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Graphite grains studded with silver nanoparticles: Description and application in promoting direct biocatalysis between heme protein and the resulting carbon paste electrode

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ABSTRACT

The impregnation of graphite grains with silver nanoparticles (AgNPs) is proposed for making a novel carbon paste electrode (CPE). The resulting material promotes direct electron transfer and direct biocatalysis of embedded heme protein. It is demonstrated that the impregnation of graphite grains with AgNPs of 16–25 nm, incorporated in a CPE, can promote measurable bio-electrochemical phenomena involving hemoglobin and myoglobin. Unlike other biosensors prepared with simple carbon, those based on carbon grains studded with AgNPs show well-defined and quasi-reversible voltammetric peak with heterogeneous electron transfer rate k_s of approximately 0.037 ± 0.007 and $0.013 \pm 0.005\ \mathrm{s}^{-1}$ for hemoglobin and myoglobin, respectively. The embedded proteins also retain their bio-catalytical activity for hydrogen peroxide and nitrite reduction with linear ranges of 0.5–3000 μM and 30–150 μM, sensitivities of $73.6 \pm 0.6\ \mathrm{nA}\ \mu\mathrm{M}^{-1}$ and $5.72 \pm 0.11\ \mathrm{nA}\ \mu\mathrm{M}^{-1}$, and detection limits close to 0.08 μM and 5.80 μM, for these two analytes respectively. These results support the viability of this preliminary approach for the development of advanced third-generation biosensors.

1. Introduction

Since the discovery of the carbon paste electrode (CPE) by Ralph N. Adams in 1958 and its introduction as an alternative to the dropping mercury electrode, particularly in anodic polarography [1], this type of electrode (and sensors based on it) have undergone considerable development. The scope for development is due to several advantages, such as low cost, low background current, wide potential window, high sensitivity, and renewable surface [2]. However, because the fabrication process involves simply mixing graphite with suitable binders to form a paste, modification of this material has branched out across several lines of innovation. Between the more relevant ones are: research into alternative binding components such as Teflon [3], sol–gel [4,5], and ionic liquid [6], modification of the paste with polymers, redox mediators and recognition elements to construct electrochemical sensors and biosensors [7–9], and the doping of the electrode with different materials such as clay [10], carbon nanotubes [11] and metal nanoparticles to enhance the analytical performance of the associated electrochemical devices [12–18]. Furthermore, in the field of enzymatic biosensors, the use of metal nanoparticles has attracted much interest in most recent years, possibly due to the advantages inherent in the properties of nanomaterials particularly for improving direct electron transfer between the co-factors and graphite grains.

Nevertheless, despite the volume of literature on carbon paste doped with nanoparticles, and its use as a basis for biosensors, only two main methods are being used for the incorporation of such particles in the material: either physical inclusion in the mixed paste or deposition onto the surface of the electrode, starting from a colloidal solution or metallic salt. However, merely deposition of nanoparticles onto the surface of the electrode does not exploit the characteristics of this material to their best advantage, especially the renewable nature of the bio-surface. Physical inclusion by mixing alone produces a biosensor in which the only way to transfer charge is in all cases through the phenomenon of percolation (enzyme-metals, enzyme-graphite and metals-graphite), as illustrated in Fig. 1A. Existing processes also have the major limitation that only a very limited concentration of nanoparticles can be incorporated, because the proportion of water/mineral oil must be kept low in order to obtain a good paste.Impregnation of graphite grains with metallic nanoparticles is a novel approach for doping the carbon paste electrode with this nanomaterial. So, advanced sensors and biosensors, in which the nano-sized metal particles are

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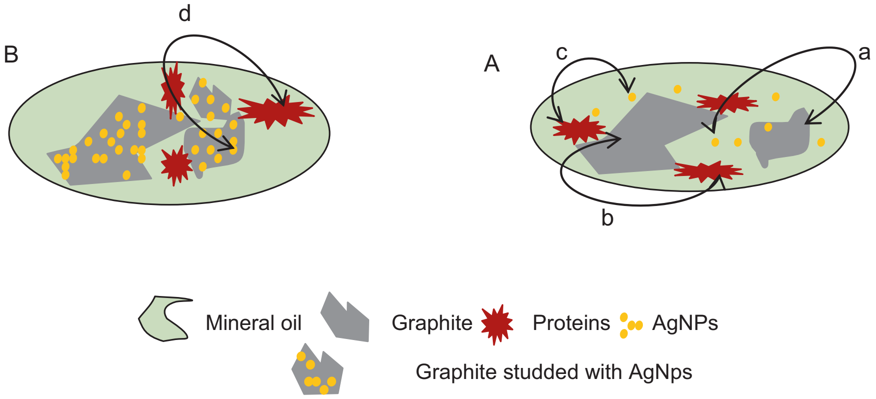

Fig. 1. Diagrams illustrating possible electron transfer in bulk modified carbon paste with nanoparticles (A) [a: APS ↔ AgNPs, b: Proteins ↔ AgNPs, c: Proteins ↔ APS], and paste based on graphite grains impregnated with nanoparticles (B): the only percolation possible is between Proteins ↔ APS – AgNPs.

in intimate contact with the graphite, can be obtained, with two significant advantages: First, a large amount of nanoparticles can be incorporated in the material; second, other mechanisms for direct electron transfer are also possible, without the need of a percolation process, which is difficult to perform in an oil media, particularly in the case of metal-graphite, as can be seen in Fig. 1B.In this paper we explore the alternative procedure of studding graphite powder particles with silver nanoparticles by an impregnation method. The resulting studded graphite grains were used in the preparation of a heme proteins-modified carbon paste electrode, and based on this, third generation biosensors for peroxide and nitrite were obtained.

2. Materials and methods

2.1. Instrumentation

Electrochemical measurements were carried out with an AutoLab®/PGSTAT20 (Ecochemie, Utrecht, Netherlands) potentiostat/galvanostat equipped with Autolab software GPES V. 4.9 for electronic waveform generation, and for data acquisition and handling. A single glass cell containing Ag/AgCl/3 M KCl, platinum wire, and carbon paste made of graphite with and without impregnation by AgNPs were used as reference, auxiliary and working electrode, respectively.

Spectra of the yellow colloidal solution of silver nanoparticles were recorded with a JASCO double-beam UV–visible spectrophotometer.

For graphite grains studded with AgNPs, transmission electron microscopy (TEM) was performed using a JEOL 2010F instrument, running High Angle Annular Dark Field software (HAADF) for particle size determination, and the INCA suite V.1.0 for elemental X-ray analysis. X-ray diffractograms were measured using an Advance-Burker D8 theta-theta X-ray diffractometer, with Cu-Kα radiation (40 kV, 40 mA) and secondary mono-chromator of graphite at an angle of 2θ from 5° to 50°; the scan rate speed and time constant were $0.02^\circ\ \mathrm{min}^{-1}$ and 2 s, respectively.

2.2. Chemicals

Graphite powder of microcrystal (APS) grade, 2–15 μM, 99.9995% (metal basis) was purchased from Alfa-Aesar (Johnson Matthey GmbH, Germany). Mineral oil, hemoglobin from bovine blood (Hb), and myoglobin from skeletal muscle (Mb) were purchased from Sigma–Aldrich (St. Louis, USA). Potassium phosphate, acetic, acetate, and tris–HCl for corresponding buffers were purchased from Fluka (Switzerland), Merck (Germany) or Panreac (Spain). Hydrogen peroxide (30% H_2O_2), sodium nitrite ($NaNO_2$) and interferents (dopamine, uric and ascorbic acids, glucose, and sucrose) were of analytical grade and purchased from Merck, Sigma or Panreac. Nanopure water was obtained by passing twice-distilled water through a Milli-Q system (18 MΩ cm^{-1}, Millipore, Bedford, MA).

2.3. Procedure

2.3.1. Preparation of APS graphite grains impregnated with AgNPs (AgNPs-APS)

First the AgNPs were prepared according to the following reaction:

$$AgNO_3 + KBH_4 \rightarrow Ag + \frac{1}{2}H_2 + \frac{1}{2}B_2H_6 + KNO_3$$

adding drop-by-drop 10 mL of 1.0 mM $AgNO_3$ to 30 mL of freshly-prepared ice-chilled 2.0 mM KBH_4 with vigorous stirring. The mixture turned pale yellow after the addition of 2 mL in drops, and become stable bright yellow at the end of the procedure. To prove the formation of AgNPs, this solution was characterized using UV–vis spectroscopy, showing an adsorption band around 390 nm with a half width of 42 nm (Supporting data 1-A-b), accompanied by the total disappearance of the related adsorption band around 300 nm for $AgNO_3$ in the absence of KBH_4 (Supporting data 1-A-a), thus it confirms the formation of the metal nanoparticles. Impregnated graphite grains (AgNPs-APS) were then obtained by adding APS graphite powder (0.2 g) to the clear yellow solution obtained with continuous stirring; heating was applied for 15 min at about 50 °C to evaporate all the solvent.

2.3.2. Preparation of biosensors

To prepare the biosensors, 50 mg of AgNPs-APS composite were mixed thoroughly with 2 mg of heme-proteins (Hb or Mb), with addition of 25 μL of mineral oil to make the paste. Then the paste was packed in the usual electrode cavity. After smoothing using satin-finish paper, the surface has a shiny appearance. The electrode is then ready for use.

In this paper, the four different carbon paste biosensors studied and compared are denoted as follows, in function of their composition: Hb/AgNPs-APS-CPE, Mb/AgNPs-APS-CPE, Hb/APS-CPE, and Mb/APS-CPE (based on hemoglobin and myoglobin mixed with APS graphite studded with silver nanoparticles, and these two proteins mixed with normal graphite, respectively).