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Talanta 67 (2005) 783–790
Talanta
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# Modified graphitized carbon black as transducing material for reagentless $H_2O_2$ and enzyme sensors

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Received 24 November 2004; received in revised form 28 February 2005; accepted 1 April 2005
Available online 21 April 2005

**Abstract**

Direct electron transfer between redox enzymes and electrodes is the basis for the third generation biosensors. We established direct electron transfer between quinohemoprotein alcohol dehydrogenase (PQQ-ADH) and modified carbon black (CBs) electrodes. Furthermore, for the first time, this phenomenon was observed for pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (PQQ-GDH). Reagentless enzyme biosensors suitable for the determination of ethanol, glucose and sensors for hydrogen peroxide were designed using CB electrodes and screen-printing technique. Aiming to create an optimal transducing material for biosensors, a set of CB batches was synthesized using the matrix of Plackett–Burman experimental design. Depending on the obtained surface functional groups as well as the nano-scale carbon structures in CBs batches, the maximal direct electron transfer current of glucose and ethanol biosensors can vary from 20 to 300 nA and from 30 to 6300 nA for glucose and ethanol, respectively. Using modified CB electrodes, an electrocatalytic oxidation of $H_2O_2$ takes place at more negative potentials (0.1–0.4 V versus Ag/AgCl). Moreover, $H_2O_2$ oxidation efficiency depends on the amount and morphology of fine fraction in the modified CBs.


*Keywords:* Bioactivity; Biosensors; Carbon black; Surface composition; PQQ-ADH; PQQ-GDH; $H_2O_2$

## 1. Introduction

In the last three decades, a new sensing concept based on biological recognition was established known as biosensors, where biologically derived molecules, organelles, whole cells or tissues are used in close contact with a transducing element [1]. Amperometric biosensors (commonly based on enzymes) have been one of the most successful and up to now has a promising future [2–4]. In this concept, the most promising approach for the development of electrochemical biosensors is to establish a direct electrical communication between the biomolecules and the electrode surface [5]. Direct electron transfer between redox enzymes and electrodes found the basis for the third generation biosensors [6]. Carbon-based electrochemical devices become most popular technological feature in the design of the biosensors [7]. During the last decade, more than 200 papers and patents have been published on the use of screen-printing technology for transducers based on carbon-paste electrodes. It turned out that the screen-printing technology is a versatile tool for the inexpensive, easy and high reproducible production of disposable biosensors [8]. Carbon materials that have been widely used in the preparation of solid electrodes include glassy carbon, carbon fibers, carbon black (CB) and several forms of graphite, from the highly oriented pyrolytic graphite to the graphite powder used in the preparation of the well-known composite carbon-paste [9]. Operation of biosensors is determined by interaction of active center of enzyme and surface of the carbon particle. Thereby, parameters of the biosensor crucially depend on parameters of carbon, used for the preparation of the paste. These should be: the amount and

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doi:10.1016/j.talanta.2005.04.004

assortment of surface functional groups, the surface roughness and heterogeneity, the spatial arrangement of graphene layers, presence of various nano-scale carbon structures (nanotubes and other formations), etc. [10].

In this work, we modified and tested a set of CB samples as transducing element in hydrogen peroxide sensors and ethanol or glucose biosensors design as well. As biological sensor components we used two types of enzymes. Pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (PQQ-GDH) was used as an well-known enzyme, which do not communicate directly with carbon surface, but acts through a number of mediating organic compounds, that possess good electrochemical reversible properties on the carbon surface [11,12]. Another enzyme—an alcohol dehydrogenase (ADH) was used as enzyme containing PQQ and hemes *c*, and possessing capability of direct electron transfer on metal [13] and carbon [14] surfaces.

## 2. Materials and methods

### 2.1. Synthesis and modification of carbon black

CB was synthesized from carbon monoxide during the Boudouard's reaction on the Fe catalyst [15]:

$$2CO(g) \xrightarrow{Fe} C(gr) + CO_2(g) \quad (1)$$

and subsequent purification with HCl [16].

The protocol of CB modification consisted of 11 technological operations (Table 1). Objectives of using these operations can be divided into three blocks:

(a) delamination of graphite structure carried out using naphthalene (X1), $H_3PO_4$ (X2), $H_2SO_4$ (X3), KOH (X4) and $H_3BO_3$ (X5) [17];
(b) tailoring of functional groups carried out in the presence of $HNO_3$ (X6), $Br_2$ + Fe (X7), $H_2O_2$ (X10) and $N_2H_4{\cdot}2HCl$ (X11) [18];
(c) modification of particle shape carried out by melting with Zn (X8) and S (X9) [19].

With an objective to screen as large as possible number of factors to identify these that may be important, a design that allows one to test the largest number of factor main effects with the least number of observations was employed. The 12-run Plackett–Burman design for 11 factors was chosen for this purpose [20]. The matrix used and the technological operations applied (with short descriptions) are presented in Table 1. Each particular batch was obtained by performing these operations, which were maked as "+1" in Table 1, and by omitting these marked as "−1". In the process of modification, the samples were purified after executing each technological operation by means of hot extraction in a Soxhlet's apparatus. Obtained batches of graphitized CB were examined by means of microscopy, acid–base titrations and nephelometry.

### 2.2. Analysis of modified carbon blacks

The amount of functional groups on the surface of CB samples was determined titrimetrically by using 0.1 M solutions of NaOH, $Na_2CO_3$, $NaHCO_3$ or HCl [21,22]. The CB samples with appropriate solutions were placed in the capped vials and mixed for 24 h in a tube rotator SC1. The aliquots were titrated under the nitrogen atmosphere using a microburette. The quantity of fine fraction in modified CB samples was established by nephelometric analysis [23], using both cationic (sodium lauryl sulphate) and anionic (tetradecyltrimethylammonium bromide) surface-active substances (SAS) and a LMF-69 instrument. The CB samples were

Table 1
Technological operations and experimental set used for the CB modification

| Operation | Short description | Runs (batches) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 |
| X1 | 30% CB + 70% naphthalene; treatment time, −6 h; temperature −100 °C | +1 | +1 | −1 | +1 | +1 | +1 | −1 | −1 | −1 | +1 | −1 | −1 |
| X2 | 10% CB + 90% $H_3PO_4$; treatment time, −6 h; temperature 100 °C | −1 | +1 | +1 | −1 | +1 | +1 | +1 | −1 | −1 | −1 | +1 | −1 |
| X3 | 10% CB + 90% $H_2SO_4$; temperature time, −6 h; temperature 80 °C | +1 | −1 | +1 | +1 | −1 | +1 | +1 | +1 | −1 | −1 | −1 | +1 |
| X4 | 50% CB + 5% KOH; treatment time 1 h; temperature 480 °C | −1 | +1 | −1 | +1 | +1 | −1 | +1 | +1 | +1 | −1 | −1 | −1 |
| X5 | 50% CB + 50% $H_3BO_3$; grinding time −0.5 h | −1 | −1 | +1 | −1 | +1 | +1 | −1 | +1 | +1 | +1 | −1 | −1 |
| X6 | 7 ml $HNO_3$ + 3 ml $H_2SO_4$ + 4 g CB; treatment time 1 h; temperature 80 °C | −1 | −1 | −1 | +1 | −1 | +1 | +1 | −1 | +1 | +1 | +1 | −1 |
| X7 | 0.8 ml $Br_2$ + 0.5 g Fe + 4 g CB; treatment time 1 h; temperature 60 °C | +1 | −1 | −1 | −1 | +1 | −1 | +1 | +1 | −1 | +1 | +1 | +1 |
| X8 | 4 g CB + 4 g Zn; treatment time 1 h; temperature 370 °C | +1 | +1 | −1 | −1 | −1 | +1 | −1 | +1 | +1 | −1 | +1 | +1 |
| X9 | 4 g CB + 4 g S; treatment time 1 h; temperature 380 °C | +1 | +1 | +1 | −1 | −1 | −1 | +1 | −1 | +1 | +1 | −1 | +1 |
| X10 | 4 g CB + 20 ml 30% $H_2O_2$; treatment time 24 h | −1 | +1 | +1 | +1 | −1 | −1 | −1 | +1 | −1 | +1 | +1 | −1 |
| X11 | 4 g CB + 20 ml 10% $N_2H_4$ 2HCl; treatment time 1 h; temperature 100 °C | +1 | −1 | +1 | +1 | +1 | −1 | −1 | −1 | +1 | −1 | +1 | +1 |

tested using an atomic force microscope Digital Instruments NanoScope III.

### 2.3. Construction of screen-printed biosensors

The screen-printed carbon electrodes were designed using CB samples mixed with the pasting liquid as described previously [24]. The pasting liquid consists of 10% polyvinyl dichloride in acetone. The PQQ-ADH (EC1.1.99.8) purified from *Gluconobacter* sp. 33 [25] (the activity was 171 U/ml) or PQQ-GDH (EC1.1.99.17) purified from *Erwinia* sp. 34-1 [26] (specific activity 12 U/mg) were immobilized on the electrode (working area 0.04 $cm^2$) by glutaric dialdehyde cross-linking (for 30 min over 25% glutaric dialdehyde water solution). The layer of an immobilized enzyme was covered with semi-permeable terrylene film.

Biosensor was installed into the flow-through three-electrode amperometric cell [24] where a working electrode forms the bottom of the flow cell. As a reference electrode, we used a silver wire (1 mm in diameter and 2 cm long) in saturated KCl. The titanium tube (2 mm in diameter and 2 cm long) on inlet was used as a counter electrode. The flow cell was supplied with a peristaltic pump. Steady state currents of biosensors were recorded on the polarographic analyzer OH-105 (Radelkis, Hungary).

Acetate buffer (0.05 M) (pH 6.0) containing 1 mM of $Ca^{2+}$ was used as a default buffer. All inorganic reagents were of analytical reagent grade and organic reagents were suitable for electron microscopy. Bidistilled and ultra-filtered water was obtained using a "Purator Bi" (Glas Keramic, Berlin, Germany).

## 3. Results and discussion

### 3.1. Characterization of modified CB

Data of CBs analyses are presented in Table 2. Predominant acidic functional groups determined among the modified CB batches are of phenolic nature, while the lactones are found only in the case of single CB sample (C4). Basic functional groups are determined as a total sum of all positive charges (pyrones, chromenes, nitrogen-containing groups, etc. [27]). The content of fine fraction determined using the anionic SAS is as a rule higher than that using the cationic SAS. Interaction between the SAS and the surface of CBs should be based on either electrostatic or van der Waals forces. In the case of prevailing of van der Waals forces, no significant difference between results using cationic or anionic SAS should be observed. The fact of significant difference between those two series of results implies to make a conclusion that in the process of formation of fine fraction electrostatic interaction prevails over the van der Waals forces.

Content of fine fraction in modified samples varies in a wide range subject to the preparation conditions. AFM micrographs (Fig. 1) show that the morphology of fine fractions from different modified CB batches is different as well. In some cases (batch C8), the structures similar to multiwall carbon nanotubes (several tenths nm in diameter) are observed. This observation correlates with the results published by other authors [28], and concerning the synthesis of multiwall carbon nanotubes by scrolling the sheets of graphene. The AFM micrographs of other modified CB batches expose much more coarse fractions, which are different as compared together as well. Rounded fractions are observed in the batch C5. Sharpe-edger structures are evident in the micrographs of the batches C3 and C4. This case may indicate the production of nanocones during the process of CB modification [29].

### 3.2. Oxidation of $H_2O_2$ on CBs

The determination of hydrogen peroxide ($H_2O_2$) plays an important role in many fields including industry, environmental protection and clinical control [30]. Horseradish peroxidase is the most commonly used enzyme for construction of $H_2O_2$ biosensors [31–34]. Hemoglobin was employed to catalyze the reduction of $H_2O_2$ as well [35,36]. An electrochemically deposited film of Prussian Blue has been found to be good catalyst for $H_2O_2$ electroreduction exhibiting even

Table 2
Amount of functional groups and content of fine fraction in modified carbon blacks

| Sample no | Amount of functional groups and standard deviation (mM/g of CB) | | | | | Content of fine fraction and standard deviation (%) | |
|---|---|---|---|---|---|---|---|
| | Total acidic | Carboxylic | Lactone | Phenolic | Basic | With anionic SAS | With cationic SAS |
| C1 | 1.86 ± 0.12 | 0.00 ± 0.03 | 0.00 ± 0.04 | 1.86 ± 0.12 | 0.66 ± 0.02 | 0.0 ± 1.9 | 0.0 ± 1.2 |
| C2 | 1.34 ± 0.17 | 0.44 ± 0.05 | 0.00 ± 0.04 | 0.91 ± 0.17 | 0.61 ± 0.01 | 51.6 ± 5.9 | 0.0 ± 0.6 |
| C3 | 0.34 ± 0.16 | 0.00 ± 0.01 | 0.00 ± 0.02 | 0.34 ± 0.16 | 0.61 ± 0.06 | 41.6 ± 7.6 | 0.0 ± 1.2 |
| C4 | 0.32 ± 0.11 | 0.00 ± 0.01 | 0.19 ± 0.06 | 0.13 ± 0.11 | 0.66 ± 0.07 | 48.3 ± 5.1 | 0.0 ± 0.9 |
| C5 | 0.66 ± 0.12 | 0.00 ± 0.05 | 0.00 ± 0.04 | 0.66 ± 0.12 | 0.59 ± 0.01 | 55.0 ± 6.2 | 30.0 ± 4.7 |
| C6 | 0.28 ± 0.02 | 0.00 ± 0.01 | 0.00 ± 0.02 | 0.28 ± 0.02 | 1.08 ± 0.09 | 17.0 ± 2.2 | 9.3 ± 2.4 |
| C7 | 1.19 ± 0.12 | 0.21 ± 0.05 | 0.00 ± 0.01 | 0.97 ± 0.12 | 0.76 ± 0.08 | 0.0 ± 1.5 | 0.0 ± 2.3 |
| C8 | 0.86 ± 0.02 | 0.00 ± 0.02 | 0.00 ± 0.04 | 0.86 ± 0.02 | 0.74 ± 0.03 | 69.3 ± 3.1 | 13.3 ± 3.1 |
| C9 | 0.92 ± 0.05 | 0.08 ± 0.03 | 0.00 ± 0.02 | 0.84 ± 0.05 | 0.72 ± 0.05 | 0.0 ± 2.0 | 0.0 ± 2.4 |
| C10 | 0.72 ± 0.14 | 0.00 ± 0.01 | 0.00 ± 0.01 | 0.72 ± 0.14 | 1.27 ± 0.01 | 0.0 ± 3.5 | 0.0 ± 0.8 |
| C11 | 0.61 ± 0.13 | 0.00 ± 0.01 | 0.00 ± 0.02 | 0.61 ± 0.13 | 1.47 ± 0.05 | 45.0 ± 4.5 | 15.0 ± 3.7 |
| C12 | 0.52 ± 0.18 | 0.00 ± 0.03 | 0.00 ± 0.01 | 0.52 ± 0.18 | 1.68 ± 0.02 | 31.6 ± 1.6 | 21.6 ± 4.6 |

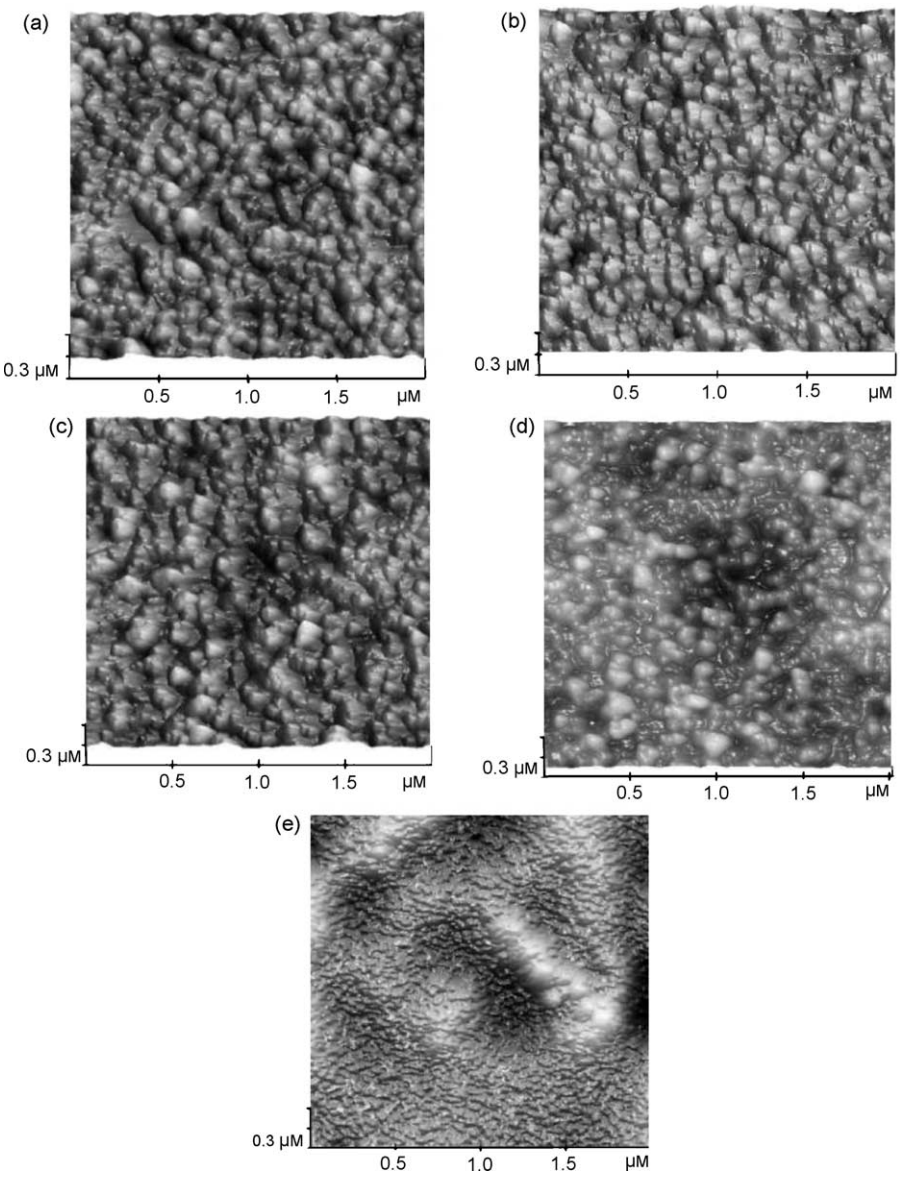


Fig. 1. AFM micrographs of modified CBs. Batch numbers: (a) C2; (b) C3; (c) C4; (d) C5; (e) C8.

better properties than horseradish peroxidase [37]. However, amperometric detection based on electrochemical oxidation of $H_2O_2$ is observed at potential range higher than 0.6 V (versus Ag/AgCl and all potential values in the text are versus this reference electrode) [38]. Unfortunately, an unspecific oxidation of various interfering compounds is observed at these potentials, too.

The modifications of CBs described in this work facilitate electrocatalytic oxidation of $H_2O_2$ at more negative potentials, as it is shown in voltammetric data presented below. Hydrodynamic voltammograms were recorded using electrodes designed on the base of different CB batches in the presence of 70 mM $H_2O_2$.

Using C2 and C4, the oxidation process starts at 0.05 V and the oxidation current is 20 and 9 nA, respectively (data not shown), whereas the oxidation on C8 and C11 electrodes proceeds at 0.15 V. The $H_2O_2$ oxidation current is 50 and 7 nA, respectively. All active CB electrodes showed good response at potential 0.4 V (Table 3). Unfortunately, modification of C1, C7 and C9 does not lead to successful oxidation of $H_2O_2$.

Therefore, the oxidation of $H_2O_2$ can be observed at the potential range 0.1–0.4 V by using corresponding modification of CB electrodes. To our knowledge, those are the lowest potentials for oxidation of hydrogen peroxide at carbon electrodes. The lower applied potential helps to decrease influence of interfering compounds. The developed surfaces open new opportunities to create specific hydrogen peroxide oxidizing systems without use of expensive metals such as gold or platinum. The oxidation currents of $H_2O_2$ sensors based on modified CB electrodes at 0.4 V are presented in Table 3. It was established that the $H_2O_2$ oxidation current

Table 3
Kinetic parameters of sensors based on modified CBs

| No. of CB batch | Maximal current of sensor (nA) | | | $K_M^{app}$ (mM) | | $K_{in}$ (min$^{-1}$) |
|---|---|---|---|---|---|---|
| | $H_2O_2$[a] | Ethanol | Glucose | PQQ-GDH | PQQ-ADH | PQQ-ADH |
| C1 | – | – | – | – | – | – |
| C2 | 530 | 2277 | 48 | 0.45 | 1.84 | $2.26 \times 10^{-3}$ |
| C3 | 84 | 6269 | 93 | 0.67 | 3.7 | $3.03 \times 10^{-3}$ |
| C4 | 375 | 5307 | 179 | 1.02 | 2.83 | $7.98 \times 10^{-3}$ |
| C5 | 370 | 1686 | 99 | 0.56 | 1.43 | $1.23 \times 10^{-3}$ |
| C6 | 62 | 1392 | – | – | 0.44 | $6.54 \times 10^{-3}$ |
| C7 | – | 36 | 91 | 0.61 | 0.22 | $9.94 \times 10^{-4}$ |
| C8 | 1200 | 2195 | 309 | 0.79 | 1.24 | $2.7 \times 10^{-3}$ |
| C9 | – | 35 | – | – | 0.13 | $2.3 \times 10^{-2}$ |
| C10 | 20 | 1342 | 68 | 0.39 | 1.87 | $1.98 \times 10^{-3}$ |
| C11 | 187 | 2487 | 159 | 3.05 | 0.99 | $1.79 \times 10^{-3}$ |
| C12 | 40 | 138 | 27 | 0.08 | 0.17 | $-2.41 \times 10^{-3}$ |

Each value is a mean value obtained using three independent biosensors.
[a] Response to 70 mM of $H_2O_2$; $K_{in}$: inactivation constant.

depends not only on the amount and assortment of surface functional groups in modified CBs but also on the amount of fine fraction in the same batches (Fig. 2).

Considering that an observed anodic current have to be proportional to the area of particle's surface and the same amount of carbon was applied for each electrode, the experimental data in Fig. 2 were fitted using the following equation:

$$y = 4\pi\left(\frac{3}{4\pi}\right)^{2/3} \times 2^{ax/3} \quad (2)$$

where $y$ is an observed anodic current (equivalent to the surface area of carbon particles), $2^{ax/3}$ is number of particles, $x$ is a fraction of fine particles, $a$ is a coefficient linking a fraction of fine particles and number of particles.

The sensor designed using C8 was found the most sensitive to $H_2O_2$ among the others. The amount of fine fraction in C8 reaches ~70% of (Table 2). As it was mentioned above, on the base of AFM image of C8 (Fig. 1d), it is possible to presume the existence of nanotubes in this sample. This presumption correlates with data obtained on carbon nanotubes

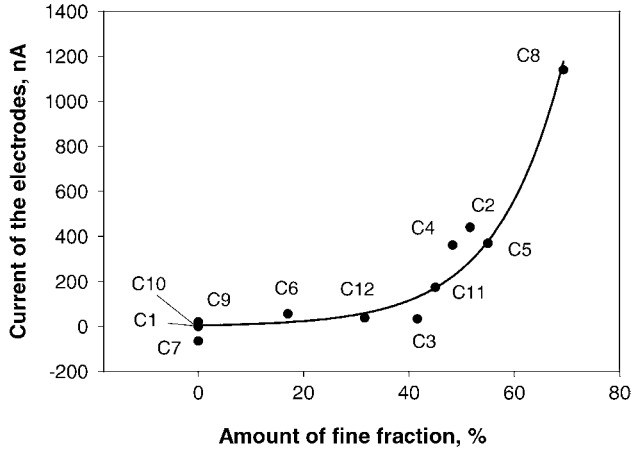


Fig. 2. Correlation between the amount of fine fraction and the sensitivity of sensors to $H_2O_2$. Response to 70 mM of $H_2O_2$. C1–C12 CBs were analyzed.

paste electrodes. The existence of nanotubes on electrode surface permits an important decrease in the overvoltage for the oxidation of hydrogen peroxide (300 mV) [39].

### 3.3. Bioelectrocatalytic behavior of PQQ-ADH-coated carbon electrodes

PQQ-dependent enzymes have two major possibilities to transfer electrons to conducting solid surfaces: either via redox mediators or via intrinsic redox chains [1,13,40]. PQQ-ADH used in this work contains three subunits: the first one contains PQQ and one heme *c* moiety; the second subunit consists of three heme *c* groups and the third small protein subunit does not contain any redox cofactor, but it is essential for the enzyme integrity. It probably joins two other subunits into one system. Electrons from a substrate are transferred via PQQ to hemes *c* as intrinsic redox moieties and then to an electrode or a mediator. An efficiency of this process can be evaluated assaying the current generated at electrode during electrocatalytic oxidation of ethanol by PQQ-ADH. A number of ethanol biosensors were prepared on a base of modified CB samples to evaluate the effect of various modifications on the efficiency of bioelectrocatalysis of ethanol. Both the maximal oxidation current and stability of sensors were tested and the apparent Michaelis constant ($K_M^{app}$) were calculated (Table 3). Voltammetric waves of ethanol oxidation observed at PQQ-ADH-CBs electrodes showed that this process starts approximately at potential range within 0.02–0.2 V depending on the modified CBs used (data not shown). The applied potential of 0.4 V was chosen to evaluate and compare all CBs samples.

Obtained kinetic parameters of ADH immobilized on modified CB electrodes are presented in Table 3. In contrast to $H_2O_2$ sensors, a clear correlation between the quantity of fine fraction and the ethanol sensors' parameters does not exist, however, in the absence of this fraction, the bioelectrocatalysis either does not occur or is very inefficient. The higher maximal current of electrocatalytic ethanol oxidation was ob-

tained on electrodes made from the modified CB batches C3 and C4. It turns to the mind that the amount of the fine fraction should be in the range between 41 and 70% for most effective operation of the PQQ-ADH-coated carbon electrodes. That could be expected, since the increasing of the fine fraction also increases the area of the carbon electrode and thereby the efficiency of the biosensor (Fig. 2). Low oxidation current of the biosensor based on carbon C7, and possessing very low fine fraction confirms this conclusion. However, we cannot explain quite high oxidation current of the biosensor based on C10, also possessing low level of the fine fraction. Probably, the shape of carbon particles and chemical groups on the surface are also responsible for the effective action of enzyme in the heterogeneous media. Furthermore, both C3 and C4 samples possess the higher values of $K_M^{app}$. The high $K_M^{app}$ values are presumably related with restricted transport of substrate to active center of enzyme due to conformational changes of protein and/or changes in environment surrounding the enzyme. It was found that a background current of the electrode depends inversely proportional on buffer capacity in the case of the C3, C4 and C10 carbon blacks (ordered according the intensity of the effect, data not shown). In the case of the C3 and C4 carbon blacks, this property correlates with the $K_M^{app}$ value and $J_{max}$. The electrodes of higher quality were obtained when the CBs contained basic functional groups in a range within 0.60–0.66 mM/g. Particularly, a higher stabilization (according to inactivation constants $K_{in}$ in Table 3), but unfortunately not higher oxidation currents were achieved when the electrodes were made from CBs containing acidic functional groups from 0.6 to 1.3 mM/g. The morphology of C3 and C4 CBs, which resulted in the highest currents of ethanol biosensors, has several distinguishing features as well. They contain sharp-edged structures clearly visible in the AFM micrographs (Fig. 1(b) and (c)). These edges may be formed as a consequence of surface curvature of graphene layers, by introducing a set of definite structural defects during the modification procedure of CBs [41]. Probably, both a set of surface functional groups and a set of nano-scale carbon structures are essential for the successful operation of PQQ-ADH-coated carbon electrodes.

### *3.4. Bioelectrocatalytic behavior of PQQ-GDH-coated carbon electrodes*

PQQ-depended glucose dehydrogenase applied in this work belongs to the group of membrane-bound glucose oxidizing enzymes [42,43,26]. It is known that these enzymes operate in the periplasm and are responsible for the alternative ways of the cell energy supply system. In contrast to PQQ-ADH, PQQ-GDH does not contain hemes and exhibits totally different electrochemical behavior on unmodified carbon or metal electrodes. This is presumably due to the fact that the active center of PQQ-GDH is buried much more deeper in enzyme globule and cannot directly transfer electrons to the surface of electrode in the absence of soluble mediators [44,45]. As it was reported previously, the reagentless glucose biosensors can be created by immobilization of PQQ-GDH on the carbon electrode modified with bioorganometallic ferrocene derivatives [46], electrochemically deposited heterocyclic compounds [12] or irreversibly adsorbed and electropolymerised napthoquinone or benzodiazepine derivatives [47]. PQQ-GDHs modified with polyarbutin or entrapped into hydrogel polymers containing electrochemically active groups have been also described [44,48]. It should be stressed that all these systems presume an operation of soluble or semi-soluble electrochemical mediator (shuttle) facilitating an electron transfer from the active center of enzyme to the electrode surface. In this work, we have tried to create the similar mediating infrastructure by modification of CBs.

A number of glucose biosensors were prepared on a base of modified CB samples to evaluate the effect of various modifications on the efficiency of bioelectrocatalysis of glucose. The glucose oxidation current as an indicator of process efficiency at the tested electrodes varied from 0 to 300 nA, depending on the used carbon (Table 3). A significant increase in electrocatalytical oxidation current of glucose was observed by using biosensors made from CBs batches C8, C4 and C11. The parameters that affect the operation quality of PQQ-GDH-coated carbon electrodes are: (i) amount of acidic surface functional groups (0.32–0.72 mM/g); (ii) the presence of sharp-edged formations in the morphology of fine fraction (Fig. 1) of modified CBs. Probably, under these conditions PQQ-GDH adsorbs on the carbon-paste electrode in the similar manner, as it is orientated in the hydrophobic cell membrane. Moreover, it is known that the membrane-bound PQQ-GDH from *Esherichia coli* has two ubiquinone-binding sites in addition to a PQQ center [49]. It is highly believable that modification of the carbon structure leads to the creation of aromatic structures interacting with ubiquinone center(s) and capable to play a mediator role in the system PQQ-GDH-electrode surface. Preliminary studies of stability of glucose biosensors based on different modification of CB showed that modification steps are critical for this parameter. The inactivation kinetics of three glucose biosensors (C8, C4 and C11) possessing the highest oxidation currents is presented in Fig. 3.

As it can be seen that the C4 biosensor lost his activity up to 30% during the first 50 h, however, 100% of initial activity remained in the case of the C8 and C11 biosensors after 200 h. The main differences between those three carbon blacks are the following: (i) the C4 CB contains a considerable level of lactonic groups which were detected only in the case of this batch; (ii) the C11 and C8 CBs contain a higher amount of ionogenic groups. This means that variety of functional groups are involved in the immobilization, enzyme stabilization and mediation of the biocatalytic process. Studies on the stability of PQQ-GDH adsorbed on modified CBs electrodes are in progress. The evaluation of the influence of each preparation step on biosensors operation quality could allow improving technologies in biosensorics.

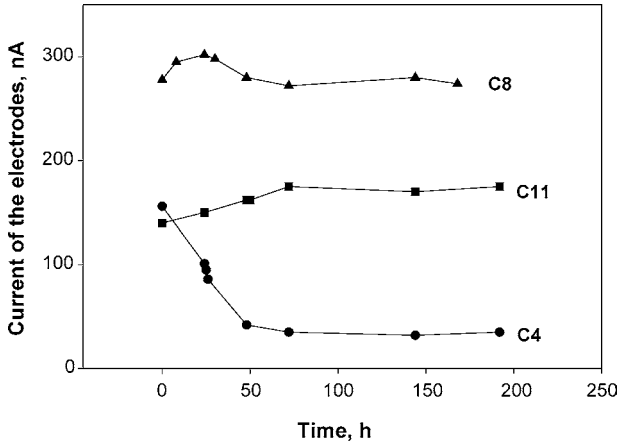


Fig. 3. Stability of glucose sensors made on a base of C8, C4 and C11 carbon blacks. Responses to 5 mM of glucose.

## 4. Conclusions

An electrocatalytic oxidation of $H_2O_2$ at more negative potentials (0.1–0.4 V versus Ag/AgCl) takes place at the modified carbon black electrodes. Moreover, $H_2O_2$ oxidation efficiency depended on the amount and morphology of fine fraction in the modified CBs.

A direct electron transfer from PQQ-dependent and heme-containing ADH to electrode was observed by using modified CBs in screen-printed biosensors.

For the first time, it was shown that PQQ-GDH communicates directly with the carbon electrode surface in the absence of soluble electron transfer mediators.

Reagentless glucose and ethanol biosensors were designed on the base of those modified carbon blacks. A definite set of the surface functional groups as well as the nano-scale carbon structures are essential for the good operation quality of enzyme biosensors.

## Acknowledgement

This work was partially supported by the Lithuanian State Science and Studies Foundation (programme: No. C-02/2004 BIOHEMAS and No. 03048 MODELITA).

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