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Amperometric glucose biosensor utilizing FAD-dependent glucose dehydrogenase immobilized on nanocomposite electrode

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ABSTRACT

Amperometric glucose biosensors utilizing commercially available FAD-dependent glucose dehydrogenases from two strains of *Aspergillus* species are described. Enzymes were immobilized on nanocomposite electrode consisting of multi-walled carbon nanotubes by entrapment between chitosan layers. Unlike the common glucose oxidase based biosensor, the presented biosensors appeared to be O_2-independent. The optimal amount of enzymes, working potential and pH value of working media of the glucose biosensors were determined. The biosensor utilizing enzyme isolated from *Aspergillus* sp. showed linearity over the range from 50 to 960 μM and from 70 to 620 μM for enzyme from *Aspergillus oryzae*. The detection limits were 4.45 μM and 4.15 μM, respectively. The time of response was found to be 60 s. The biosensors showed excellent operational stability – no loss of sensitivity after 100 consecutive measurements and after the storage for 4 weeks at 4 °C in phosphate buffer solution. When biosensors were held in a dessicator at room temperature without use, they kept the same response ability at least after 6 months. Finally, the results obtained from measurements of beverages and wine samples were compared with those obtained with the enzymatic-spectrophotometric and standard HPLC methods, respectively. Good correlation between results in case of analysis of real samples and good analytical performance of presented glucose biosensor allows to use presented concept for mass production and commercial use.

1. Introduction

Monitoring of glucose level has a wide application in areas such as medicine or food industry. For this purpose, biosensors represent suitable method due to their high specificity, short time of analyses, ability to perform measurements in situ and low fabrication expenses. Despite these advantages, biosensors still need to overcome obstacles, mainly problems arising from characteristics of the enzymes used for the fabrication.

In case of enzyme-based biosensors, oxidoreductases have been mostly used as a biological recognition element [1]. Among the oxidoreductases, glucose oxidase (GOX) is the most common used enzyme [2,3]. GOX can selectively catalyze the oxidation of glucose to gluconolactone in the presence of oxygen, and produce H_2O_2 simultaneously [4,5]. However, the response of these biosensors is susceptible to oxygen concentration in the measuring media and in cases where artificial mediators are used increasing in the O_2 concentration can lead to a decrease in the signal and underestimation of the glucose levels [6]. Moreover, if the detection is based on measuring the H_2O_2 level, the oxidation of H_2O_2 often requires higher working potential (usually over +600 mV vs. standard electrode) and thus many other electroactive compounds commonly present in biological fluids can also be oxidized and cause false current response [7,8]. Amperometric biosensors based on nicotinamide adenine dinucleotide dependent dehydrogenases can be employed to overcome this problem [9], however the necessity for a free diffusing coenzyme NAD^+ handicaps this concept. Suitable candidates for the construction of biosensors which do not require O_2 as an electron acceptor or NAD^+ as a coenzyme are pyrroloquinoline quinone- (PQQ-) [10] or FAD-dependent glucose dehydrogenase (GDH-FAD) [11]. Utilizing of GDH-PQQ in case of biosensors designed for commercial use is complicated by the fact that GDH-PQQ isolated from outer surface of the cytoplasmic membrane of suitable cells requires suitable detergents for solubilization [12]. On the other hand water soluble GDH-PQQ present in cell interior has low specificity and it can oxidize a variety of saccharides such as mannose, maltose, lactose, etc., which can cause interferences [13].

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With regard to mentioned facts and to our aim to develop novel glucose biosensor compatible with commercially available analytical device Omnilab W designed for wine makers and food industry (www.biorealis.sk/index.php?content=omnilab&lan=en), we decided to utilize the next-generation GDH-FAD produced by *Aspergillus oryzae*, a new patented product obtained from Toyobo and also GDH-FAD from *Aspergillus* sp. provided from Sekisui Diagnostics. Although the first mentioned enzyme was discovered and described as early as 1967 [14], there have been no subsequent reports on this enzyme. The gene recombination technology that producer employed in developing GDH-FAD contributed to make this enzyme more heat-resistant [15], which is also valuable for the production of biosensors with improved stability. Moreover, the next innovative step in our work was a replacement of a classic gold working electrode by nanocomposite consisting of multi-walled carbon nanotubes (MWCNT) as a cheaper alternative for a material used for the electrode fabrication. N-methylphenazonium methyl sulfate, which is known for its ability to increase the rate of electron transfer from the catalytic site of enzyme to the electrode, was used as an electrochemical mediator [16]. Finally, the presented nanocomposite-based biosensor was applied in analyses of glucose level in beverages and wines.

2. Materials and methods

2.1. Materials

Glucose dehydrogenase-FAD dependent (GDH-FAD) from *A. oryzae* (631 U mg^{-1} solid) was obtained from Toyobo (Osaka, Japan) and GDH-FAD from *Aspergillus* sp. (1160 U mg^{-1} solid) from Sekisui Diagnostic (Tokyo, Japan). Glucose oxidase (258.7 U mg^{-1} solid) was purchased from Biozyme Laboratories (Dublin, USA). Peroxidase (132 U mg^{-1} solid) was purchased from Sorachim (Lausanne, Switzerland). N-methylphenazonium methyl sulfate, N-eicosane, D-glucose, D-fructose, sodium-L-lactate, L-ascorbic acid, DL-malate disodium salt, sodium acetate, *o*-dianisidine, sulfuric acid (95–97%), hydrochloric acid (36.5–38%), anhydrous ethanol and chitosan from shrimp shells (85% deacetylated) were supplied by Sigma–Aldrich (St. Louis, USA). Potassium phosphate monobasic and potassium phosphate dibasic were purchased from Riedel-de Haen (Seelze, Germany). Water deionized by a Millipore Milli-Q purification system was used. All chemicals used were of analytical grade. A glucose stock solution was made with a Milli-Q water and stored at least 24 h to achieve mutarotation equilibrium. Multi-walled carbon nanotubes (MWCNT, d = 60–100 nm, L = 5–15 μm, 95 + % purity) were obtained from NanoAmor (Houston, USA). Planar basic sensors equipped with Ag/AgCl reference electrode (diameter 2 mm, screen-printed) deposited on the planar glass-epoxy-laminate substrate were obtained from Biorealis (Bratislava, Slovakia).

2.2. Apparatus

Electrochemical studies were performed with potentiostat model 559 from Amel (Milan, Italy), potentiostat Heka PG-284 (Lambrecht, Germany) and with electrochemical analyzer Omnilab from Biorealis (Bratislava, Slovakia).

2.3. Preparation of nanocomposites

The procedure described previously for graphite composite electrodes by Miertus et al. was adapted for the nanocomposite fabrication [17]. First, 80 mg of N-eicosane were melted at the temperature 45 °C. After this, 8 mg of MWCNT were added and the mixture was stirred vigorously with a spatula until the homogenous composition was obtained. The suspension was subsequently transferred and spread on the conductive surface layer ($d \approx 1.6$ mm) forming the working electrode on the planar basic sensor equipped with Ag/AgCl reference electrode. Finally, the layer of the nanocomposite was left to solidify and the surface was smoothed on a sheet of paper.

2.4. Preparation of biosensors

The reference electrodes were carefully cleaned with Milli-Q water and ethanol. Glucose dehydrogenase was dissolved in Milli-Q water before procedure. The immobilization of enzyme on the surface of the working electrodes was carried out by their sandwiching between (1% w/w) chitosan layers. The amounts of enzyme on the electrodes were optimized from 1 to 12 units. Each layer was deposited after the previous one was dried. The prepared biosensors were stored at room temperature in a dessicator before use.

2.5. Enzymatic-spectrophotometric assay

The determination was performed with DR 2800 portable spectrophotometer obtained from Hach Lange (Düsseldorf, Germany) using a home made kit. Briefly, glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with *o*-dianisidine in the presence of peroxidase to form a colored product. Oxidized *o*-dianisidine reacts with sulfuric acid to form a more stable pink-colored product that can be measured at 540 nm.

First, the samples were diluted to approximately 10–40 μg mL^{-1}. The 0.98 mL of phosphate buffer solution (pH 6.5) and 0.02 mL of *o*-dianisidine solution (4.5 mg mL^{-1} solution in 0.02 M HCl) was pippeted in a spectrophotometer cuvette. Then 15 units of glucose oxidase and 5 units of peroxidase were added. The mixture was gently shaken and reaction was started by adding 0.5 mL of standard solutions for calibration curve or samples for analysis. Mixtures were incubated for 30 min at 37 °C. Afterwards, reaction was stopped by adding 1.0 mL of 6 M H_2SO_4 into each cuvette. Then the developed color was measured at 540 nm, using a solution containing all the components except glucose as blank.

2.6. HPLC analysis

Reference HPLC assays of glucose were run on Waters 600E HPLC System (Waters, Milford, USA) equipped with the refractometer detector (model PU 4026, Philips, Eindhoven, Netherands). The analytical conditions were as follows: column Polymer IEX in H^+ form 250 mm × 8 mm, 8 μm in diameter (Watrex, Bratislava, Slovakia); column temperature 80 °C and pressure 300 Psi; mobile phase Milli-Q water; flow rate 1.0 mL min^{-1}. Data were collected and processed by Clarity chromatography station DataApex (Prague, Czech Republic). Samples were diluted in a mobile phase and filtered through 0.22 μm Chromafil AO filters, Macherey-Nagel (Dűren, Germany) prior to glucose analysis. Glucose was identified by comparison with retention times and coelution of authentic standard solutions.

3. Results and discussion

The principle of the presented biosensor is based on the amperometric detection of reduced electron acceptor, further referred to as mediator (Med), which is generated during the course of the GDH-FAD-catalyzed oxidation of glucose. The following reactions describe the reaction mechanism for the determination of glucose level by the proposed biosensor:

$$\beta\text{-D-Glucose} + \text{Med}_{(ox)} \rightarrow \text{D-Glucono-1, 5-lactone} + \text{Med}_{(red)} \quad (1)$$

Consequently, reduced mediator is oxidized on the electrode surface and the resulting current proportional to the analyte concentration is measured:

$$\text{Med}_{(red)} + e^- \rightarrow \text{Med}_{(ox)} \quad (2)$$

3.1. Electrochemical characterization of the nanocomposite-based electrode

The electrochemical characteristics of the nanocomposite-based electrode with N-methylphenazonium methyl sulfate (PMS) were obtained by cyclic voltammetry. Measurements were performed in 0.2 M phosphate buffer solution (PBS) at pH value of 5.5 and 7.5. In all cyclovoltammetric experiments, standard scan rate 100 mV s^{-1} was used. First, an obvious cyclovoltammetric experiment, in three-electrode arrangement was performed (Fig. 1), and significant dependence of cyclovoltammetric response on pH value was found. In case of pH 7.5 (dotted line), the peak to peak value (difference between peak potential of reduction and peak potential of oxidation of Med) was about 600 mV. In this case the peak potential of oxidation of Med was about 169 mV vs. standard calomel electrode (SCE). However by using buffer solution of pH 5.5, much better electrochemical behavior was observed. The peak to peak value was in this case only about 100 mV and the peak potential of oxidation of Med was −39 mV vs. SCE. Second, the same set of experiments by using two-electrode cyclovoltammetric experimental arrangement was investigated. The reference input of potentiostat was connected directly to counter electrode to

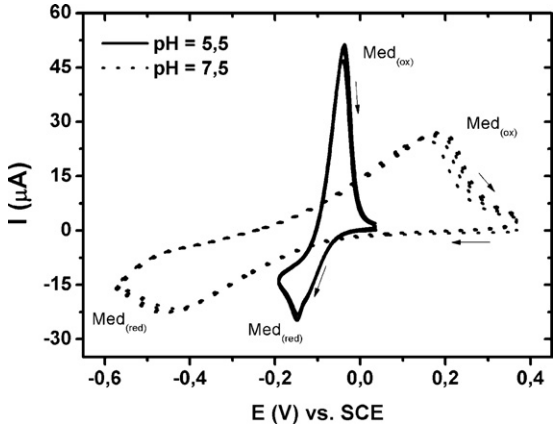

Fig. 1. Cyclic voltammograms recorded by using normal three-electrode arrangement and saturated calomel electrode as an reference electrode, at pH 5.5 (solid line) and pH 7.5 (dotted line). Experimental conditions: scan rate 100 mV s^{-1}. 0.1 M PBS, 1 g L^{-1} N-methylphenazonium methyl sulfate.

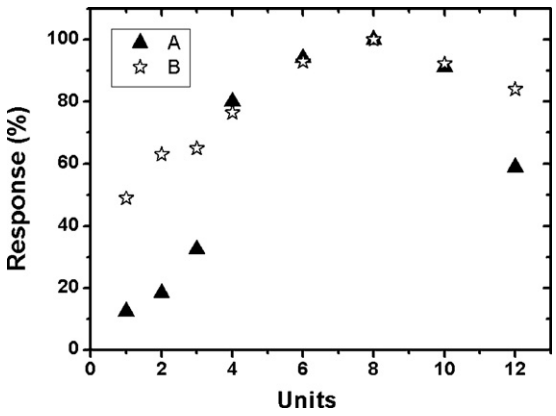

Fig. 3. Effect of enzyme loadings on the response of glucose biosensor based on glucose dehydrogenase from *Aspergillus* sp. – A and from *Aspergillus oryzae* – B (the response at 8 units = 100%). Experimental conditions: 1 g L^{-1} N-methylphenazonium methyl sulfate, 100 μM D-glucose in 0.1 M phosphate buffer at pH 5.5, applied potential +50 mV vs. Ag/AgCl.

simulate and investigate the use in commercial amperometric bioanalyser without reference electrode input. Very importantly, the voltage in voltammograms shown in Fig. 2 is not the well defined potential on the working electrode against independent reference electrode, but it is only voltage between working and counter electrode. Both voltammograms for the two-electrode arrangement are summarized in Fig. 2. In experiment at pH 5.5, new peaks at 100 mV are present, probably next oxidation reactions of species in solution. The voltage value of 50 mV between working and counter electrode in this solution seems to be the best for use in commercial amperometric bioanalyser, because it corresponds to the potential on the working electrode, which is higher, than the oxidation potential of Med, but not too high with respect to the next peaks observed in Fig. 2. That means, at this voltage, the reduced form of Med produced by catalytic reaction is immediately oxidized and current response is measured.

3.2. Optimization of working conditions

Optimization of working conditions was performed in 0.1 M PBS at pH 5.5. First, the test of reactivity to dissolved O_2 (8 mg L^{-1}) was performed. No significant changes in current response were found. This is in accordance with findings in the literature [12] and in the data from the suppliers of enzymes.

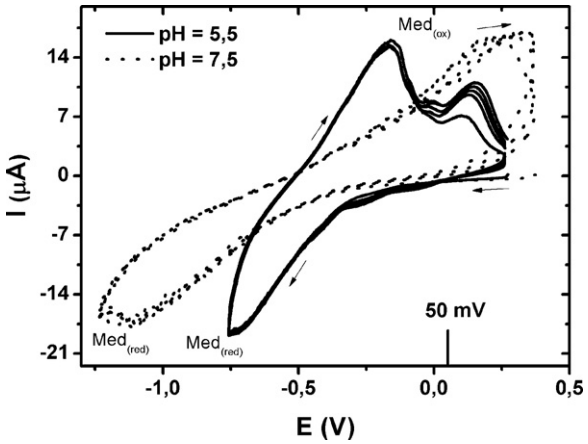

Fig. 2. Cyclic voltammograms recorded by using two-electrode method at pH 5.5 (solid line) and pH 7.5 (dotted line). Reference input of potentiostat was connected to counter electrode. Experimental conditions: scan rate 100 mV s^{-1}. 0.1 M PBS, 1 g L^{-1} N-methylphenazonium methyl sulfate.

In contrast to the biosensor concept utilizing GDH-FAD from *Asspergilus terreus* developed by Tsujimura et al. [11], ferricyanide did not work well as a mediator for our biosensors. We decided to test PMS and its optimal amount was investigated in concentration range of 0.5–2 g L^{-1} in PBS. Based on the fact that no significant changes in current response after the addition of glucose standard (10 mM) were detected, we decided to choose for further work concentration 1 g L^{-1} of PMS in a working media.

To verify the optimal working potential, chronoamperometry measurements were performed by varying of values ranging from +25 mV to +400 mV. Surprisingly, no significant differences in current response were noticed. The highest response was obtained at applied potential of +50 mV, whereas the lowest was detected at +25 mV (80% of the maximum response). Other investigated values of working potential provided responses above 90% of the highest response. Based on these results, we chose the working potential of +50 mV for next study.

Once, the optimal working potential was established, the amounts of enzyme loadings on the working electrode were tested in the range from 1 to 12 units. As it can be seen in Fig. 3, satisfactory current response started at loading of 4 units and achieved peak at 8 units for both tested enzymes. However higher enzyme loadings led to the current decrease, probably due to blockage of the surface of working electrode by immobilized protein. Hence, the amount of 8 units of GDH-FAD was used for further work.

The pH of working media is very important factor which can affect the biosensor performance. The pH dependence of the GDH-FAD based biosensor was investigated over the range from 5.0 to 8.0 in 0.1 M PBS, as shown in Fig. 4. Interestingly, the highest relative response occurred as early as at pH 5.5 and decreased as pH increased further. This is different from the biosensor utilizing GDH from *Thermoplasma acidophilus* and diaphorase co-immobilized with NAD^+ into a carbon paste electrode modified with an osmium functionalized polymer, with the optimum pH of working solution at 7.0 described by Antiochia and Gorton [18]. This difference was also caused by using PMS as a mediator, which is described in detail in Section 3.1.

3.3. Analytical performance

A calibration studies were performed by applying optimal working conditions in 1 mL of PBS in microtube under stirring at laboratory temperature by additions of 10 mM glucose solution. The resulting calibration curves (Fig. 5) were linear over the range 70–620 μM for biosensor based on GDH-FAD from *A. oryzae*

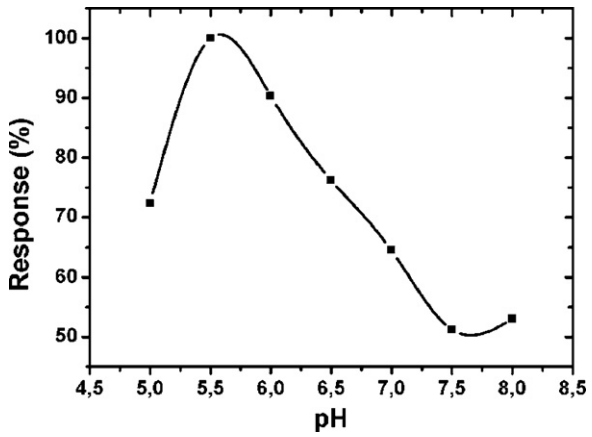

Fig. 4. Effect of pH on the response of glucose biosensor based on glucose dehydrogenase from *Aspergillus* sp. (the response at pH 5.0 = 100%). Experimental conditions: 1 g L^{-1} N-methylphenazonium methyl sulfate, 100 μM D-glucose in 0.1 M phosphate buffer, applied potential +50 mV vs. Ag/AgCl.

and from 50 to 960 μM for biosensor based on GDH-FAD from *Aspergillus* sp. with a correlation coefficients $R^2 = 0.998$ ($n = 10$) and $R^2 = 0.999$ ($n = 15$), respectively. The biosensors with immobilized GDH-FAD form *A. oryzae* showed the detection limit of 4.15 μM with the sensitivity of 2.20 nA μM^{-1}. The detection limit for the second type was 4.45 μM with the sensitivity of 2.05 nA μM^{-1}. Limits of detection are based on signal/noise = 5. The time required to reach steady-state response was 60 s for both biosensors. These results are comparable to the biosensor utilizing GOX deposited on the chitosan–Prussian blue–multiwall carbon nanotubes–hollow PtCo nanochains film. This biosensor showed a linear response to glucose range from 1.5 μM to 1120 μM with a detection limit of 0.47 μM (signal/noise = 3) [19]. Other glucose biosensor based on the NAD^+ dependent dehydrogenase (from *Bacillus* sp.) developed by Saleh et al. showed a linear range from 100 to 1700 μM. In this case, enzyme was immobilized onto nile blue covalently assembled on the surface of functionalized single-walled carbon nanotubes modified glassy carbon electrode [20]. Similar analytical characteristics showed also a glucose biosensor using methyl viologen redox mediator on carbon film electrodes developed by Ghica and Brett with the detection limit of 20 μM and linear range up to 1200 μM [21]. Significantly better characteristic were achieved by nanobiocomposite based glucose biosensor using neutral red functionalized

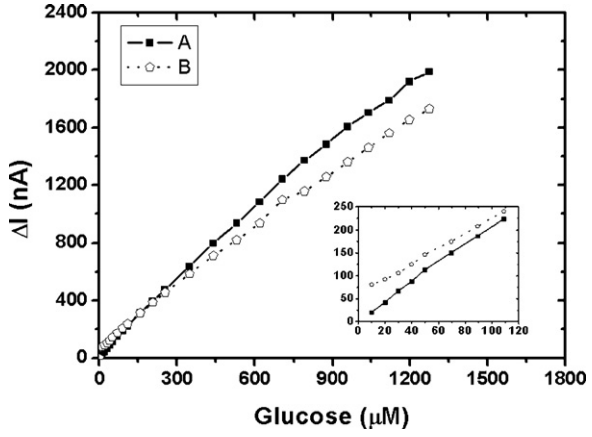

Fig. 5. Calibration curve obtained for glucose biosensor based on glucose dehydrogenase from *Aspergillus* sp. – A and from *Aspergillus oryzae* – B. Experimental conditions: 1 g L^{-1} N-methylphenazonium methyl sulfate, phosphate buffer at pH 5.5, consecutive additions of 10 mM D-glucose standard solution, applied potential +50 mV vs. Ag/AgCl.

carbon nanotubes proposed by Jeykumari and Narayanan [22]. Linear range for this biosensor was over the range 1×10^{-8}–1×10^{-3} M with a detection limit of 3×10^{-9} M and response time was only 4 s.

Based on the experimental data from Fig. 5, the apparent Michaelis–Menten constants K_M^{app} were calculated for the immobilized enzymes according to Hill equation: $I = I_{max}xC/(K_M^{app} + C)$, where I is the steady-state current, I_{max} is the maximal current and C is the glucose concentration. Results showed, that K_M^{app} of GDH-FAD from *Aspergillus* sp. for glucose was (4.53 ± 0.24) mM and I_{max} was (9.12 ± 0.39) μA. Similar values of K_M^{app} (3.09 ± 0.47) mM and I_{max} (5.84 ± 0.68) μA were obtained for GDH-FAD from *A. oryzae*. The estimated K_M^{app} values were similar to or lower than the Michaelis constant for GDH-FAD from *A. oryzae* with glucose as a substrate obtained in the presence of 0.1 mM dichloroindophenol, i.e. 25 mM [23]. This suggests the enzymes were not significantly affected by the immobilization process with a chitosan "sandwich" layer being a proper immobilization matrix for biosensor preparation. We also assume, that interaction between substrate and enzyme of GDH-FAD based biosensor is stronger than that of free GDH-FAD. As the biosensor utilizing GDH-FAD from *Aspergillus* sp. performed over a wider linear range than the biosensor based on GDH-FAD from *A. oryzae* and differences in parameters such as K_M, I_{max}, sensitivity and detection limit were negligible, for further experiments only GDH-FAD from *Aspergillus* sp. was used.

The interference studies were performed to verify the high specificity of the presented biosensor. The effect of possible interferences present in wine and juices such as ethanol (10%), sucrose (18 g L^{-1}), fructose (18 g L^{-1}), mannose (18 g L^{-1}), lactose (18 g L^{-1}), L-lactic (4 g L^{-1}), citric (4 g L^{-1}), tartaric (4 g L^{-1}), acetic (4 g L^{-1}) and L-ascorbic acid (50 mg L^{-1}) on the response of the presented biosensors was evaluated. The additions of sugars, ethanol and acids did not change the current signal over the biosensor detection limit. The effect of polyphenols was tested by using bar electrodes covered with chitosan double layer without enzymes at +50 mV using optimized measuring solution (data not shown). No significant current changes were detected, so polyphenols also do not interfere at used measuring conditions.

3.4. The reproducibility and stability

The reproducibility of measurements of the biosensor was made using standard glucose solution (10 mM) by repetitive pipetting volume of 10 μL. The average response of the biosensor was 124.31 ± 2.83 nA ($n = 12$, R.S.D = 2.28%). These findings were promising for further analysis of real food samples. Other important aspect for the commercialization is a reproducibility between batches. The average current response of six biosensors for 100 μM glucose was 120.75 ± 22.14 nA ($n = 6$, R.S.D = 18.34%). This is caused by the imperfection of fabrication process during preparation of nanocomposite. This negative fact at first sight can be overcome by in situ calibration of each biosensor before analysis, the result were calculated by "rule of three" when the current response for the addition of standard solution is measured before the real sample analysis. Thus obtained results were in a good correlation with those calculated from the equation for the calibration curve ($y = 1.657x + 83.95$, where y is a current response and x is glucose concentration).

Long-term storage stability of biosensors is one of the most important factor in case of their commercial use. Generally humidity and higher temperature are considered as the most negative factors which can affect the storage stability of enzymatic biosensors. When our biosensors were held in a dessicator at room temperature without use, they kept the same response ability at least after 6 months. Monitoring of storage stability will continue. Moreover the biosensors were also stable at 50 °C for at least 3 days.

Table 1
Determination of D-glucose in beverages and wines using biosensors, HPLC and enzymatic-spectrophotometric assay as a standard analytical method. Results are expressed in g L^{-1} ± STDEV (n = 3).

Sample	Biosensor	HPLC	Enzymatic kit
Banana juice	62.7 ± 2.5	61.42 ± 0.25	64.02 ± 1.96
Strawberry juice	37.7 ± 0.3	38.55 ± 0.10	42.39 ± 2.12
Apple juice	22.3 ± 1.7	22.54 ± 0.23	26.78 ± 0.04
Orange juice	29.2 ± 0.4	23.98 ± 0.37	26.41 ± 2.11
Orange nectar	13.7 ± 0.3	12.53 ± 0.20	12.38 ± 0.25
White wine	4.47 ± 0.19	3.77 ± 0.02	3.59 ± 0.18
Chardonnay – white wine	0.75 ± 0.03	0.78 ± 0.13	0.19 ± 0.01
La Cabernet – red wine	0.33 ± 0.02	0.59 ± 0.22	0.06 ± 0.01
Levicka frankovka – red wine	7.27 ± 0.01	6.28 ± 0.12	7.03 ± 0.06
Merlot – red wine	1.36 ± 0.07	1.27 ± 0.06	0.84 ± 0.03

The operational stability was studied by monitoring its current response with intermittent usage (every 3–6 days) and biosensor was stored at 4 °C in 0.1 M phosphate buffered saline (PBS) of pH 5.5. Surprisingly, biosensors showed no changes in activity for a 4 weeks after first use and after 5 weeks it held its activity about 82% its initial value. During the experimental period, more than 100 measurements were carried out using the same biosensor. These findings are very positive for the planned commercial purposes, as it reduces material costs and financial burden for the customer. Our results are similar to those obtained by CNT–titania–Nafion-based glucose biosensor which retained its activity about 80% its initial value after 4 weeks [24]. We assume that long storage and operation stability could be achieved thanks to the good GDH-FAD stability and to the immobilization in chitosan (CS) "sandwich" whereas amino and hydroxyl groups widely present in CS molecules provide a hydrophilic environment which is compatible with the biomolecules [25].

3.5. Real samples analysis

Although the developed biosensor based on GDH-FAD showed good analytical performance when using glucose standard solution, it was necessary to assess the performance of the biosensor with respect to real food samples and to compare the results with those obtained by standard analytical methods. This was important for verification of biosensor's ability to accurately measure real samples, which have more complex composition than standard glucose solution. Commercial fruit drinks and wines were used as real samples. Considering the linear range of the biosensor and the sugar levels in beverages and wines, some samples were diluted before the analyses. Biosensoric determination was performed by injecting 10 or 5 μL of sample and calibration solution in 1 mL of PBS of pH 5.5 containing PMS. Measuring in buffer solution and small additions of samples guaranteed that pH value of real samples could not affect biosensor performance. Samples with glucose level over 8 g L^{-1} were 10× diluted before biosensoric analysis. As it can be seen in Table 1, good correlation was obtained between results obtained by the biosensor and those obtained by HPLC or enzymatic-spectrophotometric assay. The results for chardonnay, cabernet and merlot obtained by enzymatic-spectrophotometric assay were different from those obtained with a biosensor or by HPLC, however the levels of glucose were generally very low in these samples and the enzymatic kit is probably not very accurate when measuring low glucose levels. These discrepancies found for this secondary reference method may be considered as negligible.

4. Conclusion

In summary, we have developed and characterized a new glucose biosensor based on nanocomposite electrode consisting of multi-walled carbon nanotubes. As a mediator, N-methylphenazonium methyl sulfate was used. Based on the analytical performance and results obtained in analysis of real samples, we consider both commercially available FAD-dependent glucose dehydrogenases from *A. oryzae* and *Aspergillus* sp. as suitable biocomponents for the fabrication of glucose biosensors. A novel simple immobilization method utilizing a chitosan "sandwich" assured long term operational and storage stability. Low fabrication expenses, good analytical performance and short time of analysis make the biosensor presented here based on the nanocomposite electrode suitable for mass production and commercial use. This work also showed, that the presented nanocomposite electrode is a suitable candidate for replacement of the gold planar electrode actually used for biosensors in the Omnilab W commercial analyzer.

Acknowledgment

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