

Recurrent potential pulse technique for improvement of glucose sensing ability of 3D polypyrrole

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Abstract

In this work, a new approach for using a 3D polypyrrole (PPy) conducting polymer as a sensing material for glucose detection is proposed. Polypyrrole is electrochemically polymerized on a platinum screen-printed electrode in an aqueous solution of lithium perchlorate and pyrrole. PPy exhibits a high electroactive surface area and high electrochemical stability, which results in it having excellent electrocatalytic properties. The studies show that using the recurrent potential pulse technique results in an increase in PPy sensing stability, compared to the amperometric approach. This is due to the fact that the technique, under certain parameters, allows the PPy redox properties to be fully utilized, whilst preventing its anodic degradation. Because of this, the 3D PPy presented here has become a very good candidate as a sensing material for glucose detection, and can work without any additional dopants, mediators or enzymes.

Keywords: glucose sensor, electronic materials, conducting polymers, polypyrrole, high electroactive surface area, electropolymerization

1. Introduction

Nowadays, the rapid and accurate monitoring of glucose is a fundamental concern in different areas such as the environment, biotechnology, and especially medicine [1–3]. Diabetics are one of the largest groups of patients in the world, and require constant control of their sugar level [2, 3]. The International Diabetes Federation reported that approximately 387 million people worldwide suffer from diabetes [4, 5]. Therefore, there is a need to provide an efficient way of detecting glucose.

Different techniques have been proposed in order to monitor glucose changes such as spectrometry, fluorescence [6], as well as optical [4, 7] and electrochemical [4, 6, 8] methods. The most commonly used are electrochemical sensors because of their practicability, simplicity and low cost

[2, 9], and they can be divided into enzymatic and non-enzymatic types of sensors [10]. In the enzymatic approach, glucose is oxidized to gluconolactone in the presence of a specific glucose enzyme named glucose oxidase (GO_x) [2]. This enzyme converts glucose into gluconolactone under reduction of the flavine adenine dinucleotide (FAD) prosthetic group. The cofactor is re-oxidized in the second reaction step, whereby two electrons and two protons are transferred to molecular oxygen, yielding hydrogen peroxide [2, 11]. It was reported that sensors based on this process exhibit a high glucose response and good specificity of glucose detection [4, 12, 13]. However, they also revealed instability, difficulties in the immobilization process of the enzyme to the electrode [4] and fast degradation of GO_x , which continuously needs to be constrained to pH ranges of 2–8, temperatures below 44 °C and ambient humidity levels [5]. Because of this, recent

studies have focused on the investigation of glucose biosensors based on the non-enzymatic approach [5, 14]. In the non-enzymatic type of detection, glucose is directly oxidized to gluconolactone at the electrode surface [2, 4]. However, this process varies considerably depending on the electrode material used [5]. The first sensor based on the non-enzymatic approach was investigated by Walther Loeb, who oxidised glucose electrochemically in sulphuric acid at a lead anode [5]. However, it was noticed that the kinetic of glucose oxidation at many 'bare' electrodes is very slow and produces no discernible faradic current at most commercial electrodes. This has excluded such kinds of 'bare' electrodes from use in practical applications [5]. As a result, recent studies have focused on the appropriate synthesis of nano-/microstructured materials for the non-enzymatic type of glucose detection [5, 14]. The surface of such porous materials is characterized by a high surface to volume ratio, which results in their very high electrochemical activity. The latter property is desirable for a surface-bound reaction such as glucose oxidation [5]. Therefore, in order to provide effective electrocatalytic activity in a sensor, the material used for such a purpose should be characterized by a high surface area [4, 15].

Recently, conducting polymer-based sensors have attracted the attention of many researchers from different fields of science [16, 17]. In addition to their use as electronic conductors [18], they have also been utilized as active sensing elements by coupling ligands to the backbone [19]. Especially promising among conducting polymers is polypyrrole (PPy) [20–22]. PPy exhibits very good adhesion to the substrate [18] and features high biocompatibility [22]. In addition to its use in biosensor applications, it has been investigated for many other technological applications, such as in microelectronics, electrochromic windows [20], organic lightweight batteries [23] or gas sensors [24].

This type of polymer can be synthesized electrochemically [24] or chemically [25]. However, due to the simplicity of preparation and possibility of controlling the PPy properties (for example thickness, micro/nanostructured morphology, electroactivity, conductivity), the electrochemical technique is the most frequently employed [24, 26]. It was shown that depending on the deposition conditions applied during electropolymerization, different structures and surface areas of the polymer film can be obtained [27].

Nano-/microstructured conducting polymers have many additional advantages over conventional polymers, including a high surface area and low density, along with special chemical and physical properties [28, 29]. Because of such advantages, conducting polymers were found to have superior performance relative to conventional materials, and therefore, they started to be used as a material for glucose biosensors [15]. It was reported that highly porous conducting polymers, such as nanowires, nanotubes, and microstructured polymers, enhance the sensitivity [28], speed and versatility of biosensors used in diagnostics [1]. Yang *et al* [6] proposed an amperometric, non-enzymatic glucose sensor based on over-oxidized PPy modified with nickel nanoflakes. Such a sensor exhibited a sensitivity of $1049.2 \mu\text{A}\cdot\text{mM}^{-1}\cdot\text{cm}^{-2}$ and a detection range of 0.001–4.863 mM. In comparison, the sensitivity of a

glucose sensor based on platinum nanoparticles and graphene oxide hydrogel was reported to be $137.4 \mu\text{A}\cdot\text{mM}^{-1}\cdot\text{cm}^{-2}$ [30]. Excellent electroanalytical properties were also revealed by the polypyrrole-poly(3,4-ethylenedioxythiophene)-Ag for electrochemical DNA sensing [28], PEDOT-PSS as a voltammetric sensor for the determination of caffeic acid [31] or PEDOT-Palladium particles in a voltammetric sensor for hydrogen peroxide [32].

Many studies have also been performed in the field of enzymatic glucose sensors, in which conducting polymers can also act as a matrix for enzyme immobilization [13, 33–35]. There is a lack of reports in the literature related to non-enzymatic glucose biosensors based on conducting polymer materials.

It should be noted that in most studies, the use of conducting polymers as a sensing material needs the incorporation of additional additives such as mediators, metallic nanoparticles or enzymes. This is related to the fact that conducting polymers reveal poor sensing stability because of their anodic degradation (over-oxidation). The over-oxidized polymer irreversibly loses its unique properties, such as electrochemical activity, charge storage ability, conjugation, conductivity, mechanical properties and adhesion to the substrate. In fact, all the beneficial features of the conducting polymers are destroyed by this process [36]. This is especially seen in the case of the amperometric approach where a constant potential is applied to the polymer.

In this work, a new recurrent potential pulse approach using high surface area PPy conducting polymer as a sensing material for glucose detection is proposed. This technique, under certain parameters, allows the PPy redox properties to be fully utilized whilst preventing its anodic degradation. Because of this, the 3D PPy presented here becomes a very good candidate as a sensing material for glucose detection and can work without any additional dopants, mediators or enzymes. For this purpose, PPy film is electrochemically synthesized under different conditions from an aqueous solution of lithium perchlorate and pyrrole. The influence of the synthesis conditions on the PPy structure is investigated. The stability, electroactivity and electroactive surface area of PPy are studied. Different possibilities for the usage of PPy as a glucose biosensor are also discussed and presented.

2. Experimental

All measurements are performed in a one-compartment cell with a commercial three-electrode system (AC1W2R2, BVT Technologies), using a potentiostat VersaSTAT 4. Ag/AgCl is used as the reference electrode and platinum as the counter electrode. The working electrode is a platinum screen-printed electrode with a surface area of 0.008 cm^2 .

The Pt electrodes are rinsed with deionised water and propanol before each experiment. After each electropolymerization, the electrodes are rinsed with deionised water and air-dried.

If not otherwise stated, the PPy films are potentiostatically ($+0.85 \text{ V}$ versus Ag/AgCl) synthesized in a one step process from an aqueous solution of 0.1 M lithium perchlorate and 0.15 M pyrrole (standard condition) (all from Sigma Aldrich)

with a deposition time limited by a charge of 5, 10, 20, 35, 75, 150 or 328 mC. Moreover, the PPy structures are prepared at +0.85 V versus Ag/AgCl in an aqueous solution of 0.15/0.5 M pyrrole and 0.4/0.1 M lithium perchlorate, respectively, with the deposition time limited by a charge of 328 mC.

The stability (electrochemical activity) of the PPy film is studied by cyclic voltammetry in a monomer-free aqueous solution of 0.1 M lithium perchlorate at a potential scanned between -0.7 and 0.7 V versus Ag/AgCl with a scan rate of 50 mV s^{-1} (200 cycles). The electroactive surface area of PPy/Pt is determined by performing the cyclic voltammetry of this electrode in $5 \text{ mM K}_3\text{Fe}(\text{CN})_6/0.1 \text{ M KCl}$ solution with a potential range: from -1.2 V to 1.2 V relative OCP and a scan rate of 50 mV s^{-1} .

The high surface area PPy film synthesized with a limited polymerization time by a charge of 328 mC is studied as a sensing material for glucose detection. For this purpose, two approaches are investigated: a voltamperometric and a recurrent potential pulse which are both performed in a phosphate buffer saline (2.7 mM KCl , 140 mM NaCl and 10 mM phosphate , pH 7.4) (Sigma Aldrich) aqueous solution with different D-(+)-glucose (Sigma Aldrich) concentrations ($0\text{--}3 \text{ mM}$). In order to study the voltamperometric response of PPy for the detection of various glucose concentrations, the current density is determined at $+0.8$ V based on the cyclic voltammetry measurement (-0.6 to 0.8 V versus Ag/AgCl, 50 mV s^{-1}) [37]. In the recurrent potential pulse approach, the potentials -0.45 V versus Ag/AgCl (cathodic) and 0.85 V versus Ag/AgCl (anodic) are applied alternately to the polymer for different periods of time (modes: $1 \text{ s}/1 \text{ s}$, $3 \text{ s}/3 \text{ s}$, $9 \text{ s}/3 \text{ s}$, $9 \text{ s}/9 \text{ s}$) (10 or 130 cycles). The sensitivity of the PPy/Pt electrode is determined based on the current density/glucose concentration graph (linear approximation). In the case of the recurrent potential pulse approach, this graph is obtained based on the first measurement points recorded during the oxidation step (0.85 V versus Ag/AgCl) within the first cycle, for each glucose concentration.

L-ascorbic acid (AA), glycine (Gly) and urea (U) (all Sigma Aldrich) with a concentration of 0.1 mM are used in order to study the selectivity of the PPy-based glucose sensor.

All measurements were conducted at room temperature (25°C). A scanning electron microscope FEI QUANTA FEG 250 was used to examine the morphology of the deposits.

3. Results and discussion

3.1. Properties of electropolymerized 3D polypyrrole structures

High surface area 3D PPy film is successfully electrosynthesized in an aqueous solution of 0.15 M pyrrole and 0.1 M lithium perchlorate (328 mC). The morphology of this polymer is characterized by a cauliflower-like structure, typical for this kind of polymer [38], with randomly located 3D PPy forms (figure 1(A)).

Depending on the deposition charge, the amount of 3D structures and the film porosity level are different. It is noticed that at the beginning of the polymerization, a strongly

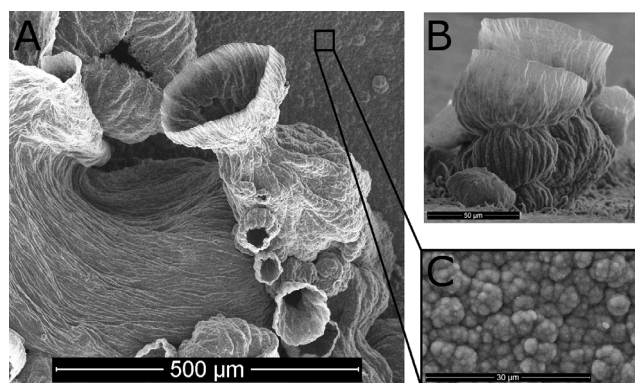


Figure 1. Scanning electron microscopy image of the 3D highly porous PPy synthesized with the electropolymerization time limited by a charge of 328 mC (A) and (C) and 10 mC (B).

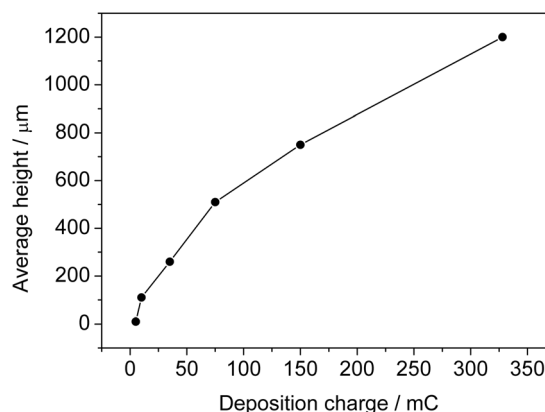


Figure 2. The average height of the 3D PPy structures obtained under different deposition charges.

adherent black PPy film is formed with a typical globular structure (figure 1(C)). With increasing deposition charge, there is a formation of PPy fibres, which later begin to create 3D polymeric structures (figures 1(A) and (B)). The detailed mechanism of PPy growth is presented in our previous work [39].

Based on the lateral scanning electron microscopy (SEM) images, the average height of the 3D PPy structures for each deposition charge is determined (figure 2).

The height of the PPy structures increases with the deposition charge. This indicates that such structures grow gradually with the polymerization time. In the case of the biosensor, it is also very important that the electrode material reveals a high electroactive surface area. The latter can result in more effective electrocatalytic activity of the sensor [4, 15]. Therefore, the electroactive surface area (ESA) of 3D PPy for different deposition charges ($5, 10, 35, 75, 150, 328 \text{ mC}$) is investigated. ESA determination is based on the Randles–Sevcik equation. This is a common electrochemical method for estimating the ESA of different electrode materials [39, 40]. For this purpose, the cyclic voltammetry of a PPy/Pt electrode in $5 \text{ mM K}_3\text{Fe}(\text{CN})_6/0.1 \text{ M KCl}$ solution was performed (potential range: from -1.2 V– 1.2 V versus OCP, 50 mV s^{-1}).

Examples of some voltammograms obtained for PPy synthesized with the polymerization time limited by a charge of $10, 75, 328 \text{ mC}$ are presented in figure 3.

The redox peaks related to the oxidation/reduction of the ferrocyanide ions occur in a potential range between -0.9 and $+0.5$ V versus Ag/AgCl [41]. The remaining peaks are related to the polymer reactions, which is typical behaviour in the case of conducting polymer materials [21].

The effective surface area of PPy synthesized for the different deposition charges is calculated according to this equation [40, 41]:

$$A_{\text{eff}} = I_{\text{pa}} / (2.69 \cdot 10^5 \cdot n^{3/2} \cdot D^{1/2} \cdot \nu^{1/2} \cdot C_0)$$

where A_{eff} is the effective surface area (cm^2), I_{pa} is the anodic current peak of ferricyanide oxidation (A), n is the number of electrons participating in the redox reaction (here equal to 1), D is the diffusion coefficient of the molecule in solution (here equal to $4.34 \cdot 10^{-6} \text{ cm}^2 \text{ s}^{-1}$), ν is the scan rate of the potential perturbation (V s^{-1}), and C_0 is the concentration of the $\text{K}_3\text{Fe}(\text{CN})_6$ in bulk solution (mol cm^{-3}).

Table 1 presents the ESA for PPy film synthesized under a deposition charge of 5, 10, 35, 75, 150 and 328 mC. It can be observed that with an increasing deposition charge, the ESA of the PPy increases. The highest value is obtained for 328 mC, which is in agreement with other studies. It should be noted that the polymeric film synthesized here reveals a very high surface area (1.47 cm^2) compared to the bare Pt electrode (0.008 cm^2). PPy increases the electrode surface area approximately 184 times in comparison to a pure platinum electrode. This is much higher than the values presented in the literature [27, 42], and is very desirable not only in the case of bio-sensing technology, but also in many other applications, such as microelectronics, energy storage [27] and gas sensors [26].

Moreover, the influence of the monomer and electrolyte concentration on the PPy structure is studied. It is observed that changing the monomer (Py) and the supporting electrolyte (lithium perchlorate) concentrations during PPy synthesis results in different polymer structures. Figure 4(A) presents the structure of a PPy film synthesized in the presence of 0.5 M Py and 0.1 M lithium perchlorate. The PPy structure obtained here differs from that obtained in standard conditions (figure 1(A)). Here, it is visible that the fibres agglomerate; however, they do not create 3D PPy structures in the further stages of electropolymerization. Therefore, it can be found that increasing the monomer concentration (from 0.15 to 0.5 M) inhibits the formation of 3D polymer structures. When the lithium perchlorate concentration is increased (figure 4(B)), the size and the shape of the PPy deposit (fibres and 3D high surface area structure) change and grow irregularly (here, the average height of the 3D structure is approximately $700 \mu\text{m}$) compared to the standard condition of PPy synthesis ($1200 \mu\text{m}$, figure 2).

It was noticed that electropolymerization of the polymer for a higher deposition charge (than 328 mC) results in very poor adhesion and detachment of the polymeric film from the substrate. The optimized electropolymerization charge which provides the highest ESA of PPy synthesized in an aqueous solution of pyrrole and lithium perchlorate is 328 mC. The electroactivity of this polymeric material is additionally studied by a cyclic voltammetry measurement in a

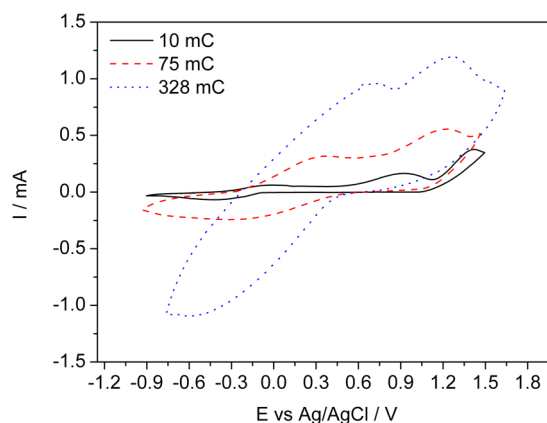


Figure 3. Examples of cyclic voltammograms of PPy synthesized with the polymerization time limited by a charge of 10, 75 and 328 mC in 5 mM $\text{K}_3\text{Fe}(\text{CN})_6/0.1$ M KCl solution.

Table 1. The electroactive surface area of a PPy film obtained under different deposition charges.

| Deposition charge (mC) | 5 | 10 | 35 | 75 | 150 | 328 |
|------------------------|------|------|------|------|-----|------|
| ESA (cm^2) | 0.04 | 0.09 | 0.29 | 0.51 | 0.8 | 1.47 |

monomer-free aqueous solution of 0.1 M lithium perchlorate at a potential scanned between -0.7 and 0.7 V versus Ag/AgCl with a scan rate of 50 mV s^{-1} (200 cycles) (figure 5).

High electroactivity is observed in the case of the high surface area PPy film (328 mC), which is studied here. The shape of i/E hysteresis indicates that 3D PPy (328 mC) reveals a highly capacitive nature with a good ion response, which is stable for up to 200 cycles. The changes in the shape of the hysteresis are associated with ion trapping during the polymer oxidation and reduction processes [43].

3.2. Three-dimensional PPy structures as a sensing material for glucose detection (328 mC)

Because of its high electroactivity and very high surface area, 3D PPy film can be a very good candidate as an electrode material for glucose detection. Figure 6 presents the response of PPy film (328 mC) to different glucose concentrations in an aqueous solution of phosphate buffer saline (PBS, pH 7.4). This response is determined by cyclic voltammetry (CV) measurements as a current at 0.8 V (the typical potential of glucose oxidation in PBS aqueous solution [37]).

The response of the PPy film (328 mC) to changes in glucose concentration is virtually linear and reproducible. The sensitivity of this material is determined to be $28.5 \text{ mA mM}^{-1} \cdot \text{cm}^{-2}$ (correlation coefficient 0.994) which is an order of magnitude higher in comparison to the literature reports [3, 30]. The detection limit is determined to be $100 \mu\text{M}$. In comparison to the literature reports, the detection limit obtained here is not satisfactory. The detection limit for the previously available amperometric glucose sensor is in the range of $0.2\text{--}9 \mu\text{M}$ [4, 6, 46]. Therefore, a further study will be performed in order to improve this parameter by changing the PPy properties.

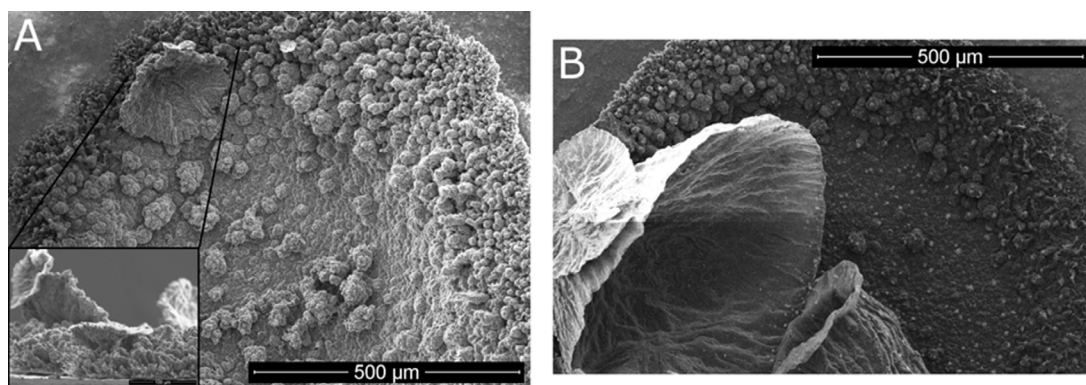


Figure 4. An SEM image of the PPY film synthesized in aqueous solution of 0.5 M Py and 0.1 M lithium perchlorate (A) or in aqueous solution of 0.15 M Py and 0.4 M lithium perchlorate (B) with a deposition time limited by a charge of 328 mC.

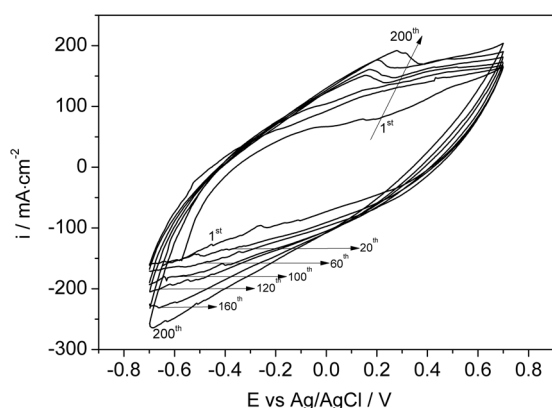


Figure 5. A cyclic voltammogram of PPy/Pt in a monomer-free aqueous solution of 0.1 M lithium perchlorate at a potential scanned between -0.7 and 0.7 V versus Ag/AgCl with a scan rate of 50 mV s^{-1} (200 cycles).

It was noticed that the same PPY material (328 mC), but with the incorporated glucose oxidase (GO_x) enzyme, reveals higher electrocatalytic properties in a range of $0\text{--}2$ mM glucose concentration compared to the PPY without the enzyme [37]. The response of the PPY/ GO_x sensor to a glucose concentration higher than 2 mM decreases. This is related to the fact that such a material does not detect glucose changes anymore due to the material degradation caused by the low stability of the enzyme [36]. The latter is the main reason why recent studies have focused mainly on the investigation of the material for a non-enzymatic approach [5, 14].

In the non-enzymatic approach, very often the sensors are based on the amperometric/voltamperometric approaches [5, 6]. However, these approaches induce the fast degradation of the sensor if the sensing material is a conducting polymer. It is noticed that the signal response of the 3D-PPy-based glucose sensor studied here diminishes after a certain measurement time as a result of the anodic potential, which is applied to the polymer. The latter results in the anodic degradation of the PPY (over-oxidation) which is the biggest issue for conducting polymers [21, 43]. The over-oxidation process leads to an irreversible loss of polymer electroactivity, as oxidation at positive potentials interrupts conjugation by the formation of hydroxyl and carbonyl species [44]. After this process, the polymer loses its desirable electrical properties and becomes

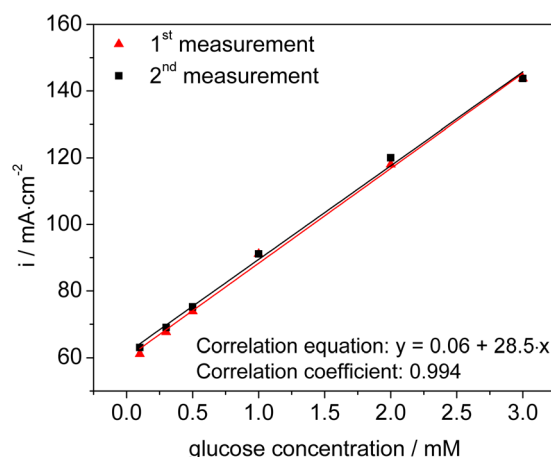


Figure 6. The current determined from the CV curve at 0.8 V for different concentrations of glucose in PBS.

an isolator. Therefore, the amperometric/voltamperometric glucose detection based on conducting polymers is not an appropriate approach in the case of this material.

Generally, PPY belongs to the group of redox polymers, and exhibits redox switching ability between its reduced and oxidized states [21]. During synthesis, a positively charged PPY backbone is formed and the polymer becomes oxidized. This is accompanied by the incorporation of counter-anions into the polymer structure in order to achieve charge neutrality. After synthesis, the polymer can be re-oxidized (doped) or reduced (de-doped) by applying anodic or cathodic potential, respectively [21]. The redox ability of certain polymers depends on the synthesis conditions and the solution/electrolyte used during measurements. The redox activity of conducting polymers is a big advantage in their use as, for example, an electrode material in neurological science [27] or as a material for an electrochemical capacitor [45].

Therefore, the new approach (recurrent potential pulse) of using PPY as a material in a glucose sensor is proposed. For this purpose, the potentials -0.45 V versus Ag/AgCl (cathodic) and 0.85 V versus Ag/AgCl (anodic) are applied alternately to the polymer for different periods of time (1 s/1 s, 3 s/3 s, 9 s/3 s, 9 s/9 s). Different times are studied to evaluate the most suitable oxidation/reduction time, for which PPY reveals stable redox properties and response to glucose

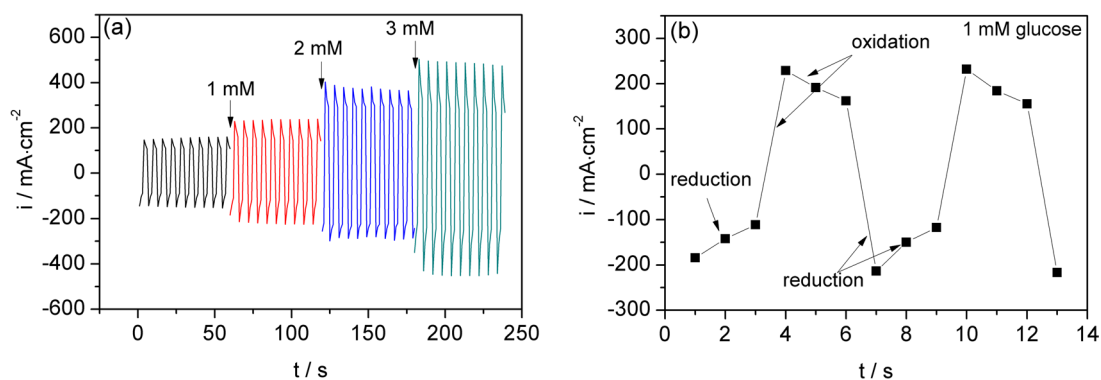


Figure 7. The recurrent potential pulse responses of 3D PPy/Pt (328 mC) in PBS with the addition of differently concentrated glucose in time (ten cycles) (a); the response of 3D PPy/Pt (328 mC) in a PBS solution of 1 mM glucose in time (two cycles) (b).

changes in PBS aqueous solution. It is noticed that the PPy subjected to the cathodic potential for 3 s and anodic potential for 3 s, respectively, exhibits the most stable glucose response (figure 7).

It can be observed that 3D PPy correctly detects the glucose changes in time. The signal response is much higher (by approximately 2.5/3.5 times depending on glucose concentration) and more stable compared to the signal response obtained when PPy is used as a voltamperometric sensor (figure 6). In the approach presented here, two different potentials are applied to the polymer-based sensor within one cycle (figure 7(b)). As after the synthesis process PPy is in its oxidized state, the cathodic potential is applied first. This prevents the possible anodic degradation of the polymer. It can be seen that the oxidation and reduction processes are approximately at the same absolute current levels, which indicates the high redox stability of the polymer [21]. This allows for appropriate glucose detection by the PPy in the PBS aqueous solution.

The detection mechanism of glucose strongly depends on the type of sensing material [5]. Here, a possible mechanism for the detection of glucose by a high surface area PPy film might be as follows:

Reduction part (-0.45 V versus Ag/AgCl):

- (1) Adsorption of glucose to the electrode surface
- (2) Reduction of PPy: $[\text{PPy}^{y+}(\text{ClO}_4^-)_y]_n + n\text{ye}^- \leftrightarrow (\text{PPy})_n + n\text{yClO}_4^-$

Oxidation part ($+0.85$ V versus Ag/AgCl):

- (1) Direct oxidation of glucose to gluconolactone
- (2) Oxidation of PPy: $(\text{PPy})_n + n\text{yClO}_4^- \leftrightarrow [\text{PPy}^{y+}(\text{ClO}_4^-)_y]_n + n\text{ye}^-$

Two processes are observed during the reduction part: adsorption of glucose to the active polymer sites and reduction of the PPy. The latter prevents fast over-oxidation of the polymer. Here, it is assumed that the redox properties of the PPy film are mainly determined by the anionic species [21]. During the following step, oxidation of the glucose and PPy film can be seen and new glucose molecules can be absorbed into the PPy in the next reduction step. It can be noticed that the current density for both oxidation and reduction parts remains at the same level and is related to the concentration

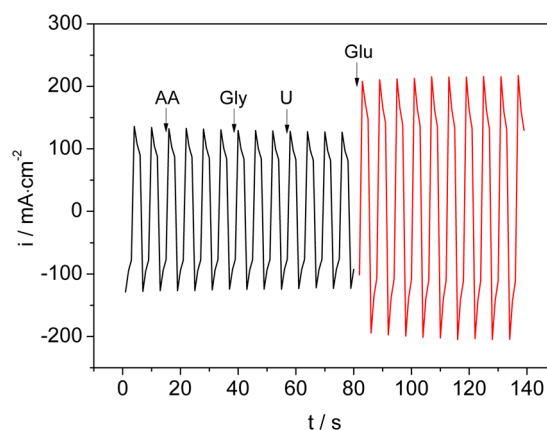


Figure 8. The recurrent potential pulse responses of 3D PPy/Pt (328 mC) in PBS in the presence of 1 mM glucose (Glu) and the potential interferents: 0.1 mM ascorbic acid (AA), 0.1 mM glycine (Gly) and 0.1 mM urea (U).

of glucose. It should be noted that the detection mechanism proposed here is only schematic and will be studied in more detail in a further work.

The sensitivity of this sensor is determined to be $123 \text{ mA mM}^{-1} \cdot \text{cm}^{-2}$ (correlation coefficient 0.982). The great enhancement of the sensitivity compared to the voltamperometric 3D PPy sensor ($28.5 \text{ mA mM}^{-1} \cdot \text{cm}^{-2}$) is attributed to the excellent electrocatalytic properties of the polymer resulting from its high electroactive and stable redox properties. The approach proposed here of using PPy as a sensor prevents its fast over-oxidation and allows the use of a conducting polymer as a sensing material. The detection limit is determined to be $100 \mu\text{M}$. The detection range is 0–3 mM glucose, which is comparable with other PPy-based sensors additionally modified with supporting metallic molecules [30]. The signal to noise ratio (S/N) for a PPy-based glucose sensor, for 1 mM glucose concentration, is determined to be 33, which is much higher compared to other non-enzymatic electrochemical glucose sensors [46]. This indicates the high precision of glucose detection performed by the recurrent potential pulse approach proposed here.

Ascorbic acid, glycine and urea derivatives are electroactive species in biological samples and may interfere with the detection of glucose. Therefore, the selectivity of the PPy-based glucose sensor is also studied (figure 8). It is noticed

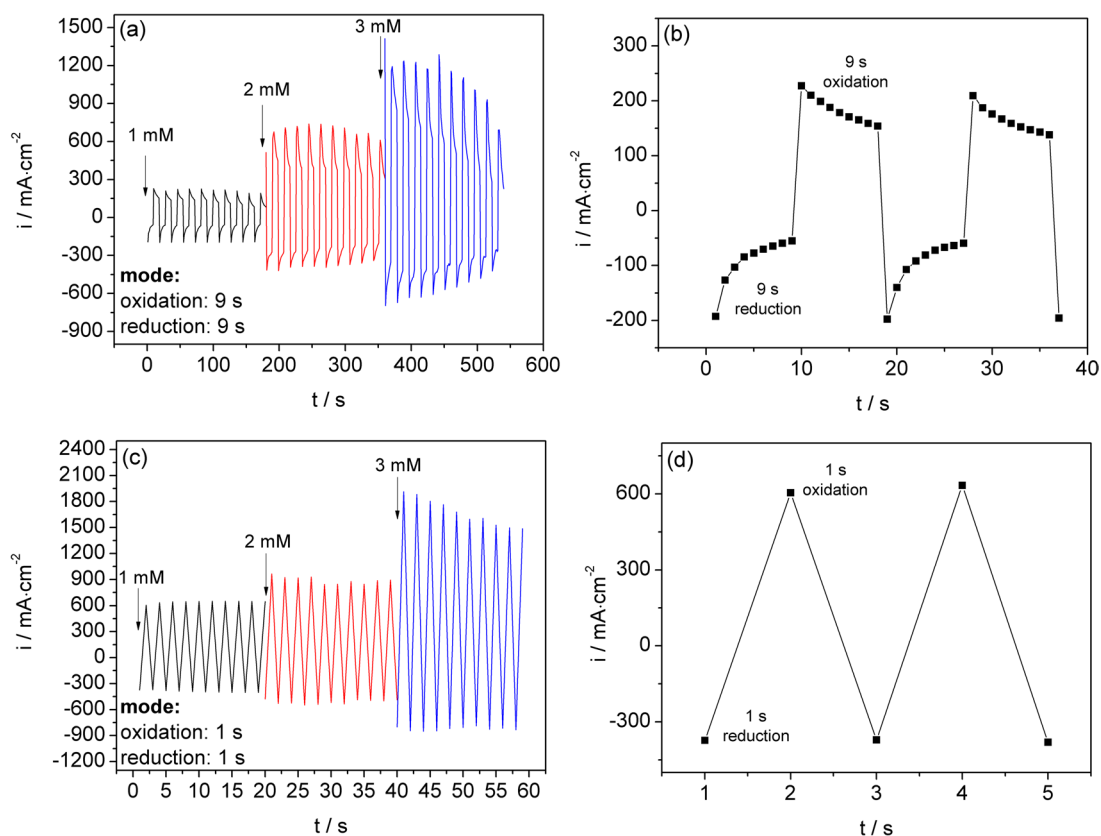


Figure 9. The recurrent potential pulse responses of 3D PPy/Pt (328 mC) in PBS with the addition of differently concentrated glucose in time (ten cycles) (a, c); the response of 3D PPy/Pt (328 mC) in PBS solution of 1 mM glucose in time (two cycles) (b, d).

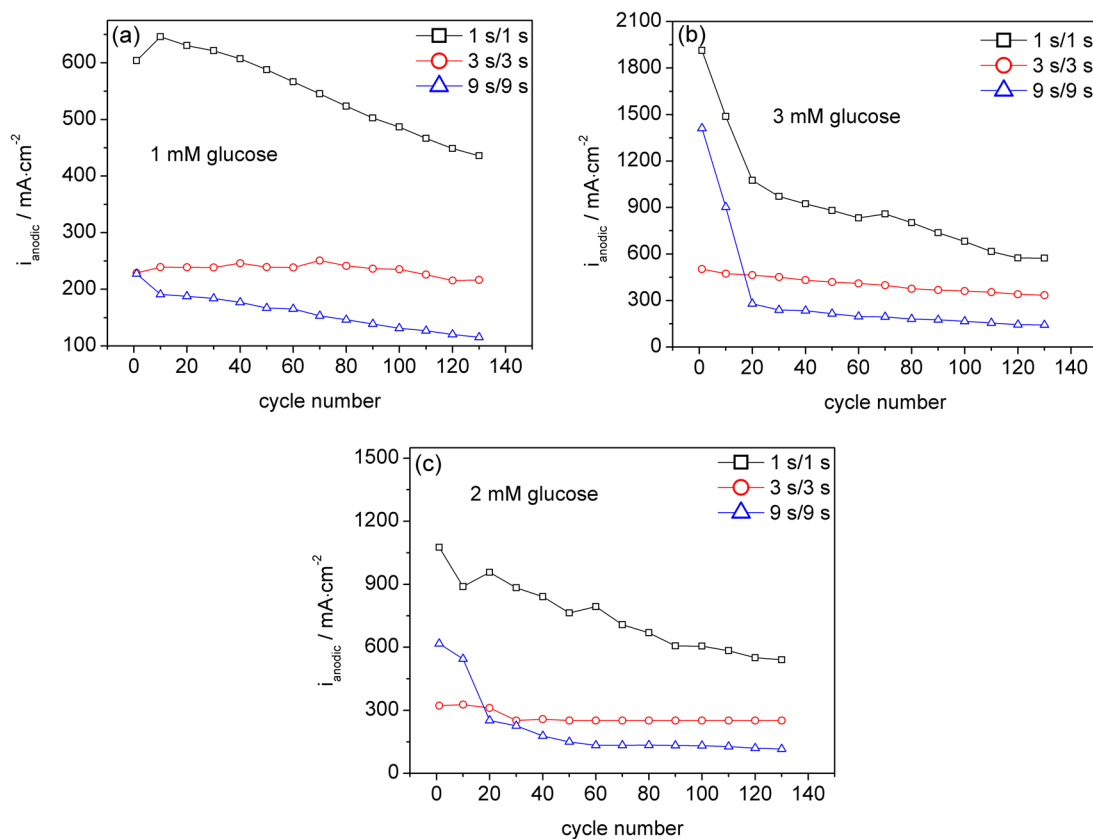


Figure 10. The anodic current response of 3D PPy/Pt (328 mC) working in different operational modes.

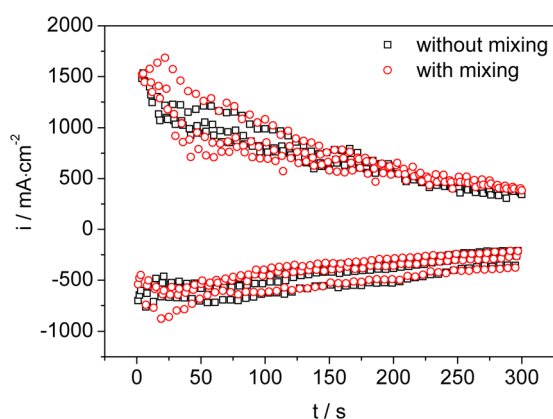


Figure 11. The recurrent potential pulse response of 3D PPy/Pt (328 mC) in PBS + 4 mM glucose, with and without solution mixing.

that virtually no changes in the signals are obtained when interferences are added to the PBS solution. The addition of glucose results in a significant increase of the current.

The recurrent pulse 3D PPy sensor is also studied for other oxidation/reduction times. Figure 9 presents the sensor's response in glucose solutions with different concentrations by applying -0.45 and 0.85 V versus Ag/AgCl for different oxidation (1 s, 9 s) and reduction times (1 s, 9 s), respectively (ten cycles). The use of the PPy sensor in other operational modes, 9 s/9 s and 1 s/1 s, also allows for the appropriate detection of glucose changes (figures 9(a)–(d), respectively). However, it can be easily noticed that such responses are not as stable as in the case of the 3 s/3 s mode (figure 7). This is especially visible in the case of the 3 mM glucose concentration, in which after the first cycle, anodic and cathodic current drifts are observed. These are due to the inappropriate selection of oxidation/reduction times. Such times do not allow for the correct functioning of the 3D-PPy-based glucose sensor and result in a decrease in the electrochemical activity of the polymer. This is especially pronounced for the 1 s/1 s mode (figure 9(d)), in which the anodic current (upper) envelope is almost twice as large as the cathodic (lower) one. This indicates that the polymer has not been sufficiently reduced at the beginning, which later on causes its degradation.

Applying the oxidation potential for 9 s and a further reduction for 3 s (9 s/3 s), causes very rapid degradation of the sensor (not shown). In this case, the electroactivity of the PPy is gradually lost after the first cycle, which is due to the high anodic potential applied for 9 s. This period of time appears to be too long for the correct functioning of the polymeric sensor.

The stability of the PPy sensor in the presence of glucose with different concentrations is also studied and is presented in figure 10. These plots are obtained based on the maximum anodic current in each cycle recorded during the recurrent potential pulse measurements of PPy (figures 7 and 9). It is observed that the highest stability of the PPy sensor is obtained in the case of the 3 s/3 s operational mode. The response of the sensor for the other modes (1 s/1 s and 9 s/9 s) decreases in time for each glucose concentration. This is related to the

gradual degradation of the polymeric film due to an inappropriate selection of oxidation/reduction times.

In the case of the 3 s/3 s mode, a slight decrease in response in the presence of 3 mM glucose solution can be observed. This can be related to two processes: the limiting diffusion of the glucose molecules from the bulk solution to the electrode or the limiting adsorption of the glucose molecules on the polymeric surface, which block the active sites of the polymer and cause difficulties in glucose detection. Therefore, it is important to check which processes, diffusion or adsorption, control the detection of glucose by 3D PPy. This can be studied by performing the recurrent potential pulse measurement of PPy in PBS aqueous solution of glucose, with and without mixing the solution. For this purpose, a higher glucose concentration is chosen (4 mM), which allows the pronounced instability to be observed.

Figure 11 shows that mixing the solution has no influence on the detection process of PPy. This indicates that the detection process of glucose by 3D PPy is not kinetically controlled but is mainly controlled by the adsorption process. This is also observed by other authors in the case of high surface area materials [5]. It was reported that in most cases, for highly porous micro-/nano-structured materials with high electroactivity, diffusion is negligible relative to the adsorption reaction. Therefore, the active adsorption sites are probably blocked, which decreases the response of the sensor at certain glucose concentrations. With an increasing glucose concentration (from 3–4 mM), the decrease of the sensor signal response is faster. This may also be related to the fact that the material can undergo contamination from other species in PBS solution. In particular, this applies to chloride, which can strongly chemisorb to the electrode surface [5].

4. Conclusions

In summary, 3D highly porous PPy structures are successfully synthesized on a Pt screen-printed electrode. It exhibits a high ESA and high electrochemical stability, which result in it having excellent electrocatalytic properties. Studies show that using the typical voltamperometric technique for a conducting polymer results in the poor sensing stability of these materials. Therefore, a recurrent potential pulse approach is proposed in order to use PPy as a glucose biosensor. It is shown that this technique, under certain parameters, results in an increase in the sensing stability of PPy, and allows its redox properties to be fully utilized whilst preventing its anodic degradation. The detection mechanism of this sensor is mainly based on the adsorption process of the glucose molecules to the active polymeric sites, glucose oxidation and PPy redox reactions. After appropriate optimization, the 3D PPy structures studied here can become a very good candidate material for different applications including bio or gas sensors.

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