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Flow injection catalase activity measurement based on gold nanoparticles/carbon nanotubes modified glassy carbon electrode

Rasha Mohamed El Nashar[a,b,*]

[a] Faculty of Pharmacy and Biotechnology, The German University in Cairo, New Cairo City, Egypt
[b] Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt

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ABSTRACT

Amperometric flow injection method of hydrogen peroxide analysis was developed based on catalase enzyme (CAT) immobilization on a glassy carbon electrode (GC) modified with electrochemically deposited gold nanoparticles on a multiwalled carbon nanotubes/chitosan film.

The resulting biosensor was applied to detect hydrogen peroxide with a linear response range 1.0×10^{-7}–2.5×10^{-3} M with a correlation coefficient 0.998 and response time less than 10 s. The optimum conditions of film deposition such as potential applied, deposition time and pH were tested and the flow injection conditions were optimized to be: flow rate of 3 ml/min, sample volume 75 μl and saline phosphate buffer of pH 6.89.

Catalase enzyme activity was successfully determined in liver homogenate samples of rats, raised under controlled dietary plan, using a flow injection analysis system involving the developed biosensor simultaneously with spectrophotometric detection, which is the common method of enzymatic assay.

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1. Introduction

The three antioxidant enzymes, namely, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH.Px), represent critical defense mechanisms for preventing oxidative modifications of DNA, proteins and lipids by blocking the chain reactions of free radicals produced in the human body. Free radicals are also known to be responsible for many previously unexplained diseases as rheumatoid arthritis, Alzheimer's, hypertension, myocardial ischchemia, liver cells injury and carcinogenesis [1,2]. Superoxide dismutase (SOD), catalyses the conversion of $O_2^{\bullet -}$ to H_2O_2 and H_2O, while catalase (CAT) will then convert H_2O_2 to H_2O and O_2, and glutathione peroxidase (GSH.Px) reduces H_2O_2 to H_2O beside acting on ROOH (hydroperoxides).

Through the past few years, enzymatic antioxidants as SOD, Cat and GSH.PX, and non-enzymatic antioxidants as vitamin E, β carotene and ascorbic acid have grasped the attention of many biologists and physicians for their effects on the aging process besides preventing or delaying of complications of many diseases. This has created a demand of having a reliable, inexpensive, fast, easy and standardized method(s) for the assay of the antioxidant enzymes in human blood samples and tissues taking in account the wide variations in antioxidant enzyme concentration as the later is highly affected by age, gender, life style, and even dietary or environmental factors [3].

In this work, a new third generation biosensor for the assay of enzymatic activity of one of these antioxidant enzymes, namely catalase is developed and optimized. Catalase enzyme (EC 1.11.1.6), CAT, is a heme protein belonging to the class of oxido-reductases with ferriprotoporphyrin-IX at the redox center, it catalyses the disproportionation of hydrogen peroxide into oxygen and water without the formation of free radicals. Catalase is determined indirectly either by: following oxygen production in the system using Clark's oxygen electrode [4] (this method has limited applications as it is not easy to control the conditions of the system besides the ease of escaping of oxygen) or by the spectrophotometric measurement of the disappearance of hydrogen peroxide at 240 nm although this method has a relatively low sensitivity and cannot be used in turbid tissue extracts [5–8].

Recently, electrochemical measurements based on third generation biosensors involving the direct electron transfer between an enzyme and the electrode has been widely applied [9–16]. Unfortunately, the direct absorption of enzymes on the electrode surfaces leads to denaturation and loss of bioactivity of the enzyme, thus there is a need of immobilizing enzyme on active particles without loss of enzymatic activity [17,18]. The immobilization of enzyme on the surface of three dimensional structures such as gold nanoparticles or multiwalled carbon nanotubes is thought to help the protein to assume favored orientation besides offering conductive channels

* Correspondence address: Faculty of Pharmacy and Biotechnology, The German University in Cairo, New Cairo City, Egypt.
E-mail address: rasha.elnashar@guc.edu.eg

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between the electrode surface and the active sites of the enzyme thus aiding the charge transfer between the electrode and enzyme without denaturation of the enzyme [14,19].

Gold nanoparticles, are well known of being biocompatible materials being of large specific surface area, strong adsorption ability as well as good conductivity, so they strongly interact with biomaterials and on combination with carbon nanotubes, which are known to have unusual electronic, chemical and mechanical properties and were applied for many biosensors preparations, in a film on the electrode surfaces will lead to synergism between the properties of these nanoparticles [20,21]. Chitosan is found to be an excellent candidate for films incorporating nanoparticles mainly due to being a cationic macromolecule with primary amines which posed high affinity for gold and carbon nanoparticles [22]. It is also reported in literature to be an excellent film for catalase immobilization without affecting the enzyme activity [23,24].

The aim of the present work is to develop and optimize the working conditions of a new catalase biosensor based on immobilization of the enzyme on glassy carbon electrode modified with electro-chemically deposited gold nanoparticles on a multiwalled carbon nanotubes/chitosan film. The developed electrode is then integrated with a spectrophotometer in a flow injection analysis system, and applied to measure the catalase activity in liver homogenate for rats raised under controlled dietary plans.

2. Experimental

2.1. Reagents and chemicals

Bovine liver catalase (EC 1.11.1.6), Chitosan-C3646 – from shrimp shells, ≥75% (deacetylated), multiwalled carbon nanotubes MWNT's-659258 >90% carbon basis, $D \times L$(110–170 nm × 5–9 μm), chloroauric acid tetrahydrate ($HAuCl_4{\cdot}4H_2O$), percloric acid ($HClO_4$), acetic acid and 30% hydrogen peroxide were purchased from Sigma (Germany). 10 mM sodium phosphate buffer saline was used. All other reagents used are of analytical grade and Milli Q water was used for solution preparation. Solutions were de-aerated by bubbling high purity argon gas (99.90%) before measurements.

2.2. Apparatus and measurements

All cyclic voltammetry measurements were performed using a CHI 800B electrochemical analyzer (USA) with a conventional three electrode system composed of a 3 mm diameter glassy carbon electrode (GCE) as working electrode, a saturated Calomel electrode (SCE) as reference and a 0.5 mm platinum wire as auxiliary electrode (model CHI 115). A 3 cm diameter circular Teflon was used, with 3 opening (6.35 mm each) for placing the electrodes and two 2 mm openings for purging Argon gas in and out during measurements with total volume 150 μl. The test hydrogen peroxide solutions were prepared in PBS, and deoxygenated by purging argon gas for 5 min before measurements.

An FIAlab-2500 system (provided by FIA lab Instruments, USA), consisting of a four-channel peristaltic pump, six-port injection valve, and a spectrophoto-meteric Z-shaped flow cell was connected via two P200-2-UV-VIS fiber optics to a USB2000 mini-spectrophotometer. The system was controlled with FIA lab software, which was used to select the wavelength and to record the absorbance readings of the samples and the electrochemical Teflon flow cell was kept under argon atmosphere throughout all the measurements. A schematic diagram for the FIA setup is given in Fig. 1. Phosphate buffer was used as carrier on doing the optimization of the working conditions of the electrochemical measurements, while on measuring liver homogenate samples, a solution that is10 μM H_2O_2 prepared in PBS was used as carrier.

All the measurements were carried out at controlled room temperature of 25 °C as the activity of the enzyme is highly affected by temperature and it is almost denaturated at temperatures' higher than 37 °C [25].

2.3. Catalase activity assay

Aebi's method was applied for measurement of catalase enzyme activity for the enzyme before immobilization on the Chitosan/MWNT's/Au nanoparticles modified GC electrode [5]. The method is based on the direct spectrophotometric measurement of the decrease in the absorbance of a 10 mM H_2O_2 solution at 240 nm due to its reaction with 100 mg enzyme prepared in PBS of pH 6.89 at 25 °C.

In order to determine the catalase activity in liver homogenate samples, a calibration curve is constructed based on a 10 mM H_2O_2 solution to which is added variable amounts of catalase (25, 50, 75 and 100 units/ml), and then the value obtained for liver homogenate samples before any dietary control was compared to this calibration curve to get the average of the initial catalase activity and then all the other measured samples of the control group

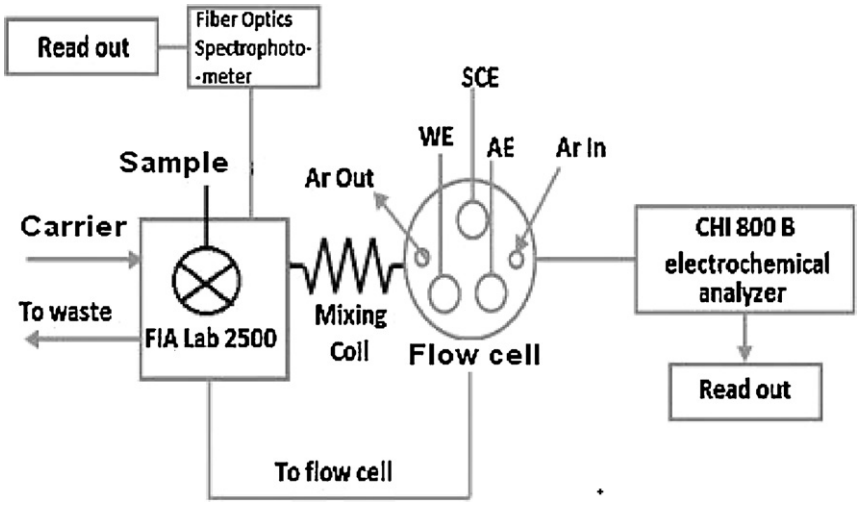

Fig. 1. Schematic diagram for the simultaneous electrochemical and spectrophoto-metric detection flow injection analysis system applied for biological samples measurements.

and the two other groups were measured in comparison to the references and accordingly the results represented as % catalase activity.

2.4. Preparation of MWNT's/chitosan solution

A 0.5% (wt) chitosan solution was prepared by dissolving 50 mg chitosan flakes in 10 ml of 1.0% acetic acid solution followed by stirring for 2 h until complete dissolution at room temperature. 3 mg of MWNT's are then dispersed in 3 ml of the 0.5% chitosan solution with the aid of ultra-sonication for 2 h to obtain chitosan/MWNT's suspension.

2.5. GC electrode conditioning, synthesis of gold nanoparticles and immobilization of catalase on the electrode surface

The glassy carbon electrode is first polished with 0.05 μm alumina powder, followed by ultrasonication for 15 min in absolute alcohol to remove any absorbed particles followed by 15 cycle scans in the potential range −2.0 to +2.0 V versus reference electrode in a solution of 1 M H_2SO_4 [26,27].

Layer by layer strategy [28,29], was applied to prepare the biosensor where first, a 15 μl of the chitosan/MWNT's is cast on the surface of the GC electrode and dried in air for 24 h followed by electrochemical deposition of Gold nanoparticles on the electrode via chronoamperometry in 1% (w/w) $HAuCl_4$ solution containing 0.5 M $HClO_4$ at a potential of 0.18 V for 3 min until the coating of the glassy carbon electrode turns red indicating the formation of gold nanoparticles. Finally, for the immobilization of catalase, consecutive cyclic potential scans (10–15 scans on average) in the potential range −1.5 to +1.5 V with a scan rate of 50 mV per second were performed for the modified GC electrode while immersed in 5 mM catalase solution prepared in PBS until a stable voltammogram is obtained, indicating the saturation of the membrane surface by catalase enzyme. The modified electrode was then removed from catalase solution and washed with PBS, followed by distilled water and stored in PBS in refrigerator at 4 °C.

2.6. Animals and dietary optimization

Sixty five male Sprague Dawley rats, weighing between 200 and 250 g, bred up in the animal house of the German University in Cairo, were used in this study. They were given free access to water and food, and maintained on 12 h light/dark cycle. All experimental procedures were carried out in compliance with the guide for care and use of laboratory animals published by the US National Institutes of Health and in compliance with the Local Animal Ethics Committee of the German University in Cairo.

Rats were divided into three groups with controlled dietary plan for 8 consecutive weeks, for group I (20 rats), dietary supplement, Stress tab Plus®, containing high potency vitamin B complex with vitamins C and E, and beta-carotene, selenium and zinc was added to the food. Each tablet contains 10,000 IU beta-carotene, 0.4 mg folic acid, 15 mg vitamin B1, 10.2 mg B2, 100 mg niacinamide, 11 mg vitamin B6, 6 mcg B12, 28.5 mg pantothenic acid, 600 mg vitamin C and 100 IU E, as well as 50 mcg selenium and 22.5 mg zinc, was crashed and added to the daily food. For group II (20 rats), the diet was based only on whole grains and vegetables with high carotene, lecopene and flavones. Group III, control group (25 rats), non specific dietary plan. Before the start of dietary control, 5 rats from the control group were scarified and the level of initial catalase activity in their liver homogenates was measured, then every 2 weeks, 5 rats from each group were scarified and the levels of catalase activity were followed up in comparison to the initial activity.

2.7. Preparation of liver homogenate samples

The liver tissues (500 mg) were then placed 5 ml in phosphate buffer saline containing 15 μl protease enzyme that breaks the long chainlike molecules of proteins into shorter fragments, followed by ultra-centrifugation at 1500 rpm for 15 min to remove erythrocytes. The clarified supernatant was divided into 1 ml aliquots and stored at −80 °C until ready for use [30].

3. Results and discussion

3.1. Optimization of the electrochemical properties of the modified GC electrode

Commonly, when catalase is immobilized in chitosan matrix, glutaraldhyde is used as a cross linker, but on combining chitosan with carbon nanotubes and gold nanoparticles no cross linker is needed, this is mainly because chitosan is a positively charged natural polymer that can entrap negatively charged nanomolecules like gold nanoparticles and the carboxylic groups present in MWCNT's to form a porous network nanostructure, that can provide electron transfer for immobilized enzyme [22,29,31,32]. Thus, the depositing time of gold nanoparticles is a very important variable to be optimized as it affects both the binding of the enzyme to the electrode and the electron transfer process. Cyclic voltammograms at different deposition time intervals were compared and it was found that sensitivity and stability increase with time and almost remain constant after 3 min of deposition indicating the saturation of the surface of the chitosan/MWNT's membrane with gold nanoparticles, thus the deposition time of 3 min was used throughout this work.

The electrode behavior was tested during the preparation steps by comparing the cyclic voltammograms obtained in phosphate buffer pH 6.89 solution, at a scan rate of 50 mV S^{-1} in the potential range −400 to +400 mV, of chitosan/MWNT's electrode, Catalase/chitosan electrode, Catalase/chitosan/MWNT's electrode and Catalase/Chitosan/MWNT's/Au nanoparticles electrode as given in Fig. 2. Quasi-irreversible voltammogram with a remarkable increase in reduction current while the oxidation current was not observed at the electrode indicating that the integration of gold nanoparticles with MWNT's have a synergetic effect improving the electron transfer and increasing the electroactive surface area of the modified GC electrode leading to catalytic ability of reduction of dissolved oxygen, especially on testing low concentrations of H_2O_2, this change in the catalytic behavior of enzymes in

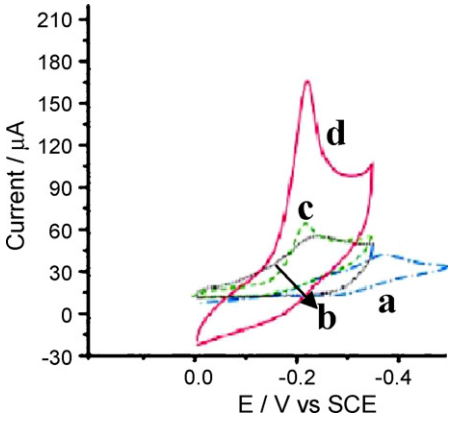

Fig. 2. Cyclic voltammograms in phosphate buffer pH 6.89 solution, at a scan rate of 50 mV S^{-1}, (a) Chitosan/MWNT's electrode, (b) Catalase/Chitosan electrode, (c) Catalase/Chitosan/MWNT's electrode, and (d) Catalase/Chitosan/MWNT's/Au nanoparticles electrode.

MWNT's and Au Nanoparticles was also reported from some other enzymes in literature [19,29,33,34]. Electrochemical contacting of redox enzymes with electrodes is a key process in the construction of third generation enzyme electrodes. While enzymes usually lack direct electrical communication with electrodes due to the fact that the active centers are surrounded by thick insulating protein shells, yet the conductivity properties of metallic nano particles at nanoscale dimensions enhances the electron transfer between the active centers of enzymes and electrodes acting as electron transfer mediators [35].

3.1.1. Electrocatalytic catalase activity towards hydrogen peroxide using the modified electrode

To observe the catalase activity at the Catalase/Chitosan/MWNT's/Au nanoparticles modified GC electrode, cyclic voltammograms were also measured for solutions of different hydrogen peroxide concentrations in the range 1.0–6.0 mM and it was found that there is a linear relationship between the increase in hydrogen peroxide concentration and the obtained current values as shown in Fig. 3 indicating that the direct correlation between the enzyme activity and the hydrogen peroxide concentration is not affected by immobilization in the Chitosan/MWNT's/Au nanoparticles matrix.

Chronoamperometric measurements were also performed at different H_2O_2 concentrations, and it was found that the electrode response was linear in the range 1.0×10^{-7}–1.0×10^{-3} M with LOD 8.5×10^{-8} M with a correlation coefficient of 0.996. when the concentration of H_2O_2 is higher than 1.0×10^{-3} M, a response plateau was observed indicating a typical characteristic Michaelis–Menton kinetic mechanism. The apparent Michaelis–Menton Constant (K_m^{app}) was calculated to evaluate the enzyme-substrate kinetics using Lineweaver–Burk equation [26,36]:

$$\frac{1}{I_{ss}} = \frac{1}{I_{max}} + \frac{K_m^{app}}{I_{max}} \cdot \frac{1}{C}$$

where I_{ss} is the steady state current after the addition of substrate, C is the bulk concentration of the substrate and I_{max} is the maximum current measured under saturated substrate solution. The apparent Michaelis–Menton Constant (K_m^{app}) was found to be 9.85 μM indicating the high affinity of catalase for its substrate and the fast kinetics of their reaction on immobilization in the Chitosan/MWNT's/Au nanoparticles matrix which can be interpreted by improvement of exchange rate due to the presence of both the MWNT's and the gold nanoparticles compared to 1.7 mM indicated before for catalase immobilized only in chitosan/MWNT's matrix [26].

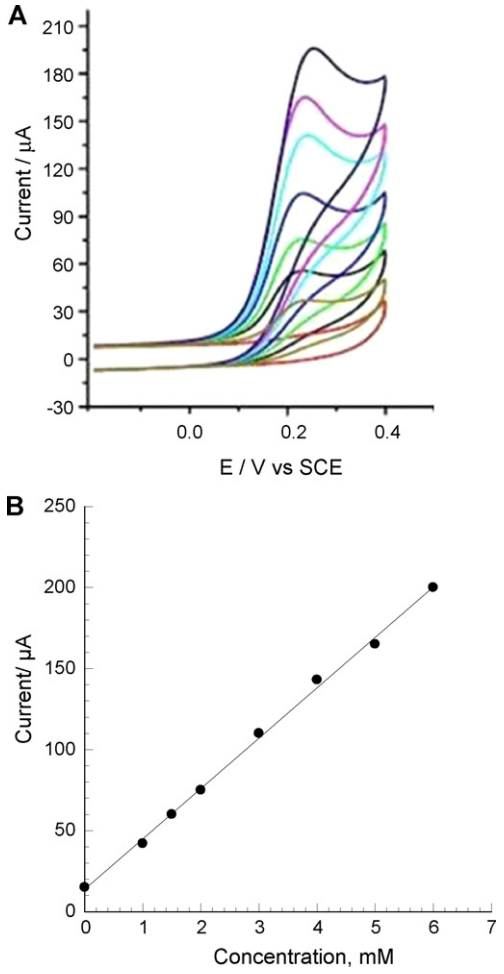

Fig. 3. Cyclic voltammograms of Catalase/Chitosan/MWNT's/Au nanoparticles electrode in PBS of pH 6.89 containing concentration 1.0–6.0 mM H_2O_2 (scan rate 50 mV S^{-1}).

3.1.2. Effect of pH

The effect of the pH 0.1 M PBS (5.50–8.00) on the amperometric response of Catalase/Chitosan/MWNT's/Au nanoparticles modified GC electrode to 1.0 mM H_2O_2 solution in PBS was investigated. Though the results should to some extent higher current at pH 7.00, yet, a pH of 6.89 was used throughout this work which is exactly the physiological pH of the liver tissue samples to be tested. Fig. 4 represents the effect of pH on the electrode response in 0.1 M PBS containing 1.0 mM H_2O_2. The decrease in the current above and below this pH value is mainly attributed to the decrease in the catalase enzyme activity.

3.1.3. Interference

Chronoamperometry was used to the measure the effect of interference on the amperometric response of 0.1 mM H_2O_2 solution to which 1.0 mM glucose, uric acid or ascorbic acid is added. It was found that influence of these interferences is negligible, though their concentration was 10 times that of H_2O_2 solution indicating high selectivity of the electrode.

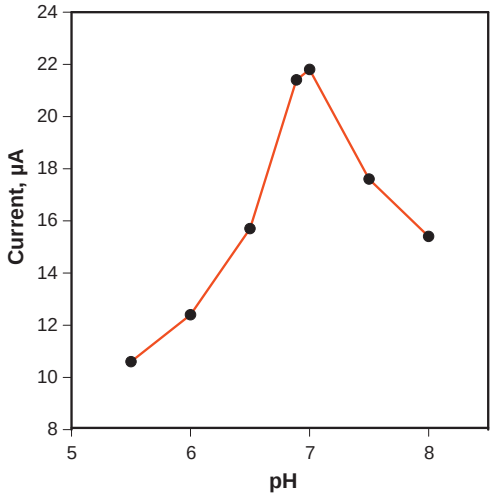

Fig. 4. The effect of the pH of 0.1 M PBS (5.50–8.00) on the amperometric response of Catalase/Chitosan/MWNT's/Au nanoparticles modified GC electrode to 1.0 mM H_2O_2 solution.

3.1.4. Repeatability, reproducibility and stability of the electrode

The reproducibly of the electrode preparation was tested by preparing four electrodes and it was found that, they all exhibited the same linear working range. A concentration of 0.1 mM H_2O_2 was repeatedly measured for 10 consecutive times, to investigate response current repeatability with a RSD 2.1% and a coefficient of variation of 2.89% ($n = 10$).

The electrode stability was tested by measuring a concentration that is 0.1 mM H_2O_2 daily for 1 month and the response was found to be stable up to 20 days and decrease to around 85% after 25 days. When not in use, the electrode should be stored soaked in a 0.1PBS solution of pH 6.89 in a refrigerator at 4 °C, to maintain the enzymatic activity which is highly affected by temperature increase.

3.2. Optimization of the flow injection analysis conditions

The influence of flow rate and sample volume on the amperometric response of the Catalase/Chitosan/MWNT's/Au nanoparticles modified GC electrode was studied. Amperometric response towards 0.1 mM H_2O_2 was measured at flow rates ranging from 0.25 up to 5.0 ml/min, the obtained current peaks were found to increase with flow rate until a flow rate of 3.0 ml/min is reached were the peaks start to decrease, this can be mainly result from incomplete reaction of catalase with H_2O_2, thus, a flow rate of 3.0 ml/min was chosen for the measurements involving standard H_2O_2 solution. Sample volumes of 50 to 300 μl were tested and it was found that when the volume of sample is increased, the time required for interaction with catalase, and in turn analysis, increases with an increase in the amperometric signal, also, the cell washout process requires longer time. A sample loop of 75 μl was chosen, which requires a short time of interaction and less consumption of both sample and enzyme.

The distance between the injection valve and the flow cell was optimized to be 150 cm (0.03 mm i.d.) PTFE tube in the form of a coil, to allow enough interaction between the sample and the carrier before reaching the detector.

Fig. 5 represents the amperometric response for successive injections of 75 μl solution of H_2O_2 of concentrations 10–100 μM at a flow rate of 3.0 ml/min. the calibration plot exhibits linear proportionality between the current signal and the H_2O_2 concentrations which is also linearly correlated to catalase activity [37,38]. The regression analysis of the calibration graph was found to have a slope of 7.5 μA mol L^{-1} with a correlation coefficient of 0.996. The apparent Michaelis–Menton Constant (K_m^{app}) was found to be 15.64 μM indicating that the affinity of catalase for its substrate and the kinetics of their reaction is comparable to batch condition and is not affected to a great extent on applying flow injection analysis [26,30].

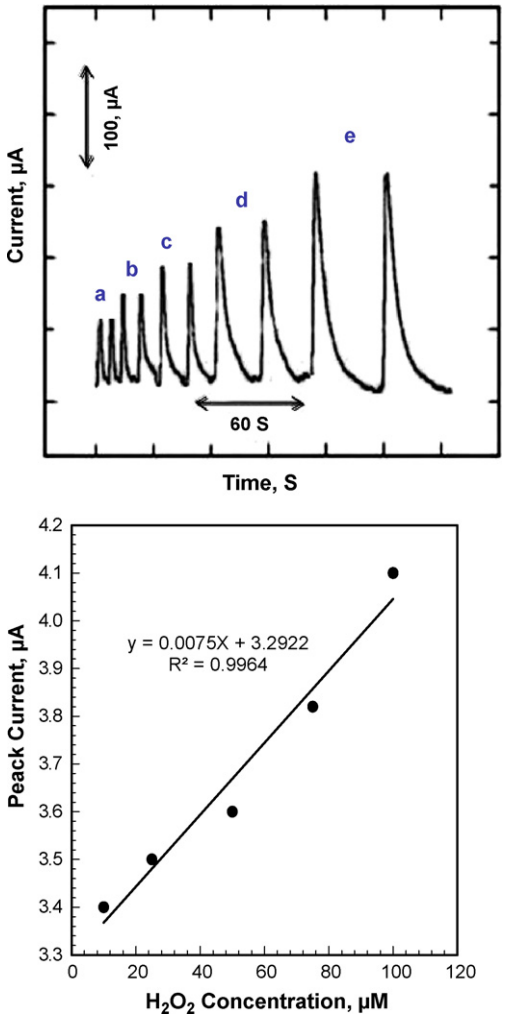

Fig. 5. Flow injection measurements of 75 μl solution of concentrations, 10 μM (a), 25 μM (b), 50 μM (c), 75 μM (d) and 100 μM H_2O_2 at a flow rate of 3.0 ml/min.

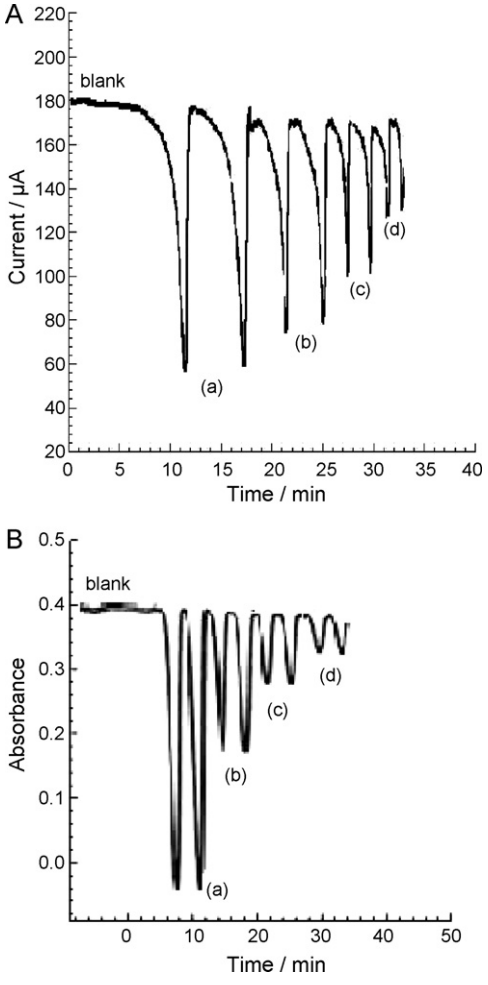

Fig. 6. Calibration graphs obtained for amperometric (A) and spectrophotometric (B) measurements on using a 10 μM H_2O_2 prepared in PBS as carrier, at a flow rate of 1.0 ml/min on injecting 75 μl of 25 units/ml (a), 50 units/ml (b), 75 units/ml (c), and 100 units/ml (d) catalase.

Table 1
FIA catalase enzymatic activity results for liver homogenate samples of controlled dietary rats in comparison to the standard method [5].

	2 weeks			4 weeks			6 weeks			8 weeks		
	Biosensor	Spectro.[a]	Simult.[b]	Biosensor	Spectro.[a]	Simult.[b]	Biosensor	Spectro.[a]	Simult.[b]	Biosensor	Spectro.[a]	Simult.[b]
	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD	(%) ± RSD
Group I	68.5 ± 2.8	68.0 ± 3.5	69.1 ± 2.8	70.3 ± 3.3	69.7 ± 5.5	70.9 ± 2.7	72.1 ± 1.6	71.3 ± 2.7	72.9 ± 2.0	76.9 ± 2.3	75.5 ± 2.6	77.5 ± 1.9
Group II	69.2 ± 4.5	68.4 ± 3.7	70.0 ± 2.7	74.3 ± 3.6	72.8 ± 6.2	75.1 ± 2.3	79.6 ± 4.2	78.7 ± 5.4	80.4 ± 2.8	86.7 ± 4.9	84.1 ± 3.9	87.4 ± 2.4
Group III	45.0 ± 1.7	45.5 ± 4.8	44.8 ± 1.5	49.2 ± 2.6	48.4 ± 3.1	49.8 ± 3.7	51.6 ± 6.2	49.8 ± 4.9	51.0 ± 4.6	50.9 ± 6.3	50.5 ± 4.4	51.6 ± 4.5

Group I: dietary supplement, Stress tab Plus®, containing vitamin B complex with vitamins C and E, and beta-carotene, selenium and zinc.
Group II: diet based only on whole grains and vegetables with high carotene, lecopene and flavones.
Group III: control group, non specific dietary plan.
The initial catalase activity before any dietary control is 38.2 ± 2.6 units/mg sample.
RSD for five determinations.
[a] Standard spectrophotometric detection [5].
[b] Simultaneous spectrophotometric FIA detection.

3.3. Catalase activity measurement in liver homogenates samples

In order to test the catalase activity in liver homogenate samples, some modi-fications in the FIA system were made, where instead of using PBS as a carrier, 10 μM H_2O_2 prepared in PBS was used as carrier and the decrease in current and the absorbance signals of this solution was followed on injecting 75 μl of liver homogenate samples. The flow rate was also decreased to 1.0 ml/min to allow enough contact time between the samples and hydrogen peroxide. Fig. 6 represents the calibration graphs obtained for the decrease in the amperometric and spectrophotometric signals of 10 μM H_2O_2 solution used as a carrier under the above mentioned flow conditions on injecting 75 μl of 25–100 units/ml) catalase samples.

The initial catalase activity in liver homogenates for five rats from the control group scarified before the start of dietary control was found to be equivalent to 38.2 ± 2.6 units/ml in comparison to the peak heights of the calibration graphs. For obtaining the level of catalase activity for the three study groups, 5 rats from each of the studied groups were scarified every 2 weeks for two consecutive months and the levels of catalase activity were followed up in comparison to this initial activity.

It was noticed that on comparing the results of FIA measurements with those obtained from the batch spectrophotometric measurements [5], it was found that the FIA were in most cases higher by 1.0–2.0%, this increase can be attributed to the catalase enzyme immobilized on the modified GC electrode which is also reacting with H_2O_2. Accordingly, in order to overcome this difference, a last modification in measurements was made, where a FIA system with simultaneous amperometric and spectrophotometric detection was employed. The sample is first passed by the modified Catalase/Chitosan/MWNT's/Au nanoparticles GC electrode used for amperometric detection, and then is directed to the spectrophotometric flow cell to be measured at once.

From the results given in Table 1, its clear that, the catalase enzyme activity is highly affected by the control of the dietary intake of rats where, the catalase activity was found to increase by time in case of groups I (dietary supplement) and group II (whole grains and vegetables with high carotene, lecopene and flavones) in comparison to Group III, control group (non specific dietary plan). Also, on comparing between group I and Group II, it was found that natural dietary support had a better impact on the catalase activity compared to getting tablets of dietary supplement and this agrees with previously reported literature [39–44].

4. Conclusion

In this work, a new catalase biosensor based on catalase enzyme (CAT) immobilization on a glassy carbon electrode (GC) modified with electrochemically deposited gold nanoparticles on a multi-walled carbon nanotubes/chitosan film was developed.

The biosensor response was fully characterized both in batch and FIA condition and applied in measurements of H_2O_2 samples and then detection of catalase enzyme activity in liver homogenate samples of controlled dietary intake using simultaneous electrochemical and spectrophotometric detection. The results obtained using the developed biosensor are comparable to those of the recommended literature method of catalase activity [5], which indicates the possibility of applying such biosensor for catalase enzymatic activity assay.

It was also found that catalase activity is highly improved in case of having whole grains and vegetables with high carotene, lecopene and flavones enriched diets compared to commercially available supplements.

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