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# A New Biosensor for Glucose Based on Carbon Paste and Enzyme Immobilized onto the Polyaniline Film

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Conductive polymer polyaniline (PANI) was electrochemically synthesized by controlled potential cycling as a film at the surface of a carbon paste electrode (CPE) from acidic aqueous solution containing 1 M HCl and 0.25 M aniline. The characterization of the PANI-CPE was carried out with the aid of cyclic voltammetry in 1 M HCl, obtaining three couple of peaks from which the two pairs were distinct, having proved the electroactivity of the PANI film. Then, the PANI-CPE system was for the first time used and tested as glucose biosensor, when the respective enzyme was potentiostatically immobilized onto the surface from 0.1 M phosphate buffer (pH 7.5) with 0.75 mg/mL GOD. Optimal conditions for the enzyme immobilization were investigated and the biosensor tested for glucose sensing within the concentration range of 1-500 mgL-1 D-glucose. All preliminary assays with the GOD/PANI-CPE configuration have shown favorable operational features of this new carbon paste biosensor whose further development and adaptation for flowing streams are also outlined.

**Keywords:** Biosensor, carbon paste, polyaniline film, glucose oxidase, glucose

## 1. INTRODUCTION

During recent years, electro-conducting polymers have been extensively investigated. One of the fields attracting considerable attention is the (bio)sensor preparation [1]. Among the most studied polymeric materials, one can present the polyaniline (PANI), a remarkably conducting material with challenging electrochemical and optical properties [2] widely applicable in configurations of electrochemical biosensors (see e.g. review article [3] or even some newer papers [4-8]), secondary batteries [9], special gas sensors [10], and some anti-corrosive coatings [11,12].

Preparation of PANI by polymerization of the aniline monomer can be made either electrochemically or chemically; usually, in a simple and inexpensive way. Electrochemical polymerization involves different approaches performed under galvanostatic [13], potentiostatic [14], or the most preferred potentiodynamic conditions [15,16]. The most significant advantage of electrochemically prepared PANI is that the polymer is deposited directly on the electrode surface, when its thickness and properties can be controlled via the quantity of electrical charges consumed during polymerization [17]. Moreover, PANI provides a very suitable environment for enzyme immobilization.

In this way, carbon paste electrode (CPE) has been shown a convenient type of working electrode / substrate for such electropolymerization and many studies regarding electropolymerization on the surface of CPE were done. Among frequently and repeatedly studied polymers, one can assign various polyaniline derivates: poly(N-methylaniline) [18,19,20,21], poly(N,N-dimethylaniline) [22], and poly(2,5-dimethylaniline) [23]. Furthermore, polyglycine [24] or poly Eriochrome Black T [25] modified CPE could also be used for parallel detection of dopamine and ascorbic acid. For the determination of folic acid in biological samples or pharmaceutical preparations, a poly( -anisidine) film-coated CPE has been proposed [26]. In other studies, poly(o-aminophenol) [27,28], poly(m-toluidine) [29] or poly(1,5-diaminonaphthalene) [30] films were also used in combination with CPEs and utilizing the Ni(II) / Ni(III) redox system in order to enhance the electrocatalytic oxidation of methanol or formaldehyde, respectively. Polyaniline its self has been electrochemically prepared at different electrodes materials such as platinum, glassy carbon, carbon fiber micro electrodes [31], indium tin oxide [32] and also on the surface of lately popular nanoparticles [33,34]. As stated above, the polymeric layer should represent a suitable environment for the effective enzyme immobilization. In this respect, a CPE-based biosensor was prepared coated with the electropolymerized layer of poly(o-phenylendiamine) and hosting the immobilized chloro-peroxidase for amperometric detection of 2,4,6-trichlorophenol [35].

In the biosensor configurations, glucose oxidase (GOD) is undoubtedly one of the most frequently used enzymes thanks to its wide availability, inexpensiveness, as well as a good stability. Its enzymatic activity is well-known [36,37], giving rise to hydrogen peroxide which can further be reduced or oxidized; both pathways being used for the detection in the amperometric mode. Anyway also non-enzymatic electrochemical glucose sensors are well known [38]. Most importantly, GOD acts principally in the determination of glucose, enabling to monitor the glucose level in whole blood of the patients with Diabetes Mellitus. Otherwise, glucose biosensors can also be used for the determination of glucose in food or agricultural products.

In this paper, the fabrication and characterization of a new type of polyaniline biosensor for glucose is presented employing specifically incorporated GOD. A novelty is the use of the carbon paste substrate in combination with electro-polymerized aniline, as the housing matrix of choice for the GOD immobilization.

## 2. MATERIAL AND METHODS

### *2.1. Chemicals*

Analytical grade aniline and glucose oxidase were purchased from Aldrich. HCl (32% m/m); $NaH_2PO_4 \cdot H_2O$; $NaH_2PO_4 \cdot 2H_2O$ and D(+) glucose were purchased from Merck. Water used throughout the experimental work was doubly distilled and finally purified in a cartridge system (Nanopure, Barnstead).

### *2.2. Instrumentation*

The working electrode was prepared by thoroughly hand-mixing of 0.5 g spectroscopic graphite powder (RW-B, Ringsdorff, Germany) with 0.3 mL highly viscous silicone oil (SO, type: LUKOIL MV 8000; Lučební závody Kolín, Czech Republic). After homogenization, the mixture prepared was packed into the piston-driven carbon paste electrode holder (with a surface diameter of 3 mm) designed in our laboratories [39] The reference electrode was Ag/AgCl (3M KCl); a Pt-wire serving as the counter.

### *2.3. Electropolymerization of Aniline*

Electropolymerization of the PANI film was performed by potential cycling in the regime cyclic voltammetry using an electrochemical workstation (model "BAS 100B"; Bioanalytical Systems, USA). (Hydro)amperometric experiments were carried out using a portable potentiostat / galvanostat (PalmSens, The Netherlands).

In the proper procedure, the surface of CPE was first freshly renewed by smoothing with a wet filter paper, the electrode then placed into a solution containing 0.2 M aniline in 1M HCl and exposed to a potential cycling (n = 10) between -400 and +1200 mV vs. ref. at a scan rate of 100 mV $s^{-1}$. After polymerization, the electrode was rinsed with water and placed into a 0.1 M phosphate buffer (pH 7.5), where a potential of -500 mV was applied to remove the reaction products from the film. The resultant configuration, the CPE with polyaniline film ("PANI-CPE"), could then be used for direct GOD immobilization.

### *2.4. Immobilization of Glucose Oxidase*

The PANI-CPE was placed into 0.1 M phosphate buffer (pH 7.5) containing 0.75 mg $mL^{-1}$ glucose oxidase. Afterwards, the film was oxidized at the potential chosen (always within 400–1000 mV) for a given period (in an interval of 5–60 minutes) and GOD potentiostatically attached. Again, the freshly prepared GOD/PANI-CPE electrode was carefully rinsed with distilled water to remove non-attached enzyme.

## 3. RESULTS AND DISCUSSION

### *3.1. Electrochemical Synthesis of PANI at CPE*

A typical cyclic voltammogram of the formation of PANI at a CPE is shown in Fig. 1. Three subsequent responses with the parent peaks can be observed in the potential range studied, indicating the existence of the respective redox couples. And, at the same, the replicate scans show that the electrode oxidation of aniline occurs at slightly higher potentials because of deposition of the polymer onto the surface of CPE support. According to the previously reported mechanism behind this process, the first peak appears at a potential of ca. +220 mV, representing the oxidation of leucoemeraldine into emeraldine [8,40]. This corresponds to the oxidation from the fully reduced form into the partly oxidized polyaniline. An anodic peak occurring at approx. +800 mV then corresponds to the transformation of emeraldine into perningraniline – the fully oxidized form of polyaniline. Finally, a couple of small waves between the two evident peaks can be attributed either to p-benzoquinone [16,41] or to some other degradation products [42,43].

In order to prove that the PANI film is electroactive and the electron transfer takes place across the polymer chain, a new series of cyclic voltammograms was recorded at different scan rates (from 10 to 200 mV $s^{-1}$) in a solution of 1M HCl. It was found that the oxidation peaks were shifted towards the higher potentials with the increased scan rate, whereas the reduction peaks moved towards the lower potentials. And the respective "IP vs. $v^{2}$" ($mV^{2}.s^{-2}$) dependence resulted in a straight line, revealing the surface-confined process.

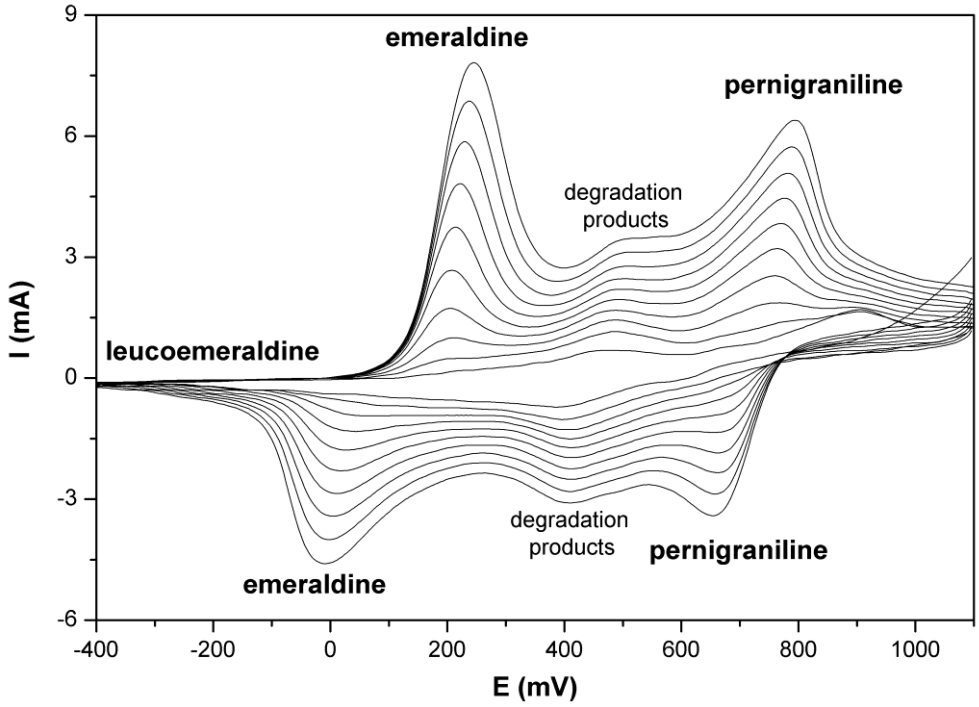


**Figure1.** Electrochemical synthesis of PANI. Experimental conditions: 0.2M aniline monomer in 1MHCl. Cyclic voltammetry; potential limits: $E_{low}$ = -400 mV; $E_{high}$ = +1200 mV, initial potential,$E_{init}$ = +1200 mV; scan rate, v = 100 mV s-1; number of cycles, n = 10.

*3.2. Preparation of PANI-CPE Based Biosensor*

Glucose oxidase was immobilized potentiostatically as described in Experimental, when the immobilization potential and time had to be optimized. The effect of potential was investigated in the range from +400 mV to +1000 mV and the result is illustrated in Fig. 2. The graph shows a maximum at around +700 mV, indicating that the potential value of +700 mV is the most effective for immobilization of GOD on the PANI-CPE surface under the experimental conditions chosen.

Fig. 3 shows the dependence of the immobilization time on the current response of glucose. As seen, the maximal efficiency was reached after ca. 30 min. of continuously applied potential previously found as the optimum. Since longer periods did not result in any further improvement of the current response, it could be concluded that the CPE substrate exhibited a limited binding capacity for the enzyme whose extensive immobilization had already become contra-productive, blocking the active sites at the electrode surface.

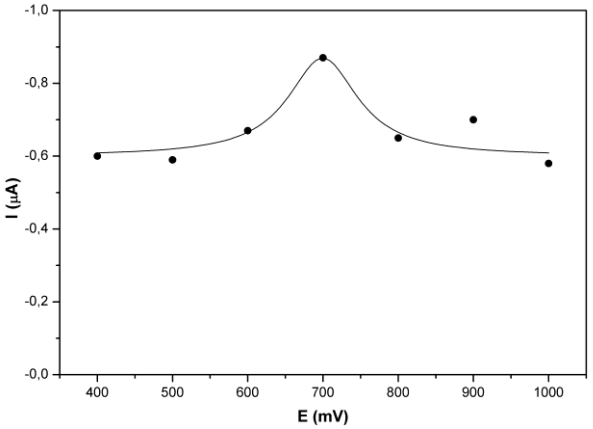


**Figure 2.** Effect of applied potential on the GOD immobilization. Experimental conditions: GOD immobilization conditions: Eimb = +400 to +1000 mV vs. ref., $t_{imb}$ = 30 min; c(GOD) = 0.75 mg $mL^{-1}$ in phosphate buffer (pH 7.5); Test solution: phosphate buffer (pH 7.5), c(GLU) = 100 mg $L^{-1}$; detection potential, $E_{det}$ = -300 mV.

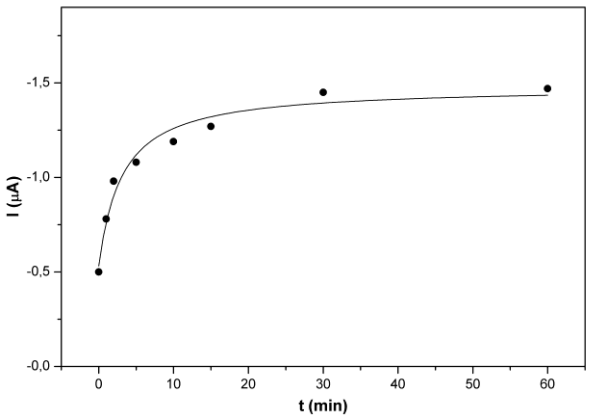


**Figure 3.** Effect of time on the GOD immobilization. Experimental conditions: Eimb = +700 mV; $t_{imb}$ = 0-60 min. $E_{det}$ = -400 mV. For other conditions, see Fig.2 and legend.

*3.3. Electrochemical Characterization of the GOD/PANI- CPE Biosensor*

At first, the amperometric response was studied by using hydrodynamic amperometry (HA) for which the operational potential is a crucial parameter. Fig.4 shows the current response in dependence of the potential applied, illustrating that the current has rapidly decreased with the increasing potential. Also, as found for potentials higher than -100 mV, no significant current response was observed. Despite the fact that applications of more negative potentials gave rise to somewhat higher current responses, useful operational potentials for the glucose sensing seemed to be between -200 and -400 mV; the upper limit being chosen for further studies. (The reason was that, in the case of lower potentials, the HA curves were less reproducible in consequence of higher background and a noise.)

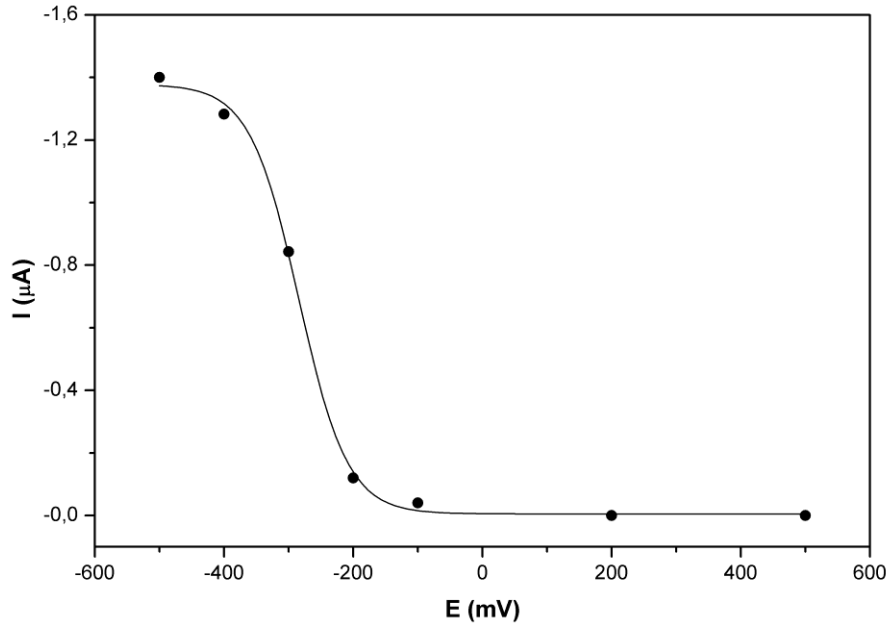


**Figure 4.** Effect of potential on the CPE/PANI/GOD sensor response. Experimental conditions: $E^{det}$ = -500 to +500 mV. For other conditions, see Fig.2.

As another important experimental parameter, the optimal pH value for the measurements with the GOD/PANI-CPE biosensor was sought when considering that the target sample could also be the whole blood with physiological pH. In contrast to the previous reports thatthe electroactivity of the PANI film decreases at higher pHs (see e.g. [44]), the PANI film in combination with the GOD (both housed in the CPE substrate) has exhibited the optimal function in non-acidic solutions, as depicted in Fig. 5 and by the respective relationship between the current response to glucose in dependence of pH. As can be seen, the highest signal was achieved in a phosphate buffer (pH 7.5), which corresponds to the above-mentioned preferential choice of pH for sensing of biological substances. Rather unexpected activity of the GOD/PANI system at neutral pH is a new observation requiring the continuing investigations; nevertheless, it can already be stated that this behavior may further expand the applicability of such sensors in analysis under physiological conditions.

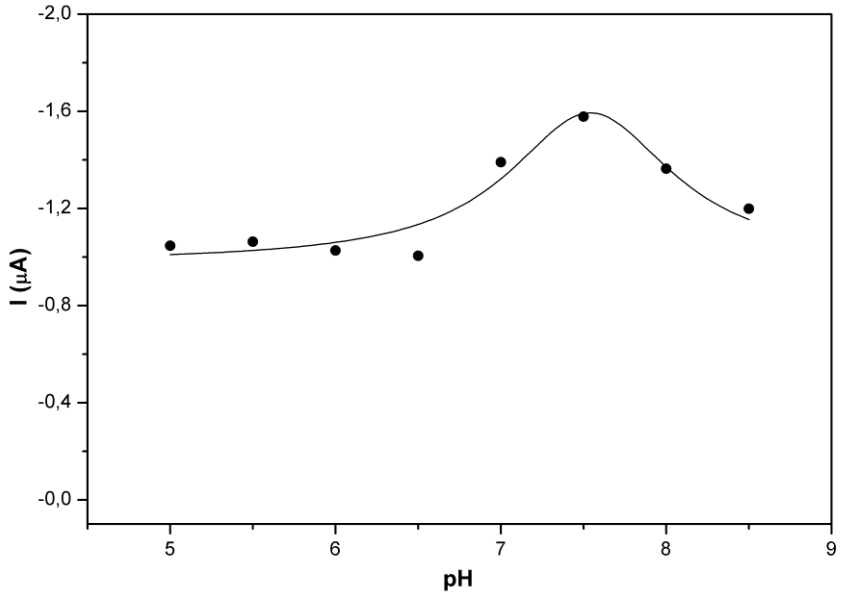


**Figure 5.** Effect of pH on the CPE / PANI / GOD sensor response. Experimental conditions: $E_{det}$ = -400 mV; a set of phosphate buffers (pH 5.0 – 8.5). For other conditions, see Fig.2.

Finally, a series of calibration experiments had been carried out, when the current response of the GOD/PANI-CPE was studied over a wide concentration range of 1-500 mg $L^{-1}$ D-glucose.

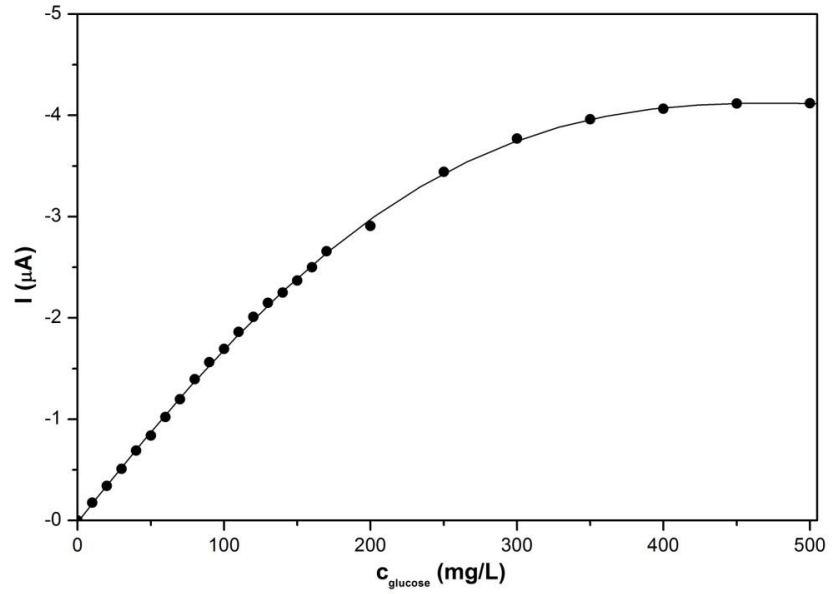


**Figure 6.** Relation between current response and glucose concentration. Experimental conditions: c(GLU) = 1-500 ppm; Edet = -400 mV; phosphate buffer (pH 7.5). For other conditions, see Fig.2.

As shown in Fig. 6, the linear dependence could be observed in the range from 1 to 200 mg $L^{-1}$, indicating that the principal enzymatic reaction was of the first order. (At higher glucose

concentrations – namely, between 200–400 mg $L^{-1}$, the current response started to deviate from linearity remaining constant above 450mg $L^{-1}$ due to saturation at the electrode surface.) By averaging of 10 replicates ($s_{n-1}$ = 9.8 nA, RSD = ±5.6%), the sensor of interest was able to detect about 1.8 mg $L^{-1}$ D-glucose with the corresponding limit of quantification (LOQ; 3σ) of about 6.5 mg $L^{-1}$.

Regarding the actual GOD/PANI-CPE configuration, it employs the piston-driven CPE holder devised mainly for batch experiments [34] and due to this, initial tests had to be carried out in the HA mode which might suffer from somewhat higher background. A possible rearrangement of the GOD/PANI-CP biosensor into a design suitable for FIA is now intensively examined in combination with recently proposed "groove electrode" (GrE [45]). (The latter is a planar and miniaturized construction of a CPE handled and operated in similar way like machine-manufactured screen-printed electrodes, SPEs, designed predominantly for measurements in flowing streams.)

## 4. CONCLUSIONS

In previous sections, a new type of carbon paste-based biosensor for glucose sensing has been proposed having combined for the first time the advantages of electrodeposited polyaniline film with unique properties of carbon paste matrix for hosting the natural enzyme; in this case, glucose oxidase. The actual configuration of the GOD/PANI-CPE, tested herein in the hydrodynamic amperometric mode, has been subjected to the basic characterization and optimization of key experimental parameters in an effort to define typical operational conditions, such as linearity within the concentration range of 1-200 mg $L^{-1}$ D-glucose and a LOQ at the low ppm level. Apparently one of the most interesting features of the CP-biosensor developed is the dual function of the PANI film, providing suitable conditions for the effective, one-step enzyme immobilization (i.e., without use of a cross-linking agent) and, at the same, acting as a mediator. In addition, this utility could be accomplished under hitherto non-recommended conditions of neutral pH [44], being typical for analyses in physiological solutions. And if some enzymatic system would require even more effective mediating, it can be devised as well – via the bulk-modification of carbon paste underneath the film with enzyme [45]. Last but least, as quoted at the end of the Results & Discussion part, the continuing work on the GOD/PANI-CPE is now focused on possible rearrangement into the GOD/PANI-GrE and GOD/PANI-SPE analogues used as detectors for FIA and related measurements.

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