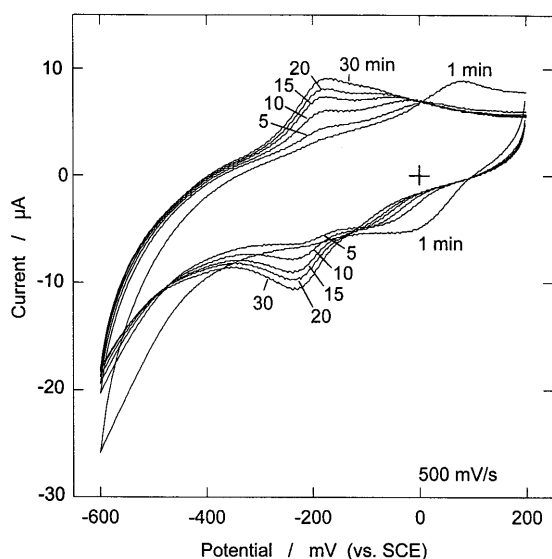
In both cases in Fig. 2 the peak currents diminish gradually by keeping the electrodes in a buffer solution, thus indicating a leaching of the mediator from the deposited layer of ZrP. In both cases, however, a relative stabilization of the peak current after 30–40 min is observed. By integration of the stabilized cathodic peaks, a surface coverage of ca. 1 nmol/cm^2 can be obtained from the data such as in Fig. 2. This means that only around one or a few monolayers of NB manifest themselves as surface-bound redox-active species for ZrP·NB-modified GC electrodes, in contrast to unmodified or modified
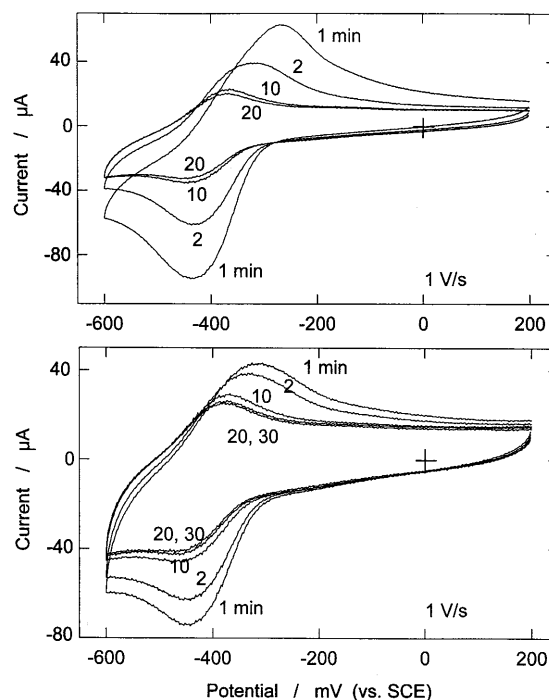
Fig. 2 Cyclic voltammograms (CVs) obtained at different time moments after immersion into buffer solution (as indicated, in minutes) for ZrP·NB-modified GC electrodes, prepared by method A (*top*) and method B (*bottom*)

with ZrP graphite electrodes, where a surface coverage up to a few tens of monolayers was detected [26]. Closely similar to the results shown in Fig. 2 were those obtained for another redox mediator tested (TB), by the use of both electrode modification procedures as in Fig. 2. Also, quite similar results were obtained by using method C for immobilization of both NB and TB; however, less uniform coatings by ZrP were obtained because of the droplet evaporation procedure used. In all cases, poor mechanical stability was obtained because of low adhesion of a ZrP layer to the surface of the GC electrode.
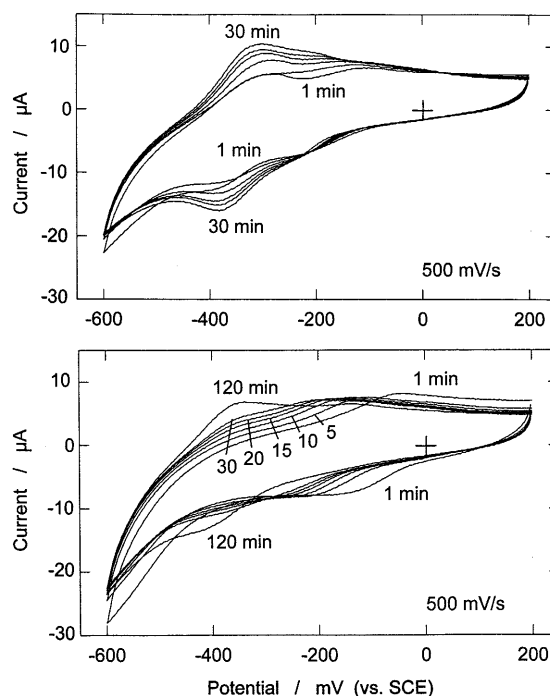
Some different results were obtained for GC electrodes modified by separately synthesized ZrP (methods D and E). Figure 3 shows CVs for the electrode modified with the ZrP·TB complex following method E. Immediately after immersing into the supporting electrolyte, a cathodic wave and an anodic peak, characterized by an E_m of $+0.03$ V, are observed. By keeping the electrode in a buffer solution, both waves diminish in height and shift to negative potentials. At the same time, another pair of cathodic and anodic peaks appears and grows in height around -0.2 V (Fig. 3). After 30–40 min, the first redox process, observed initially at higher potentials, disappears completely, leaving the second one, appearing at an E_m of -0.203 V. The value obtained is closely related to the standard redox potential of TB ($E^{\circ\prime} = -0.211$ V [27]). It may be concluded from these observations that some kind of rearrangement of the ZrP·TB composite takes place in a few tens of minutes after immersion of the ZrP·TB-modified GC electrode into the buffer solution. As a result of this rearrangement, the redox system initially observed at higher electrode potentials returns to lower potentials, characteristic for TB solution, and the growth of both i_{pc} and i_{pc} is observed (Fig. 3). In contrast to the ZrP-modified carbon paste

electrodes studied [19, 20, 21, 22, 23], the modified GC electrode does not show stable current peaks for any redox couple shifted to positive potentials. The integration of cathodic peaks on the CVs, as in Fig. 3, yields approximately monolayer coverage of the electrode surface by the TB adsorbate.
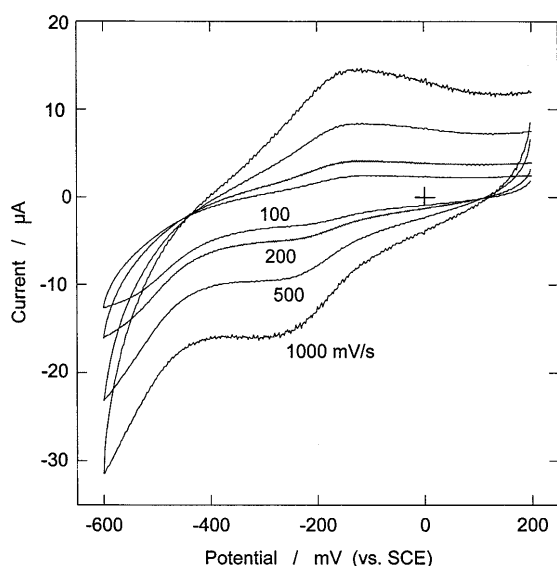
A similar picture was obtained for NB (Fig. 4). For electrodes modified by evaporation of a droplet of ZrP gel, followed by adsorption of NB, the shape of the CV obtained depends on the duration of keeping the electrode in the NB solution. By performing the adsorption for a short time, e.g. 1 min, a pair of cathodic and anodic peaks is seen in the CVs at relative high potentials, e.g. in the range 0.0 to -0.2 V after immersion of the resulting electrode into the buffer solution. By keeping this electrode in the buffer solution, the pair of peaks in the range of higher potential values gradually disappears, and a new pair of peaks appears in the usual potential range characteristic for NB. By performing the adsorption of NB for a longer time period, e.g. exceeding 10 min, only one pair of peaks is obtained, positioned in the usual (lower) potential range. Figure 4 (top) presents CVs obtained for the electrode prepared by adsorption of NB for 3 min. Two pairs of current waves or peaks, initially placed at E_m values of ca. -0.19 V and -0.34 V, are transformed gradually to one pair of peaks, centered at ca. -0.36 V, i.e. close to the standard redox potential of NB [27]. By the use of method E, again, a pair of peaks at higher potential is observed, transforming spontaneously into a low-potential pair (Fig. 4, bottom).

Fig. 3 CVs obtained at different time moments after immersion into buffer solution (as indicated, in minutes) for a ZrP·TB-modified GC electrode, prepared by method E

Fig. 4 CVs obtained at different time moments after immersion into buffer solution (as indicated, in minutes) for ZrP·NB-modified GC electrodes, prepared by method D by performing the adsorption for 3 min (*top*) or method E (*bottom*)

It may be concluded from the results obtained (Figs. 3 and 4) that, after immediate contact of NB or TB with ZrP, especially in the case when ZrP gel is prepared in the presence of the redox dye, a high-potential redox system is produced, showing redox transformations in the potential range of 0.0 to approx. –0.2 V for NB, or +0.1 to 0.0 for TB, thus shifted by approx. 0.2 V to the positive direction compared to standard values for these redox couples. By contacting with the buffer solution, these redox systems disappear spontaneously during a few tens of minutes, and new redox systems appear which correspond obviously to the native redox couples of NB and TB. The reasons for these changes are unknown. Particularly, it may be supposed that if GC has some higher affinity to the redox dyes studied, as compared to the ZrP host matrix, the readsorption onto GC proceeds following a slow desorption from ZrP. Also, some rearrangements of the ZrP matrix may be supposed to take place.

The nature of the high-potential redox couple, observed in a short time after immersion of the modified electrode into the buffer solution, cannot be determined from the results obtained. However, some behavior of this redox system can be studied during the relatively short time of its existence. Figure 5 shows CVs obtained at different potential scan rates for a ZrP·NB-modified electrode in a short time interval of approx. 3–5 min after immersion. The E_m value of (–0.220 $\pm$ 0.010) V was obtained from these CVs in the v range of 500–10,000 mV/s; thus the redox couple appears shifted to higher potential values by ca. 0.15 V. The dependence of a cathodic peak current on v cannot be linearized, neither vs. $v^{1/2}$, as would be expected for a diffusion-controlled process, nor vs. v, characteristic for a surface-bound redox couple. Thus, the data were linearized in double logarithmic coordinates and, from the slopes, n values (relating to the dependence of $i_p \sim v^n$) of 0.766 and 0.706 were obtained for the electrodes prepared by methods E and A, respectively. The values obtained show that ZrP·NB- as well as ZrP·TB-modified GC electrodes possess mixed behavior, ranging between that characteristic for a diffusion-controlled process and that characteristic for a surface-bound redox couple. This mixed behavior can be caused by the superposition of the charge carrier diffusion through the ZrP layer, and the thin layer behavior of small solid particles placed directly onto the GC surface [28].

Conclusions

In the present work we have tried to modify the surface of a glassy carbon electrode with a layer of microporous zirconium phosphate. A few methods were used for this purpose, including chemical synthesis on the surface and gel droplet evaporation. Some known redox dyes were adsorbed on these modified electrodes, and the resulting electrochemical behavior was quite different from that observed at unmodified carbon-based electrodes. The stability of the modified electrodes prepared, however, was found to be unsatisfactory for their possible application in electrocatalytic systems, and some new modification procedures should be elaborated for this aim.

Fig. 5 CVs obtained for a ZrP·NB-modified GC electrode, prepared by method E, obtained after 3–5 min after immersion of the electrode into buffer solution

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