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Novel Electrochemical Sensors for Safety and Control in Fermentation Processes

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We report on the development of enzyme-free glucose electrochemical sensors for safety and control application in fermentation industry, manufactured by printing Au/TiO_2 composites onto gold based planar electrodes. The electrocatalytic materials were prepared in two steps: firstly, colloidal solutions of Au nanoparticles (NPs) ranging from 2 to 40 nm, were synthesized by reduction of an aqueous solution of $HAuCl_4$; secondly, the Au NPs were deposited on the surface of TiO_2 by chemical wet impregnation. The use of different reducing agents allowed to control the size of Au NPs, which plays an important role in their electrocatalytic behaviour. Results showed the high electrocatalytic activity of Au NP -modified gold electrodes towards glucose oxidation in alkaline solution; instead no response was given towards ethanol suggesting the possibility to use these kinds of sensors for fermentation process monitoring in industrial technology. The good sensing properties of Au NP-embedded TiO_2 composites may be ascribed to the electrocatalytic activity of Au NPs stabilized on TiO_2.

1. Introduction

Lack of reliable sensors to measure on-line variables related to concentration is one of the main issues in industrial chemical plants (Ampelli et al., 2013a). In fermentation industry, process control and safety assessment are currently assured using highly sophisticated (and expensive) analytical methods, often performed off-line and characterized by a delay not compatible with the timing of the process. Therefore, there is an urgent need to develop new industrial technologies based on simple, low-cost electrochemical sensors for on-line monitoring and safety in fermentation processes (Si et al., 2013).

Modern fermentation industry requires quick solutions for process control and monitoring as many parameters as possible. While most bioprocesses are controlled by measuring traditional parameters (pH, temperature, CO_2 and O_2 concentrations), a demanding issue is to follow on-line the concentrations of raw substrates and fermentation products. The direct monitoring of chemical species, which could be tracers in the process, may strongly improve the bioprocess performances. Several techniques have been employed in this direction, but a number of difficulties are still encountered in their implementation (Yeung et al., 1999).

Among the various entities to be monitored during fermentation, glucose is the most one of interest. The determination of glucose level is an important prerequisite of fermentation process control and the easiest and cheapest way to monitor it is represented by the use of electrochemical biosensors (Karube, 1984). Previous works reported the possibility of using enzymatic biosensors for monitoring of glucose in fermentation processes (Brooks et al., 1987). However, there are some issues related to the instability of the enzyme. The device must probe a wide range of glucose levels and monitor periods from hours to several days, local oxygen tension is unstable and inherently restricted by the low solubility of oxygen in

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the fermentation media. Moreover, all the parts of the enzyme electrode in contact with the fermentation broth must be sterile or aseptic. The development of non-enzymatic electrochemical sensors for glucose monitoring may be an effective alternative to overcome the above issues. The ability to perform the electrochemical oxidation of glucose in the absence of enzyme, has already been presented in literature. Noble metals, metal oxides, carbon based materials and nanostructured materials have shown as very promising for the realization of non-enzymatic biosensors (Toghill and Compton, 2010). Nanomaterials have demonstrated excellent performance for the electrochemical non-enzymatic sensing of glucose (Genovese et al., 2013a). The advantages of these materials arise from their unique physical, chemical, electrical properties such as high surface-to-volume ratio, high index facets, large specific surface area, good electrical conductivity and high electrocatalytic activity (Genovese et al., 2013b).

In this contribution we report the development of an enzyme-free glucose electrochemical sensor based on Au nano-particles (NPs) dispersed on titania. In order to obtain Au NPs with different dimensions, ranging from 2 to 40 nm, we started with the synthesis of colloidal solutions of Au NPs by reduction of an aqueous solution of $HAuCl_4$ performed with different reducing agents. By varying some parameters, such as the i) nature of reducing/capping agent, ii) ratio of gold ion concentration to stabilizer/reductant and iii) reaction temperature, a high grade of control on size distribution may be obtained. In a second step an appropriate volume of Au colloidal solution was contacted with TiO_2 powder (P25 Degussa), in order to have an Au loading of 0.5 wt. % by wet impregnation.

The ability of gold to oxidize directly glucose is already well known (Adzic et al., 1989). Furthermore, recent studies show that Au NPs exhibit extraordinary catalytic activity towards oxidation of glucose. Many types of metal oxide, such as SiO_2, CeO_2, MnO_2 and TiO_2 nanoparticles, have been used to construct biosensors. Among them, TiO_2 nanoparticles have attracted considerable interest due to the superior properties, such as their large specific surface area, high uniformity, and excellent biocompatibility (Passalacqua et al., 2012). TiO_2 is also suitable as an excellent and low cost substrate for the dispersion of catalytic metal particles (Ampelli et al., 2012a).

Planar electrochemical sensors were fabricated starting from gold based compact disk (G-CDs) and modifying by casting the Au/TiO_2 powder onto the working electrode surface. The electrocatalytic activities of bare gold electrode, TiO_2 and Au-TiO_2 electrodes towards glucose were studied in alkaline electrolyte. The properties of the sensors based on these electrodes, such as sensitivity, detection limit and selectivity in the glucose detection were also investigated. The final aim is to develop an electrochemical sensor for the control of industrial processes, for example in the conversion of glucose to ethanol in yeast fermentation tanks (Brooks et al., 1987). Integrated in an automation platform, the sensor allows the monitoring in real time of the concentration of glucose in the bioreactor and, through a control loop, can improve the safety of the fermentation process with cost saving and improved yields.

2. Experimental

2.1 Synthesis of the electrocatalytic materials

Au NPs-embedded TiO_2 composites were prepared by wet impregnation of TiO_2 P25 Degussa with Au NPs colloidal solutions.

In order to obtain Au NPs with different dimensions in the composites (ranging from 2 to 40 nm), the colloidal solutions were prepared according to three different procedures. In the first procedure, a freshly prepared aqueous solution of $NaBH_4$ (2 mM, 30 ml) was cooled in an ice bath. Next, a solution of $HAuCl_4 \cdot 3H_2O$ (1 mM, 10 ml) was added drop to drop to the reducing solution while stirring. The solution turned pink immediately after adding $NaBH_4$, indicating particles formation (Lee and Meisel, 1982). This solution is indicated hereinafter as AuNPs@SB. In the second procedure, a solution of $HAuCl_4 \cdot 3H_2O$ (1 mM, 90 ml) was heated at 100°C for 15 min in an oil bath, then a solution of sodium citrate (38.8 mM, 10 ml) was added. The mixture was maintained under reflux condition for 30 min. The oil bath was removed and stirring was continued for an additional 15 min (Grabar et al., 1995). This solution is indicated hereinafter as AuNPs@SC. In the third procedure, Au NPs were stabilized by Polyvinylpyrrolidone (PVP). $HAuCl_4 \cdot 3H_2O$ (16 mM, 25 ml) was added to 106 mg of PVP aqueous solution (300 ml) with stirring for 30 min. An aqueous solution of $NaBH_4$ (100 mM, 5 ml) was rapidly added and the mixture was stirred for 3 hours (Yogi et al., 2011). This solution is indicated hereinafter as AuNPs@PVP.

In order to characterize the dimension of Au NPs, the thus obtained aqueous colloidal solutions were characterized by UV-visible absorption spectroscopy using a JASCO V570 UV-VIS-NIR spectrophotometer.

In a second step of the preparation, an appropriate volume of the Au NPs colloidal solutions was contacted with TiO_2 powder (P25 Degussa), in order to have an Au loading of 0.5 wt. % by wet impregnation at 90 °C

(Ampelli et al., 2013b). With reference to the colloidal solution used in the preparation, Au NPs-embedded TiO_2 composites are indicated hereinafter as AuNPs@SB-TiO_2, AuNPs@SC-TiO_2 and AuNPs@PVP-TiO_2.

2.2 Sensors fabrication and electrochemical tests

The electrochemical sensors were manufactured starting from commercial G-CDs by removing the protective layers present and exposing the thin reflective layer of gold. The support was worked by means prototyping system for micromachining Protomat S103 (LPKF). The excess of gold was then removed in order to obtain the desired geometry for the conductive tracks of the electrochemical sensor prototype. Realized sensors were constituted by a plastic support (35 mm x 10 mm) with three-electrode configuration. The size of the working electrode surface was 7 mm^2, while the counter and the reference electrodes had a surface of 14.7 mm^2 and 4.5 mm^2, respectively. The working electrode surface was modified by printing a paste of synthesized materials dispersed in ethanol. In order to ensure the adhesion to the gold electrode, Nafion (5% wt) was added during the paste preparation.

All the electrochemical experiments were performed with a home-made potentiostat (Leonardi et al., 2012; Leonardi et al., 2013). Cyclic voltammetry (CV) and amperometric measurements were carried out in order to evaluate the performance of the realized sensors. CV was performed at 100 mV/s scan rate, in the potential range ±0.8 V. All the tests were carried out at room temperature in 0.1 M KOH solution. For the amperometric measurements, the working electrode was maintained at a appropriate constant potential while a precise volume of glucose solution was injected in 0.1 M KOH solution by means of micro syringe. During the operation, the solution was kept under stirring to ensure the homogeneity of the concentration of the analyte.

3. Results

3.1 Characterization

Figure 1 shows the absorption spectra of three different size of Au NPs in the colloidal solutions. Gold NP solutions show a clearly visible plasmon absorption peak at 511 nm for AuNPs@SB, 520 nm for AuNPs@SC and 530 nm for AuNPs@PVP, attributed to a diameter of NPs of 2 nm, 5 nm and 35 nm, respectively. A high grade of control on dimension and size distribution is obtained by changing the nature of reducing and capping agent, the ratio of gold ion concentration to stabilizer/reductant and the reaction temperature, as evidenced by the plasmon red-shift from 511 to 530 nm.

Once deposited on TiO_2 supports, Au NPs maintained the average diameter as confirmed by Transmission Electron Microscope (TEM) analysis.

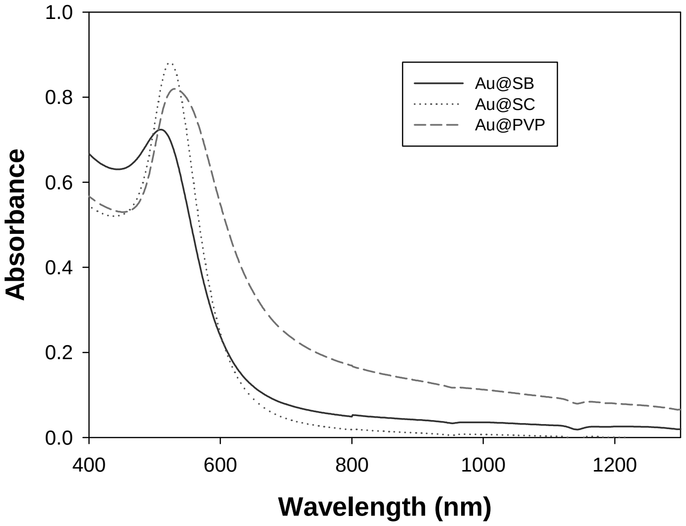


Figure 1: UV-visible absorption spectra for three different size of Au NPs in the colloidal solutions (AuNPs@SB, AuNPs@SC and AuNPs@PVP).

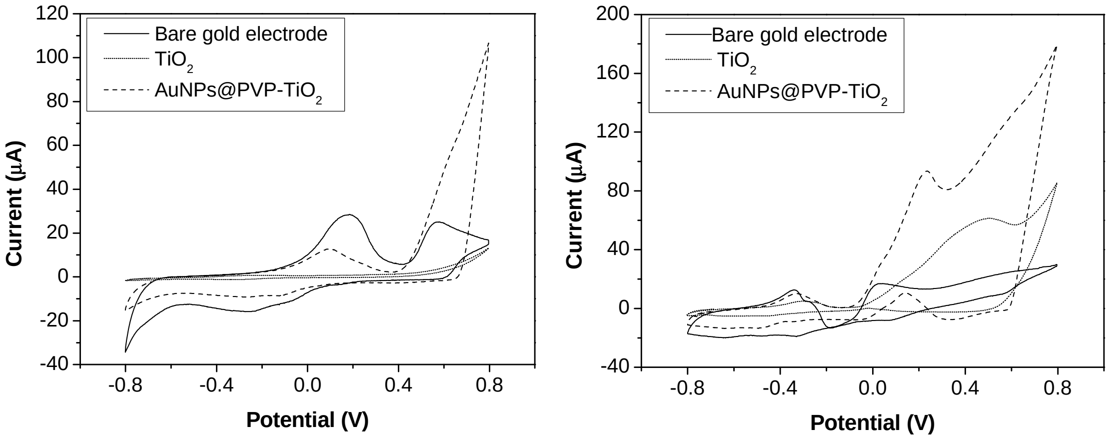
3.2 Electrocatalytic oxidation of glucose

Cyclic voltammetry (CV) was employed to study the electrochemical behaviour of bare gold electrode, TiO_2 and Au-TiO_2 modified electrodes. In this preliminary study the AuNPs@PVP-TiO_2 was investigated. Figure 2a shows CV responses for all the samples (potential range ±0.8 V, scan rate 100 mV/s) in 0.1 M KOH. Bare gold electrode cycled in alkaline solution during positive scan, showed two anodic peaks at 0.17 and 0.6 V due to the formation of a monolayer of adsorbed hydroxyl species OH^- and surface oxide (Au_2O_3) formation. During reverse scan two slight cathodic peaks were observed at negative potentials due to the dissolution of Au_2O_3 and hydroxyl group reduction (Burke, 2004). Similar behaviours were observed for Au/TiO_2 NPs modified electrodes. However, a lower intensity of the oxidation and reduction peaks was observed compared to the bare gold electrode, due to the low loading of Au NPs on titania. Instead, no electrochemical reaction was observed for pure TiO_2 modified electrode in the potential range investigated.

Figure 2b shows CV analyses carried out in presence of 1 mM glucose in 0.1 M KOH electrolyte solution. Very low electrocatalitical activity towards glucose was shown by bare gold electrode. Two weak oxidation processes were indeed observed around -0.3 V and 0 V. Pure TiO_2 modified electrode showed a large oxidation peak at the potential of 0.5 V. The weak oxidation peak observed at the potential of -0.3 V may be attributed to the presence of exposed basal gold electrode. Regarding the sample containing Au NPs, CV profile shows multiple oxidation peaks. These processes are commonly attributed to the formation of intermediate products and their subsequent oxidation (Pasta et al., 2010). In the specific, Au/TiO_2 samples showed a clear increase in the intensity of the oxidation peak, triggered at the potential of -0.11 V with maximum to 0.2 V, observed during the anodic scan. The lower anodic potential and higher intensity of this peak compared to pure TiO_2, is explained considering the high electroctalytic activity of Au NPs (Ben Aoun, 2013).

For practical purposes, where it is required ease of use, the electrochemical sensors should work by maintaining a constant potential and monitoring, as the only parameter, the current related to the oxidation or reduction process of the analyte species. Amperometric tests were carried out for all the fabricated sensors in alkaline solution. Figure 3 shows the current response vs. time recorded after successive additions of glucose in the concentration range of 0.03 – 1.7 mM, while working electrodes potential were maintained at 0.2 V. The low working potential allowed to avoid the interference from other electroactive species that might be oxidized at higher potential.

The sensor with AuNPs@PVP-TiO_2 modified electrode showed a fast response (7- 10 s) upon the addition of glucose in solution, suggesting a rapid electron transfer process to the electrodes. Furthermore, an excellent signal to noise ratio ensures the possibility to observe very small current variations.



(a) (b)

Figure 2: Cyclic voltammetry profiles for all the samples a) without glucose and b) with 1 nM glucose (potential range ±0.8 V, scan rate 100 mV/s, 0.1 M KOH).

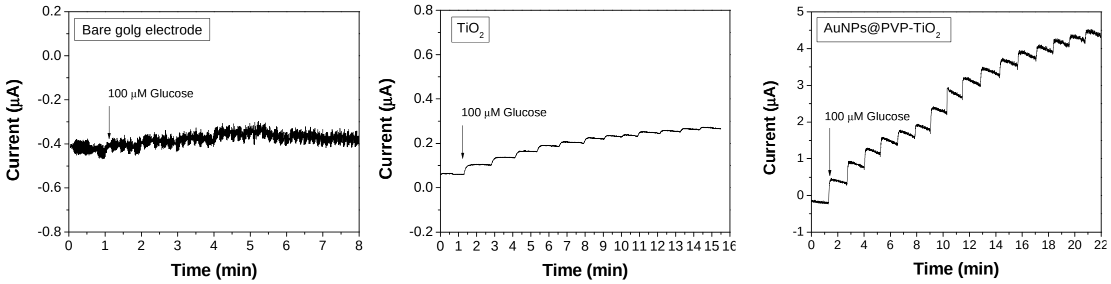


Figure 3: Current response vs. time after successive additions of glucose in the concentration range of 0.03 – 1.7 mM (0.2 V).

On the contrary, the bare gold electrode and TiO_2 modified electrode, operating at the same potential, showed a low increase of current. Moreover, after two successive additions of glucose, greater than 1 mM, no further response was observed suggesting a possible phenomenon of self-poisoning of the working electrode.

Figure 4 shows the calibration curves extrapolated from the above cronoamperometry results. In according to what already observed during the voltammetric analysis, the sample AuNPs@PVP-TiO_2 evidenced the highest sensitivity with a lower detection limit estimated lower than 0.03 mM.

In order to assess the actual possibility of using the sensor for monitoring fermentation processes, the possible interfering effect of ethanol (commonly present in these processes in combination with glucose) was also verified and no responses were observed for successive addictions of ethanol.

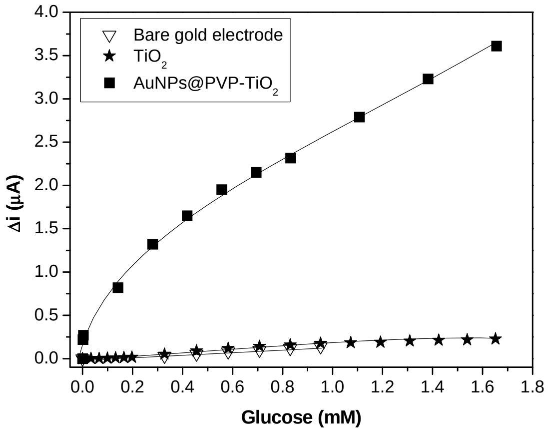


Figure 4: Calibration curves for the investigated electrodes.

4. Conclusions

Enzyme-free electrochemical sensors were fabricated by starting from gold based compact disks as planar electrodes and casting on them Au/TiO_2 composites as electrocatalytic materials. The electrodes are low costly, easily manufacturable and show excellent electrocatalytic properties towards the oxidation of glucose, without any interference due to the presence of ethanol.

The electrocatalytic activity of Au TiO_2 may be ascribed to the large surface area of Au NPs on the TiO_2 surface (Ampelli et al., 2011; Ampelli et al, 2012b). Furthermore, titania stabilizes Au NPs and new catalytic sites are formed at the interface between Au and TiO_2. This joint effect significantly enhances the electrocatalytic activity towards the selective oxidation of glucose, suggesting the use of the fabricated sensor for monitoring industrial fermentation processes.

Work is in progress to evaluate the effect of different preparation methods, crystallite size and crystalline habit of Au NPs on the sensing characteristics. Moreover, will be deposited Au NPs on different kinds of titania, as nano-structured TiO_2 arrays prepared by anodic oxidation (Ampelli et al., 2009) to further improve the electrocatalytic responses of our sensors.

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