Development of nanostructured gold-containing electrodes for glucose oxidation∗

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Keywords: gold nanoparticles; glucose oxidation; polyoxometalate

Abstract: We have demonstrated electrocatalytic activity towards glucose oxidation in phosphate buffer (pH 7) of phosphomolybdate-capped gold nanoparticles deposited on multiwalled carbon nanotubes (MWNT/AuNPs-PMo12). The system is an alternative of higher durability to enzymatic electrodes containing glucose oxidase (MWNT/GOx). Under applied conditions the MWNT/AuNPs-PMo12/GC electrode is more active towards electrooxidation of glucose than the MWNT/GOx/GC system. The process starts at more negative potentials and registered currents are of higher value, when compared to the performance of a typical enzymatic catalyst. Moreover, for the proposed inorganic hybrid system, the mediator is not required and hydrogen peroxide as an intermediate is not generated. The gold nanoparticles synthesized on multiwalled carbon nanotubes have been subjected to physical characterization, including Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) to verify their structure and morphology.

1. Introduction

Conversion of chemical energy into electrical energy from biofuels like glucose has attracted the attention of many electrochemists over the last years. Both bioanodes comprising glucose oxidase enzyme as the biocatalyst and systems employing gold centers have found numerous applications in the fields like implementable devices, waste water treatment, drug delivery, and biosensors (Wang et al., 2012; Bullen et al., 2006). Although, among other fuel cells, biofuel cells comprising glucose oxidase are highly selective and able to operate at physiological media and ambient temperature they suffer from poor stability due to short enzyme lifetime (Sarma et al., 2009).

∗The authors would like to thank Professor Pawel Kulesza from our research group for support and helpful discussion.
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Enzymatic glucose oxidation mechanism:

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\text{Glucose} + \text{GOx(FAD)} + 2\text{H}^+ \rightarrow \text{gluconolactone} + \text{GOx(FADH}_2) \\
\text{GOx(FADH}_2) + \text{O}_2 \rightarrow \text{GOx(FAD)} + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^{-}
\]

After glucose oxidation \(\text{O}_2\) acts as a mediator for \(\text{FADH}_2\) regeneration (active enzyme center) and hydrogen peroxide is produced as a by-product (Bankar et al., 2009). \(\text{H}_2\text{O}_2\) presence is highly deleterious for enzymes since it causes denaturation of the protein shell. Moreover, the active center of glucose oxidase is deeply hidden in the protein shell and the use of mediators providing electric contact between the active center and the electrode is needed. Nevertheless, the use of a mediator is not preferred because it can leak and may not be biocompatible with the enzyme. Therefore, gold nanocenters have attracted our attention as alternative efficient catalysts towards glucose oxidation.

Glucose oxidation on a metal surface:

The mechanism of glucose electrooxidation suggested in literature (Hasio et al., 1996; Adzic et al., 1989; Makovos and Liu, 1986) occurs through a different pathway and up to now is not unequivocal. If the interaction between glucose and the gold electrode is relatively weak the mechanism may look as follows:

1. The first step of the glucose electrochemical oxidation is the formation of \((\text{OH})_{\text{ads}}\) species on gold;
2. The second step involves the oxidation of the hydrogen atom from the hydroxyl group \(\text{bounded}\) to the \(\text{C}_1\) carbon atom of the glucose substrate. Free radical species are formed and one electron is transferred to the electrode;
3. Radical species are further \(\text{oxidized}\) by the removal of the residual hydrogen atom. During this process the second electron is transferred to the electrode substrate with gluconolactone formation;
4. The last step involves the hydrolysis of gluconolactone.

During electrochemical oxidation of glucose on catalytic active centers on a metal surface hydrogen peroxide is not produced. Gold nanoparticles exhibit specific chemical and physical properties which depend on their size, shape and stabilizing agents. Decreasing the size of nanoparticles develops the catalytic active surface of \(\text{Au}\), improving the accessibility of the substrate to active sites (Daniel and Astruc, 2004; Haruta, 1997). The three low index surfaces of gold \(\text{Au}(110), \text{Au}(111)\) and \(\text{Au}(100)\) are reactive in the glucose electrooxidation process and the last one is the most active. To improve durability of the high surface-to-volume ratios of small gold nanoparticles and to keep the so-called quantum-size effects generated by electrons confined within a small volume, nanoparticles are immobilized within multiwalled carbon nanotubes. Nanotubes provide a porous structure and are characterized by a highly active surface that improves the kinetics of the electrocatalytic process. Herein we use a simple approach to prepare a hybrid electrode of improved catalytic activity towards glucose electrooxidation able to operate in physiological \(\text{pH}\). We utilize multiwalled carbon nanotubes decorated with polyoxometalate (phosphododecamolybdates \(\text{PMO}_{12}\))-modified gold nanoparticles (AuNPs) prepared according to the modified procedure.
described earlier (Zoladek et al., 2011). The proposed concept of gold nanoparticles synthesis has numerous advantages, inter alia, it is simple, single-phase and it occurs in a water solution. The resulting material is highly porous and characterized by high catalytic activity. Compared to other stabilizers (Blicharska et al., 2013), polyoxometalates occupy only a small part of gold surfaces and do not block catalytic centers (Zoladek et al., 2011). The inorganic catalytic system MWNT/AuNPs-PMo$_{12}$ is characterized by high durability in contrast to enzymatic electrodes whose stability is limited by enzyme lifetime.

2. Experimental design

To prepare catalytic materials we used reagents listed in subsection 2.1. In order to determine the physicochemical and electrochemical properties of the received catalysts we used the apparatus described in the second subchapter. The procedure for preparing and the method of modifying the electrode surface are described in subsection 2.3.

2.1. Reagents

All chemicals were of analytical grade purity, and they were used as received. Glucose oxidase from Aspergillus Niger (GOx, EC 1.1.3.4, 192 U/mg) and Bovine serum albumin (BSA) were obtained from Sigma. Glutaraldehyde (GA, 50 %) and Hydroquinone (HQ) were obtained from Sigma-Aldrich. Multiwalled carbon nanotubes (MWNT), Nafion (5 wt % in lower aliphatic alcohols and 15 – 20 % water), hydrogen tetrachloroaurate (III) trihydrate (HAuCl$_4$3H$_2$O), sodium borohydride (powder NaBH$_4$), phosphomolybdic acid hydrate (H$_3$PMo$_{12}$O$_{40}$·H$_2$O) were purchased from Aldrich. D-Glucose was obtained from POCh, glucose solutions were stored overnight to allow them to reach mutarotational equilibrium before use. The phosphate buffer (pH 7) contained KH$_2$PO$_4$ and K$_2$HPO$_4$ from Sigma. Solutions were prepared using triply-distilled and subsequently deionized (Millipore Milli-Q) water. They were deoxygenated by bubbling with ultra-pure argon. Experiments were carried out at room temperature.

2.2. Apparatus

Electrochemical measurements were carried out by CHI 760 D electrochemical working station (CH Instruments Inc., Austin, USA). A standard three-electrode cell was used. A saturated calomel electrode (SCE) and glassy carbon wire were used as counter and reference electrodes, respectively. A glassy carbon disk (0.071 cm$^2$) and a glassy carbon rotating disk (0.247 cm$^2$) were used as working electrodes. Current densities were calculated against the surface area of the electrodes. All the electrochemical experiments were conducted at the temperature of about 20 ± 2 °C. Before modification, the glassy carbon disk electrode and the rotating disk electrode were polished on a Buehler polishing cloth with aqueous alumina slurries of successively finer grain sizes (5 – 0.05 µm). RDE voltammetric measurements were taken using a variable speed rotator, Pine Research Instrumentation, USA. Transmission Electron Microscopy (TEM) experiments were carried out with Libra 120 EFTEM (Carl Zeiss) operating at 120 kV. The samples were prepared by placing one drop of ethanol diluted catalyst suspension (but without Nafion) onto a nickel grid (Agar Scientific).
The morphology of deposited layers was also investigated by Scanning Electron Microscope (SEM) with Roentec model M1, EDX analyzer (Germany) integrated with LEO, model 435 VP with accelerating voltage 15 keV.

2.3. Preparation of electrocatalytic films

MWNT were subjected to purification with concentrated HCl, rinsing, and interfacial functionalization by being exposed to 3 M nitric acid as reported earlier (Skunik and Kulesza, 2009). The whole synthesis was carried out in water. The phosphomolybdinate-modified gold nanoparticles were prepared following the procedure reported by Zoladek that involved a reaction between partially reduced phosphomolybdic heteropolyblue and the gold precursor (H AuCl₄). MWNT/AuNPs-PMo₁₂ were prepared by adding 10 mg of MWNT to a solution of phosphododecamolybdic heteropolyacid (H₃PMo₁₂O₄₀) and sonifications. Then a stoichiometric amount of freshly prepared 0.016 M water solution of sodium tetrahydridoborate (NaBH₄) was added. After 4 h of stirring, an equivalent volume of 7.5 mM water solution of the precursor (H AuCl₄) was added. The resulting colloidal suspension was centrifuged, and the supernatant solution was removed and replaced with water. 5 µl of the resulting suspension was cast on a glassy carbon electrode and then covered by a deposition of 0.5 µl of 0.5 % Nafion and left to dry. The enzyme-based ink of GOx modified MWNT was prepared using the above described procedure with some modifications. 30 µl of the suspension of 10 mg of functionalized MWNT in 2 ml of water was mixed with 6.5 µl of the enzymatic suspension composed of 1.5 mg of glucose oxidase, 18 µl of phosphate buffer and 2 µl of 0.5% glutaraldehyde. Then 16.25 µl of the resulting suspension was cast on the RDE disk. Before performing each electrochemical experiment, all bioelectrocatalytic films were subjected to potential cycling (5 cycles) in a phosphate buffer (pH 7) in the potential range from -0.55 V to 0.8 V vs Ag/AgCl/3M KCl at a scan rate of 50 mV s⁻¹.

Physicochemical characterization:

TEM micrograph of phosphomolybdate modified gold nanoparticles obtained through Zoladek’s approach shows uniformly distributed nanoparticles of regular hexagonal shape with an average size of 30 nm (Fig. 1A). When the nanoparticles were prepared on multiwalled carbon nanotubes they changed their spherical shape for more longitudinal with an average size of 40 nm (Fig. 1B). It is also apparent from Fig. 1B that gold nanoparticles are uniformly distributed on oxidized carbon nanotube walls. SEM image (Fig. 1C) reveals the highly porous nature of the catalytic material.

Electrochemical characterization:

Voltammetric curves of the GC electrode modified with MWNT/GOx recorded in 0.1 M phosphate buffer of pH 7 in the absence and presence of 50 mM glucose are shown in Fig. 2. Two pairs of well defined peaks at formal potentials of about -0.055 V and 0.14 V are related to the presence of carbon-oxygen functionalities on the carbon material surface (e.g. carboxylic groups, phenolic groups, lactone groups, and ether groups) which undergo redox reactions at these potentials. At the formal potential of -0.477 V a pair of well defined and symmetric peaks (anodic and cathodic peak separation 32 mV) is observed. These peaks result from direct electron transfer of the GOx FAD/FADH₂ prosthetic group (Zhao et al., 2006; Guiseppi-Elie et al., 2002).
Oxidation of glucose starts at about 0.4 V but registered catalytic currents are only slightly higher than the background currents. The low activity of the enzyme-based electrode is assigned to insufficient electron transport between the active center and the electrode surface during glucose electrooxidation.

As presented in Fig. 3, when hydroquinone is incorporated into the enzymatic layer (MWNT/HQ/GOx), a pair of symmetric peaks with $E_f = 0.055$ V is observed in the buffer solution. Herein similarly to the MWNT/GOx system (Fig. 2) at the formal potential of -0.48 V a pair of well defined and symmetric peaks (anodic and ca-
Thodic peak separation 34 mV), originating from the conversion between GOx (FAD) and GOX (FADH$_2$), was observed. These peaks also indicate direct electron transfer from the prosthetic group of FAD/FADH$_2$ of the GOx as was in the case of the MWNT/GOx system (Zhao et al., 2006; Guiseppi-Elie et al., 2002). For the MWNT/HQ/GOx catalyst glucose oxidation starts at 0 V and the catalytic current is much higher in comparison to the system without the mediator.

![Fig. 3. Voltammetric oxidation of glucose on a glassy carbon electrode modified with MWNT/HQ/GOx. Experiments were carried out in the presence (grey curves) and absence (black curves) of 50 mM glucose in deoxygenated 0.1 M phosphate buffer of pH 7. Scan rate: 10 mVs$^{-1}$](image)

In turn, voltammetric curves of the GC electrode modified with MWNT/AuNPs-PMo$_{12}$ in 0.1 M phosphate buffer of pH 7 in the absence and presence of 50 mM glucose are presented in Fig. 4. The cathodic peak on voltammetric curve at potential of about 0.52 V (Fig. 4) is assigned to the reduction of superficial gold oxide formed in the anodic cycle above the potential 0.54 V. When glucose is added to the electrolyte, two oxidation peaks centered at potential about 0.33 V and 0.35 V, in the forward and backward scan respectively, are observed. According to the mechanism presented and discussed in the literature the first peak is associated with the oxidation of the hydrogen atom from the hydroxyl group bounded to the C1 carbon atom of the glucose substrate. As a result, free radical species are formed. The second peak is associated with further oxidation of radical species with gluconolactone formation. Since the AuOH sites on Au surfaces are the active sites for glucose oxidation, the catalytic activity would be strongly dependent on their number. The anodic curve of glucose oxidation consists of two partially overlapping peaks, the first one seen as a pre-peak at potentials between -0.2 V and 0.1 V (subsequently increasing during cycling in the presence of glucose – data not shown) and the second, characterized by
much higher currents, between 0.1 V and 0.5 V. It may be postulated that the process appearing at lower potentials is related to the formation of a new phase of gold with different catalytic activity towards glucose oxidation. In comparison to enzymatic electrodes (MWNT/GOx and MWNT/HQ/GOx), the proposed inorganic system is characterized with much higher values of catalytic current and the oxidation process starts at more negative potentials.

![Cyclic voltammograms](image.png)

Fig. 4. Cyclic voltammograms at 10 mV s$^{-1}$ of MWNT/AuNPs-PMo$_{12}$ registered in deaerated 0.1 M phosphate buffer of pH 7 in the absence (black line) and presence (grey line) of 50 mM glucose.

When it comes to applications as a glucose sensor high sensitivity is needed. Our preliminary chronocamperometric results that will be the subject of our next publication clearly imply that MWNT/AuNPs-PMo$_{12}$ material is attractive as a glucose sensor. The linear response range of current to glucose concentration was observed up to more than 17 mM with sensitivity of 20.2 $\mu$A mM$^{-1}$ cm$^{-2}$.

3. Conclusions

We demonstrated here a MWNT/AuNPs-PMo$_{12}$ electrode comprising inorganic (AuNPs) active centers, exhibiting high catalytic activity towards glucose oxidation. The PMo$_{12}$ modifying metal nanoparticles prevent their agglomeration, keep the particle structure and do not affect the catalytic ability towards glucose electrooxidation. Preparation of AuNPs-PMo$_{12}$, directly on multiwalled carbon nanotubes stabilizes the active metal surface area. The highly porous network of three-dimensionally distributed nanotubes with gold centers is formed. As voltammetric results indicated, the glucose electrooxidation on the MWNT/GOx system is ineffective since the active center of the enzyme is deeply hidden in the protein shell hindering efficient electron
The introduction of hydroquinone (HQ) to the system as a mediator results in the increase of the glucose oxidation currents. The mediator, however, can leak from the layer and undergo other reactions. Therefore the proposed inorganic electrode MWNT/AuNPs-PMo$_{12}$/GC seems to be an interesting alternative to the discussed enzyme-based catalysts. The oxidation currents are much higher and the process starts at lower potentials. Moreover, the inorganic system MWNT/AuNPs-PMo$_{12}$/GC is more stable in time. Further research is underway in our laboratory to fabricate other effective electrodes containing metal nanoparticles (for example bimetallic nanoparticles) for biofuel cells applications.

References


