

Construction and Analytical Characterization of Prussian Blue-Based Carbon Paste Electrodes and Their Assembly as Oxidase Enzyme Sensors

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This paper reports on the development and characterization of Prussian Blue-modified carbon paste electrodes. New methods of matrix modification with Prussian Blue are reported. Two different carbon pastes have been prepared, using mineral oil or solid paraffin, thus obtaining different types of sensors whose behavior toward the electrochemical reduction of hydrogen peroxide has been fully characterized. Results obtained with Prussian Blue-modified electrodes showed a long operational lifetime, an excellent stability in a wide range of pH (3–9), a high sensitivity, and a fast response time. In addition, the coupling of solid carbon paste modified with Prussian Blue and the enzymes glucose oxidase and choline oxidase led to the assembling of biosensors that showed an optimum working range at alkaline pH.

Potassium iron(III)hexacyanoferrate(II) or Prussian Blue (PB) is an inorganic polycrystal with well-known electrochromic and electrocatalytic properties. Since it was deposited on solid electrodes by Neff,¹ it has been the subject of many studies: because of its changes in color (colorless when reduced to Prussian White, yellow when oxidized to Prussian Yellow), this compound has been employed in the development of electrochromic devices;^{2,3} it can be used as a power source in rechargeable batteries,⁴ and its electrocatalytic properties have been investigated by Itaya and co-workers, who first described an electrochemical method for growing PB films and their electrochemical behavior.^{5,6} They showed that the reduced form of PB, the Prussian White, does have a catalytic activity for the reduction of O₂ and H₂O₂ and also the oxidized form of PB shows a catalytic activity for the oxidation of H₂O₂.⁶

Others PB analogues, as Cu, Co, Cr, and Ni hexacyanometalates, have been used as electrocatalysts for developing electro-

chemical sensors for the detection of H₂O₂^{7–9} and other compounds such as perborate,¹⁰ persulfate,¹¹ hydrazine,^{12,13} and cytochrome *c*.¹⁴ In fact, these transition metal hexacyanometalates are able to reduce the overpotential necessary for nonmodified electrodes to reduce or to oxidize of the previous mentioned species.¹³

The ability of PB in reducing H₂O₂ has attracted the attention of the biosensor community, and recently, in some papers,^{15–17} PB has been defined as an “artificial peroxidase” because of its analogy with the biological family of peroxidase enzymes, responsible in nature for reduction hydrogen peroxide.¹⁸

This reduction, in fact, occurs at electrodes modified by PB in different ways at a potential range between –200 and 0 mV (vs Ag/AgCl), defined as the “range offering the most sensitive and interference-free detection”¹⁸ for amperometric biosensors. In fact, this range is low enough to reduce the signal of the electrochemical interfering species often present in real matrixes. In addition, PB does not lack in long-term stability and relatively high costs which are often related to the use of biological components.

Recently, a considerable number of papers has appeared in the literature, mainly based on glassy carbon or graphite electrodes modified by a film of electrodeposited PB, showing the preparation of biosensors for the detection of glucose,^{19–25}

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glutamate,^{16,17} amino acids,²⁰ and alcohols.²² In addition, PB analogues have been used for electrochemical glucose biosensors^{26–29} and for optical biosensors in conjunction with various enzymes.³⁰

Drawbacks related to this kind of electrode are mainly the operational stability of the electrocatalyst, limited to 3 h in FIA systems,¹⁶ its high decrease of sensitivity at neutral and alkaline pH, and the interference of dissolved oxygen.

Almost the same drawbacks are shown in sensors assembled with PB chemically synthesized from solutions containing ferrous ions.^{4,7,10,11,17}

Some of these problems can be partially circumvented, for example, by carefully controlling the operative conditions, in the case of the electrochemical deposition of PB,^{6,31} or substituting the PB with its analogues,^{7–9,11,26–29} but still some problems have to be solved.

Prussian Blue films have been deposited on a variety of surfaces, the most common being glassy carbon,^{6,12,14–17,19,21,22} graphite,^{13,20,23,24} Pt,^{5,9} and carbon fiber.²⁵ It has been also incorporated into polypyrroles.^{30,32} Also, carbon paste has been used in electrode assembling, because it is easy to prepare and to handle. In addition, the direct mixing technique has opened a very simple and effective way to add inorganic, organic, or biological modifiers to the electrode material.^{18,33,34}

Despite this, very little attention has been given to carbon paste electrodes modified with PB.^{7,10,11,36} This could be due in part to the poor mechanical strength of the paste, which can cause the leakage of the modifier, but also to a supposed lower stability of the PB in the presence of a liquid, as the mineral oil.¹⁰ Moreover, just one paper deals with the preparation of a glucose CP biosensor, but in this case, the authors used its analogous cupric hexacyanoferrate.²⁸

Recently the replacement of the hydrophobic oil with solid paraffin brought to the preparation of new, so-called solid carbon paste or carbon wax electrodes, with improved mechanical stability, reproducibility, and rapid response rate compared to the classical oil-based CP electrodes. Also, these probes were used to incorporate enzymes and modifiers.^{35,37,38}

This paper reports an efficient and yet very simple way to prepare PB-modified carbon paste (CP) and solid carbon paste (sCP) electrodes with improved characteristics of lifetime and

operational stability in acid and alkaline pHs. These electrodes have been also assembled with choline oxidase and glucose oxidase, obtaining biosensors whose analytical features have been described. We report a new method of graphite powder modification with “in situ” chemically synthesized PB, which resulted in a very active and very stable matrix, suitable to be used for CP and sCP electrodes and related biosensors but, in our opinion, useful also for other electrode preparations.

EXPERIMENTAL SECTION

Apparatus. Amperometric measurements were carried out using a VA 641 amperometric detector (Metrohm, Herisau, Switzerland), connected to a *X-t* recorder (L250E, Linseis, Selb, Germany) and equipped with a conventional three-electrode system, consisting of the CP- or sCP-modified electrode as working electrode, 3-mm diameter; an Ag/AgCl/KCl (3 mol/L) reference electrode; and a Pt electrode as counter. Cyclic voltammetry (CV) was performed using an Autolab electrochemical system (Eco Chemie, Utrecht, The Netherlands) equipped with PGSTAT 10 and GPES software (Eco Chemie). All the measurements were carried out in batch mode in a cell (Amel, Milano, Italy) connected, when necessary, with nitrogen gas for deaeration.

Reagents and Solutions. The enzyme glucose oxidase (GOx) (EC 1.1.3.4, type VII, 195 units/mg), hydrogen peroxide, and ascorbic acid were obtained from Sigma (St. Louis, MO).

Choline oxidase (ChOx) (EC 1.1.3.17, 11 units/mg), mineral oil, solid paraffin, graphite particles (powder ≤ 0.1 mm), and commercial PB with two main different specifications, soluble and insoluble, were obtained from Fluka (Buchs, Switzerland). All the inorganic salts, glucose, choline, and other reagents were of analytical grade. A 0.1 mol/L phosphate buffer solution, potassium based, was used as the supporting electrolyte.

Synthesis of PB. The PB was also synthesized in the laboratory according to Boyer et al.¹⁰ briefly, 0.11 mol of FeCl₃ was dissolved in 60 mL of water, and 20 mL of concentrated HCl was added to obtain a clear solution. Then 0.051 mol of K₄Fe(CN)₆, previously dissolved in 80 mL of water, was slowly added under vigorous stirring for 1 h. The precipitate was collected by centrifugation and washed with hot water adjusted to pH 2 with HCl. When the water became colorless (this was obtained with at least 10 cycles of washing), the precipitate was dried in the dark over solid NaOH under vacuum, in a desiccator for 3 days.

Graphite Pretreatment. The graphite powder was cleaned with acetone, rinsed with water, and then activated with aqua regia for 30 min. After this treatment, the particles were washed with distilled water until a neutral pH was reached and then dried at 400 °C for 4 h.

Preparation of Graphite Modified with PB. The activated graphite (1 g) was suspended in 10 mL of a solution of K₃[Fe(CN)₆] 0.1 mol/L in 10 mmol/L HCl and then a further 10 mL of a 0.1 mol/L solution of FeCl₃·6H₂O in 10 mmol/L HCl was added, and the resultant mixture was stirred for 10 min. The particles with adsorbed PB were then collected by filtration and washed with 10 mmol/L HCl until the washing solution became colorless and then dried in oven at 100 °C for 1.5 h. The modified graphite (referred as PB-adsorbed graphite) was stored dark in a desiccator at room temperature).

Preparation of PB-Modified CP and sCP Electrodes. The carbon paste mixture was prepared in different ways: (a) mixing

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graphite (either PB-adsorbed or simply activated with aqua regia) and mineral oil (66 and 34%, respectively). (The electrodes prepared following this procedure will be referred as PBads-CP or CP electrodes.). (b) mixing the aforementioned graphites with solid paraffin (66 and 34%, respectively) (referred as PBads-sCP or sCP electrodes). In this case, the paraffin was melted at 55 °C before mixing with the graphite powder. (c) mixing CP with different percentages of commercially available or chemically synthesized PB.

The pastes were carefully hand-mixed in a mortar and then packed into a hole (3-mm diameter, 2-mm depth) at the end of a Teflon tube. The electrical contact was made by a copper wire connected to the paste in the inner hole of the tube. The pastes were kept at room temperature in a desiccator until used.

Electrodeposition of PB on CP and sCP. A total of 2 g each CP or sCP, prepared as described before, was carefully pressed in the bottom of a homemade Teflon cell of 3-cm internal diameter with the help of a flat pestle of the same diameter. A copper wire was connected to the bottom of the cell for the electrical contact. In this case, all the paste acted as working electrode when filled with the PB growing solution. An Ag/AgCl reference electrode and a Pt counter electrode were inserted in the same growing solution, and after 20 min of deaeration with nitrogen, a constant potential of 400 mV was applied for 120 s. The growing solution was made by addition of 2 mmol/L FeCl₃ and 2 mmol/L K₃[Fe(CN)₆] solutions in the supporting electrolyte, consisting in 0.1 mol/L KCl + 0.1 mol/L HCl solution.²¹ This procedure resulted in the deposition of a PB film on the surface of the pressed pastes. Then the solution was aspirated and the surface washed with 10 mmol/L HCl to remove the adsorbed PB. The paste was removed, accurately mixed, and pressed again in the bottom of the cell in order to expose a new surface for the electrodeposition. The whole procedure was repeated 10 times to homogeneously mix PB in the bulk of the paste. (The electrodes prepared following this procedure will be referred as PBelect-CP or PBelect-sCP electrodes.)

Preparation of Enzyme-Modified sCP Electrodes. The PB-adsorbed graphite was carefully hand-mixed in a mortar with different percentages of enzymes (GOx or ChOx). Then the mixture was added to the melted paraffin, and the mixing was continued until a homogeneous paste was obtained. An aliquot of the enzyme-modified paste filled the tip of the electrode; the remaining was kept in an aluminum foil at 4 °C until reused.

RESULTS AND DISCUSSION

Usually PB films are prepared by electrochemical reduction of solutions containing Fe(III) and hexacyanoferrate(III) ions.^{2,3,5,6,9,14–18,20–25,31,32} In addition, PB is chemically synthesized directly from solutions containing Fe(III) and either hexacyanoferrate(III)^{1,12} or hexacyanoferrate(II) ions.^{4,7,10,11,36}

Attempts carried out at the beginning of the work to prepare CP electrodes using commercially available PB or PB synthesized as in ref 10 (from solutions containing Fe(III) and hexacyanoferrate(II) ions) were not successful. These sensors did not show the typical voltammogram of PB, even if the PB percentage added to the paste was increased from 10 to 25%.

In our work, two different approaches have been followed: the first was the PB chemical synthesis, starting from solutions

containing Fe(III) and hexacyanoferrate(III) ions, in the presence of activated graphite particles; the second was the electrochemical deposition and bulk enrichment on CP and sCP matrixes already prepared.

In the case of chemical synthesis, an autoreduction or a catalytic reduction of the highly reactive ferric–ferricyanide complex, which presumably is initially formed, seems to occur.⁵ In ref 5 (and in the references herein reported), Itaya and co-workers pointed out that a solution of ferric–ferricyanide in acidic media is a very strong oxidant, showing an open circuit potential highly positive (0.98 V vs SCE for a Pt disk electrode). This potential cannot be obtained in solutions of either ferric chloride or potassium ferricyanide, respectively. Moreover, the nonelectroplating method of PB deposition seems to be highly dependent on the nature of the substrate surface.⁵

For all these reasons, our procedure of chemical synthesis starts from both ferric precursors and the process is made to happen in the presence of graphite powder activated with aqua regia. The latter is a strong oxidant, known to form carboxylic groups on carbon. The presence of these groups seems also to contribute to the formation of PB films.²³ Moreover, the presence of graphite microparticles during the PB formation allowed its capillary adsorption. Thus, we obtained a homogeneous and highly efficient modified carbon matrix, which was successively mixed with mineral oil or solid paraffin.

The electrochemical deposition on the CP and sCP electrode surface is derived from the most used procedure of PB deposition found in the literature for solid electrodes. Although the film formed on the surface of CP and sCP electrodes was effective on these matrixes, it showed a very poor operational stability, limited to few minutes when measured under stirring. Then, we decided to increase the amount of the electrodeposited PB in the bulk of both pastes, either increasing the surface dimension or renewing several times the surface itself in order to expose new portions of pastes to the electrodeposition.

We obtained in this way four kinds of modified sensors whose voltammograms are reported in Figure 1. The typical two pairs of redox waves showing the oxidation of Prussian Blue to Prussian Green as well as the reduction to Prussian White are present for all the sensors, proving the effective presence of the modifier in the paste. The effectiveness of these electrodes in reducing H₂O₂ is shown by the dotted voltammograms in the same figure, where the increase of the cathodic wave in the presence of this analyte is evident. In addition, an increase in the anodic peak related to the oxidation of hydrogen peroxide can be observed at a potential higher than 0.7 V (vs Ag/AgCl).

The effect of the potential scan rate (v) on the peak reduction current (i_p) of the four kinds of modified sensors has been investigated in the range 2–50 mV/s. As expected,³⁶ the peak current plotted versus the square root of the scan rate ($v^{1/2}$) for the redox reaction between Prussian Blue and Prussian White (in the absence of hydrogen peroxide) gave linear results. This linearity could indicate that the diffusion of potassium ions within the pasting phase is rate limiting,³⁶ because the concentration of this ion in the bulk solution is 0.1 mol/L.

Hydrodynamic voltammograms of all the sensors have been also carried out, to choose the most suitable applied potential for the H₂O₂ reduction. Two examples for adsorbed and electrode-

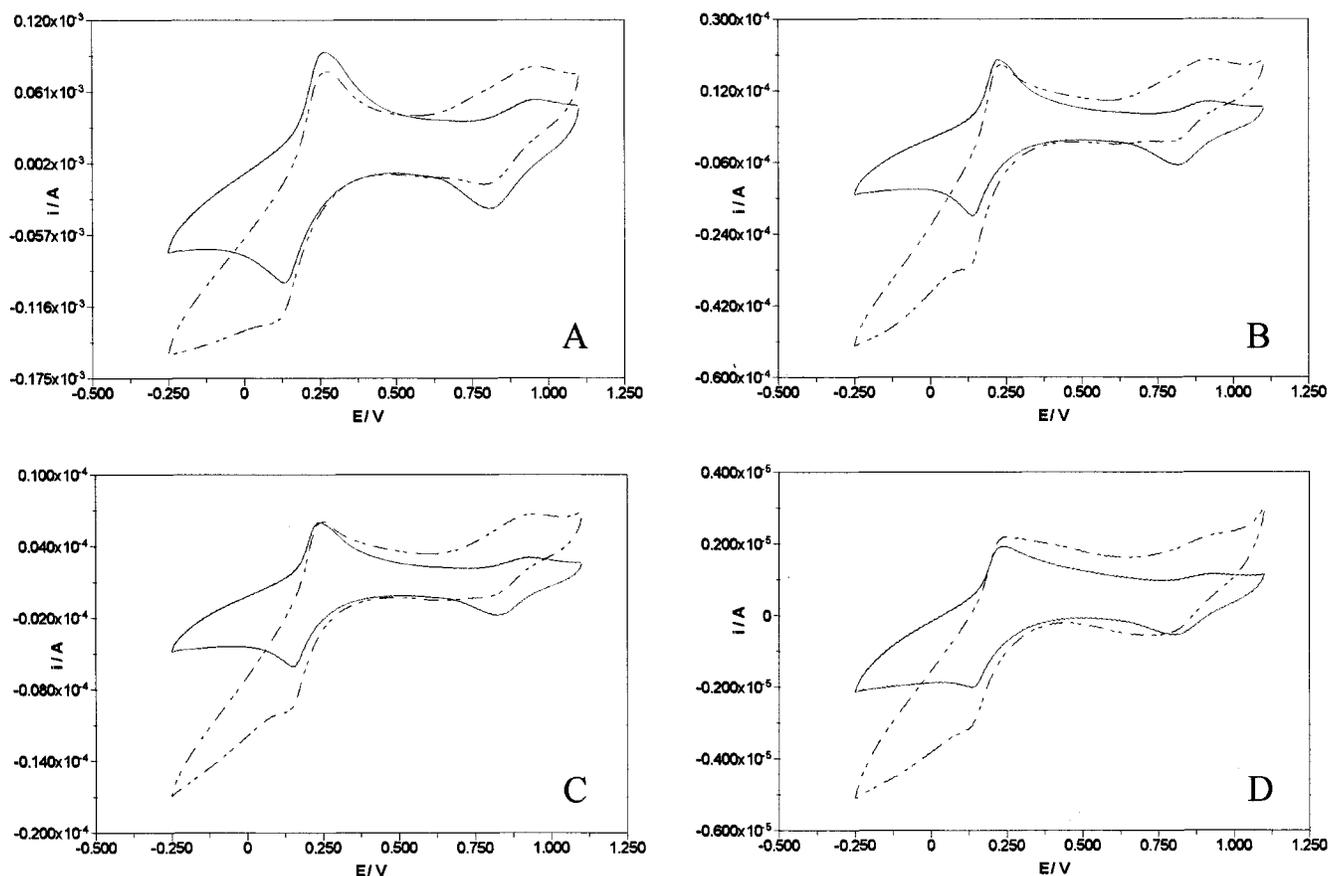


Figure 1. Cyclic voltammograms of the PB-modified sensors in the absence (continuous line) and in the presence (dotted lines) of 10 mmol/L H_2O_2 : phosphate buffer 0.1 mol/L, pH 6.0, scan rate 50 mV/s. (A) PBads-CP; (B) PBelect-CP; (C) PBads-sCP; (D) PBelect-sCP.

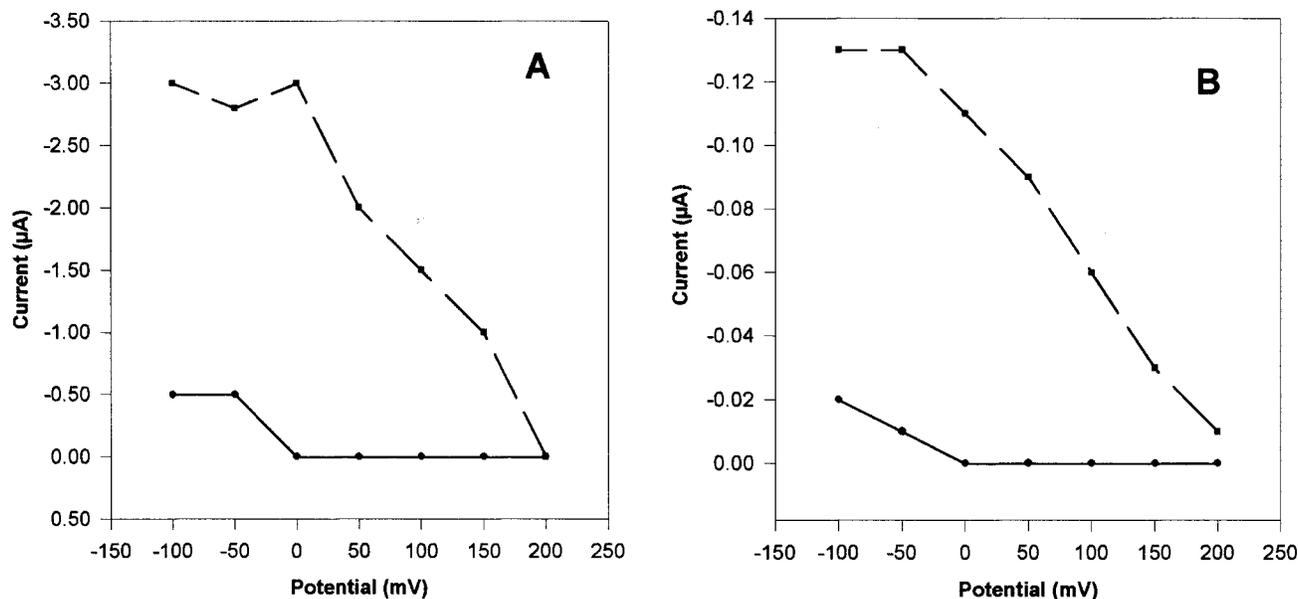


Figure 2. Hydrodynamic voltammograms of (A) PBads-CP and (B) PBelect-CP electrodes in the absence (continuous lines) and in the presence (dotted lines) of 0.1 mmol/L H_2O_2 . Phosphate buffer 0.1 mol/L, pH 6.0.

posited PB-modified CP electrodes are reported in Figure 2, where the continuous line represents the baseline current values and the upper dotted line represents the response to the addition of 0.1 mmol/L H_2O_2 solution. It can be noted that near 0.0 V (vs Ag/AgCl) the current baseline is very low, approaching to zero, while at potentials lower than this value it starts to increase. This

behavior is probably due the influence of the oxygen dissolved in solution, but also entrapped in the pastes, which can hardly be removed under nitrogen bubbling.

The current response to H_2O_2 additions increases with the decrease of the applied potential, starting from 0.2 V, and reaches a plateau at ~ 0.0 V. These results are very similar to the ones

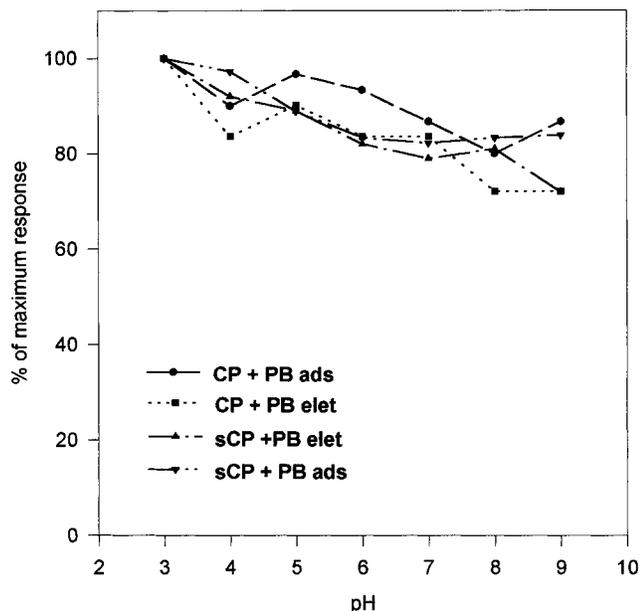


Figure 3. Response profile of the modified sensor in the range of pH 3–9. H_2O_2 additions 0.1 mmol/L. Applied potential 0.0 mV (vs Ag/AgCl).

reported in the literature for glassy carbon electrodes modified by electrodeposited PB.²¹

The value of 0.0 mV (vs Ag/AgCl) was chosen as a compromise between the highest response to H_2O_2 and the lowest current background. Similar results were obtained for PB-modified sCP electrodes.

pH, Stability, and Interference Studies. All the experiments reported above were carried out in 0.1 mol/L phosphate buffer at pH 6. However, in the literature, is clearly reported that PB is unstable and dissolves at pH values above 7, suggesting a limited use of PB-modified electrodes in acidic solutions.^{6,7,9,24,25,31}

Studies of the pH effect on these sensors have been undertaken, and we could verify a very appreciable stability of all four types of sensors tested (Figure 3). Starting from pH 3 and giving to the current for the response to 0.1 mmol/L H_2O_2 at this pH a value of 100, we observed a constant current response until pH 9. The figure shows just a random variation of less than 15% of the mean value and with no appreciable difference between the four types of electrodes. This behavior suggests that the structure of PB crystals is protected in the pastes and not influenced by the proton and the hydroxyl ion concentration in the bulk solution.

These results were subsequently confirmed by experiments carried out to verify the operational stability of the PB probes. One of these sensors, the PB-adsorbed sCP electrode, was continuously kept in a stirred buffer solution at different pHs for 8 h and the current values due to the addition of a 0.1 mmol/L solution of H_2O_2 were measured every hour. The results are reported in Figure 4 where for all the pH values tested (5, 7, and 9) a good stability can be observed during the whole experiment, the fluctuations being within the sensitivity variation of this kind of electrode. The same experiment was repeated the second day with the same sensor, and a similar behavior was observed, thus indicating the improved operational stability of these sensors. This could be due to the carbon paste matrix, which acts as a reservoir of PB, and to the solid paraffin, which acts as a barrier restricting

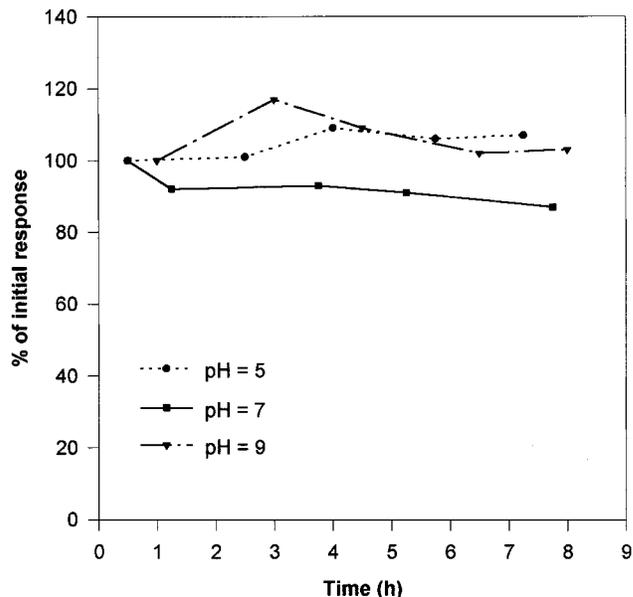


Figure 4. Long-term study of the PBads-sCP electrode at different pHs. H_2O_2 additions 0.1 mmol/L. Applied potential 0.0 mV (vs Ag/AgCl).

Table 1. Lifetime of the Prepared Matrixes, Stored Dark in the Desiccator at Room Temperature

matrix	lifetime
PBads-graphite	more than 5 months
PBelect-CP	more than 8 months
PBelect-sCP	more than 6 months

any PB leaking and highly decreasing the pH effect. Indeed, cyclic voltammograms performed after these experiments confirmed the presence of PB in CP and sCP electrodes, but the shape of the peaks was not really well defined, and therefore, any calculation of the current recorded as function of coverage of PB was not undertaken.

It is interesting to note that the graphite powder with adsorbed PB, as well as the CP and sCP with the electrodeposited PB, was stable for several months stored in a desiccator at room temperature. Also a little variation in sensitivity was observed. The lifetimes reported in Table 1 were limited only by the fact that all the stock matrixes were used for the experiments.

The same PBads-sCP electrode has been tested for interference studies. The experiments carried out showed that at selected applied potential of 0 mV (vs Ag/AgCl) the most common interfering compounds such as ascorbate, urate, and acetaminophene at a concentration of 0.1 mmol/L gave no response. Only ascorbate at a concentration of 1 mmol/L showed an oxidation current negligible (less than 1%) at 0 mV (vs Ag/AgCl) and small (4.8%) at +50 mV (vs Ag/AgCl) compared to the reduction current of 1 mmol/L hydrogen peroxide (100%) at the same applied potentials.

Sensitivity and Detection Limits. Table 2 summarizes the analytical characteristics toward the H_2O_2 reduction of the four kinds of electrodes tested: i.e., detection limits (reported as 2 times the background noise); linearity ranges; sensitivity variations, related to the slope variations of calibration curves continuously carried out along 8 h; and reproducibility, reported as the RSD of

Table 2. Analytical Data of the H₂O₂ Reduction at the Sensors^a

electrode	detection limit (mol/L)	linearity range (mol/L)	sensitivity (nA mmol ⁻¹ L ⁻¹)	sensitivity variation during 8 h (% RSD)	reproducibility (% RSD, <i>n</i> = 6)	response time (90% of steady state) (s)
PBads-CP	5 × 10 ⁻⁷	5 × 10 ⁻⁷ –5 × 10 ⁻³ <i>r</i> ² = 0.9999	30200	14	14	less than 15
PBelect-CP	2.5 × 10 ⁻⁷	2.5 × 10 ⁻⁷ –2 × 10 ⁻⁴ <i>r</i> ² = 0.9998	1240	13	18	less than 60
PBads-sCP	5 × 10 ⁻⁶	5 × 10 ⁻⁶ –10 ⁻² <i>r</i> ² = 0.9997	3150	9	10	less than 15
PBelect-sCP	5 × 10 ⁻⁷	5 × 10 ⁻⁷ –5 × 10 ⁻⁴ <i>r</i> ² = 0.9998	125	6	16	less than 60

^a All the measurements were carried out in 0.1 mol/L phosphate buffer pH 6 at an applied potential of 0.0 V vs Ag/AgCl. See text for explanations.

Table 3. Analytical Characteristics of PBads-sCP Glucose Biosensors, in 0.1 mol/L Phosphate Buffer pH 6, and PBads-sCP Choline Biosensors in 0.1 mol/L Phosphate Buffer pH 8^a

electrode	detection limit (mol/L)	linearity range (mol/L)	sensitivity (nA/mM)	sensitivity variation during 8 h (% RSD)	<i>K_m</i> (mM)
sCP + PBads + 5% GOx	10 ⁻⁴	10 ⁻⁴ –2 × 10 ⁻² <i>r</i> ² = 0.9998	160	14%	15
sCP + PBads + 5% ChOx	2 × 10 ⁻⁵	2 × 10 ⁻⁵ –2 × 10 ⁻³ <i>r</i> ² = 0.9998	250	14%	1.2

^a Applied potential: 0.0 V vs Ag/AgCl.

the current values due to the H₂O₂ reduction by six different sensors obtained from the same paste matrix.

The detection limits are sufficiently low to allow a sensitive detection of H₂O₂ and range between 0.1 μmol/L for the PB-electrodeposited CP electrode and 5 μmol/L for the PB-adsorbed sCP electrode. This latter shows again the barrier effect of the solid paraffin to the mass transport. The linear ranges cover 3–4 orders of magnitude, the upper limit being also in this case higher for PB-adsorbed sCP electrodes and influenced by the limited diffusion rate. The same table shows a difference in sensitivity of ~10 times between both CP and sCP electrodes, probably because of the same insulating effect of the solid paraffin in the paste. The sensitivity decreases also with the method of PB deposition, the adsorbed PB being much more active than the electrodeposited PB. This last effect could be ascribed to a greater loading of PB, due to a very deep adsorption on the graphite powder prepared with the proposed deposition method.

The same effect could explain the difference in the response time found among the different sensors prepared. In Table 2, it can also be observed that the matrixes prepared using the PB-adsorbed graphite, either in CP or in sCP, showed a very fast response time and required less than 15 s to reach 90% of the steady-state response, proving the high catalytic efficiency of the modifier. On the contrary, both the electrodeposited matrixes required almost 1 min to achieve the same result, probably because of a minor PB loading.

Since the procedure of PB electrodeposition on CP and sCP was long and time-consuming, and because of the lower noise, better background stability, better reproducibility, and very fast response time shown by the PB-adsorbed sCP electrodes, these latter were chosen for further modification with enzymes to assemble biosensors.

GLUCOSE AND CHOLINE BIOSENSOR

Different percentages of GOx and ChOx enzymes, ranging from 2.5 to 7.5%, have been mixed with the PB-adsorbed graphite powder before its mixing with the melted paraffin (the inverse procedure gave worse results). The enzyme choline oxidase was chosen because its optimum activity is in the alkaline range.^{39–41} The sensitivity of the probes increased almost linearly with the enzyme loading, but amounts higher than 5% gave worse results both in the background noise and in the current signal response. This effect could be due to the lower conductivity of the paste, because of the increase of nonconducting components as the solid paraffin and the enzyme itself. Further studies were then carried out with 5% of enzyme in the solid paste. Table 3 summarizes the principal analytical characteristics of these biosensors, in terms of detection limit, linear range, sensitivity, and sensitivity variation during 8 h of continuous measurements. The detection limit and the linear range of the choline biosensors were one decade lower than the correspondent glucose biosensor. Furthermore, the glucose biosensor showed an apparent Michaelis–Menten constant (*K_M*) of 15 mmol/L (concentration calculated as *I_{max}*/2), much higher than that calculated for other PB-based glassy carbon glucose biosensors.^{20,21} On the contrary, the choline biosensor presented an apparent *K_M* of only 2 mmol/L, quite similar to that of the enzyme in solution (1.2 mmol/L at pH 8).^{39–41} The variation in sensitivity during 8 h of continuous measurements was similar in both cases and, as in the case of H₂O₂ measurements, did not

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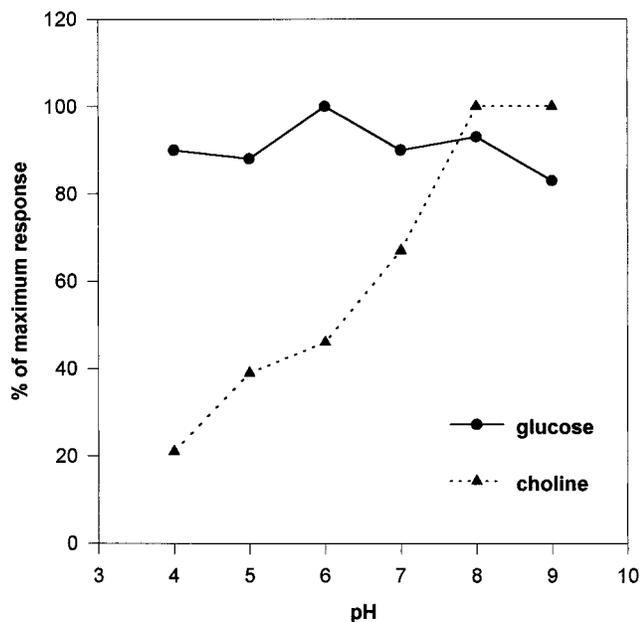


Figure 5. Response profile of the glucose biosensor and of the choline biosensor at different pHs. Glucose and choline additions 1 mmol/L. Applied potential 0.0 mV (vs Ag/AgCl).

present a defined bias. This could be an effect of electrode surface modification, due to the swelling of the paste and/or to the leakage of the modifiers.

The response time was also similar for both biosensors, which required ~30 s to achieve 90% of the steady-state current response.

pH and Stability Studies. Figure 5 reports the results of pH tests for both the biosensors. As can be observed, the glucose biosensor is almost insensitive to the pH effect in the whole range studied (from pH 3 to pH 9), while the choline biosensor follows more closely the behavior of the free enzyme (the response increases with the pH up to a maximum plateau of pH 8–9).

This behavior could be explained as follows: glucose is a neutral molecule, which can diffuse through the paraffin phase containing GOx and PB. Thus, the electrode response is limited by the rate of the diffusion of glucose in the pasting phase. For this reason a high value of apparent K_M (15 mmol/L) was obtained, which allows us to use this biosensor for glucose measurements in the diabetic range. Choline is a charged molecule and cannot

diffuse through the paraffin phase because of its hydrophobicity. Thus, choline reacts only with the enzyme present at the interface of electrode–solution, yielding an apparent K_M and an optimum pH similar to those observed with the free enzyme.

Both the biosensors showed a quite good storage stability: the PB-adsorbed sCP, mixed with the enzymes and stored at 4 °C, has been used during at least one month with practically no loss of sensitivity.

CONCLUSION

We described a new procedure for PB deposition on graphite powder, which has been used for the preparation of CP and sCP electrodes. Their performances have been compared with a more traditional procedure of electrodeposition of PB, also adapted to be used with CP and sCP electrodes. Both PB-modified CP and sCP sensors showed improved characteristics of storage stability, and the PB-modified sCP showed also an operational stability at alkaline pHs which is, in our knowledge, not yet reported in the literature.

The PB-adsorbed graphite powder is suitable for other applications, and its use in the production of PB-modified screen-printed electrodes is at present in progress in our laboratory with preliminary encouraging results.

Abbreviations: CP, carbon paste obtained by mixing graphite activated with aqua regia and mineral oil; sCP, solid carbon paste obtained by mixing activated graphite and solid paraffin; PBads-CP, carbon paste obtained by activated graphite with previously adsorbed PB and mineral oil; PBads-sCP, solid carbon paste obtained by activated graphite with previously adsorbed PB and solid paraffin; PBelect-CP, carbon paste modified with PB obtained by electrodeposition and bulk enrichment of the paste; PBelect-sCP, solid carbon paste modified with PB obtained by electrodeposition and bulk enrichment of the solid paste.

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