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Original scientific paper

A new amperometric glucose biosensor based on screen printed carbon electrodes with rhenium(IV) - oxide as a mediator

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Abstract

Rhenium(IV)-oxide, ReO_2, was used as a mediator for carbon paste (CPE) and screen printed carbon (SPCE) electrodes for the catalytic amperometric determination of hydrogen peroxide, whose overpotential for the reduction could be lowered to -0.1 V vs. Ag/AgCl in flow injection analysis (FIA) using phosphate buffer (0.1 M, pH=7.5) as a carrier. For hydrogen peroxide a detection limit (3σ) of 0.8 mg L^{-1} could be obtained.

ReO_2-modified SPCEs were used to design biosensors with a template enzyme, i.e. *glucose oxidase, entrapped in a Nafion membrane. The resulting glucose sensor showed a linear dynamic range up to 200 mg L^{-1} glucose with a detection limit (3σ) of 0.6 mg L^{-1}. The repeatability was 2.1 % RSD (n = 5 measurements), the reproducibility 5.4 % (n = 5 sensors). The sensor could be applied for the determination of glucose in blood serum in good agreement with a reference method.*

Keywords

Biosensor; glucose; hydrogen peroxide; rhenium(iv)-oxide; blood serum

Introduction

Nowadays, sensors and biosensors research, an ever expanding field of analytical chemistry, has been attracting scientists from many related disciplines such as biology, material sciences *etc.* [1]. Chemical sensors and biosensors are offering alternative solutions, capable of satisfying the increasing demand for precise and fast analytical information through devices that require relatively simple instrumentation [2-4].

Electrochemical biosensors often rely on the use of oxidases or dehydrogenases as biological recognition element [5-8]. When using oxidases, hydrogen peroxide is often formed as an electrochemically active intermediate, whose detection is the crucial point of the design of corresponding biosensors.

H_2O_2 can be oxidized or reduced at rather high positive or negative potentials with inherent risk to co-oxidize or co-reduce components of complex matrices, such as blood, urine or other biological fluids or extracts. In order to overcome the problem, mediators are used which react chemically with hydrogen peroxide and reduce high over potentials [6-9].

In many cases transition metal compounds are used which may change easily their oxidation states such as hexacyanoferrates [8-17], platinum metals or their oxides [18], and manganese dioxide [6-9,19-21].

Rhenium shows quite a few oxidation states which could be involved in a reaction cycle where hydrogen peroxide is involved [22]. The main goal of this work was to investigate the electroanalytical behavior of ReO_2 in terms of acting as a catalyst for the determination of hydrogen peroxide as a basis for oxidase - based biosensors. In particular, glucose oxidase was used as a template enzyme.

Experimental

Apparatus

Batch cyclic voltammetry and chronoamperometric measurements were performed using a potentiostat (PalmSens, Electrochemical Sensor Interface) connected to a laptop computer. Carbon paste electrodes CPEs (modified and unmodified) were used as working electrodes. As a reference electrode an Ag/AgCl electrode was used (3M KCl). All potentials referred to in this paper are against this reference electrode. A platinum wire served as a counter electrode**.**

The flow injection system consisted of a high performance liquid chromatographic pump (510 Waters, Milford MA, USA) in connection with a system controller (Waters 600E), a sample injection valve (5020 Rheodyne, Cotati, CA, USA), and a thin layer electrochemical detector (LC 4C, BAS, West Lafayette, Indiana, USA) with a flow through cell (spacer thickness 0.19 mm; CC-5, BAS) in combination with an electrochemical workstation (BAS 100B). SPCE was used as the working electrode and an Ag/AgCl-electrode (3 M KCl) as the reference. The steel back plate of the thin layer cell served as the auxiliary electrode.

Reagents and solutions

Phosphate buffer (0.1 mol L^{-1}) was prepared by mixing aqueous solutions (0.1 mol·L^{-1}) of sodium dihydrogen phosphate and disodium hydrogen phosphate to produce solutions of the required pH (7.5).

A stock solution of hydrogen peroxide (1000 mg L^{-1}) was prepared freshly every day and solutions of lower concentrations were prepared immediately before use.

Also a stock solution of glucose (1000 mg L^{-1}) was prepared with the corresponding working buffer solution, kept at room temperature overnight to facilitate α-ß-mutarotation, and stored at 4 ^{o}C when not in use. Lower concentrations were prepared immediately before use. All chemicals were of analytical purity grade (*p.a.,* Fluka).

Preparation of working electrodes

Carbon paste electrodes (unmodified); 1 g graphite powder and 360 μL paraffin oil were mixed in an agate mortar by gently stirring with a pestle until uniformity and compactness. For modified CPE 50 mg of ReO_2 were added per gram of graphite powder.

Screen printed carbon electrodes (modified): 0.05 g of rhenium(IV)-oxide was added to 1 g carbon ink (Electrodag 421 SS, Acheson); the oxide-ink mixture was stirred for 10-30 minutes with a glass-rod or stainless steel spatula and finally sonicated for 30 minutes in an ultrasonic bath (Transsonic 700/H, Elma®)**.** The resulting mixture was immediately used for electrode fabrication using the semi-automatic screen printer (SP-200, MPM, MA-USA) and aluminum oxide support. The printed electrodes were dried at room temperature overnight before using for measurements.

To prepare enzyme casting solutions, required volumes of ethanol (80 μL), Nafion (5 % w/w in lower alcohols, 40 μL), water (80 μL) and GOD solution (5 % w/w, 40 μL) were mixed in the order listed in a plastic vial (1.5 mL micro centrifuge tubes, 616.201, Greiner Labor Technic). Five μL (unless otherwise specified) of the resulting mixture were directly applied onto the active area of the SPCE surface (≈0.40 cm^2 area) and air dried. The electrode was inserted into the thin layer cell; electric contact was made with a crocodile clamp.

Procedures

Cyclic voltammograms were recorded from an initial potential of 0.80 V to a vertex potential of -1.00 V. The scan rate was 20 mV s^{-1}; usually three cycles were recorded.

Hydrodynamic amperograms were recorded at potentials from -500 mV to 100 mV in increments of 100 mV. Flow injections analyses were done at a potential of -100 mV if not stated otherwise. The flow rate of the pump was 0.40 mL min^{-1} and the injection volume of hydrogen peroxide and glucose solutions was 200 μL.

Analyses of samples

Blood samples were taken manually with a syringe and put in the plastic tubes containing EDTA (1.9 mg per mL of blood) as an anticoagulant. 1 mL of each sample was transferred into a 10 mL sterile PP-tube, diluted 10 times and centrifuged for 20 minutes at 4500 rpm. From the centrifuged supernatant serum the analyte solutions were prepared by diluting 40 and 100 times with phosphate buffer (0.1 M, pH 7.5). Measurements were done in FIA mode using the standard addition method by sequentially adding three times an amount of glucose standard (100 mg L^{-1}) in phosphate buffer solution (0.1 M, pH 7.5), which corresponds roughly to half of the original glucose amount in the sample. After each addition the sample was re-measured. A commercial glucometer Ascensia BRIO was used for glucose reference measurements using whole blood.

Results and discussion

The studies were done with a few working electrodes: unmodified and ReO_2 - modified carbon paste electrodes (CPE) and screen printed electrodes (SPCE) modified with ReO_2 as a mediator and glucose oxidase as a biocomponent (entrapped in a Nafion film).

Studies with Hydrogen peroxide

Cyclic Voltammetry

H_2O_2 is electrochemically active with carbon paste electrodes, but shows high overpotentials for its reduction and also its oxidation as can be seen in Fig. 1. At positive potentials H_2O_2 does not show any particular electrochemical activity at unmodified carbon paste electrodes.

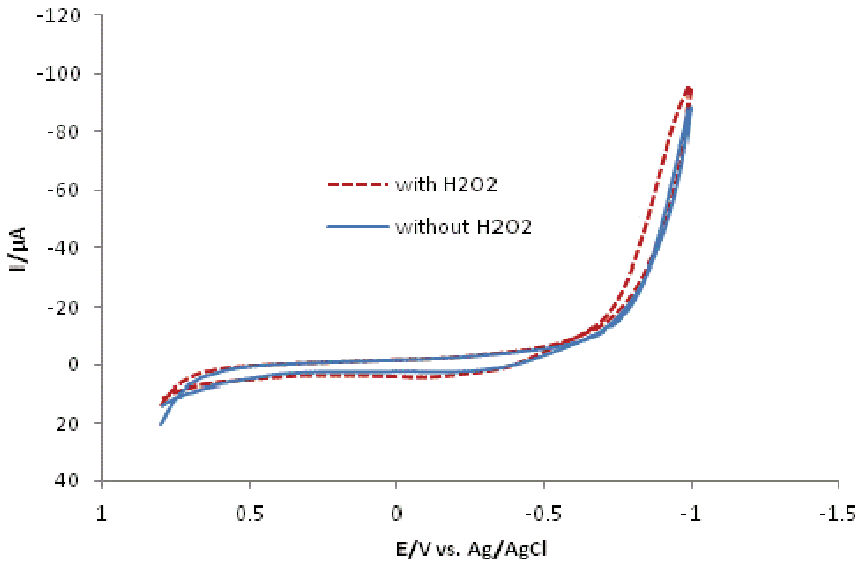

Figure 1. *Cyclic voltammograms of H_2O_2 at an unmodified CPE; phosphate buffer solution 0.1 M, pH 7.5;Fill curve blank, broken curve with 200 mg L^{-1} H_2O_2; conditions:* $E_{initial}$ *= 0.80V,* E_{final} *=-1.00V, scan rate 20 mV* s^{-1}.

At negative potentials, similar behavior can be noticed. Direct reduction of hydrogen peroxide occurs at rather negative potentials only.

According to our knowledge, rhenium (ReO_2) has not yet been used as a modifier for carbon pastes or carbon inks.

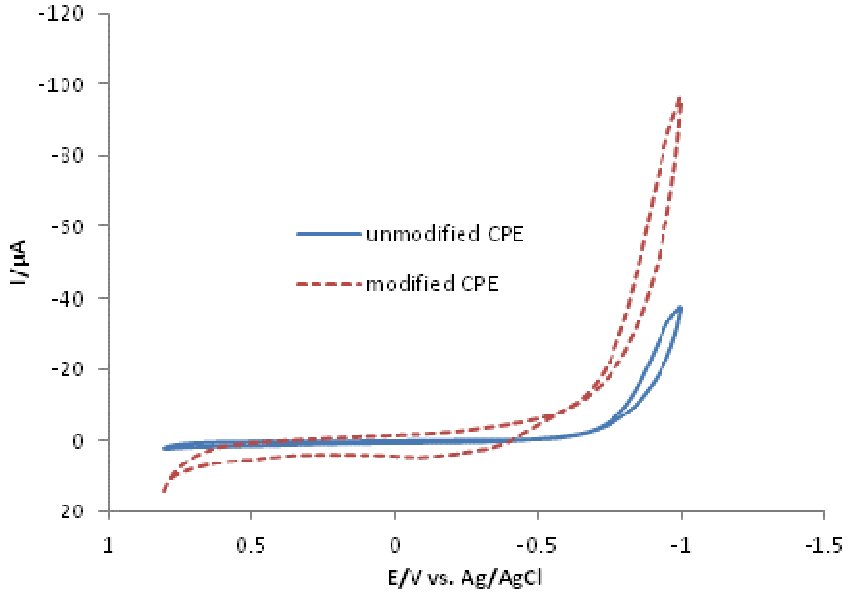

Figure 2*.Cyclic voltammogram of a ReO_2-modified CPE;* $E_{initial}$ *= 0.80 V,* E_{final} *= -1.0 V; scan rate 20 mV* s^{-1}*; support electrolyte 0.1 M phosphate buffer, pH 7.5.*

Figure 2 shows the cyclic voltammogram of a rhenium(IV)-oxide - modified carbon paste electrode. No distinct peaks can be discerned in the investigated potential range. Reduction occurs at positive potentials already and dominates at more negative values. Re-oxidation on the other hand begins at slightly negative potentials already, but in fact does not get dominant even up to 0.7 V. The reduction current can be assigned to the reduction of Re(IV) to Re(III) (probably present as Re_2O_3 in slightly alkaline medium or even as $RePO_4$). Re-oxidation in phosphate buffer solution seems electrochemically rather irreversible because it is much smaller than reduction showing a very broad signal extending from -0.3 V onward.

In the presence of hydrogen peroxide a small additional reduction current occurs in cyclic voltammetry (Fig.3).

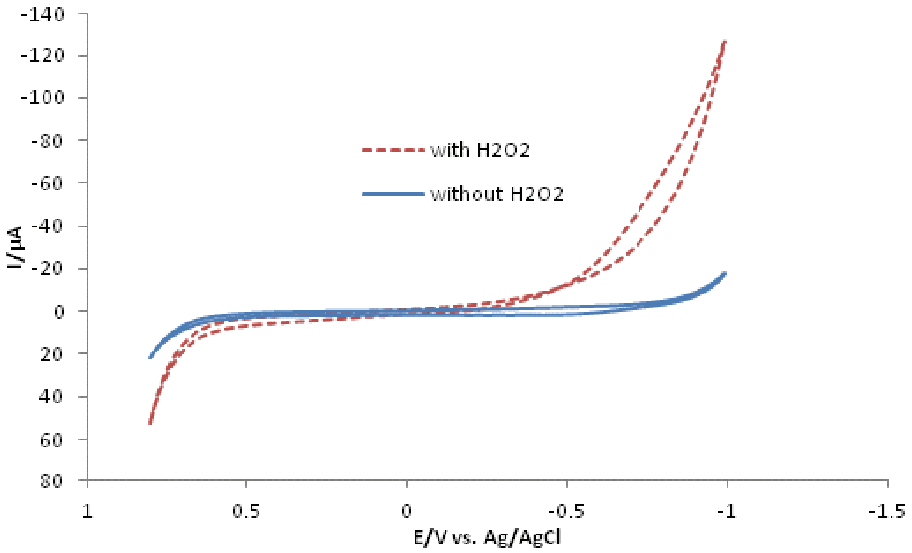

Figure 3 *Cyclic voltammograms of a CPE modified with ReO_2 in the absence and in the presence of hydrogen peroxide; H_2O_2 200 mg L^{-1}; conditions: $E_{initial}$ =0.80V, E_{final} = -1.00V, scan rate 20 mV/s, support electrolyte 0.1 M phosphate buffer, pH 7.5.*

Although the effect of the modifier is small in CV (but more pronounced in amperommetry, see below) it may be noted that in fact a catalytic effect on the reduction of H_2O_2 exists.

Basically two mechanisms can be assumed: rhenium(IV)-oxide (in the oxidation state +4) is reduced to trivalent rhenium (Re_2O_3) which is oxidized to ReO_2 by hydrogen peroxide again (mechanism A, Fig.1). Thus, virtually more modifier (ReO_2) is present at the surface creating a higher reduction current.

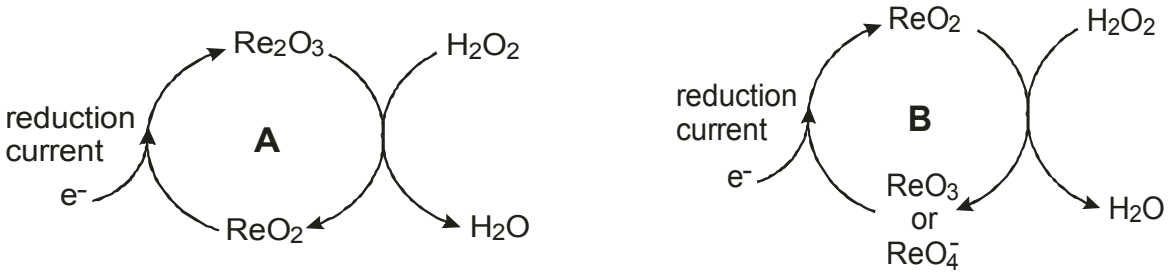

Fig.4. *Models for the catalytic action of ReO_2 on the reduction of H_2O_2; catalytic redox cycle of rhenium(IV)-oxide via trivalent rhenium and the action of H_2O_2 (A); catalytic redox cycle of rhenium(IV)-oxide via hexa- or heptavalent rhenium and the action of H_2O_2 (B).*

On the other hand it is basically also possible that ReO_2 is oxidized to ReO_3 or even perrhenate, ReO_4^-, a reaction which is known to proceed rather slowly in aqueous solution. In this case hydrogen peroxide would react with the original modifier, ReO_2, first, and then the reduction of the Re(VI) or Re(VII)-species would occur (mechanism B, Fig.4). According to the fact that at negative potentials, where catalytic action can be noticed, reduction of ReO_2 is observable at the modified electrode alone, mechanism A seems more probable.

Hydrodynamic Amperommetry

Figure 5 summarizes the hydrodynamic voltamperogram of the catalytic current caused by ReO_2 as a modifier compared with an unmodified CPE. The measerments were performed with stirred solutions as batch experiments, and the current change after addition of hydrogen peroxide was recorded. High signals can be obtained at potentials beyond -400 mV, but at such values the risk of co-reduction of other compounds in complex matrices is also high. Nevertheless, at a potential of -100 mV there are significant current responses observable already, which can be exploited for quantitative analytical purposes. In fact, even higher operation potentials up to +100 mV could be used, but the current is significantly lower compared to the signal at -100 mV. With unmodified electrodes reduction currents of H_2O_2 can be observed under the given experimental conditions only at -400 mV and below (Fig.5 curve b).

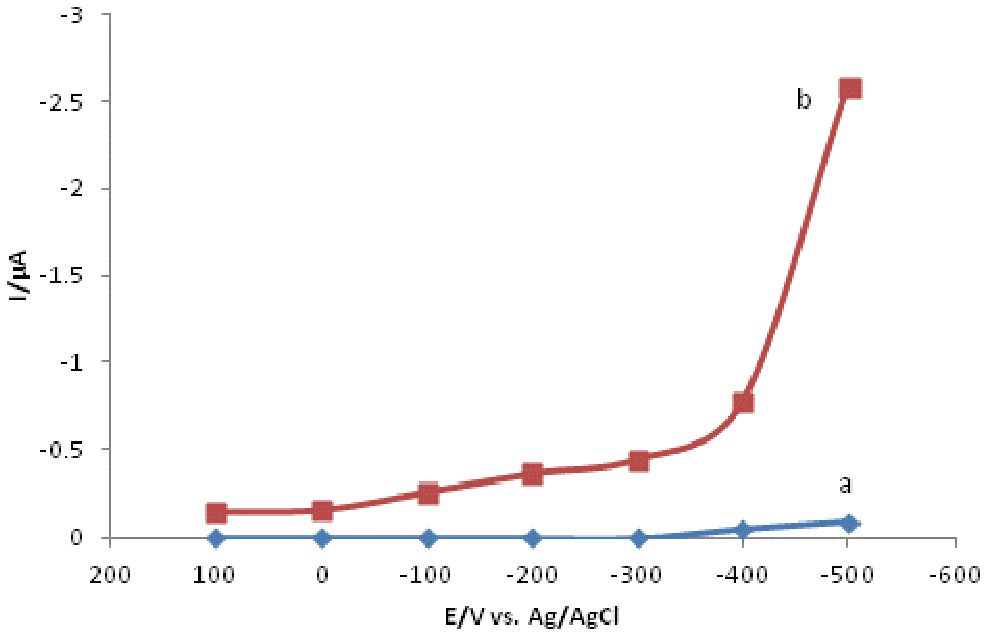

Figure 5. *Reduction current of H_2O_2 in hydrodynamic voltamperometry at a CPE modified with ReO_2 (a) compared to an unmodified CPE (b), supporting electrolyte sodium phosphate buffer (0.1 M, pH 7.5); hydrogen peroxide concentration 50 mg L^{-1}.*

Flow Injection Analysis (FIA)

FIA was used to study the effect of ReO_2 on the reduction of H_2O_2 in detail and to optimize the experimental parameters.

As screen printed carbon electrodes show higher robustness towards mechanical and chemical stress, but are otherwise comparable to carbon paste sensors due to their heterogeneous character concerning the electrode material, this type of electrode was used for further studies. Figure 6 shows a typical amperogram with injection of hydrogen peroxide using a ReO_2-SPCE as a detector.

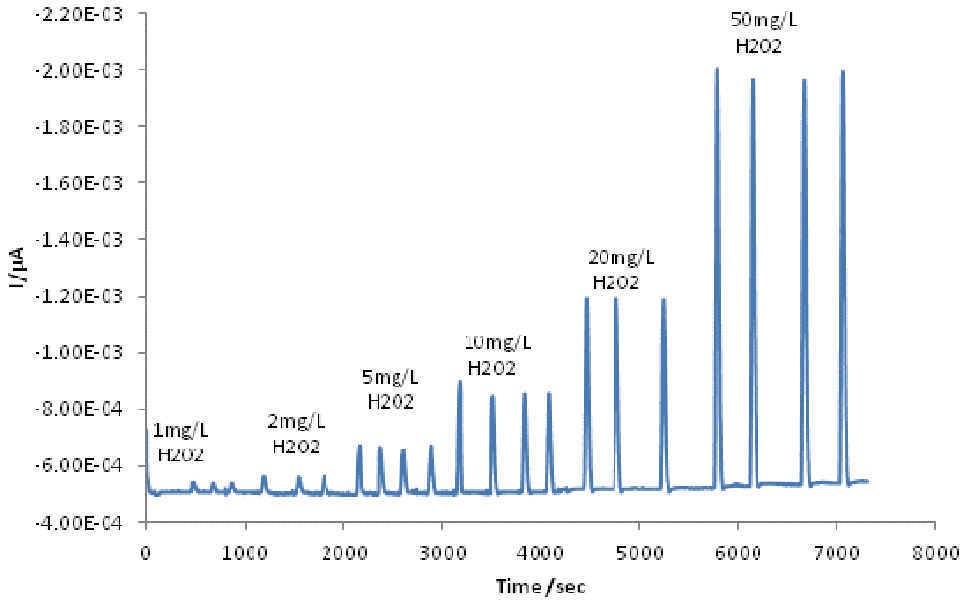

Figure 6. *Amperogram obtained by FIA with a SPCE modified with ReO_2; injection volume of H_2O_2 solution 200 µL; operating potential -100 mV; flow rate 0.4 mL min^{-1}; carrier phosphate buffer (0.1 M, pH 7.5).*

The rhenium(IV)-oxide modified electrode responds very well and reproducible to hydrogen peroxide concentrations even in the low mg L^{-1}-range.

For optimization of the operating potential a hydrodynamic voltammperogram was recorded under FIA conditions (Fig.7).

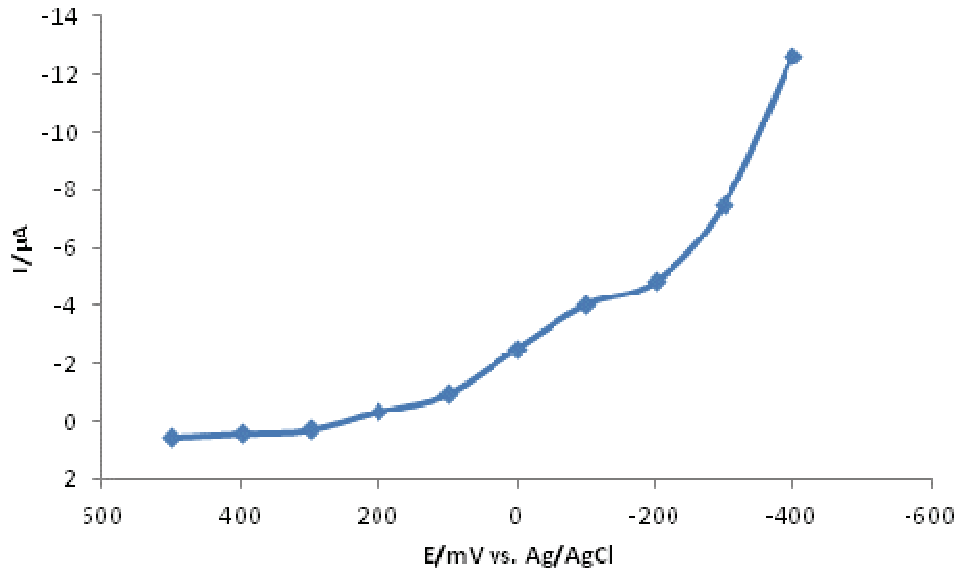

Figure 7. *Dependence of the peak height of H_2O_2 on the working potential in FIA with SPCEs modified with ReO_2 as detector, flow rate 0.4 mL min^{-1}, carrier 0.1 M phosphate buffer, pH 7.5, H_2O_2-solution 100 mg·L^{-1}; injection volume 200 µL.*

Reduction currents can be observed up to even 200 mV. When decreasing the potential to more negative values the signal (peak height of the reduction current) increases. Apart from the reasons discussed already (increased risk of interference with increasing negative potentials) there is another disadvantage connected with highly negative operating potentials. The background

current strongly increases below -200 mV (not show) and even tends to vary during operation that the repeatability is significantly deteriorated. The signal-to-background ratio gets unfavorably low because the latter exceeds even the height of the signal. For these reasons -100 mV was chosen as the most favorable operating potential.

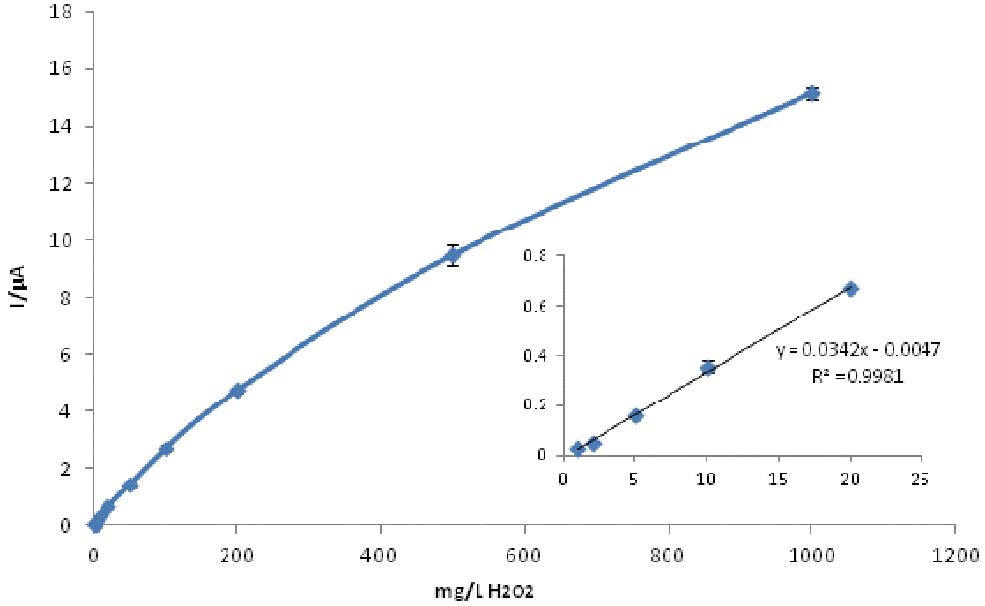

Figure 8. *Calibration curve for H_2O_2 within concentration 1-1000 mg·L^{-1} at modified SPCE at -100 mV. Supporting electrolyte phosphate buffer 0.1 M, pH = 7.5.*

A corresponding calibration curve for hydrogen peroxide is displayed in Fig. 8. The linear dynamic range of the sensor (concentration range with linear relation between signal and concentration) is restricted to the lower concentrations of the analyte. Linearity between concentration of hydrogen peroxide and signal exists from 0.6 to 20 mg L^{-1}, with a correlation coefficient over 0.99.

The detection limit (3σ), estimated from the standard deviation of FIA-peaks at 5 mg L^{-1} H_2O_2, is 0.2 mg L^{-1}.

The repeatability of measurements for 100 mg L^{-1} is 3 % RSD (n = 5 measurements), and the reproducibility for 100 mg L^{-1} at different working electrodes (n = 5 electrodes) is 5.7 %.

Studies with glucose

In order to check if the ReO_2 modified electrode may serve as a basis for the design of first generation- biosensors with oxidases producing H_2O_2, glucose oxidase was used as a template enzyme. It was immobilized on the surface of the ReO_2-modified SPCEs using a Nafion membrane [23]. The sensor responds well to injections of glucose under FIA conditions.

A typical calibration curve of the biosensor is shown is shown in Figure 9.

The range with a linear relation between current and concentration of glucose was found to extend up to 100 mg L^{-1} (Fig. 9 insert) with a correlation factor R^2=0.9999. The linear regression function is given in eqn. (1).

$$i \,/\, \text{nA} = 0.4867\, c \,/\, \text{mg L}^{-1} + 2.4184 \qquad (1)$$

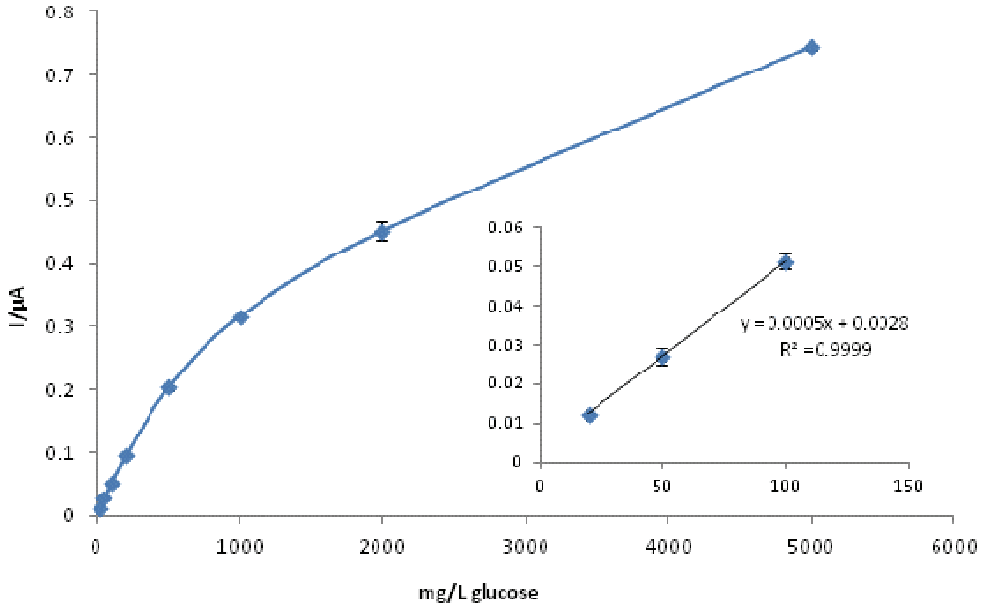

Figure 9. *Calibration of glucose with a glucose oxidase/ReO_2-modified SPCE; working potential: -100 mV; flow rate: 0.4 mL/min; 100 µL injection volume.*

The detection limit (3σ) estimated from the standard deviation of FIA-peaks for 20 mg L^{-1} glucose concentration is 0.6 mg L^{-1}. The repeatability of measurements for 200 mg L^{-1} glucose was 2.1 % RSD (n = 5 measurements), and the reproducibility was 5.4 % RSD (n = 5 electrodes).

Table 1. *Comparison of limit of detection of different used modifiers*

Modifiers	Detection limit of H_2O_2 mg L^{-1}	Detection limit of glucose mg L^{-1}	References
IrO_2	0.24	0.9	[26]
Fe_3O_4	0.2	0.5	[23]
MnO_2	0.045	0.087	[24]
SnO_2	15	6.8	[27]
PtO_2	0.03	2.0	[26]
CuO	-	0.03	[23, 25]
ReO_2	0.2	0.6	this work
PdO	0.8	0.83	[26]

Table 1 compares the LOD values for sensors and biosensors modified with different metal oxides for the detection of H_2O_2 and glucose. It may be stated that ReO_2 is capable of detecting very low concentrations of hydrogen peroxide and glucose; it is comparable to Fe_3O_4 [23] and super cedes platinum metal oxide [26].

Interferences

Four common interferences, vitamin C, paracetamol, gentisic acid, and uric acid were tested if they interfere with the detection of glucose using the ReO_2-based biosensor. All investigated interferents give significant responses in the investigated concentration ratio. Nevertheless, their concentration in blood may be expected to be much smaller so that the extent of influence on the signal of the analyte, glucose, is expected to be negligible (Table 2).

Table 2. *Relative signals of possible interferences in the current response of the biosensor in the absence of glucose; 100 % corresponds to the current of 200 mg L^{-1} glucose. Flow rate: 0.40 mL min^{-1}, operating potential -100 mV* vs. *Ag/AgCl; carrier: phosphate buffer (pH=7.5; 0.1 M), injection volume 100 µL*

Interferent	50 mg L^{-1}	100 mg L^{-1}	500 mg L^{-1}
	%	%	%
Ascorbic acid	46.1	95.6	304
Uric acid	38.0	45.4	166.0
Paracetamol	21.8	21.5	34.7
Gentisic acid	19.2	26.7	61.6

Analysis of blood plasma samples

The glucose biosensor described in this work was used to determine the glucose level in human serum. Two samples (ST1, SA1) were investigated. Analyses were done with the standard addition method basically for two reasons: first to exclude effects of the matrix and interferences, and second to investigate if the linear relation between signal and different concentrations of glucose in the plasma is maintained.

Glucose concentrations obtained with the biosensor (blood plasma) were multiplied with a factor of 1.15 in order to convert them to whole blood concentrations [28, 29].

The results between the method employing the new biosensor and the reference are in very good agreement (Table 3). Sample SA1 is blood from a healthy person in the morning, sample ST1 from the same person after eating a piece of sweet cake.

Table 3 *Comparison of glucose concentrations of two human serum samples measured using the new biosensor and the pocket Glucometer Ascensia Brio.*

Sample	ReO_2 –based biosensor	Glucometer Ascensia Brio
SA1	993 ± 26 mg L^{-1}	980 ± 10 mg L^{-1} mL
ST1	1396 ± 32 mg L^{-1}	1360 ± 20 mg L^{-1} mL

Conclusion

The work presented here has clearly demonstrated that heterogeneous carbon sensors (carbon paste, screen printed carbon electrodes) with rhenium(IV)-oxide as a mediator exhibit good performance for the determination of hydrogen peroxide because the modifier lowers the overpotential of the analyte.

A biosensor based on thick film technology with glucose oxidase as a template enzyme was developed. When using the screen-printed biosensor with flow injection analysis using phosphate buffer (0.1 M, pH 7.5) as a carrier with a flow rate of 0.4 mL min^{-1}, a detection limit of 0.2 mg L^{-1} glucose could be achieved with a linearity range up to 20 mg L^{-1}. Thus, glucose can be determined in blood with this method.

The influence of possible interferences (ascorbic acid, uric acid, paracetamol, and gentisic acid) on the determination of glucose was estimated. The extent of all investigated interferences is not fatal for the determination of glucose in human blood plasma.

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