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## Accepted Manuscript

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**Effective electrochemical sensor based on screen-printed electrodes modified with a carbon black - Au nanoparticles composite**

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**Abstract**

A screen-printed electrode (SPE) modified with a carbon black (CB) - Au nanoparticles (AuNPs) composite is assembled and tested. Electrochemical and morphological investigations highlight the physico-chemical properties of the resulting AuNP-CB-SPE amperometric device with respect to SPEs modified with a single component of the nanocomposite. The effective performance of such a modified electrode in activating electrocatalytic processes, consisting both in oxidation and reduction reactions, is demonstrated. In particular, electrochemical tests on analytes such as glucose, hydrogen peroxide, hydroquinone, and ascorbic acid, evidence that the composite possesses electrocatalytic performance well superior with respect to the relevant mono-component modified SPE. As a consequence, a meaningful lowering of the peak potentials and improvement of the sensor sensitivities is observed when using AuNP-CB-SPEs with respect to both CB-SPEs and AuNP-SPEs. In the case of H<sub>2</sub>O<sub>2</sub> reduction, the occurrence of the electrochemical process at less negative potentials is coupled to an improvement of sensor sensitivity of about one order of magnitude. Concurrently, lower limit of detections, ranging from 20 to 99 % less, have been obtained for the major part of the analytes studied, i.e. glucose, hydrogen peroxide and

hydroquinone. Preliminary results reported here indicate that AuNP-CB-SPE can be proposed as an efficient amperometric sensor to be used in many analytical applications.

**Keywords:** screen-printed electrodes; carbon black; gold nanoparticles; nanocomposite; amperometric sensors; electrocatalysis.

## 1. Introduction

Screen-printed electrodes (SPEs) are acknowledged to be very effective devices for the development of disposable sensors to be used in electroanalysis. They are highly-versatile, easy to use, cost-effective analytical tools, also suitable to miniaturization. In order to improve their electrochemical performance, SPEs have been coated with Au, Bi, and Hg films [1-3]. The recent development of nanosized materials has opened to the possibility to take advantage of their peculiar properties arising from the formation of nanostructured surfaces [4]. The particular reactivity of atoms located in correspondence to edge-plane defects of the nanostructure, i.e., suffering from incomplete coordination sphere, may lead to i) less extreme potentials required for the electrode process to occur, thanks to higher reversibility degree of the charge transfer (electrocatalysis); ii) increase of the relevant currents, in part due to the same effect on the charge transfer and, in part, to the more effective diffusion regime that operates at a nanostructured surface [5]. By taking advantage of these characteristics, SPEs modified with carbon nanotubes [6], Ag [7] or Au nanoparticles (AuNPs) [8] have demonstrated to achieve an electroanalytical performance well superior to those of bare SPEs, in terms of activation of electrocatalytic processes, improvement of sensitivity and lower limit of detection.

Recently, we have reported the advantages arising from the use of SPEs modified by carbon black (CB) [9-12], namely CB-SPE: electrode coatings obtained from a dispersion of commercially available CB N220 show marked electrocatalytic properties with respect to oxidation of several species of analytical interest. The advantages of CB modified electrodes with respect to bare electrodes in the development of very efficient amperometric sensors have been extensively demonstrated by us and by other authors [10-16]. Furthermore, the CB modified electrodes have shown competitive electrochemical performance even when compared with electrode modified with carbon nanotubes [12,13,15].

CB coatings were also proved to be efficient in combination with metallic NPs. In particular, CuNPs-CB composite shows marked electrocatalytic activity for detection of aminoacids [15], whereas AuNPs-CB coatings are suitable for As(III) detection by stripping analysis [17]. In both cases, CB allows the homogenous distribution of NPs on the electrode surface.

In particular, AuNPs represent interesting nanosized materials capable to effectively activate electrocatalytical processes [4]. The main problem associated to their use in electroanalysis lies in the strong interaction among metal cores that induces aggregation phenomena, occurring both in solution and on the electrode surface. We could recently verify that AuNPs encapsulated by  $\text{Cl}^-$  ions, deriving from  $\text{HAuCl}_4$  reduction, are well stable in solution [18,19]. They can be homogeneously deposited on polymer-based nanostructured surfaces, preserving at best their individuality and, as a consequence, their electrocatalytic activity [18]. The presence of  $\text{Cl}^-$  ions allows the metal cores to be closely approached by species in solution.

In the present work, we highlight, for the first time, the electrocatalytic effectiveness of AuNP-CB modified SPE (AuNP-CB-SPE) in electrochemical processes involving different electroactive species, namely glucose, hydrogen peroxide, hydroquinone (HQ), and ascorbic acid (AA).

A relatively large number of species was investigated, in order to perform a preliminary overview of the potentiality of the bi-component system in comparison with SPEs modified with one of the individual components, i.e., either with CB (CB-SPE) or AuNPs (AuNP-SPE). Due to

the goal of this study, despite the lower sensitivity with respect to pulse techniques, cyclic voltammetry (CV) was the technique chosen. CV, in fact, is also capable to clearly evidence different aspects of the electrode process, such as the reversibility degree of the charge transfer and the stability of the product formed.

Morphological characterisations have been also performed, in order to give reason of the best performance found in the case of AuNP-CB-SPE.

## 2. Material and Methods

Electrochemical tests were performed using an Autolab PGSTAT-30 electrochemical system (Eco Chemie, Utrecht, The Netherlands).

SPE was produced with a 245 DEK (Weymouth, UK) screen-printing machine. The electrochemical cell was printed on flexible polyester foils (Autostat HT5) obtained from Autotype Italia (Milan, Italy). 48 SPEs for each foil were produced. Graphite based ink (Electrodag 423 SS) from Acheson (Milan, Italy) was used to obtain the working and counter electrodes. The working electrode finally possesses an electroactive area of  $3.6 \text{ mm}^2$ . Silver ink (Electrodag 477 SS) was used to print the pseudo-reference electrode. An Ag/AgCl, 3 M KCl, reference electrode was used when working in alkaline media, due to the low stability of Ag pseudo-reference electrode in these conditions.

All reagents were of analytical grade and supplied by Sigma-Aldrich; all solutions were obtained with ultrapure water ( $18 \text{ M}\Omega\text{cm}$  resistance). Commercial CB N220 (19-25 nm diameter,  $124 \text{ m}^2/\text{g}$  surface area) was obtained from Cabot Corporation (Ravenna, Italy). AuNPs were synthesized by slightly modifying the procedure previously reported [19]; they were characterised

by recording TEM images (JEOL 2010, equipped with energy filter) and UV-Vis spectra (Perkin Elmer Lambda 650 spectrophotometer).

CB-SPEs were prepared accordingly to previous papers [11,12], by modifying the bare working electrode of a SPE with three subsequent drops (2  $\mu\text{L}$  each) of 1 mg/mL CB dispersion in a 1:1 DMF:H<sub>2</sub>O mixed solvent. In these conditions we could verify [11] that the electrode surface was largely coated by CB and the nanosized material is stably anchored at the electrode surface. AuNP-CB-SPEs were prepared by dropping 2 to 14  $\mu\text{L}$  of AuNPs solution onto the CB-SPE. AuNP-SPEs were prepared by depositing three drops (2  $\mu\text{L}$  each) of AuNPs solution onto a bare SPE. In all cases, the solvent was evaporated at room temperature. All SPEs modified by AuNPs coatings underwent five subsequent CV runs from 0.0 to +1.5 V, 0.1  $\text{Vs}^{-1}$  potential scan rate, in 0.1 M H<sub>2</sub>SO<sub>4</sub>, before use; a similar treatment was found useless for CB-SPE.

The electroactive area of all SPEs was determined by voltammetric tests recorded in the same 1 mM [Fe(CN)<sub>6</sub>]<sup>3-</sup>, 0.1 M phosphate buffer solution (PBS); a bare GC electrode possessing a previously computed electrochemical area was used as the reference from which to calculate the electrochemical area of the differently modified SPEs. Similarly, the Au surface area of AuNP-SPE and AuNP-CB-SPE exposed to the solution was determined by CV curves recorded in a 0.1 M H<sub>2</sub>SO<sub>4</sub>, by considering a bare Au electrode with known electrochemical area as the reference; in particular, the charge spent in the peak of the backward trace, due to the reduction of surface Au oxides during the scan toward positive values, is considered [20].

The electrocatalytic efficiency of AuNPs in AuNP-CB coatings was preliminarily tested in 1 mM glucose, 0.1 M KOH solution. The performance of all modified SPEs for their possible use them as sensors for the determination of several analytes at mM level was tested in 0.1 M PBS, pH 7.2. Four similarly modified SPEs were used under the same experimental conditions, aiming at comparing the relevant responses by t-Student significance tests, at a confidence limit of 5%.

The morphology of the working electrode surfaces was investigated by a Nova NanoSEM 450 scanning electron microscope (SEM, FEI Company) working in high vacuum conditions, equipped with an Energy Dispersive Spectrometer (EDS, Bruker QUANTAX-200).

### 3. Results and discussion

#### 3.1 Electrochemical and morphological characterisation of AuNP-CB-SPE

AuNPs encapsulated by  $\text{Cl}^-$  ions were obtained by using  $\text{HAuCl}_4$  as the precursor, reduced at room temperature by  $\text{NaBH}_4$ . UV-Vis spectra of the resulting AuNPs show a plasmon band always centred at 514 nm wavelength, indicating the good reproducibility of this synthetic procedure. TEM images reveal that the major part of NPs possesses a core diameter ranging from 2 to 7 nm. An exemplificative TEM image of the  $\text{Cl}^-$  encapsulated AuNPs, together with the relevant size distribution, has been reported in the Supporting Information.

Different volumes of AuNPs solution were deposited onto various CB-SPEs to define the conditions for most effective modification of SPEs with the AuNP-CB nanocomposite. Not surprisingly, the electrochemical area ascribed to AuNPs according to the method described in the Experimental section, increases at increasing the amount of AuNPs deposited. A limiting value is reached by addition of 10  $\mu\text{l}$  of AuNPs solution. However, the best reproducibility, as ascertained on four AuNP-CB-SPEs exhibiting a relative standard deviation of 9%, was found using 6  $\mu\text{l}$  of AuNPs solution. This volume was, hence, selected to assemble the AuNP-CB-SPEs. As observed in Fig. 1A, the voltammetric peaks relative to Au oxides formation and reduction indicate that the mean electroactive Au surface area results significantly higher than that obtained by directly depositing similar volume of AuNPs solution onto bare SPE. The relevant values, calculated on the



basis of four electrodes obtained by following the same preparation procedure, are reported in Table 1. Furthermore, repeated CV traces collected in H<sub>2</sub>SO<sub>4</sub> aqueous solution demonstrate that AuNPs are more stably anchored on CB modified SPEs than that on bare SPEs.

CV responses have been recorded on the [Fe(CN)<sub>6</sub>]<sup>3-</sup>/[Fe(CN)<sub>6</sub>]<sup>4-</sup> typically reversible system, using the differently modified SPEs (Fig. 1B). In all cases, a linear trend between the forward cathodic peak intensity and the square root of the potential scan rate in the 0.02 to 1.00 V/s interval was observed (see inset of Fig. 1B), indicating the occurrence of a diffusion-controlled electrode process. All the modified SPEs lead to voltammetric traces exhibiting negligible iR drops (see Table 1), at variance with bare SPE, that exhibits a peak-to-peak separation of ca. 450 mV, due to an inner resistance of the electrode system [21]. From the slopes of  $i_p$  vs.  $\sqrt{v}$  plots, repeated on four modified SPEs for each kind of electrode coating, it was possible to estimate that, in any case, the deposition of nanosized materials on the electrode surface results in a significant enhancement of the total electroactive area with respect to the bare SPE. Furthermore, we can conclude that no meaningful difference is evident between the computed total electroactive area of CB-SPE and of AuNP-CB-SPE (see Table 1), whereas the electrochemical area of AuNP-SPE results smaller.

As a support to these electrochemical conclusions, SEM images collected on various regions of AuNPs-CB-SPE and CB-SPE (see Fig. 2A and 2B, respectively) evidence that the morphology of the two electrode surfaces is, actually, quite similar. Repeated EDS spectra acquired in fairly wide regions of the coating evidence that AuNPs are homogeneously distributed on the AuNP-CB electrode surface. Furthermore, EDS spectra collected in very small portions of the coating testify that the small white spots present in Figs. 2A and 2C actually consist of Au deposits (see inset of Fig. 2A). It is thus well evident, from a comparison between these two images, that the AuNPs deposited on CB coatings are less prone to aggregation or coalescence phenomena; this aspect may indicate that, in principle, they better preserve the electrocatalytic activity.

### 3.2 Electroanalytical applications of AuNP-CB-SPE

The performance of AuNP-CB-SPE, CB-SPE, and AuNP-SPE has been tested and compared with respect to the determination of the listed analytes in 0.1 M PBS. In Table 1 the values of the significant parameters calculated on the CV responses, at equal concentration of each analyte, are reported. In Table 2 the values of slope and intercept of relevant calibration straight lines are given, together with the relevant limits of detection (LOD).

The electrocatalytic efficiency of AuNPs fixed on the CB matrix has been preliminarily tested by using glucose in strongly alkaline media, under Ar atmosphere: a characteristic voltammetric response [22] is reported in Fig. 3A. No oxidation peaks are evidenced on CB-SPE, confirming the key role played by AuNPs in the glucose oxidation mechanism. The response on AuNP-CB-SPE results repeatable over more than 20 subsequent potential scans, indicating that AuNPs preserve their electrocatalytic efficiency even when deposited on CB and that they do not suffer from fouling phenomena.

Noteworthy, when considering the possible application of AuNP-CB-SPE for the determination of glucose in real samples, an electrolytic medium at neutral pH values represents an environment closer to the actual ones. In addition, untreated samples even require the presence of oxygen. For these reasons, tests were performed in PBS (pH 7.2), from which oxygen was not removed. The voltammetric curves recorded, limitedly to the forward scan, are reported in Fig. 3B. Complete voltammetric traces collected at the different modified SPEs have been reported in the Supporting Information. The shape of these traces is very similar to that recorded in the alkaline solution, but the sensitivity results quite lower. Such a behaviour was expected on the basis of the mechanism of glucose oxidation at Au surfaces [23,24], which does require the presence of hydroxides. The sensitivity of sensors based on chemically synthesised AuNPs is generally so low in PBS, that, to the best of our knowledge, only few papers report that glucose oxidation is possible at neutral pH [25-27]. On the other hand, it is important to evidence that the choice to perform measurements in

PBS allows the extension of the limit of linearity up to 30 mM, i.e., more than one order of magnitude higher with respect to that observed when using strong alkaline medium.

In order to compare the performance of such a composite modified electrode with respect to that only consisting of one component, similar experiments have been also carried out at AuNP-SPE. In view of the lower Au surface calculated (see Table 1), it is not surprising that the sensitivity results lower in this last case (see Table 2). However, when the values are normalised with respect to the relevant electroactive Au areas, no meaningful difference between the two electrodes can be inferred from the resulting current density. We can conclude that the role of CB, in this case, lies in inducing an increase of the Au surface active for electrocatalytic interaction with glucose in solution. Nevertheless, a slight decrease of the LOD was observed in the case of AuNP-CB-SPE.

Different results are obtained in the case of  $\text{H}_2\text{O}_2$  reduction. This is a further electrochemical process that shed light to the essential role played by AuNPs in activating electrocatalytic processes [18]. Fig. 4A reports the CV traces obtained with the AuNP-CB-SPE in the absence and in presence of 1.0 mM  $\text{H}_2\text{O}_2$ , respectively; as observed, the electrochemical reduction of this analyte starts occurring at a not particularly low cathodic potential, i.e., at ca. -0.20 V. Fig. 4B reports a comparison between the behaviour of the three modified electrodes; for the sake of clarity, only forward traces, subtracted by the relevant background signal, are reported in this case. A marked shift of this cathodic process to less negative potentials is evident in the case of voltammetric traces collected at the AuNP-CB-SPE. Moreover, when comparing calibration plots calculated at -0.3 V (see Fig. 4B, inset, and the relevant parameters in Table 2) in the concentration range from 0.5 to 4.0 mM, it is well evident that the superior performance of this electrode with respect to AuNP-SPE cannot be merely ascribed to an increase of Au electroactive area. Concurrently, the values of LOD are strongly conditioned by the use of the AuNP-CB composite material. A synergic action between these two components of the material in the electrocatalytic mechanisms involved has to be invoked. These data are in agreement with the literature, in which a synergetic effect of CuNPs and CB for amino acid detection is reported [15].

Synergic action between nanomaterials is a phenomenon already reported in the literature [28-30]. It is generically ascribed to an increment of the number of edge-plane defects at the electrode|solution interface, occurring in coatings based on a composite material with respect to the mono-component ones. On the other hand, it is plausible that the combination of two materials at a nanometric level affects their energy levels, finally resulting in superior electrocatalytic performance to ascribe to the individual components. Unfortunately, investigations aimed at elucidating electro-co-catalytic mechanisms are quite hard. The complete definition of the mechanisms involved in this charge transfer process is also complicated by the fact that the formation of a composite material can also affect the morphology of the coating, inducing different diffusion regimes [5].

Finally, we could also verify that the presence of  $O_2$  in the solution does not affect the performance of the sensor in  $H_2O_2$  detection: very similar voltammetric traces have been obtained either in absence or in presence of  $O_2$  in the solution. This aspect is of utmost importance for the application of the sensor, since de-oxygenation of an  $H_2O_2$  solution constitutes a problematic issue.

The electrocatalytic performance of AuNP-CB-SPE, compared with those of CB-SPE and of AuNP-SPE, has been also tested with respect to electroactive species undergoing non-reversible oxidation, namely HQ and AA. Similarly to glucose and  $H_2O_2$ , it is well known that the relevant electrode processes show drawbacks at a bare SPE, as well as at conventional metal electrodes, such as marked overvoltage and dramatic surface fouling. Nevertheless, the quantitative determination of the two species is of great analytical interest in many applicative areas.

As to HQ oxidation, meaningful differences between voltammetric curves collected at the three modified SPEs are well evident (see Fig. 5A and Table 1). Very interesting results are obtained when comparing voltammetric curves recorded at AuNP-CB-SPE and CB-SPE, i.e., electrode systems possessing similar electroactive area and similar inner resistance: the higher electrocatalytic efficiency of the composite material leads to a significant shift of the voltammetric responses to less positive potentials and with a concomitant increase of the relevant current values. The calibration

plots (see inset of Fig.5A and Table 2), calculated in the 0.1-1.0 mM concentration range, testify the particularly high sensitivity of AuNP-CB-SPE with respect to CB-SPE and to AuNP-SPE, not merely ascribable to the difference observed in the dimension of electroactive surfaces of the three electrodes. The improvement of sensor sensitivity also results in a slight decrease of the LOD.

A quite interesting test used to prove the effectiveness of coatings based on AuNP-carbon composite consists in the simultaneous determination of HQ and catechol (CT) present in the same solution [31-33]. As a quite interesting effect of the peak shift observed at AuNP-CB-SPE, it was observed that the signals relative to HQ and CT oxidation can be only resolved when using such a bi-component modified electrode (see Fig. 5B). As observed from the inset of this same figure, HQ oxidation is not affected by the contemporary presence of CT in the same solution. This indicates that the concurrent determination of the two species is possible when using AuNP-CB-SPE.

Even clearer evidence of the performance of AuNP-CB coatings in oxidation processes, with respect to the individual mono-component ones, is obtained when considering AA. As is well evident from Fig. 6A, the electrocatalytic properties of AuNP-CB-SPE result in an oxidation peak significantly higher and located at less anodic potential values. The relevant values of sensitivity (Fig. 6A, inset and Table 2), calculated in the 0.17-3.0 mM concentration range, again demonstrate that the superior performance of this modifier is not merely related to the value of the electroactive area. However, the higher values of standard deviation of both slope and intercept of the calibration equation, most probably associated to the higher number of steps required to deposit the electrode coating, induce a slight increment of the LOD value.

The marked potential shift observed for AA oxidation at AuNP-CB-SPE results in well resolved voltammetric peaks for dopamine (DP) and AA oxidation (Fig. 6B), a further electrochemical process that can put in light the effective electrocatalytic properties of AuNP-carbon nanocomposites [34-37]. The inset of this same figure demonstrates that the oxidation of AA is unaffected by the contemporary presence of DP. Even more important in view of a possible application of the sensor, determination of very low amounts of DP is possible in the presence of

fairly high AA concentrations, which constitutes the situation encountered in biological matrices. Due to the peculiar conditions explored in this case, the determination of such a low DP concentration is only possible by using a pulsed voltammetric technique, in order to improve the sensitivity.

The results obtained when considering the oxidation processes of HQ and of AA further evidence the excellent performance of AuNP-CB electrode coatings. Once more, the results indicate an effective synergic action of the two nanosized materials in the electrocatalytic mechanisms involved in the electron transfer processes in charge of these species.

As a further remark, the increase the electroactive area obtained when using nanostructured surfaces is often associated to an increase of the noise present in the signal, i.e., of the standard deviation of both slope and intercept of the calibration equation and, as a consequence, of the relevant LOD. However, when considering the performance of the AuNP-CB-SPE with respect to AuNP-SPE, the higher sensitivity values observed are, in most cases, associated to a lower uncertainty of the regression parameters. It is not easy to give a rationale to this finding; however it constitutes a further advantage of the bicomponent electrode system.

The storage stability of AuNP-CB-SPE was finally tested, because it represents one of the key characteristics of the relevant sensor systems. We could verify that the electrodes can be used up to one month after the deposition of AuNPs, if stored in air and at room temperature. Due to the partial oxidation of Au surface, an electrochemical pre-treatment in 0.1 M H<sub>2</sub>SO<sub>4</sub> before the use is necessary, in order to restore the active electrode surface (see Experimental section for further details). Furthermore, we could ascertain that AuNP-CB-SPEs can be continuously used over an entire working day.

#### 4. Conclusions

SPEs modified by a coating consisting of CB and AuNPs have been developed. The synergic action of the two nanosized materials forming a composite film results in an electrocatalytic efficiency much higher than that exhibited by SPEs modified either with CB or with AuNPs. The effective application of this composite in the frame of amperometric sensors has been demonstrated for some anodic and cathodic processes of different complexity.

In particular, the occurrence of well effective electrocatalytic processes at AuNP-CB coated electrodes allows the quantification of the considered analytes at less extreme potential values. This property leads to a better resolution between voltammetric peaks deriving from different electroactive species present in the same solution. As demonstrated in the case of HQ and CT, as well as of AA and DP, the simultaneous determination of multiple analytes is possible at this SPE.

For similar reasons, values of sensitivities well superior to those predictable on the basis of a mere increase of the surface area are observed at the bicomponent sensor system. Since this property is not associated to a concomitant increase of the noise present in the signal, it also results in lower LOD values observed in many of the cases studied in the present article.

Excellent repeatability of subsequent voltammetric traces demonstrates that poisoning phenomena, most often affecting bare electrode surfaces, are avoided when using AuNP-CB modified electrodes.

All these properties, combined with quite interesting values of both life time and storage stability, indicate that AuNP-CB-SPEs are suitable for possible application in many analytical frames.

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**Figure Captions**

**Figure 1** – Steady state potential scans registered in A) 0.1 M H<sub>2</sub>SO<sub>4</sub> ( $v = 0.1 \text{ Vs}^{-1}$ ) and B) 1 mM [Fe(CN)<sub>6</sub>]<sup>3-</sup>, 0.1 M PBS ( $v = 0.05 \text{ Vs}^{-1}$ ) at AuNP-CB-SPE (blue solid line), CB-SPE (red dotted line) and AuNP-SPE (green dash-dotted line).

**Figure 2** - SEM images of A) AuNP-CB-SPE, B) CB-SPE and C) AuNP-SPE obtained from secondary electrons. Two exemplificative EDS spectra has been reported in the insets of A.

**Figure 3** - CV traces registered at AuNP-CB-SPE A) in absence (dotted line) and in presence (solid line) of 1.0 mM glucose, 0.1 M KOH; B) different concentrations of glucose in 0.1 M PBS (for the sake of clarity, only the forward potential scan traces are reported in the figure). Inset of B reports the trend of  $i_p$  at *ca.* 0.075V, subtracted for the relevant background signal, of AuNP-CB-SPE and AuNP-SPE.

**Figure 4** - A) CV traces of AuNP-CB-SPE in the absence (dotted line) and in the presence (solid line) of 1 mM H<sub>2</sub>O<sub>2</sub>; B) relevant forward trace subtracted by the blank signal for AuNP-CB-SPE (blue solid line) compared with similar responses obtained at CB-SPE (red dashed line) and AuNP-SPE (green dash-dotted line). 0.1 M PBS (pH 7.2), 0.05 Vs<sup>-1</sup> potential scan rate. Inset of B reports the  $i$  vs. [H<sub>2</sub>O<sub>2</sub>] plots obtained with the different electrodes by sampling the current at -0.3 V.

**Figure 5** - Steady state CV curves registered at AuNP-CB-SPE (blue solid line), CB-SPE (red dashed line) and AuNP-SPE (green dash-dotted line) in 1 mM HQ, 0.1 M PBS solution, A) in the absence and B) in the presence of 1 mM CT. The calibration plots for HQ oxidation obtained at the three different electrodes are reported in the inset of A. Inset of B reports CV traces obtained at

AuNP-CB-SPE in 1 mM HQ in the absence (solid line) and in the presence of 0.10 (dashed line) and 0.28 mM (dotted line) CT.

**Figure 6** - Steady state CV curves registered at AuNP-CB-SPE (blue solid line), CB-SPE (red dashed line) and AuNP-SPE (green dash-dotted line) in 1 mM AA, 0.1 M PBS solution A) in the absence and B) in the presence of 0.1 mM DP. The relevant calibration plots are reported in the inset of A. Inset in B reports DPV curves recorded at AuNP-CB-SPE in 1 mM AA in the absence (solid line) and in the presence (dashed line) of 2  $\mu\text{M}$ ; pulse potential = 10 mV,  $\Delta E = 4$  mV, pulse time = 0.15 s, 0.01  $\text{Vs}^{-1}$  potential scan rate.

### Table captions

**Table 1.** Electrochemical parameters calculated from CV traces collected with modified SPEs in the presence of different electroactive species. Confidence intervals for  $n=4$  and  $\alpha=0.05$ .

**Table 2.** Values of slope and intercept of regression curves calculated with modified SPEs in the presence of different electroactive species. The relevant CV curves have been collected in 0.1 M PBS at 0.05  $\text{Vs}^{-1}$  potential scan rate.

**Table 1** - Electrochemical parameters calculated from CV traces collected with modified SPEs in the presence of different electroactive species. Confidence intervals for  $n=4$  and  $\alpha=0.05$ .

	CB-SPE			AuNP-SPE			AuNP-CB-SPE		
Au surface area ( $\text{mm}^2$ )*	-			3.8 ± 0.8			5.4 ± 0.7		
Total electroactive surface area ( $\text{mm}^2$ )**	9.3 ± 1.2			6.7 ± 0.9			9.5 ± 0.6		
	$\Delta E$ (V)	$E_p$ (V)	$i_p$ ( $\mu\text{A}$ )	$\Delta E$ (V)	$E_p$ (V)	$i_p$ ( $\mu\text{A}$ )	$\Delta E$ (V)	$E_p$ (V)	$i_p$ ( $\mu\text{A}$ )
1 mM $[\text{Fe}(\text{CN})_6]^{3-}$	0.088±0.018	0.061±0.024	12.3±1.6	0.089±0.013	0.063±0.006	8.8±1.2	0.079±0.021	0.078±0.014	12.4±0.8
10 mM Glucose	-	-	-	-	0.124±0.025	0.42±0.08	-	0.095±0.010	0.34±0.09
1 mM Hydroquinone	0.084±0.007	0.065±0.010	27.5±5.2	0.120±0.014	0.083±0.014	14.0±5.3	0.063±0.012	0.045±0.013	43.3±7.2
1 mM Ascorbic Acid	-	0.056±0.023	5.9±0.5	-	0.380±0.045	4.1±0.4	-	-0.064±0.034	8.0±0.7

\*Calculated on the basis of CV curves in 0.1 M  $\text{H}_2\text{SO}_4$ , 0.1 V/s potential scan rate.

\*\* Calculated on the basis of CV curves in 1 mM  $[\text{Fe}(\text{CN})_6]^{3-}$ , 0.1 M PBS, 0.05 V/s potential scan rate.

**Table 2** - Values of slope (m) and intercept (q) of regression curves calculated with modified SPEs in the presence of different electroactive species; the relevant standard deviation (s) is also reported inside brackets. LOD are calculated from the calibration plots by considering  $y_{\min} = q + 3s_q$ . The relevant CV curves have been collected in 0.1 M PBS at 0.05 Vs<sup>-1</sup> potential scan rate.

	CB-SPE			AuNP-SPE			AuNP-CB-SPE		
	$m / \mu\text{AmM}^{-1}$	$q / \mu\text{A}$	$LOD (mM)$	$m / \mu\text{AmM}^{-1}$	$q / \mu\text{A}$	$LOD (mM)$	$m / \mu\text{AmM}^{-1}$	$q / \mu\text{A}$	$LOD (mM)$
Glucose	-	-	-	0.036 (0.001)	0.004 (0.012)	1.00	0.052 (0.001)	-0.015 (0.015)	0.87
H <sub>2</sub> O <sub>2</sub>	0.87 (0.08)	3.81 (0.19)	660	0.25 (0.12)	1.97 (0.27)	3.24	5.47 (0.14)	1.02 (0.33)	0.18
Hydroquinone	22.13 (0.19)	0.37 (0.11)	0.015	11.29 (0.43)	0.56 (0.24)	0.064	33.01 (0.24)	0.38 (0.13)	0.012
Ascorbic acid	7.15 (0.02)	0.05 (0.03)	0.013	4.86 (0.04)	0.22 (0.07)	0.043	8.69 (0.03)	0.61 (0.06)	0.021



## Biographies

Fabiana Arduini is researcher in analytical chemistry at the Chemical and Technology Department of the University of Rome “Tor Vergata”. She graduated “cum laude” in chemistry (2004) and obtained her Ph.D. degree in chemical science (2007). Her research interests include the development of electrochemical sensors and biosensors and their application in the field of clinical, food and environmental analytical chemistry. She was involved in several National and European projects. She is author of more than 30 papers in international and national scientific journals and books and of more than 50 poster and oral presentations at National and International Congresses.

Chiara Zanardi is researcher in Analytical Chemistry at the University of Modena and Reggio Emilia. Her research interests are in the field of electroanalysis, particularly dealing with the development of new materials as electrode coatings possessing electrocatalytic and antifouling properties; these materials found application in the formation of sensors and biosensors for the quantification of meaningful analytes in food and environmental matrices. She is author of 1 book dealing with modified electrodes in electroanalysis, 2 book chapters, more than 50 papers printed on international journals, and 1 patent application.

Fabio Terzi is researcher in Analytical Chemistry at the University of Modena and Reggio Emilia. His research activities are focused on synthesis and characterisation of nanomaterials, modification of electrode surfaces using conducting polymers, self assembled monolayers and nanomaterials, spectroscopic and microscopic characterisation of the electrode coatings and development and testing of amperometric sensors based on the systems realised. Co-author of 1 book dealing with modified electrodes in electroanalysis, 2 book chapters, and more than 40 articles in international peer-reviewed journals.

Danila Moscone is full professor in analytical chemistry at the Chemical and Technology Department of the University of Rome “Tor Vergata”. She has been involved in the field of biosensors for about 30 years, and she is an expert in electrochemical biosensor and immunosensors assembling, their analytical evaluation and application for solving analytical problems. She is actually involved in European projects and is responsible for National projects. Her scientific activity is summarized in more than 150 papers on international and national scientific journals and books, and in more than 300 oral and poster presentations at scientific meetings.

Giuseppe Palleschi, full professor of analytical chemistry, has been the Head of the Department of Chemical Science and Technology of the University of Rome “Tor Vergata” for 1995–2007. In 2000 he obtained the “Laurea Honoris Causa” from the University of Bucharest. Prof. Palleschi’s research over the last 30 years has been focused on the development of chemical sensors as well as bio- and immunosensors for use in the areas of biomedicine, food and environmental analysis. He is the author of more than 200 papers in international scientific journals and an invited speaker in many International Congresses.

Renato Seeber is Full Professor of Analytical Chemistry at the University of Modena and Reggio Emilia. His actual research interests are in the field of modification of electrodes with intrinsically and redox conducting polymers, metal nanoparticles, graphene, and relevant composites. They are applied for the detection of specific analytes or as elements of electronic tongues, in the frame of blind analysis. The analytical application has always been coupled to an as deep as possible electrochemical, spectroscopic, and microscopic characterization of the electrode system. He is co-author of 1 book dealing with modified electrodes in electroanalysis, 5 book chapters, more than 200 papers printed on international journals, and 1 patent application.

Figure 1

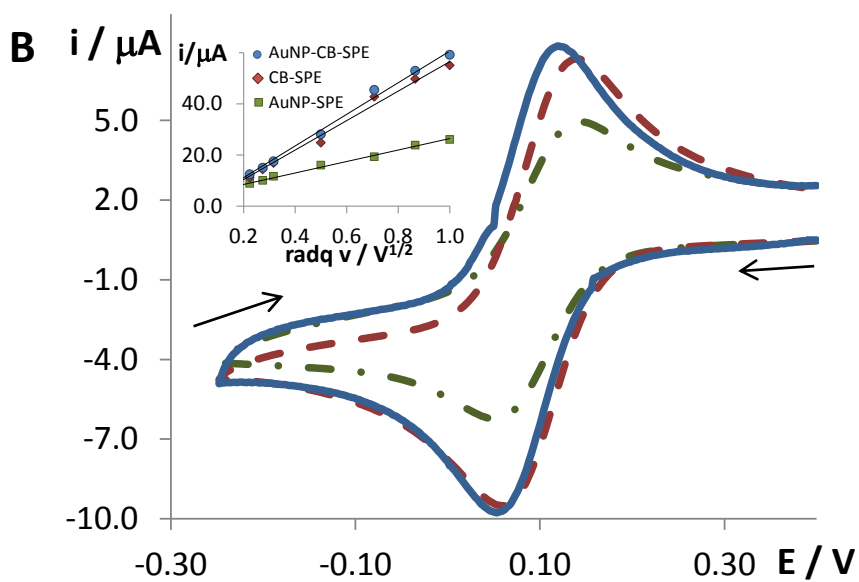
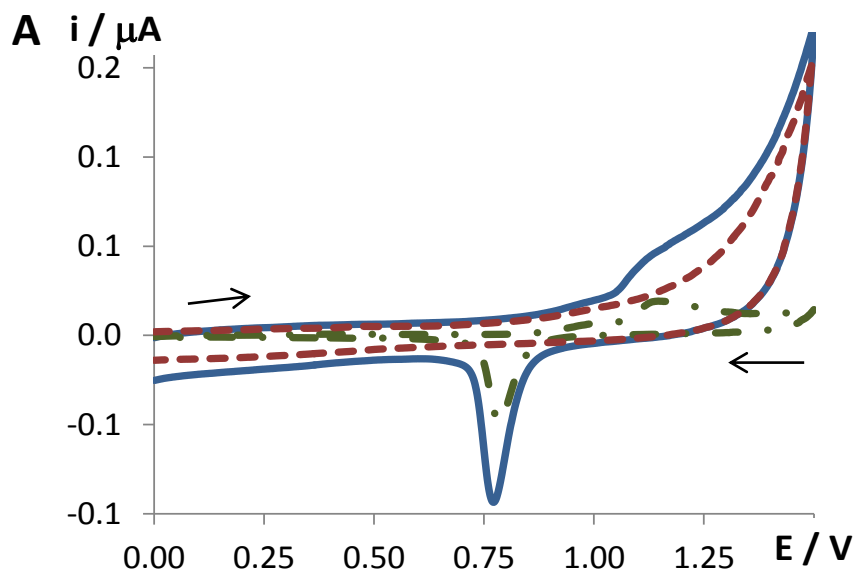


Figure 2

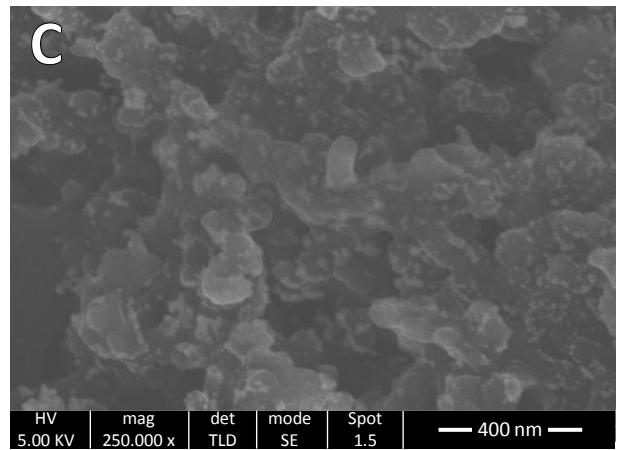
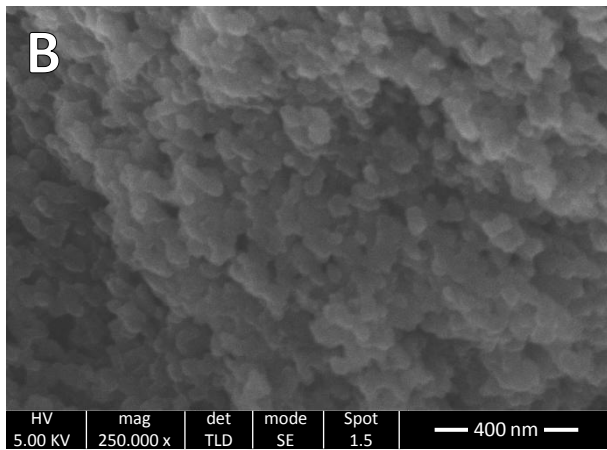
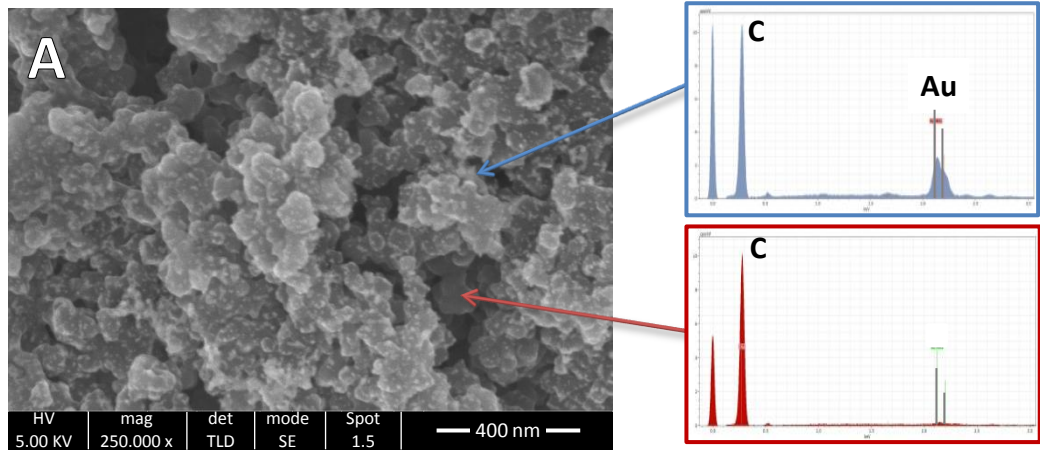


Figure 3

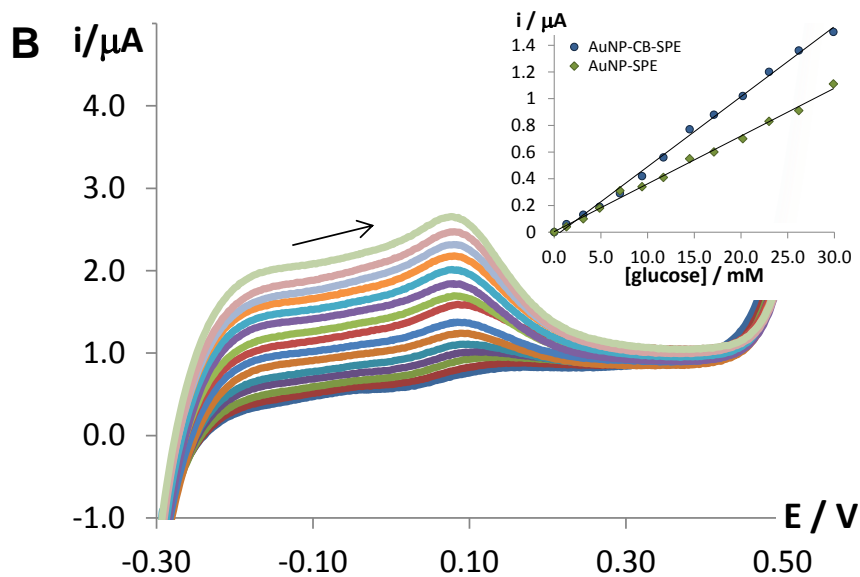
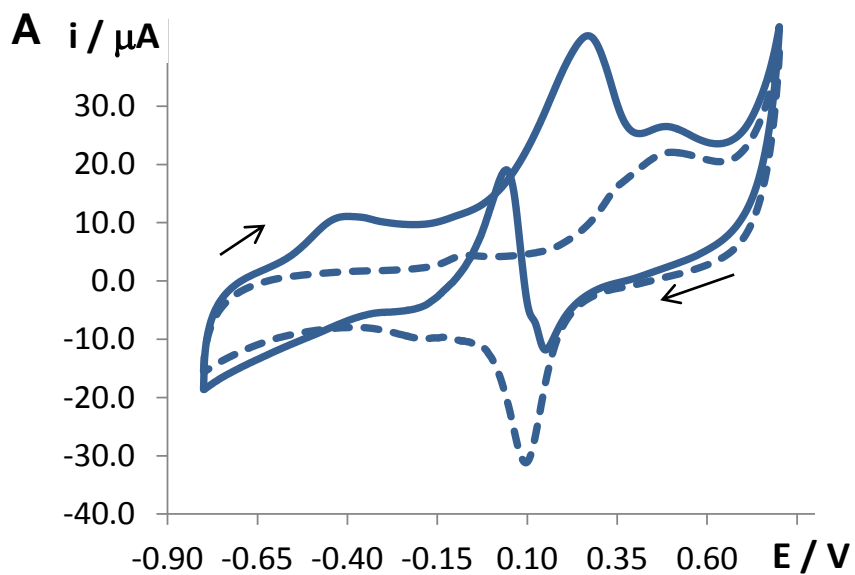


Figure 4

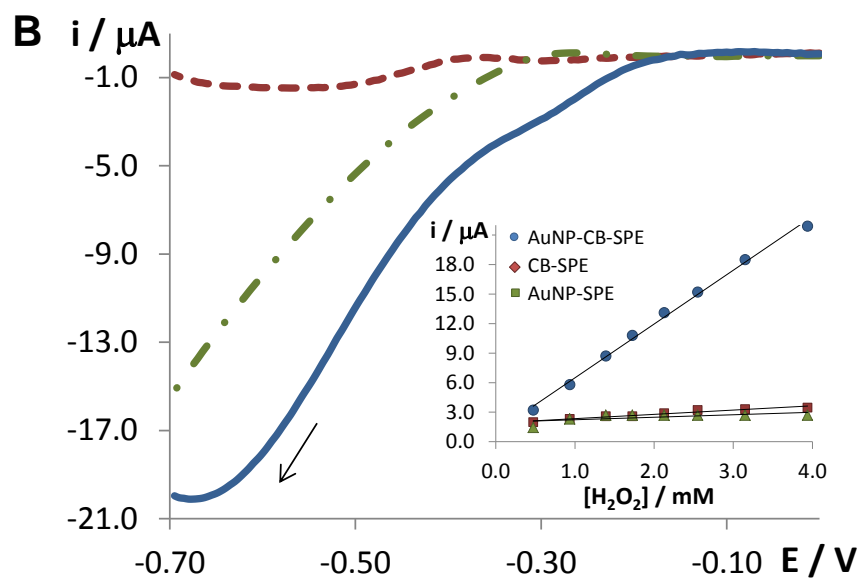
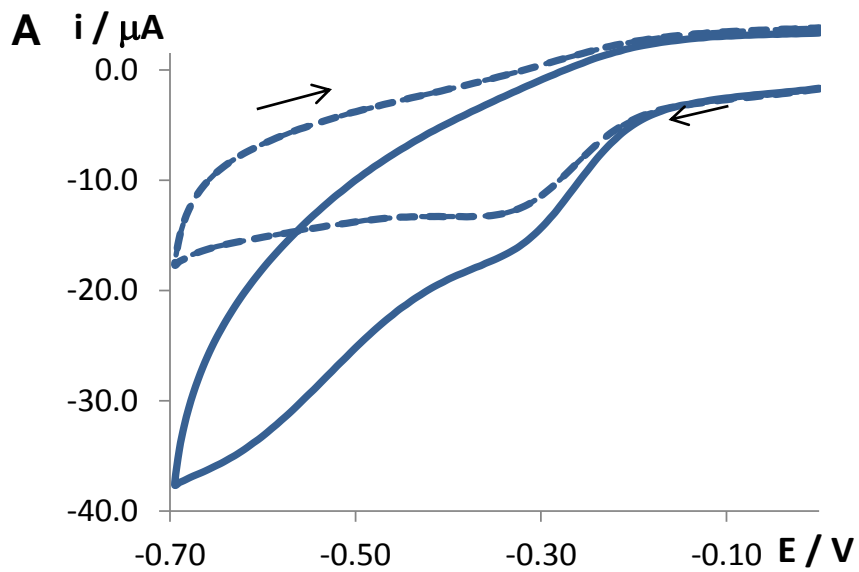


Figure 5

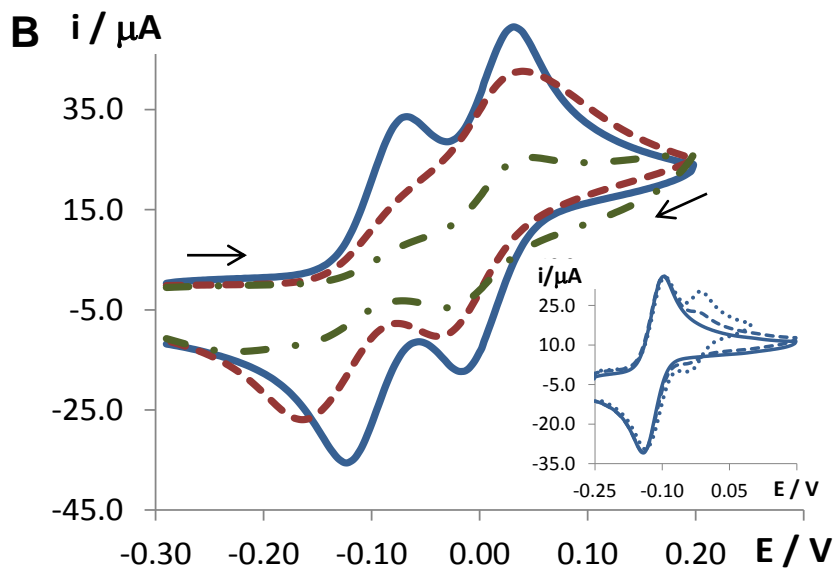
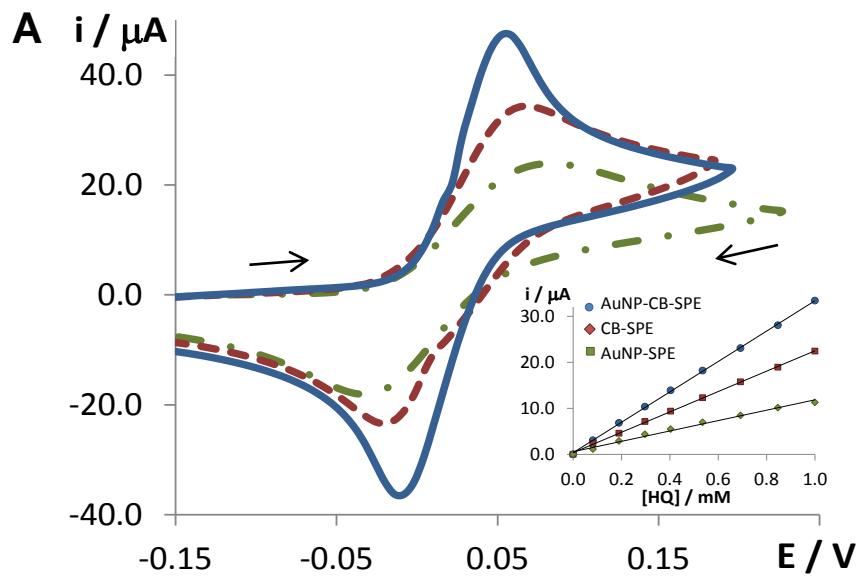


Figure 6

