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Oxides of platinum metal group as potential catalysts in carbonaceous amperometric biosensors based on oxidases

Petr Kotzian[a], Petra Brázdilová[a], Kurt Kalcher[b], Karel Handlíř[c], Karel Vytřas[a,*]

[a] Department of Analytical Chemistry, University of Pardubice, Nám. Čs. legií 565, CZ-532 10 Pardubice, Czech Republic
[b] Institute of Chemistry—Analytical Chemistry, Karl-Franzens University, Universitätsplatz 1, A-8010 Graz, Austria
[c] Department of General and Inorganic Chemistry, University of Pardubice, Nám. Čs. legií 565, CZ-532 10 Pardubice, Czech Republic

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Abstract

Oxides of four platinum metals (PdO, OsO_2, IrO_2, PtO_2) were incorporated into carbonaceous materials such as carbon paste or carbon ink to study their catalytic properties towards oxidation or reduction of hydrogen peroxide, which is known as a product of biocatalytic reactions in which enzymes of the oxidases participate. In the second step, glucose oxidase – the typical representative of the group – was immobilized in a Nafion film onto the surface of screen-printed carbon electrodes and investigated for its ability to serve as a detector of glucose in flow injection analysis. From all the metal oxides selected, IrO_2 was found the best, because it was capable to operate at low applied potentials (around -0.2 V) at which the undesirable effects of common interfering species (both ascorbic and uric acids) were significantly eliminated.

Keywords: Carbon paste electrodes; Screen-printed carbon electrodes; Biosensors; PdO; OsO_2; IrO_2; PtO_2; H_2O_2; Glucose; Amperometry; Flow injection analysis

1. Introduction

As evident from the number of scientific papers, various reviews and monographs, research on the field of biosensors has attracted the activities of the world's scientific community in the last few decades and still represents a very active area of science. The success of the last world congress devoted to biosensors [1] demonstrated well their popularity and significance.

The widespread in the group are amperometric enzyme biosensors, which are often based on the oxidation of target analyte with appropriate oxidase, where formation of hydrogen peroxide is monitored [2,3]. Glucose oxidase represents one of the most exploited enzymes of the oxidases group. Of course, its importance consist in monitoring glucose in blood of patients with diabetes mellitus [4] as well as in quality control applications in food industry [5], but appreciative is also a fact that it is relatively cheap, available and stable and, for those reasons, being very suitable for all beginners starting experiments on that field. Despite the high specificity of the enzyme, the response of corresponding sensors may still be influenced by common electroactive interfering substances such as ascorbic acid and uric acid. Therefore, the main aim is to reduce the high overpotential of hydrogen peroxide (offering usually around +0.7 V at bare electrodes) by means of a mediator introduced as a modifier to the electrode surface or into the electrode bulk. Several excellent reviews dealing with the matter can be found in the literature [6–11].

Concerning heterogeneous carbon materials, they have a long tradition as more than two thousand of original papers were published since their first application (see [12]). The most significant benefit of using carbon paste electrodes (CPEs) is the simple and effective renewal of their surface. CPEs also give a wider choice in the geometry of the surface area and offer the possibility of specific modification of control of electrochemical properties by judicious choice of the carbon paste composition. On the other hand, screen-printed carbon electrodes (SPCEs) are ideally suitable for decentralized clinical, environmental or industrial testing and monitoring. SPCEs can be proposed as a solution to the lack of commercial sensors for decentralized monitoring, while CPEs continue to play a major role in the development of new analytical procedures in laboratories [13,14].

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* Corresponding author. Tel.: +420 466 037 512; fax: +420 466 037 068.
E-mail address: karel.vytras@upce.cz (K. Vytřas).

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As regards mediators, we have demonstrated that various metal oxides are suitable as modifiers of both carbon pastes and carbon inks used in CPEs and SPCEs. In principle, they are two ways, which may be followed. The first is the use of more frequent oxides such as MnO_2 [15–19], Fe_3O_4 [20], FeO [21], SnO_2, CuO, Fe_2O_3 [22], etc. The second one is the use of oxides of platinum group metals; they are more expensive (anyway, their consumption is not too high) but the resulting sensors are less affected by various side reactions (dissolving in highly acidic media, etc.). For that reason, we started with investigations of catalytic properties of RuO_2 [23,24] and RhO_2 [25]; both of them were applied in biosensors for determination of hydrogen peroxide, glucose and other biologically important compounds. Oxides of the remaining group of platinum metals (PdO, OsO_2, IrO_2 and PtO_2) were subjected to similar studies more recently and the results are presented in this paper.

2. Experimental

2.1. Chemicals, reagents and solutions

Glucose oxidase (EC 1.1.3.4. from *Aspergillus niger*, specific activity 210 U mg^{-1}; GOx), Nafion (5% m/m solution in lower aliphatic alcohols) and metal oxides (IrO_2, PtO_2 and PdO) were obtained from Aldrich. OsO_2 was home-synthesized through ammonium hexachloroosmate from pure osmium (Aldrich) according to the procedure described by Dwyer and Hogarth [26]; after alkalizing the solution with Na_2CO_3, a precipitate of OsO_2 was isolated, filtrated and air-dried. All chemicals used for the preparation of buffer, stock and standard solutions were of analytical reagent grade and purchased from Lachema (Brno, Czech Republic). Phosphate buffer was prepared by mixing aqueous solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate (both 0.1 M) to achieve the required pH. The glucose stock solution (2.5 g L^{-1}) was prepared and diluted appropriately. Solutions of both ascorbic and uric acids (both Aldrich, 50 mg L^{-1}) were prepared immediately before use.

2.2. Instrumentation

A modular electrochemical system, AUTOLAB, equipped with modules PGSTAT 30 and ECD (Ecochemie, Utrecht, Holand) was used in combination with corresponding software (GPES, Ecochemie) under Windows XP®.

The flow injection system consisted of a peristaltic pump (Minipuls 3, Gilson SA., France), a sample injection valve (ECOM, Ventil C, Czech Republic), and a self-constructed thin layer electrochemical flow-through cell. The working electrode was fixed via rubber gaskets (thickness 0.6 mm) directly to the back plate of the thin layer cell with a Teflon support as a holder. The reference electrode was Ag/AgCl/3 M KCl (RE-6, BAS, USA), the stainless steel back plate represented the counter electrode of the cell. The responses were evaluated using the peak heights (differences between background and response current of the analyte).

For pH measurements, a portable pH-meter (model CPH 52, Elteca, Turnov, Czech Republic) equipped with a combined glass pH-sensor (OP-0808P, Radelkis, Budapest, Hungary) was used; the measuring cell was calibrated with buffer solutions of the conventional activity scale [27].

2.3. Electrode preparation

Unmodified carbon paste was prepared by adding 72 μL paraffin oil (Uvasol, Merck) to 0.2 g of carbon powder (CR-5, Maziva Týn nad Vltavou, Czech Republic). When metal oxide-modified CPEs were prepared, part of carbon powder (5%) was substituted by the corresponding metal oxide and mixed with the same amount of the paraffin oil as above. The mixture was properly homogenized in a mortar and resulting paste was packed into the piston-driven electrode holder of own construction [28]. The end-hole of this holder was 3 mm in diameter, giving the active surface area of ca. 7 mm^2.

To fabricate SPCEs, carbon ink (0.95 g, Gwent C50905D1, Pontypool, UK) and the corresponding metal oxide (0.05 g, Merck) were thoroughly mixed manually for 5 min and subsequently sonicated for 30 min; the resulting mixture was used immediately. Working electrodes were prepared by screen-printing the modified ink onto an inert laser pre-etched ceramic support (113 mm × 166 mm × 0.635 mm, No. CLS 641000396R, Coors Ceramics, Chattanooga, TN, USA). Thick layers of the modified carbon ink were formed by brushing the ink through an etched stencil (thickness 100 μm, electrode printing area 105 mm^2) with the aid of the squeegee of the screen-printing device (SP-200, MPM, Franklin, MA, USA) onto the ceramic substrates. The resulting plates were dried at 60 °C for 2 h.

For the enzyme entrapment, 1 mg of enzyme (GOx) was dissolved in 20 μL of 0.1 M phosphate buffer (pH 7.5) and mixed with an equal amount of Nafion solution neutralized to pH ∼ 7 with ammonia. The resulting mixture (5 μL) was applied directly onto the active area of the SPCE surface and air-dried for 30 min.

3. Results and discussion

3.1. Electrochemical response to hydrogen peroxide

All operational parameters (applied potential, pH of phosphate buffer and flow rate) influencing the H_2O_2 determination were optimized. For preliminary studies, carbon paste electrodes bulk-modified with metal oxides were used in batch mode. An advantage is that such a type of electrode allows simple and effective change of the corresponding modified paste within few minutes and consequently, more paste mixtures may quickly be tested. To verify catalytic properties of metal oxides for oxidation of H_2O_2, potential of +0.5 V was applied (Fig. 1). As seen, the highest sensitivity occurred at CPEs modified with palladium oxide, followed by those modified with IrO_2, OsO_2 and PtO_2. No or very low response was observed when unmodified CPE was used.

For more detailed studies of operational parameters, SPCEs were applied. First, the operational potential was investigated