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Examination of performance of glassy carbon paste electrode modified with gold nanoparticle and xanthine oxidase for xanthine and hypoxanthine detection

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Abstract

A composite electrode was prepared by modifying glassy carbon microparticles with gold nanoparticles (Au-nps) and xanthine oxidase enzyme (XOD) for xanthine (X) and hypoxanthine (Hx) detection. After the optimization of the system for X, the biosensor was characterized for X and Hx. A linearity was obtained in the concentration range between 5.00×10^{-7} and 1.00×10^{-5} M for X with equation of $y = 0.24x + 0.712$ and 5.00×10^{-6} to 1.50×10^{-4} M for Hx, with equation of $y = 0.014x + 0.575$, respectively. Obtained results were compared to X and/or Hx biosensors including/not including Au-np in the structure. The developed system was also applied for detection of Hx in canned tuna fish sample and very promising results were obtained.

Keywords: Gold nanoparticle; GCPE; Xanthine; Hypoxanthine; Biosensor

1. Introduction

The development of a sensor for xanthine (X) and hypoxanthine (Hx) is of medical and biological importance [1,2]. On the other hand, the levels of these compounds are generally used in the food industry as an index for evaluating meat or fish freshness [3]. Methods like chromatography and spectrophotometry were utilized for the detection of X and Hx [4,5]. However, since these methods are costly and laborious, alternative systems like amperometric biosensors have been developed for this particular application. In these systems, the level of X or Hx is monitored electrochemically by following H_2O_2 and/or uric acid oxidation or O_2 consumption produced by the XOD-catalyzed reaction depending on the charge of applied potential [6].

$$\text{Hypoxanthine} + O_2 \xrightarrow{\text{XOD}} \text{Xanthine} + H_2O_2$$

$$\text{Xanthine} + O_2 \xrightarrow{\text{XOD}} \text{Uric acid} + H_2O_2$$

Recent developments about attractive properties of nanoparticles increase interest about their electrochemical behavior and applicability of these materials in biosensing areas [7]. Gold nanoparticle (Au-np) is a kind of nanomaterial where it can help proteins to retain their biological activity upon adsorption [8,9]. Its large surface area and good electronic properties [10] and its utilization for the study of direct electron transfer of redox proteins [10–12] increase their usage in biosensor construction [12–14].

Pingarron and co-workers [6] have used Au-nps for various kinds of sensors and biosensors. They prepared glucose oxidase (GO_x) biosensors by immobilizing GO_x onto different tailored Au-nps modified electrode surfaces [15]. In another work they obtained colloidal gold–carbon paste electrodes (CPEs) by using CPEs modified with cysteamine SAMs [16]. Recently, a xanthine oxidase (XOD) biosensor, based on a carbon paste electrode (CPE) modified with electrodeposited Au-nps, for the amperometric determination of hypoxanthine (Hx) is reported by the same group [6].

Another novel material that was used for X and Hx biosensor construction is glassy carbon microparticles. Combination

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of this material with mineral oil results with the formation of glassy carbon paste electrode (GCPE). It has been demonstrated that this electrode has better electrochemical reactivity towards the oxidation of hydrogen peroxide compared to conventional CPE [17]. In our previous work, GCPE was modified with xanthine oxidase enzyme (XOD) to obtain X and Hx biosensor and very promising results with low detection limit were obtained [18].

In the present work, for the first time GCPE was modified with Au nanoparticles and XOD for obtaining proper composite structure that can be used as transducer for X and Hx biosensor. After the optimization of the system with X, the biosensor was applied for Hx detection in canned tuna fish. The performance of the biosensor was also compared with other X and Hx biosensors.

2. Experimental

2.1. Apparatus

Chronoamperometric experiments were carried out with the AUTOLAB PGSTAT 12 electrochemical measurement system from ECO CHEMIE Instruments B.V. (The Netherlands) driven by GPES software. The experiments were conducted in a voltammetric cell (Metrohm), at room temperature (25 °C), using a three-electrode configuration. A platinum electrode was served as an auxiliary electrode and an Ag/AgCl as a reference electrode (Metrohm). Electrodes were inserted into the cell through its Teflon cover.

2.2. Reagents and materials

Xanthine oxidase from buttermilk (XO, 0.06 units/mg solid) was obtained from Sigma. Glassy carbon spherical powder (20–50 μM) was purchased from Alfa Aesar, while mineral oil was obtained from Aldrich and gold colloid from Sigma (0.75 A_{520} units/mL; 10 nm ~0.001% as $HAuCl_4$). Phosphate buffer (50.0 mM, pH 7.5) was served as supporting electrolyte. One millimolar Hx and X solutions were prepared freshly by dissolving the appropriate amount of Hx with 2 M NaOH and diluting to the mark with 50.0 mM phosphate buffer solution of pH 7.5 while stock X solutions were prepared by dilution of proper amount of this reagent with working buffer solution.

2.3. Electrode preparation

XO-based GCPE was prepared by hand mixing of 80:20 (% w/w) glassy carbon spherical powder/mineral oil with 0.3 mg XOD and 2 μL Au-np. A portion of the resulting paste was then packed firmly into the electrode cavity (3.0 mm diameter and 5.0 mm depth) of a PTFE sieve where electrical contact was established via a copper wire. Resulting electrode was put into desiccator for about 15 min for allowing the dryness of the surface. Then electrode surface was covered by a pre-soaked dialysis membrane by means of rubber o-ring in order to prevent leakage of the paste into the solution.

2.4. Procedure

Prior to its use, the surface of the composite electrode was smoothed with a weighing paper. The three electrodes were immersed into the 10 mL electrochemical cell. Chronoamperometric measurements were carried out in 50.0 mM phosphate buffer (pH 7.5) medium under the operating potential of +700 mV while the solution was stirred. The duration of each analysis was 125 s and the transient current decayed to a steady state value after 50 s in the presence of supporting electrolyte. Cyclic voltammetric experiments were conducted with same supporting electrolyte between −500 and +1250 mV; at 50 mV/s with a frequency of 50 Hz, amplitude of 20 mV and potential step of 20 mV. The electrode surface was renewed before the beginning of every measurement.

2.5. Sample application

Canned tuna fish sample (Dardanel) was purchased from a local market. The sample was divided into two parts. One part was immediately used while the other part was allowed to stand at room temperature for 15 days.

Canned tuna fish was chopped and homogenized until a fine paste was obtained after addition of 5 mL 0.5 M $HClO_4$. Obtained denatured samples were mechanically stirred for 10 min and then centrifuged at 4000 rpm for 5 min. The pH of the supernatant was adjusted to 7.5 with concentrated NaOH and diluted 10 times before applying standard addition method for analytical assays.

Amperometric measurements in stirred solutions were performed after transferring the corresponding analytical solution to the electrochemical cell with a potential of +700 mV versus Ag/AgCl. Determination of Hx involved two to three successive additions of samples and denatured samples which included known amount of Hx (80 μM), respectively.

3. Results and discussion

3.1. The effect of Au-np on electrochemical response of nanocomposite biosensor

Au-np has received so much attention in the field of biosensors due to its physico-chemical character [19,20]. Au-np can be defined as good biocompatible material since modification of electrode surfaces with this particle provides a suitable microenvironment similar to that of redox protein. Hence, it gives the protein molecules more freedom in orientation [21].

In order to examine its electro catalytic behavior, two biosensors; one with Au-np and other without Au-np were prepared and used for X detection. Fig. 1 displays the cyclic voltammograms at 50 mV/s of biosensors (A) without Au-np and (B) with Au-np for (a) background and (b) 10 μM X solution. Both electrodes display a defined electrochemical response upon addition of X. However, as can clearly bee seen from the voltammograms, the enzymatic reactions at the XOD–Au-np–GCPE electrode begins at a substantially lower potentials (around 0.5 V), compared to XOD–GCPE (0.75 V) counterpart. Besides, the XOD–Au-

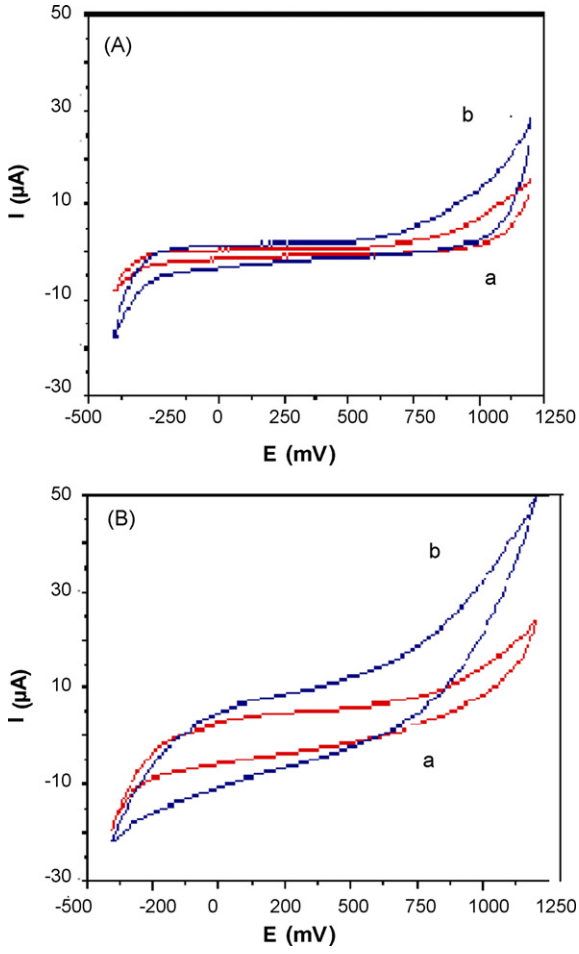

Fig. 1. Cyclic voltammograms recorded for composite biosensors (A) without Au-np and (B) with Au-np for (a) background and (b) 10 μM X solution. Conditions: supporting electrolyte, 50 mM phosphate buffer (pH 7.50) and scan rate 50 mV s^{-1}.

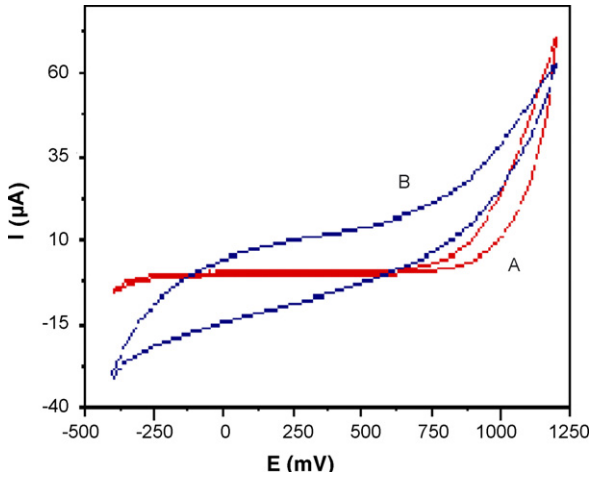

Fig. 2. Cyclic voltammograms of 10 mM H_2O_2 at (A) plain GCPE and (B) Au-np modified GCPE. Conditions: supporting electrolyte, 50 mM phosphate buffer (pH 7.50) and scan rate 50 mV s^{-1}.

np–GCPE nanocomposite yields also a substantially higher sensitivity over the entire potential range.

In Fig. 2, the response of (A) plain GCPE, (B) Au-np modified GCPE on the 10 mM H_2O_2 solution is demonstrated. As it can clearly be seen from the voltammograms, both electrodes display almost same electro catalytic behavior upon H_2O_2 solution. On the other hand, it is claimed that at lower potentials XOD enzyme reaction products could be both hydrogen peroxide and uric acid [6]. So this increase might be due to catalytic effect of Au-np to uric acid. However, it is stated that plain Au-nanoclusters have almost no catalytic activity towards uric acid [22]. Moreover to enhance the catalytic activity Au-np was used with polypyrrole film [22]. As a result, it is clear that presence of Au-np facilitates the enzymatic reaction. Au-np reduces the insulating effect of the protein shell by providing direct electron transfer through the conducting tunnels of gold nanocrystals [15]. It is claimed that the penetration of nanomeric edges of gold particles decreases the distance between the electrode and biomolecular redox sites for electron transfer [15].

3.2. The effect of applied potential

It is known that the Au-np has been used as modifier to catalyze the electrochemical reaction and direct electron transfer of some molecules like H_2O_2 [12]. In order to examine this effect optimum operating potential was searched. As shown in Fig. 3, the performance of the sensor was tested between 200 and 1000 mV with increment of 100 mV for 10 μM X solution. Although highest current value was obtained at +900 mV, +700 mV was chosen as optimum potential since 77% of maximum current was attained at this potential.

Substances like ascorbic acid (AA) may present together with X and Hx in real samples and can be electrochemically oxidized at this potential value. In order to observe this effect, developed system's chronoamperometric responses at +700 mV to AA were monitored. As a result, up to 500 μM no chronoamperometric signal was obtained for AA. For this reason, it can be concluded that with developed nanobiocomposite electrode and under these working conditions, AA does not interfere to our measurements.

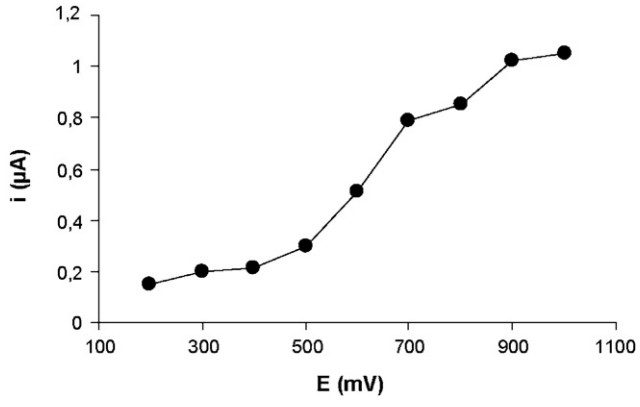

Fig. 3. Effect of working potential (200–1000 mV) on the electrode response in phosphate buffer, 50 mM at pH 7.0, for 10 μM X with 5 μL Au-np.

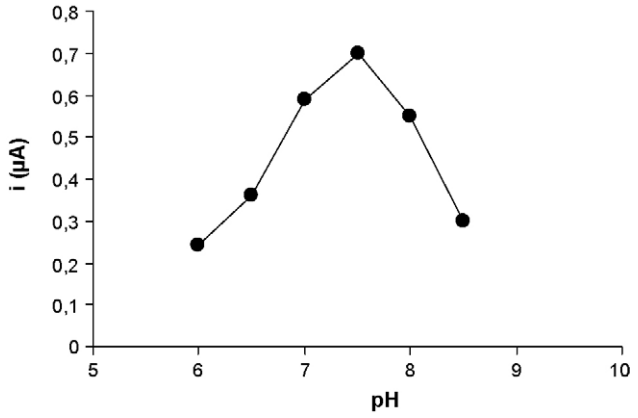

Fig. 4. Effect of pH (pH 6.0–8.5) on the biosensor response; phosphate buffer (50 mM), +700 mV, 10 μM X, with 5 μL Au-np.

3.3. The pH effect

The effect of pH on the electrode response was investigated by using phosphate buffer systems (50.0 mM) between pH 6.5 and 8.5 with an increment of 0.5 (Fig. 4). The response current of the enzyme electrode to 10 μM X increases significantly from pH 6.5 to 7.5, and then a decrease is obtained at pH values higher than 7.5. As a result pH 7.5 was chosen as optimum pH for further studies.

3.4. The influence of Au-np amount

Fig. 5 demonstrates the effect of Au-np amounts (0.5, 1, 2, 5, 10 μL) on the current response of 10 μM X. As clearly can be seen from the figure, the current increases sharply from 1 to 2 μL and then a sharp decrease is obtained for volumes greater than 2 μL. The shape of the curve is in accordance with other biosensor studies containing Au-np modification [7]. According to Liu et al. [7] this decrease can be attributed to increment of resistance and capacitance that are caused by double layer of the electrode since the active sites of the graphite decreases due to the increase in Au-np amount.

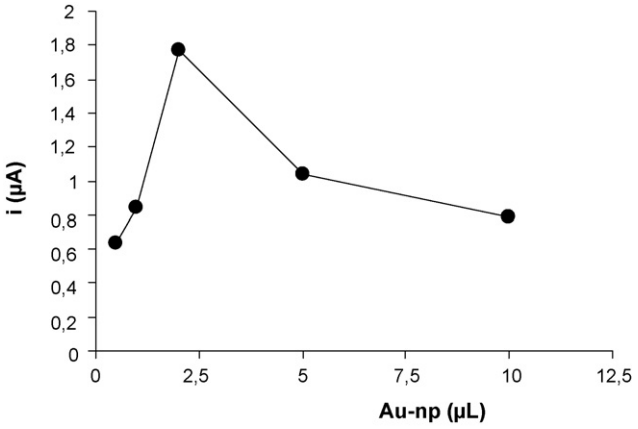

Fig. 5. The effect of Au-np amount (0.5, 1.0, 2.0, 5.0 and 10.0 μL) for 10 μM X, at pH 7.5 at +700 mV.

3.5. Surface imaging

Fig. 6 indicates the SEM images of XOD–Au-np–GCPE composite material under the optimized conditions. At (A) with 1.84 k× magnification highly packed non-porous surfaces characterized the GCPE [17] with small dots on to the spherical microparticles in the dark part. On the other hand, concerning the dimensions of the materials like glassy carbon microparticles

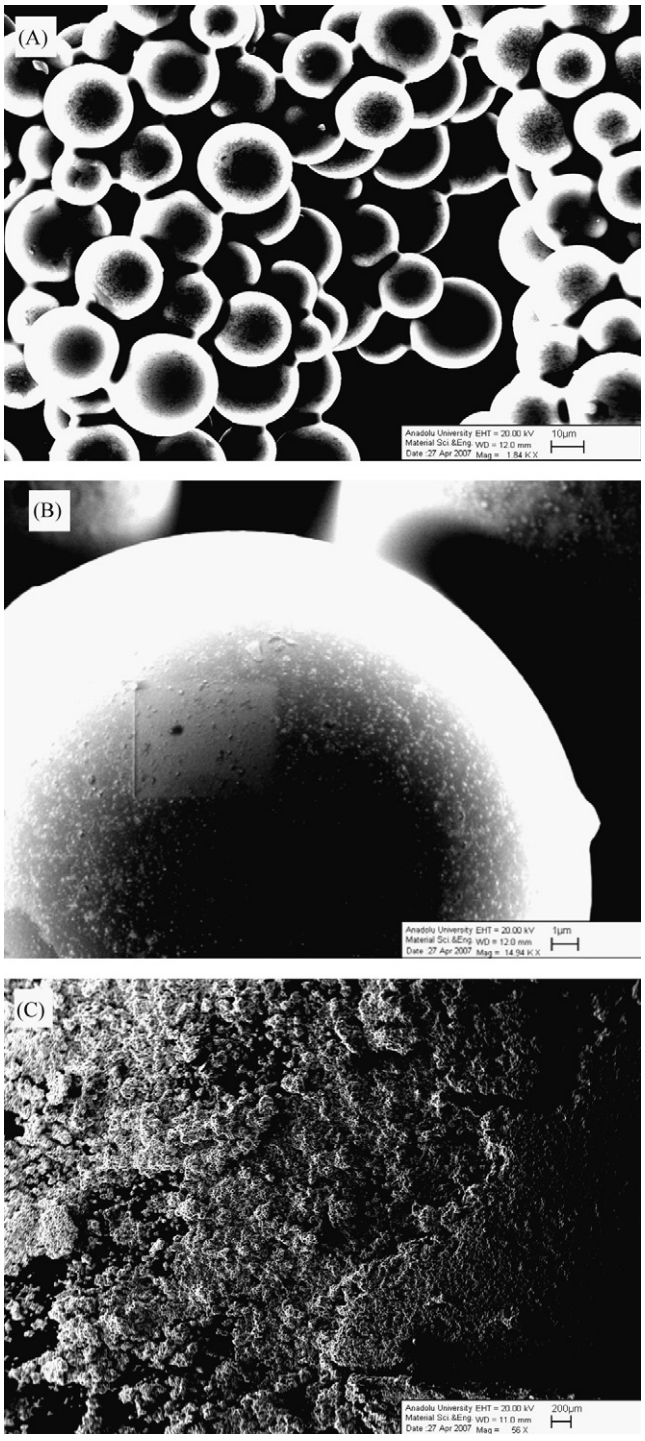

Fig. 6. The SEM images of XOD–Au-np–GCPE composite with magnification of (A) 1.84 k×, (B) 14.94 k× and (C) 36 k× accelerating voltage 20.00 kV.

20–50 μm and Au-np 10 nm, the dots at the dark part might be Au-np with XOD enzyme in it (at (B) with 14.94 k× magnification). At (C) with 36 k× magnification, a sponge-like structure of the composite can be seen.

3.6. Analytical characteristics

A linearity for the biosensor was obtained in concentration range between 5.0×10^{-7} and 1.0×10^{-5} M for X with equation of $y = 0.24x + 0.712$ and 5.0×10^{-6} to 1.50×10^{-4} M Hx, with equation of $y = 0.014x + 0.575$, respectively. R^2 values were equal to 0.99 for both reagents. For comparison, these experiments were repeated by using plain GCPE with XOD. For X, the linear range was between 2.0×10^{-6} and 1.0×10^{-5} M with equation of $y = 3.10^{-5}x + 0.0033$ while for Hx the linear range was between 20.0×10^{-6} and 80.0×10^{-6} M with an equation of $y = 0.0206x + 0.2111$. R^2 values were equal to 0.99 for X and 0.97 for Hx. As it is clearly be seen Au-np included structure possess wider linear range with better R^2 values. Also when compared with other Hx biosensors that has Au-np (modification of CPE with electrodeposited Au-np [6]) wider linear range is obtained with XOD–Au-np modified GCPE. This could be due to more efficient electrochemical oxidation of H_2O_2 and/or uric acid at this electrode potential (+700 mV). The presence of Au-np as a part of composite structure may prevent accumulation of enzymatic reaction products and hence subsequent enzyme inhibition.

The repeatability of the biosensor was tested in terms of R.S.D. values for 5 μM ($n = 5$) X and 80 μM ($n = 5$) Hx and calculated as 6.31 and 3.57%, respectively.

Storage stability was also checked and the enzyme electrode was kept at +4 °C between the measurements. Only 18% of activity was diminished after 1 week.

3.7. Sample application

Hx level in meat and marine products are important as a food quality control index. Canned tuna fish samples which are divided two parts (one part was used as a fresh sample and the other part that was labeled as decomposed sample was waited at room temperature for 15 days before its usage) were used for Hx detection. Standard addition assay was also applied to evaluate the sample matrix effect.

As already mentioned in Section 2, perchloric acid was used for deproteinisation of samples by providing denaturation of proteins. After centrifugation, clear supernatant solution was obtained. Standard HX solution was added to one part of this supernatant solution to obtain final concentration of 80 μM while other part used without any Hx standard solution inside. Both solutions were subjected to standard addition in other words, samples with and without standard HX were used as stock substrate solutions and added to the reaction cell after equilibration. Hx amount in samples was calculated from calibration curve.

The recovery values for fresh and decomposed samples were calculated as 102.5 and 106%, respectively. The samples that did not contain standard Hx solution did not give any signal. Higher recovery value for decomposed sample can be attributed to interferences of other compounds which are caused by the microbial contamination. On the other hand, the recovery of the fresh sample was close to 100%. Since the obtained signals from spiked samples were very close to the standard solutions, it can be concluded that the nature of sample does not affect the measurement.

3.8. Comparison of performance of biosensor with other Au-np contained HX biosensors

Au-np modified electrode surfaces can be prepared in different ways: (1) by binding gold nanoparticles with functional groups of self-assembled monolayers (SAMs); (2) by direct deposition of nanoparticles onto the bulk electrode surface; (3) by incorporating colloidal gold into the electrode by mixing the gold with the other components in the composite electrode matrix. Biosensors can then be constructed by immobilizing the biomolecules via adsorption onto the nanoparticles, by cross-linking them with bifunctional agents such as glutaraldehyde, or by mixing them with the other components of composite electrodes [23].

Pingarron and co-workers [6] compared the response of some of other Hx biosensors in the presence/absence of Au-np in structure and found that highest current value (87.4 nA for 5 μM Hx) was obtained with glutaraldehyde (GA)–BSA cross-linked XOD electrodeposited Au colloidal CPE. When using the same enzyme amount and under the same operating potential (+600 mV), the XOD–Au-np–GCP electrode exhibited 91 nA ($n = 3$) for same amount of Hx. The slightly higher current value can be attributed to the fact that the immobilized enzyme retained higher bioactivity into the composite electrode material, giving rise to fast, stable and sensitive responses to the concerning substrate. Being more practical and providing effective signals, makes developed biosensor a robust tool for Hx detection.

4. Conclusion

XOD–Au-np–GCPE provides easy to prepare and useful biosensor for X and Hx detection. It can simply be prepared by mixing proper amount of XOD, Au-np and GC microparticles and mineral oil. From the results, it can be concluded that especially for Hx detection, more practical and efficient biosensor has been obtained. This can be explained by the composite structure that contains GC microparticles with Au-np. Au-np catalyzes the enzymatic reaction by allowing direct electron transfer between the electrode and enzyme active center by means of the conducting tunnels of gold nanocrystals [16]. On the other hand, the combination of these two materials into a composite structure rather than usage of Au-np as an immobilizing reagent, may cause the immobilized enzyme to have higher bioactivity that results fast, stable and sensitive responses to the concerning substrate.

Congratulations

To Dr. Wang,

"Anyone who stops learning is old, whether at twenty or eighty. Anyone who keep learning stays young. The greatest thing in life is to keep your mind young. . ." Henry Ford.

Happy Birthday! I know that you will always stay young...

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