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Poly-L-Lysine-modified boron-doped diamond electrodes for the amperometric detection of nucleic acid bases

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Abstract

Boron-doped diamond (BDD) is a very promising supporting material used in the construction of biosensors for molecular recognition. The direct immobilization of structurally-organized huge molecules, such as poly-L-Lysine (PLL) provides the possibility of determining organic molecules, e.g. nucleic acid bases (e.g. adenine, guanine) or peptides and proteins. This paper describes the direct method for chemical and electrochemical modification of the BDD electrode surface with organic molecules, including the potential application of such modified electrode for detecting selected nucleic acid bases, e.g. adenine and guanine.

Keywords: Boron-Doped Diamond, Direct Surface Amination, Polymer Modification, Differential Pulse Voltammetry, Nucleic Acid Bases

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1. Introduction

In recent years, different kinds of chemically modified electrodes have been applied to investigate the direct electrochemistry of nucleic acids (NA) [1–3]. Both miniaturization of the electrochemical measuring systems and the application of advanced electrode materials belong to the key steps in the development of devices applicable to practical analyses such as genetic assays, DNA diagnostics, DNA damage monitoring, etc. [4].

Oliveira-Brett *et al.* [5,6] have firstly reported electrochemical oxidation mechanism of guanine and adenine at glassy carbon microelectrode and cyclic and differential pulse voltammetry.

Furthermore, they demonstrated also application of ultrasound in combination with differential pulse voltammetry in a reliable analytical procedure for thymine and cytosine measurements avoiding electrode fouling and maintaining the electrode characteristics [7]. Finally, Oliveira-Brett's team [8] showed for the first time that equimolar mixtures of all DNA bases, nucleosides, and nucleotides could be quantified by differential pulse voltammetry

Teh *et al.* studied the electrochemistry of adenine on a sol–gel carbon composite electrode by differential pulse stripping adsorption voltammetry, a method useful for simultaneous analysis of adenine and guanine from denatured DNA [1]. Wu *et al.* investigated the direct electrochemistry of DNA, guanine and adenine at a nanostructure film-modified electrode, which resulted in the development of a sensitive electrochemical technique for measuring native DNA [9]. Moreover, Sun *et al*. [10] applied the carbon ionic liquid electrode for monitoring the direct electrooxidation behaviours of adenine and guanine in the thermally denatured single-stranded DNA. The simultaneous detection of guanine (G), adenine (A), thymine (T) and cytosine (C) was achieved at a multiwalled carbon nanotube (MWCNT)/choline (Ch) monolayer-modified glassy carbon electrode (GCE), with its output decreasing over time [11]. Wang *et al.* [12] developed reliable method based on adsorptive stripping at an electrochemically pre-treated glassy carbon electrode (GCE) for simultaneous or individual determination of G and A in DNA. Furthermore, the Nafion composite [13], graphene-COOH modified glassy carbon electrode (GCE) [14], multi-wall carbon nanotubes (MWNT) [15], b-cyclodextrin/MWNTs [16] or the porous structure of overoxidized polypyrrole/graphene electrochemically coated onto GCE [17] have been successfully utilized as efficient electrode material for the quantitative detection of the components of DNA and RNA. Moreover, a remarkable improvement in the kinetics of the electron transfer for guanine and adenine was shown by Wang *et al.* [18] on the surface of the silver decorated graphene quantum dots (AgNPs/GQDs) modified GC electrode, by shifting negatively in anodic peak potentials and

significantly increasing the anodic peak current. Nevertheless, the fabrication of composite materials requires several separate steps or electrochemical pre-treatment, which creates difficulties with regard to the repeatable electrode production and scaling of this process.

The remarkable electrochemical properties of boron-doped diamond (BDD), e.g. low background current, wide potential window, high stability combined with biocompatibility and chemical inertness makes it a very promising material for the third-generation biosensors [19,20]. The fast response, sensitivity and selectivity of biosensors based on BBD electrodes aroused great interest among scientists. [3,21–26]. The BDD electrodes are mostly prepared by the microwave plasma enhanced-chemical vapor deposition (MW PE CVD) method, while the as-deposited surfaces of BDD are hydrogen terminated (H-BDD).

Hason *et al.* successfully applied the anodic stripping determination of purine bases in acid-hydrolysed DNA, which involved electrochemically controlled accumulation of purine-Cu(I) complexes [27]. The significant sensitivity improvements in DNA electrochemical sensing were obtained using vertically aligned diamond nanowires fabricated by reactive ion etching (RIE) with O_2 [28].

Recently, Prado *et al.* have demonstrated the possibility of detecting underivatized nucleic acids at the boron-doped diamond electrodes using cyclic voltammetry and square wave voltammetry [29]. Ivandini *et al.* [30] reported the importance of surface termination and ionic strength of electrolyte for electrochemical oxidation of nucleic acids at the diamond electrode. The authors have shown the linearity of current at concentrations between 0.1 and 8 μg mL^{-1} for both guanine and adenine residues at as-deposited BDD. As-deposited diamond film with predominantly hydrogen-terminated surface demonstrated superior performance in comparison to oxygen-terminated diamond in terms of sensitivity, but this feature was present only within the limited range of potential and pH[30].

The application of a bare BDD electrode for the determination of G and A (purine DNA bases) in biological samples was reported by Svorc *et al.* [31]. Low LOD values were obtained with the previously reported electrochemical methods for G and A detection. Compared to other electrodes, the bare BDD electrodes do not require a rather tedious modification process, however, they suffer from the interfering signals caused by common components such as non-target proteins or polysaccharides.

Different terminations on the surface of BDD electrode can be obtained by applying a fixed potential and pre-treatment [32,33]. The detection of a specific DNA nucleotide is strongly influenced by the presence of different functional groups on the BDD electrode surface[34,35].

Thus, there is a need to modify the surface of BDD electrodes because of the insufficiency of chemically reactive groups, which precludes the attachment of organic compounds to the electrode surface [36]. The most widely used approach to functionalizing diamond film surface with organic compounds is an amine functionality introduced to the surface. So far, several amination methods of diamond surface have been proposed [36–39]. In general, they require: (I) etching by NH_3 plasma in a specific reactor [39], (II) chemical modification with (3-aminopropyl) triethoxysilane [40], (III) photochemical reaction of amino molecules containing a vinyl group [41], or (IV) diazonium functionalization [42–44]. It is apparent that there is a need to develop a simple procedure with better sensitivity, and to achieve a good linear sensing range. Electropolymerized or chemically polymerized amine polymers seem to be attractive candidates to fulfil the aforementioned aims.

Gu *et al.* [45] demonstrated a one-step chemical modification of BDD by the thin polyaniline/poly (acrylic acid) (PANI/PAA) composite polymer film. The procedure did not show any non-specific DNA adsorption, and DNA probes immobilized on the BDD substrates were stable and selective to DNA sensing, while the oxidation peak potentials were lower than those

reported for the oxidation of guanine and adenine on other carbon-based electrodes [22,23] and bare BDD [3,4]. However, the electroactivity (i.e. redox behaviour) of polyaniline is strongly dependent on the pH of electrolyte, and is greatly weakened at pH > 4 [46]. Su *et al.* have fabricated the functionalized carboxyl graphene oxide (GO–COOH) at a glassy carbon electrode (GCE) and L-lysine was electropolymerized on the GO–COOH modified GCE by cyclic voltammetry (CV) [47].

Studies on the modification of BDD electrodes with poly-L-Lysine, aimed at the detection of nucleotides, have not been published until now. Therefore, we report a simple and convenient one-step method to modify the surface of boron-doped diamond (BDD) by taking advantage of the conducting nature of BDD due to the presence of electropolymerized and chemically polymerized poly-L-Lysine (BDD/PLL). The electrostatic interactions between the positively charged $–NH_2$ groups of PLL and negatively charged GO–COOH stabilize the thin film[48].

All prepared electrodes were characterized by various electrochemical systems such as $Fe(CN)_6]^{3-/4-}$, Fe^{2+}/Fe^{3+} and (Q/H_2Q) in order to determine their electrochemical behaviour. Furthermore, differential pulse voltammetry (DPV) was successfully utilized to investigate the interaction between BDD/PLL electrodes and adenine and guanine. This technique is suitable for studying biological systems since it is fast and highly sensitive.

One advantage of pulse techniques is that they are characterized by much better signal-to-noise ratio and, in many cases, by greater selectivity than steady-state techniques [49,50]. Moreover, the prepared PLL-modified BDD electrode was analysed by means of X-Ray photoelectron spectroscopy (XPS) and Scanning Electron Microscopy (SEM).

2. Experimental

2.1. Si/BDD electrode deposition

BDD electrodes were deposited in an MW PA CVD system (Seki Technotron AX5400S, Japan) on p-type Si substrates with (111) orientation. Substrates were cleaned by sonication in acetone and 2-propanol for 5 min in each solvent. Next, the substrates were seeded by means of spin-coating in a nanodiamond suspension (crystallite size of 5-10 nm), and spun three times for 60 sec at 4000 rpm [51]. The temperature of heating stage was kept at 700°C during the deposition process. In the first step of the procedure, the substrates were etched in hydrogen plasma for 1 min. The optimized power of microwave plasma for diamond synthesis was kept at 1300 W. Excited plasma was ignited by microwave radiation (2.45 GHz). The total flow of gas mixture, containing 1% of the molar ratio of CH_4-H_2, was kept at 300 sccm. All samples were doped by using diborane (B_2H_6) dopant precursor; the [B]/[C] ratio in the plasma was 10000 ppm (BDD10). The reactor chamber was evacuated to a base pressure of about 10^{-6} Torr, while the process pressure was kept at 50 Torr. The time of polycrystalline layer growth was 6h, which resulted in the thickness of the deposited films of approx. 2 μm.

The four-step pre-treatment of the deposited Si/BDD electrodes was applied to obtain H-terminated surface and etch sp^2 phase impurities, as reported elsewhere [52–54]. For all Si/BDD samples, the diamond surface was cleaned with acids and hydrogen plasma. First, metallic impurities were dissolved in hot aqua regia (HNO_3 : HCl /1:3), followed by the removal of organic impurities with hot "piranha" solution (H_2O_2 : H_2SO_4 / 1:3) at 90°C. Microwave hydrogen plasma treatment was performed using 1000W of microwave power and 300 sccm of hydrogen gas flow for 10 min. Thus, the resulting BDD surface was predominantly hydrogen-terminated [53,54].

2.2. Si/BDD electrode modification with poly- L-Lysine (PLL)

Reagents: Guanine, adenine and poly- L-Lysine solution 0.1% (w/v in water) (PLL, MW 150 000–30 000) were purchased from Sigma–Aldrich and used without further purification. All

other chemicals were of analytical grade and used without further purification. Ultra clean water was used for the preparation of phosphate buffer as the electrolyte solution.

Sample preparation: Si/BDD electrodes were used as substrates for wet chemical and electrochemical modification with poly- L-Lysine (PLL) (Fig. 1).

Wet chemical modification

The BDD electrodes were initially cleaned by immersion in "piranha" solution for ca. 15 min, then rinsed with water and methanol, and dried in a stream of nitrogen. Next, the electrodes were immersed in 0.5 ml of solution consisting of 0.1% poly- L-Lysine (PLL) dissolved in 5 ml of water. The reaction mixture was left at room temperature for 24 h under ambient atmospheric pressure. Next, the modified electrodes were removed from the solution, washed twice with methanol and water, and finally dried under a stream of nitrogen.

Electrochemical modification

The volume of 100 µl of poly- L-Lysine (PLL) was dissolved in 3ml of phosphate buffer (pH 7.4) and electropolymerized on the surface of cleaned BDD electrode by cycling the potential between 0.5 and +1.5 V versus Ag/AgCl at 100 mV/s for 30 cycles. In order to remove unreacted monomer, the PLL-modified electrode was washed following the procedure described in the wet chemical modification. Finally, the electrode was dried.

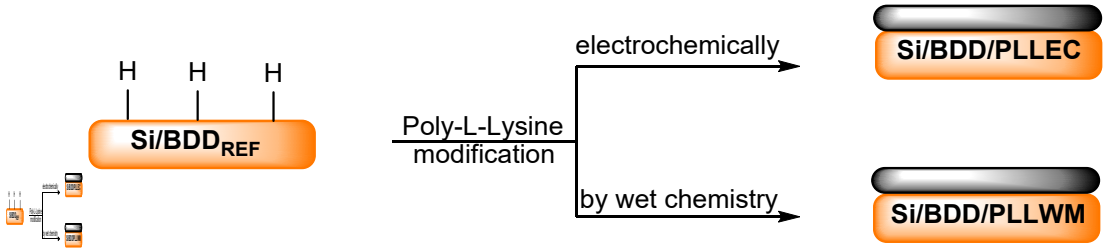

Fig. 1. Scheme of wet chemical and electrochemical modification of the boron-doped diamond electrode with poly- L-Lysine (PLL).

2.3 Surface analysis of Si/BDD/PLL electrode

SEM: Topography analysis of BDD electrodes covered with poly- L-Lysine film was carried out by means of scanning electron microscopy (SEM), with a tungsten source (S-3400N, Hitachi) and under accelerating voltage of 20kV. Additionally, the surface of the BDD samples has been analysed via graphical profiling tool of the computer software for data visualization and analysis (Gwyddion, 2.40, Czech Republic)[55].

XPS: The elemental surface composition was determined by high-resolution X-ray photoelectron spectroscopy (XPS) with the monochromatic Al Kα source (Escalab 250Xi, ThermoFisher Scientific). Charge neutralization was implemented. Spectra were recorded with an energy step of 0.1 eV and energy pass of 10 eV for highly resolved C1s and N1s peaks. The data analysis was performed by using Avantage software provided by the manufacturer.

Contact angle: The contact angle hysteresis (CAH) measurements were performed with the captive bubble method described in detail elsewhere[56]. It should be noted that the measurement of dynamic contact angles is rate-dependent at high capillary numbers. The capillary number relates inertial forces to surface tension forces and is defined as $Ca = \mu V/\gamma_{LV}$, where μ is the liquid viscosity, V denotes the characteristic contact line velocity, and γ_{LV} is the liquid surface tension. At low Ca ($<< 10^{-5}$), the dynamic contact angles are largely independent of the rate of contact line motion [57]. Deionized water (Millipore system; conductivity 0.05 μS cm^{-1}) with a pH 5.8 ± 0.1 and the surface tension γ_{LV}= 71.2 ±0.2 mN m^{-1} at room temperature T=25 ^{0}C was used as a probe liquid for CA measurements. For each studied surface, 5 to 10 measurements were

conducted at different locations on the substrate to evaluate the surface spatial homogeneity. The obtained measurement errors were within 1^0.

Electrochemical measurements: All electrochemical measurements, including cyclic voltammetry (CV) and differential pulse voltammetry (DPV), were performed using potentiostat (Autolab, PGSTAT30, Netherlands) equipped with GPES 4.9 software. A conventional three-electrode system was used with a platinum wire as counter electrode and an Ag/AgCl (0.1 M NaCl) as reference electrode. The working electrode was the modified BDD electrode with a radius of 8 mm. Working solutions were freshly prepared prior to use. The measuring cell was placed at room temperature during all the measurements.

Cyclic voltammetry (CV) measurements were carried out in Na_2SO_4 solution containing reference redox systems: $[Fe(CN)_6]^{3-/4-}$. The concentration of each component in the solution was set at 5 mM, while the scan rate at 100 mV/s. The other redox reference system quinone/hydroquinone (H_2Q/Q) and Fe^{2+}/Fe^{3+} redox couple were carried out in Na_2SO_4 solution where the concentration of each component was set at 5 mM, while the scan rate at 100 mV/s.

Differential pulse voltammetry (DPV) was performed in phosphate-buffered solution with the potential ranging from 0.5 to 1.8V, 50 mV amplitude modulation, 70 ms pulse width, and a scan rate of $5mVs^{-1}$.

3. Results and discussion

3.1. Analysis of Si/BDD/PLL electrode surface

Figure 1 displays the SEM images of BDD samples before and after PLL modification. The images were qualitatively analysed by using a statistical procedure in Gwyddion software, which produces the root mean square roughness R_{RMS}. The image of H-terminated Si/BDD electrode, shown for reference in Fig. 2(A), exhibited a typical polycrystalline structure with the uniformly

distributed sharp-edged crystallites[58]. As revealed by SEM imaging, the difference in height (edge-to-valley) of these crystallites reached up to hundreds of nanometres, while the R_{RMS} value for the entire image area was approx. 170 nm. The PLL-modified BDD electrodes (see Fig. 2AB) were considerably smoother than those made of bare H-terminated BDD. The morphology of poly-L-Lysine films attached to BDD by means of the chemical and electrochemical process was characterized by ovoid shape. The effect of electrochemical modification can be seen in Fig. 2(C), i.e. the obtained surface is smoother compared to chemically processed BDD in Fig. 2(B).

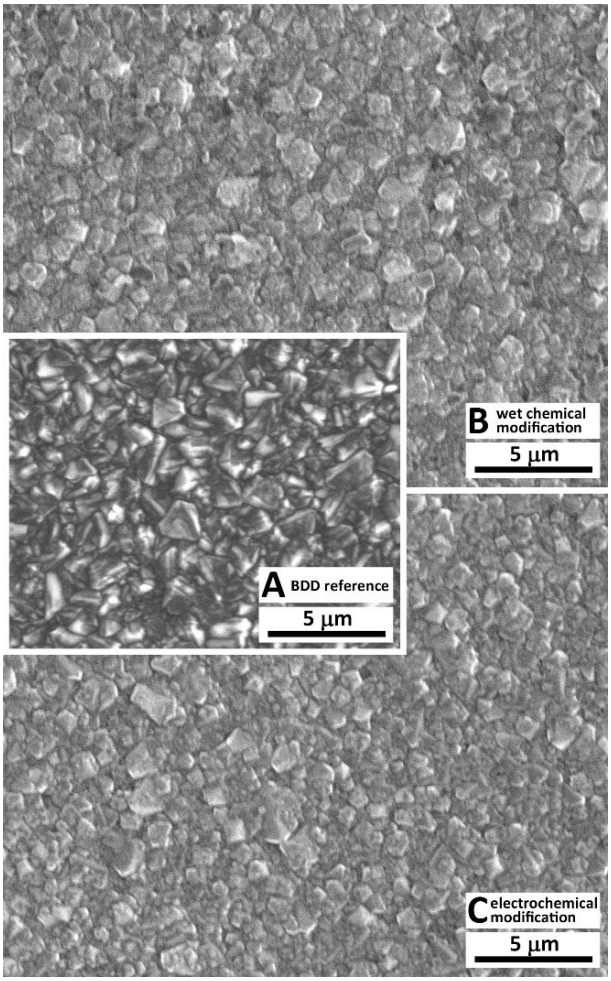

Fig. 2. SEM micrographs of BDD electrodes modified with poly- L-lysine via a) electrochemical treatment and b) chemical treatment. Magnification x5000.

Specifically, the R_{RMS} value decreased due to the modification process, reaching 150 nm and 130 nm for the chemically deposited PLL_{WM} and electrochemically deposited PLL_{EC},

respectively. The morphological analysis revealed that the electrochemical process is more efficient compared to the chemical one, and it results in a visibly more homogeneous PLL layer. The improvement associated with the use of electrochemical method is attributable to the highly positively-charged PLL that is deposited preferentially by electrostatic interactions [47]. Furthermore, the adsorption process and C-N bonding between PLL and BDD [48,49] could also play a role in the deposition of polymerized lysine. Thus, the electrode potential applied during the cyclic voltammetry deposition introduced additional activation energy which enhanced the polymerization process.

Generally, the PLL modification was clearly manifested by lowered R_{RMS} values, the flattening of the valleys, and closing of intergrain regions. This implies that the valleys and intergrain regions attract PLL. In physical terms, the sharp peaks on the surface still remained, whereas the intergrain regions and valleys became filled with PLL. This scenario could result from the smaller size of intergrain crystals and higher conductivity of intergrain regions, which introduces more centres for mechanical or chemical bonding [60–62].

The XPS analysis was performed to determine the chemical composition of PLL-modified film deposited on the Si/BDD electrode. High resolution XPS spectra performed for C1s and N1s are shown in Fig. 3. The charge shift was corrected by referencing to the adventitious C1s peak at 284.6 eV. The measurement was performed after ion gun etching for a period of 10s at low voltage (1000V) in order to remove contaminants originating from the sample exposure to the air.

Based on literature review and the analysis carried out earlier by authors for similar films deposited on titanium surface[58,63,64]., five elementary peaks were proposed for the C1s spectrum and two for the N1s spectrum to perform deconvolution. The main C1s component was located at 284.1 ± 0.1 eV, which corresponds to the C-C and C-H bonds in the BDD electrode with the [B]/[C] ratio of 10k. Poly- L-Lysine film displayed multiple peaks in the C1s spectra, ranging from 284.8

to 288.0 eV. The interpretation of the peaks has been summarized in Table 1. Additionally, a partial change in the surface termination type of BDD electrode (oxidation) under chemical or electrochemical conditions occurred as a shift by +0.6 eV with respect to the main C1s peak[65] , which can also be a part of the peak visible at 284.8 eV. The peak attributed to the C-NH bonds overlaps with the peak assigned to the C-O bonds in poly- L -Lysine film. A small peak observed for the lowest BE values should be interpreted as the C-C sp^2 bonds originating from the partial damage of BDD electrode due to boron incorporation or electrochemical oxidation process[66].

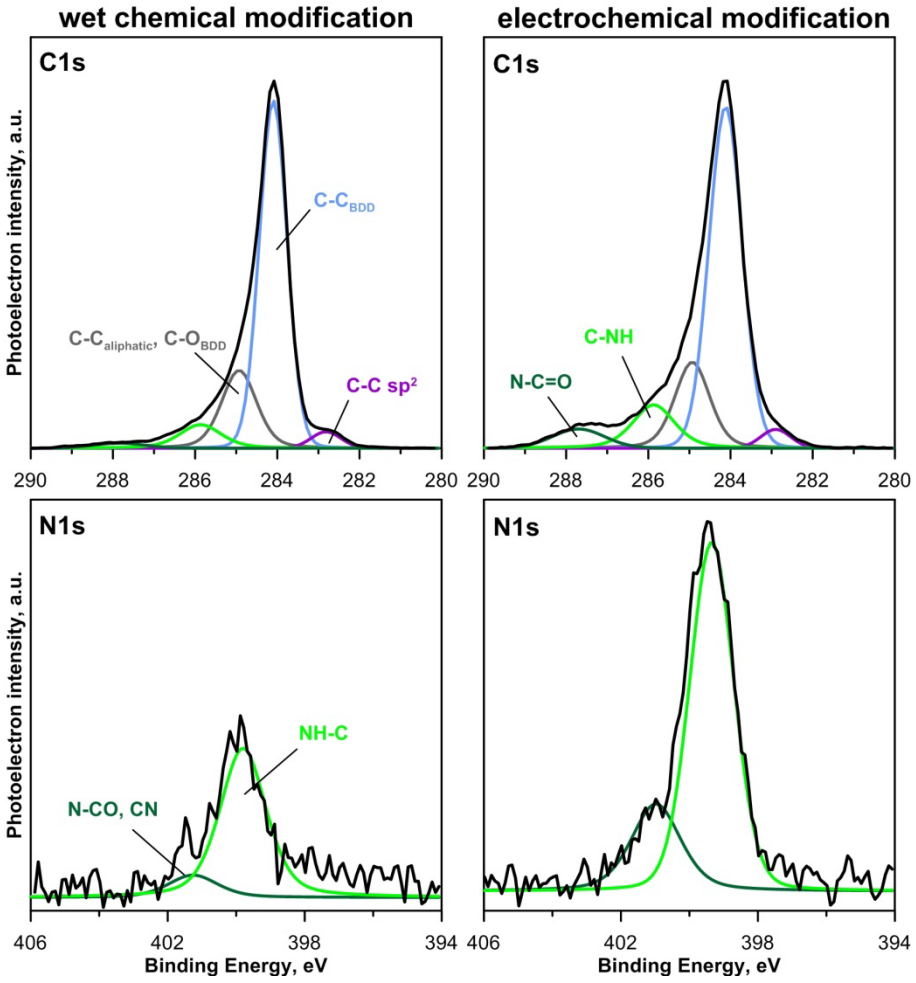

Fig. 3. High resolution XPS spectra of the C1s and N1s peaks in poly- L-Lysine freshly deposited on BDD electrodes.

Tab. 1. Percentage contribution of the C1s and N1s peaks in poly- L-Lysine film.

	C-C BDD	C-C aliphatic	C-NH	N-C=O	C-C sp^2	NH-C	N-CO
Peak BE	284.1	284.8	285.9	287.8	282.9	399.4	401.0
Si/BDD/PLL_{WM}	61.5	18.9	7.2	1.7	3.1	1.1	0.3
Si/BDD/PLL_{EC}	50.8	14.7	9.3	4.5	2.5	4.6	1.4

The N1s peak was fitted by combining sub-peaks originating from different bonds between the carbon and nitrogen atoms, namely, =C-NH_2, -C-NH, and -N-C=O. Oxygen was also a part of this analysis, contributing 12.1% to electrochemical and 6.2% to wet chemical modification of poly- L-Lysine film.

The CAH measurements were used to determine the wettability of poly- L-Lysine-modified boron-doped diamond electrodes. Wettability represents a fundamental property of any material; it reveals information about the chemical structure of the material and its surface topology. In particular, contact angle hysteresis (CAH), i.e. a difference between the advancing Θ_A and receding Θ_R contact angles is a measure of the surface "non-ideality" [67], and is intimately related to the adhesion of materials on surfaces. The adhesion between the liquid and the substratum increases with increasing CAH. The receding CA actually gives the best characteristic of the modified component of the surface and is the more important of the two CA measurements, particularly in the case of surfaces affected by the deposition of adhesive layers introducing energy barriers[68].

Both modified surfaces (Si/BDD/PLL_{EC} and Si/BDD/PLL_{WM}) and H-terminated Si/BDD_{REF} used as reference, exhibited the wettability values characteristic of hydrophilic specimens ($\Theta_Y < 90^0$).

Tab. 2. The mean values of wettability parameters for the samples in contact with distilled water (γ_{LV}= 71.2 mNm^{-1} at T= 24.8 ^{0}C; W_C= 142.4 mJm^{-2}).

Sample	Θ_Y	Θ_A	Θ_R	CAH	Π	γ_{SV}	W_A	W_S	γ_S^d/γ_{SV}
	(deg)	(deg)	(deg)	(deg)	(mNm^{-1})	(mJm^{-2})	(mJm^{-2})	(mJm^{-2})	-
Si/BDD_{REF}	39.4	50.8	23.8	27.0	20.2	53.4	116.2	-26.2	0.89
$Si/BDD/PLL_{EC}$	41.3	53.3	25.2	28.1	21.9	51.9	115.4	-29.0	0.88
$Si/BDD/PLL_{WM}$	51.5	62.5	38.3	24.2	23.0	46.9	104.1	-38.3	0.81

A contact angle hysteresis approach provides further parameters of wettability and surface energetics (see Table 2) for the studied Si/BDD/PLL electrode material with different surface characteristics for comparative purposes.

The surface modification effect was observed for both applied treatments as reflected by the following variability of CAH parameters: Θ_A, Θ_R, increased several per cent; CAH (24.2- 28.1 mNm^{-1}) and Π (21.9- 23.0 mNm^{-1}) remained almost the same; γ_{SV} and W_A decreased by a few percent; and W_S was more negative (changing from -26.3 to -38.3 and - 28.6 mJ m^{-2} for chemical and electrochemical treatment, respectively). As a result, the post-treatment surface became more hydrophobic. The surface energy parameters of water/solid surface interactions mainly exhibited hydrophobic character, i.e. the dispersive term of the surface free energy was equal to 0.89 γ_{SV} (REF) and decreased to 0.81 γ_{SV} for the chemically-treated sample whereas it remained almost unchanged (0.88) for the electrochemically modified substrate. It should be noted that the modifying polypeptide provided excessive positive charges to the electrode surface that could lead to a significant degree of additional electrostatic interactions. Moreover, the application of PLL introduces the total surface free energy leading to the drop in the dispersive interaction component, as it was found here. The electrochemical treatment changed the interfacial force balance due to different interactions, and the $\gamma_{SV}^d/\gamma_{SV}$ ratio was close to the value of reference substratum. The strength of the dispersive interactions between the substratum and water molecules was rather

strong, i.e. the $\gamma_{SV}^{d}/\gamma_{SV}$ values for hydrophobic polymer surfaces (PMMA) equalled 0.89-0.90)[69]. The surface wettability was rather attributed to the compositional changes at the interface than to the surface roughness and homogeneity since CAH remained almost unchanged, i.e. 27.0, 24.2 and 28.1° for the reference and treated samples, respectively. An increase in both Θ_A and Θ_R for the treated surface confirms this conceivable explanation. In the case of heterogeneous surface, the values of Θ_A and Θ_R indicate the most hydrophobic and most hydrophilic components, respectively[57]. The 2D surface pressure Π results from the repulsive forces between the surface active molecules adsorbed at the liquid/solid interface or organized molecules of water. Π is proportional to the Gibbs adsorption (surface excess) at the interface. It seems that the surface treatment was not affected by the interfacial organization of water and dissolved molecules in the bulk phase because the Π values were comparable (21.9 and 23.0 mN m^{-1} versus 20.2 mN m^{-1} for the reference sample). Many solid surfaces have a low surface energy, for instance, biological or synthetic organic substrates (γ_{SV}= 20-30 mJm^{-2} for polymers). If the surface originally has a higher surface energy, it may adsorb contaminants from the environment, which reduces its surface energy. Thus, it becomes more hydrophobic. The observed drop in the γ_{SV} value from 53.4 (reference) to 46.9 for the electrode modified by wet chemistry and 51.9 mJm^{-2} for the electrochemically modified electrode may also be attributed to the adsorption at the solid surface. In general, the electrochemical treatment of the electrode surface led to less pronounced changes in wettability and surface energetics compared to the wet chemical treatment.

3.2. Electrochemical modification of a BDD electrode with poly-L-Lysine

The modification of BDD electrode was performed by cyclic voltammetry in 30 scans. The evolution of cyclic voltammograms (CVs; see Fig. 4) registered during the electrochemical modification process in the solution of 3 ml of phosphate buffer containing 0.1 ml of poly-L-Lysine

indicates the formation of a stable PLL layer on the surface of BDD electrode. The differences between the unmodified and modified electrodes subjected to 1 and 30 scans are presented in Fig. 4. After modification, the peak current decreased after the initial potential cycles of scans and then became stable with the progressing electrochemical modification.

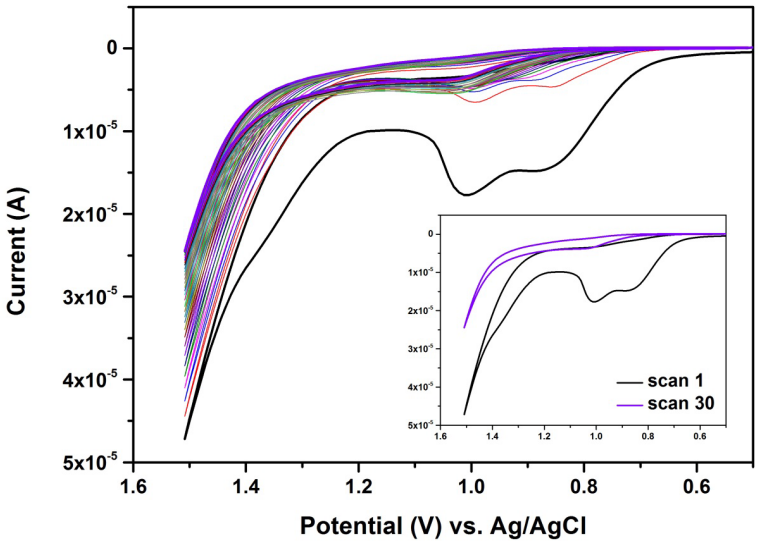

Fig. 4. Effect of cyclic voltammetry experiment on the growth of poly-L-Lysine (PLL) on the surface of BDD electrode.

3.3. Electrochemical behaviour and stability in relation to PLL modification

The authors intended to apply the widely used electrochemical systems to characterize the surface of modified electrodes, as follows: (I) electrochemical window 0.5M Na_2SO_4, (II) negatively charged $[Fe(CN)_6]^{3-/4-}$ which is a one-electron reduction/oxidation system, (III) positively charged Fe^{2+}/Fe^{3+} one-electron reduction/oxidation system, and (IV) neutral quinone/hydroquinone (Q/H_2Q) which undergoes two-electron/two-proton redox reaction.

The synthesis of polyamine layer on the BDD surface caused a significant broadening of the electrochemical window in both cathodic and anodic areas (Fig. 5 (A)). The modification with poly-L-Lysine significantly increased the measuring range of the obtained new BDD electrodes compared to the unmodified electrodes. The broadest electrochemical window was observed in the case of the BDD electrode modified with poly-L-lysine by the chemical process.

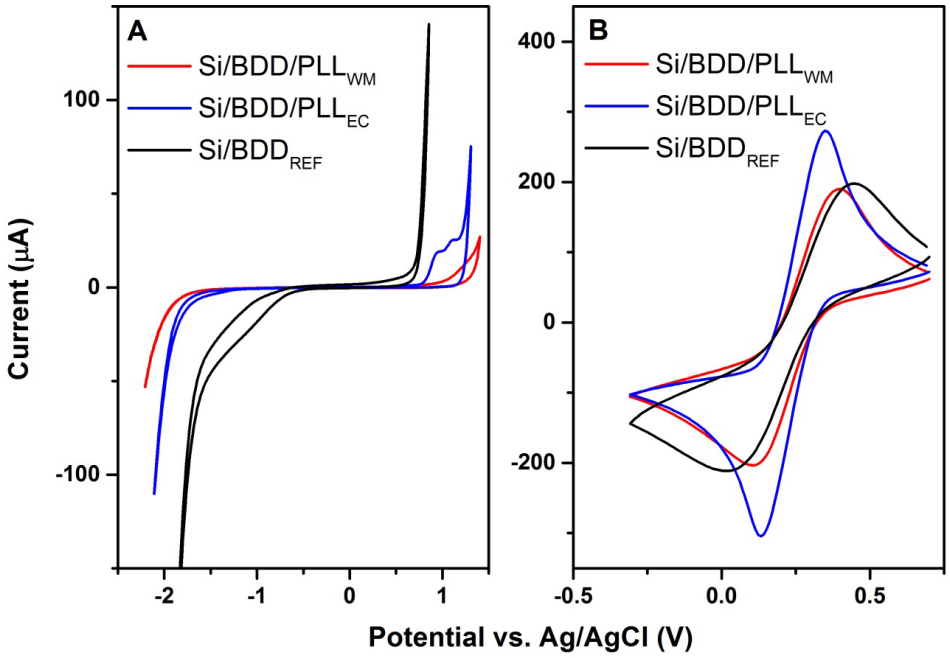

Fig. 5. A) Potential window of the modified BDD electrodes in aqueous 0.5M Na_2SO_4 solution, scan rate: 100 mV/s; B) Cyclic voltammograms of the redox reaction of $[Fe(CN)_6]^{3-/4-}$ (5 mM) in 0.5M Na_2SO_4 solution at the modified BDD electrodes, scan rate: 100 mV/s.

The $[Fe(CN)_6]^{3-/4-}$ (5 mM) redox couple was used as the electrochemical probe with a scan rate of 100 mV/s. Fig 5(B) presents the cyclic voltammograms of studied electrodes. The voltammetric curve for the Si/BDD$_{REF}$ reference electrode (black line) shows the cycle with a peak to peak separation (ΔE) of 0.396 V. After the modification with poly-L-Lysine, the shape of the curve has changed for both electrodes, i.e. Si/BDD/PLL$_{EC}$ and Si/BDD/PLL$_{WM}$. In the case of wet chemical modification (electrode Si/BDD/PLL$_{WM}$ marked with red line), the redox peak current

decreased, but Red/Ox peak separation (ΔE) equaled 0.261 V. A significant increase in the peak current was observed for the electrochemically modified Si/BDD/PLL_{EC} electrode (blue line) with a peak to peak separation ΔE of 0.198 V. In general, the reversibility of the redox process was more favoured in the modified electrodes, which were positively charged due to the presence of protonated amine groups on the electrode surface. The electron transfer of negatively charged $[Fe(CN)_6]^{3-/4-}$ system in the BDD electrodes modified with poly-L-Lysine (PLL) was less inhibited than in the Si/BDD_{REF} reference electrode. The diffusion of ferricyanide toward the surface of polyamine-modified electrodes was more pronounced.

For each of the investigated electrodes, the currents of the redox process of couples were observed. Independently of the applied method of modification, the ratio of currents I_c/I_a was close to 1 indicating that the process is diffusion-controlled and characterized by an enhanced electrochemical reversibility. By assuming that the charge equals ΔE in the function of the scan rate (10 – 1000 mv/s), it was possible to calculate the charge transfer rate constant k_o [70]. The constant k_o varies depending on the mode of layer formation on the electrode surface. An increase in the charge transfer rate constant of treated electrode in relation to the reference electrode was pronounced in the case of Si/BDD/PLL_{EC}. This may indicate a more ordered structure of the polymerized amine layer. On the other hand, the layer obtained via chemical method displayed a slightly lower value of k_o.

Tab. 3. Electrochemical parameters of the reaction of $[Fe(CN)_6]^{3-/4-}$ on the surface of modified BDD electrodes.

Sample	Modification	E_c (V)	E_a (V)	ΔE (V)	I_c (A)	I_a (A)	k_0 ($cm \cdot s^{-1}$)
Si/BDD	Reference	0.043	0.439	0.396	$-1.947 \cdot 10^{-4}$	$1.959 \cdot 10^{-4}$	$3.55 \cdot 10^{-4}$
Si/BDD/PLL_{WM}	Wet chemistry process	0.124	0.385	0.261	$-1.726 \cdot 10^{-4}$	$1.730 \cdot 10^{-4}$	$3.29 \cdot 10^{-4}$

$Si/BDD/PLL_{EC}$	Electrochemical process	0.141	0.339	0.198	$2.419 \cdot 10^{-4}$	$2.477 \cdot 10^{-4}$	$5.52 \cdot 10^{-4}$

Additionally, two other redox systems, i.e. Fe^{2+}/Fe^{3+} couple and quinone/hydroquinone (Q/H_2Q) redox system were used to fully characterize the electrochemical behaviour of the produced electrodes (Figs. 6A and 6B).

The positively charged Fe^{2+}/Fe^{3+} (5 mM) redox couple was utilized as the electrochemical probe with a scan rate of 100 mV/s. Fig. 6 (A) shows cyclic voltammograms for all examined electrodes. The reversibility of the redox process, defined as $\Delta E=E_a-E_c$, increased in the electrochemically modified $Si/BDD/PLL_{EC}$ electrode (blue line). The $Fe^{2+/3+}$ redox reaction measured at the untreated $Si/BDD/PLL_{REF}$ reference electrode (black line) resulted in the reversibility ΔE of 1.053 V. For the electrochemically modified $Si/BDD/PLL_{EC}$ electrode, ΔE equaled 0.9 V. Thus, the electrochemical modification of the BDD electrode surface resulted in the enhanced electron transfer process compared to the $Si/BDD/PLL_{REF}$ electrode.

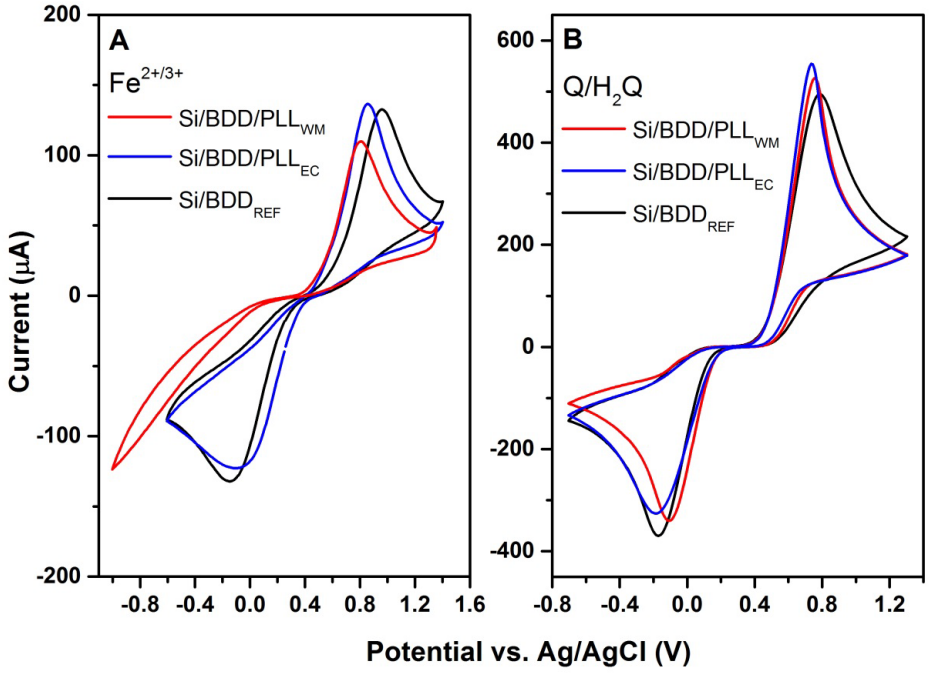

Fig. 6. Cyclic voltammograms of the modified BDD electrodes in an aqueous solution of A) Fe^{2+}/Fe^{3+} (5 mM) and B) quinone/hydroquinone (H_2Q/Q) (5 mM) in Na_2SO_4 (0.5M) at a scan rate of 100 mV/s.

In the case of wet chemical modification (electrode Si/BDD/PLL_{WM} marked with red line), the $Fe^{2+/3+}$ redox reaction was irreversible. Such outcome might have been caused by a strong interaction between the positively charged amino groups and the ions present in the electrolyte at the electrode surface.

Quinone/hydroquinone (H_2Q/Q) redox system implies the transfer of two electrons and two protons. The cyclic voltammograms of all examined electrodes in 0.5 M H_2SO_4 are shown in Fig. 6 (B). The oxidation potential at the modified electrodes containing positive charges at the surface was higher than that at the reference BDD electrode. In the case of modified Si/BDD/PLL_{WM} (red line) and Si/BDD/PLL_{EC} (blue line) electrodes, the resulting values of ΔE were 0.855 V and 0.909 V, respectively. The ΔE value for Si/BDD/PLL_{REF} (black line) reached 0.945 V. The lower ΔE observed for the modified electrodes means that these electrodes are more conductive and catalyse the H_2Q oxidation reaction more efficiently than the reference electrode.

3.3. Detection of selected nucleic acid bases using PLL-modified Si/BDD electrode

The changes in oxidation current were observed at the PLL-modified BDD electrodes as a result of the presence of nucleic acid bases in the solution. As mentioned before, the immobilization of poly-L-lysine (PLL) on the BDD electrode surface was achieved by the two independent methods, i.e. electrochemically and via wet chemistry method. These modifications mediate the oxidation process of nucleic acid bases enhancing charge transfer between the electrolyte and the electrode. As an example of their practical application, the BDD electrodes obtained via the

electrochemical (Si/BDD/PLL_{EC}) and wet chemical method (Si/BDD/PLL_{WM}) were used to detect specific nucleic acid bases.

The direct detection of adenine by differential pulse voltammetry (DPV) at pH 7.4 (physiological pH) conducted by means of the Si/BDD/PLL_{REF} reference electrode (black line) at the low adenine concentration of $2 \cdot 10^{-5}$ M did not show any changes in current. On the other hand, the application of the chemically modified Si/BDD/PLL_{WM} electrode (red line) and electrochemically modified Si/BDD/PLL_{EC} electrode (blue line) demonstrated the changes in current, allowing for the analytical identification of adenine (Fig. 7A). The distinguished changes in current were observed for the Si/BDD/PLL_{EC} electrode. The increasing adenine concentrations in the buffer solution were successfully detected which shows that the analytical capabilities of modified electrodes are at an acceptable level (Fig 7B, 7C).

Wet chemical modification of BDD electrode (Si/BDD/PLL_{WM}; red line) caused a shift in the oxidation potential of adenine towards more negative values in relation to the Si/BDD/PLL_{REF} reference electrode (black line), amounting to 38 mV, 38mV and 47 mV at adenine concentration of $2 \cdot 10^{-5}$, $3.9 \cdot 10^{-5}$ and $7.7 \cdot 10^{-5}$ M, respectively (see Fig. 7).

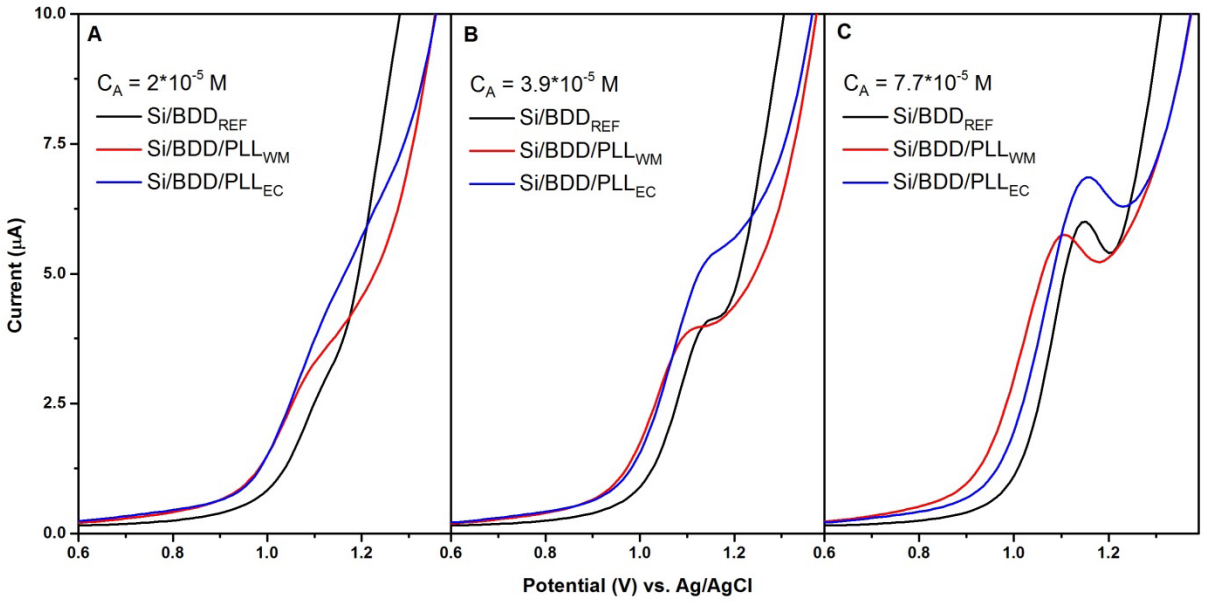

Fig. 7. Differential pulse voltammograms obtained with the Si/BDDREF reference electrode, electrochemically modified Si/BDD/PLL_{EC} electrode, and the Si/BDD/PLL_{WM} electrode modified by wet chemistry for different concentrations of adenine at pH 7.4: A) $2.0 \cdot 10^{-5}$ M/dm^3, B) $3.9 \cdot 10^{-5}$ M/dm^3 and C) $7.7 \cdot 10^{-5}$ M/dm^3.

The relationship between the oxidation peak and adenine concentration was compared for both modified electrodes (Fig. 8). The DPV results indicated that the higher limit of detection for adenine at pH 7.4 was achieved with the Si/BDD/PLL_{WM} electrode (red line). Summarizing, mainly electrochemically modified Si/BDD/PLL_{EC} electrode shows improved sensitivity to different concentrations of adenine comparing to bare BDD or Si/BDD/PLL_{WM} electrodes. The phenomenon could be result of increased effective electrode of area by the PLL modification or the electrostatic interactions between electrode surface and analytes. The next reason for that could be also the change of Gibbs energy of reaction products and by-products [71]. The BDD or other carbon electrodes modified by conductive polymers (here PLL) generally are more sensitive and selective not only due to protection from fouling but also by increase of effective area of electrode [72].

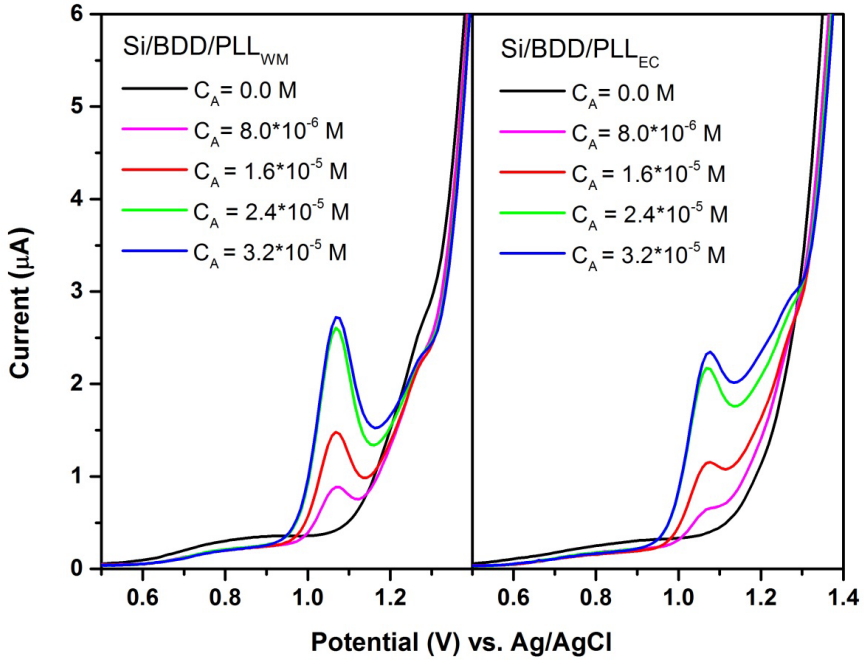

Fig. 8. Differential pulse voltammograms obtained with the electrochemically modified Si/BDD/PLL$_{EC}$ electrode and Si/BDD/PLL$_{WM}$ electrode modified by wet chemistry for different concentrations of adenine.

Based on the results of differential pulse voltammetry (DPV), the most intensive oxidation peaks were identified for guanine and adenine. The linear relationship between the concentration of adenine and peak oxidation current was observed in the concentration range 0 – $3.15 \cdot 10^{-2}$ mM, with the correlation coefficient R equal 0.978 and 0.974 for the measurements performed by means of the Si/BDD/PLL$_{WM}$ and Si/BDD/PLL$_{EC}$ electrodes, respectively (Fig. 9A). In the case of guanine, the relationship between the level of guanine and peak oxidation current was observed in the concentration range 0- $1.96 \cdot 10^{-2}$ mM, with the same value of R= 0.989 for both aforementioned electrodes (Fig. 9B). The minimum value of the detection limit for guanine and adenine measured by means of the chemically modified Si/BDD/PLL$_{WM}$ electrode was 3 µM and 4 µM, respectively. Whereas the detection limit for guanine and adenine measured with the electrochemically modified Si/BDD/PLL$_{EC}$ electrode reached 6 µM and 8 µM, respectively.

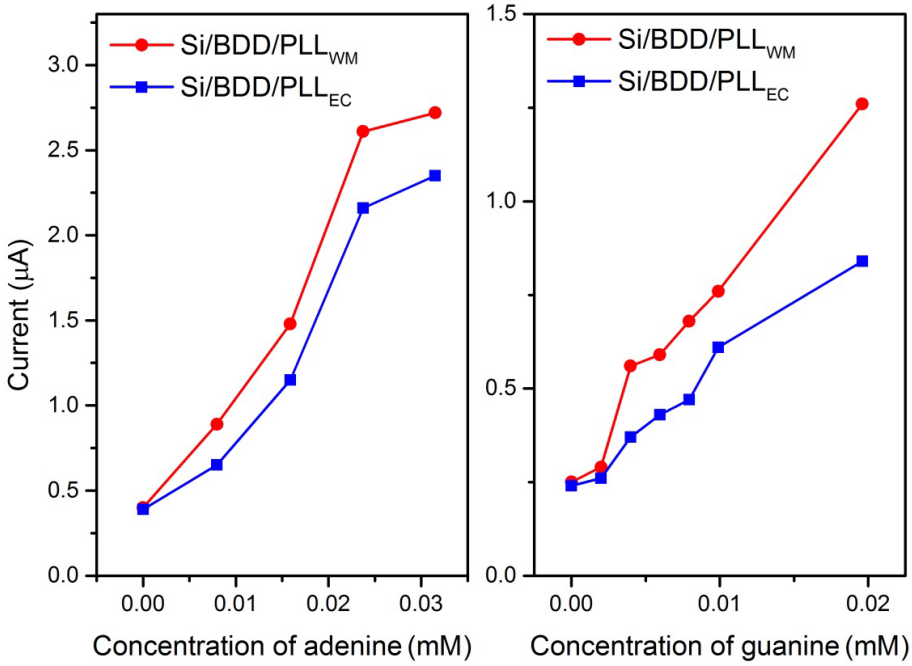

Fig. 8. Plots of Ip vs concentration of a) adenine b) guanine obtained from differential pulse voltammograms in the buffer solution pH 7.4.

The pH of a solution strongly influences the electrochemical process of nucleic acid base oxidation. Under highly acidic conditions, the basic centres of nucleic acid bases present in the solution and the amino groups of poly-L-lysine (PLL) on the BDD electrode exist in their protonated form. Electrically charged molecules of poly-L-lysine (PLL) on the surface of modified electrode have fundamental importance for the kinetics and thermodynamics of oxidation of nucleic acid bases [8]. In the present work, the influence of pH in the range 5.9-11.4 on the electrochemical oxidation process of nucleic acid bases was studied using differential pulse voltammetry (DPV). The varied electrochemical behaviour of examined nucleic acid bases in different pH values (acidic, neutral, and alkaline) is observed for all electrodes. As an example of such diversity is presented for electrode Si/BDD/PLL_{WM} obtained in wet chemical modification (Figs. 9 A and 9B). In acidic solutions, a single peak of oxidation was observed for adenine (1.27 V) and guanine (0.845 V) at pH 5.9. With increasing pH the oxidation peak potential shifted towards more negative values, while the height of peak current increased by 20-50% compared to that measured in the acidic solution. In addition, a non-specific increase in the current value for the more positive potentials during the oxidation process of guanine and adenine was observed. The differential pulse voltammograms (DPV) obtained for the pH values greater than 11 were more complex and analytically useless.

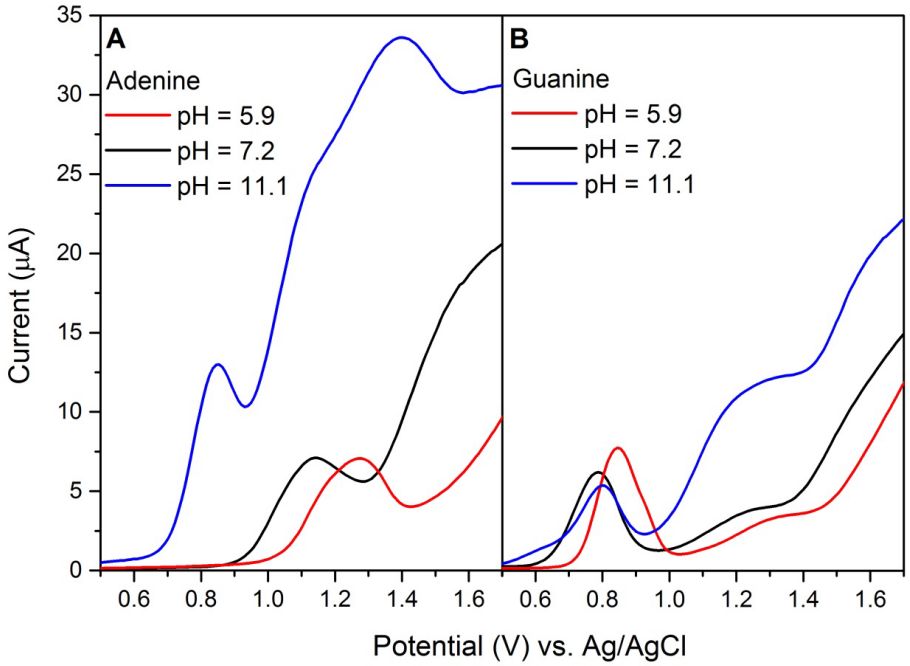

Fig. 9. Differential pulse voltammograms of $1.82 \cdot 10^{-4}$ mol/dm^{-3} of A) adenine B) guanine in a buffer solution at the pH values of 5.9, 7.2 and 11.1 for electrode Si/BDD/PLL$_{WM}$ obtained in wet chemical modification.

4. Conclusions

The presented results indicate that poly-L-Lysine (PLL) is a useful organic molecule for modifying the surface of BDD electrode by both the chemical and electrochemical procedures. The PLL-modified BDD electrodes are considerably smoother than those made of bare H-terminated BDD. The morphology of poly- L-Lysine films deposited onto the BDD substrate by means of the chemical and electrochemical processes is characterized by ovoid shape.

Furthermore, the electrochemical behaviour of BDD electrodes obtained via electrochemical (Si/BDD/PLL$_{EC}$) and wet chemical modification (Si/BDD/PLL$_{WM}$) was characterized by cyclic voltammetry and differential pulse voltammetry. Based on the collected

data, it can be concluded that the modified electrodes can be used for the detection of guanine and adenine at different pH values. The linear relationship between the concentration of adenine and peak oxidation current was observed in the concentration range 0 – $3.15 \cdot 10^{-2}$ mM, with the correlation coefficient R equal 0.978 and 0.974 for the measurements performed by means of the Si/BDD/PLL_{WM} and Si/BDD/PLL_{EC} electrodes, respectively. Moreover, the aforementioned electrode modification with PLL also enables the detection of inorganic ions.

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