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Comparison of Electrochemical Determination of Purines and Pyrimidines by means of Carbon, Graphite and Gold Paste Electrodes

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Comparison of electrochemical detection with the use of thick-film sensors with working electrodes made of graphite, carbon and gold pastes for individual determination of four nucleobases – adenine, guanine, cytosine and thymine is presented. The best results and oxidation peaks for the four nucleobases were obtained for gold paste electrodes. For purines limits of detection (LOD) and sensitivities obtained with the use of the developed gold paste electrodes were as follows: for adenine – $7.4 \cdot 10^{-7}$ M with mean sensitivity of 26 nA/μM, for guanine for two oxidation peaks – $1.3 \cdot 10^{-6}$ M and $2.6 \cdot 10^{-6}$ M with mean sensitivities 3 nA/μM and 1.6 nA/μM, respectively. Whereas, for pyrimidines, LOD and sensitivities obtained with the use of the same type of electrodes were as follows: for cytosine – $6.3 \cdot 10^{-7}$ M with sensitivity of 39.8 nA/μM, for thymine for two oxidation peaks – $1.3 \cdot 10^{-6}$ M and $6.4 \cdot 10^{-7}$ M with mean sensitivities 11.5 nA/μM and 34.1 nA/μM, respectively. Therefore, the highest sensitivities were obtained for adenine and cytosine, while the lowest values of LOD were for cytosine and thymine determination.

Keywords: electrochemical detection; nucleobases determination; differential pulse voltammetry (DPV); thick-film electrodes.

1. INTRODUCTION

DNA molecule consists of two strands coiled around each other to form a double helix. Those two strands are composed of simpler units called nucleotides. Each nucleotide is composed of a nitrogen – containing nucleobase: adenine (A), guanine (G), cytosine (C) or thymine (T), as well as monosaccharide sugar - deoxyribose and a phosphate group. Nucleobases are classified into two groups: the purines (A and G), and the pyrimidines (C and T), which are five– and six-membered heterocyclic compounds, respectively. Two DNA strands are joined together in pairs formed

by purines and pyrimidines *via* hydrogen bonds between complementary nucleobases (*i.e.* A–T or G–C) [1].

Many different methods have been used for nucleobases detection. The most commonly used are separation methods, such as: capillary electrophoresis (CE) [2-4], capillary electrophoresis/mass spectrometry (CE/MS) [5], high–performance liquid chromatography (HPLC) [6-9], capillary chromatography [10], ion chromatography [11] and others including: infrared reflection absorption spectroscopy (IRAS), making use of adsorption of cytosine, thymine, guanine and adenine on Cu(110) [12], as well as electrospray ionization mass spectrometry (ESI–MS) for purines detection [13].

Since all nucleobases could be oxidized and/or reduced at polarized electrode surface, an alternative to all above mentioned methods for purines and pyrimidines is electrochemical detection. This method has some advantages over instrumental methods such as low cost, fast response, simplicity of construction and small dimensions of the devices, small testing sample volume and the most important – high sensitivity. Therefore, intensive efforts to apply different electrode materials for detection of nucleobases and DNA oxidation were undertaken. DNA oxidation results in DNA damage occurring as guanine oxidation peak. It is due to the fact that guanine is the most electrochemically active nitrogenous base.

The conventional electrochemical detection of nucleobases in aqueous solutions is based on electroreduction/electrooxidation at renewable electrode surface. The example of such electrode is drop mercury electrode widely studied and described by Paleček [14-18] and by other authors [19, 20]. Polarographic signals coming from adenine and guanine are used in DNA biosensors to evaluate hybridization process. It is mainly due to A and G low oxidation potentials, lower than for other nucleobases - cytosine and thymine. Since then, many kinds of electrodes including disposable and regenerated ones were used for nucleobases detection *e.g.*: copper [21], highly boron–doped diamond [8, 22], as well as carbon disc-electrodes [3], carbon paste [23], pyrolytic graphite [24], gold electrodes for all nucleobases detection [25] and for individual guanine and thymine investigations [26, 27]. But the most frequently used and closely studied are glassy carbon electrodes [28-33]. There are also many types of modified electrodes, which were applied for simultaneous [34-46] or individual [47-50] nucleobases detection. The glassy carbon electrodes (GCE) were modified with silicon carbide nanoparticles (SiCNP/GC) [34], poly(alizarin red)/graphene composite film (PAR/graphene/GCE) [35], graphene/New Fuchsin – cationic dye (G/NF) films [36] or functionalized with boron–doped carbon nanotubes (BCNTs/GC) [37]. For simultaneous detection of adenine and guanine, glassy carbon electrodes were modified with nanocomposite films such as: a nano–material carboxylic acid functionalized graphene (graphene–COOH/GCE) [38], graphene-ionic liquid–chitosan [39], graphene–Nafion [40], silver nanoparticles–polydopamine–graphene [38], PbO_2–carbon nanotubes–ionic liquid [41] and with 2,6-pyridinedicarboxylic acid/multiwall carbon nanotubes (MWNTs) [42] and TiO_2–graphene nanocomposite bulk electrode [43]. For guanine and adenine determination, carbon paste electrodes were modified with cyclodextrin–modified poly(N–acetylaniline) (PNAANI) [44], multi-walled carbon nanotube–ionic liquid composite film [45]. Edge–plane pyrolytic graphite (EPPGE) electrodes were modified with single-walled carbon nanotubes (SWNT) and used in the simultaneous assays of adenine and adenosine monophosphate [46]. Thymine determination was based on carbon ionic

liquid electrode (CILE) fabricated using 1-(3–chloro–2–hydroxy–propyl)–3–methylimidazole acetate as a binder [47]. There are also described platinum electrodes for adenine determination modified with carbon nanotubes [48] and with redox polymer poly(vinylferrocenium) (PVF^+) [49]. For guanine and *ss*DNA detection a cobalt(II) phthalocyanine modification of electrodes was applied [50].

Both types of double and single stranded deoxyribonucleic acid – *ds*DNA and *ss*DNA are identified *via* individual and/or simultaneous purines and pyrimidines detection. It is useful tool for detection of DNA hybridization and damage [51]. Because pyrimidines (cytosine and thymine) oxidation peaks occur at higher positive potentials than for purines (adenine and guanine), hence DNA detection is usually based on purines oxidation [52, 53].

Moreover, Toh and Pumera reported that the electrochemical responses of DNA nucleobases in terms of peak potentials and current might be influenced by the sequences of an oligomer at standard glassy carbon and electrochemically reduced graphene oxide electrodes [54].

The electrochemical oxidation of purine and pyrimidine nucleobases has been studied widely [25, 28, 31, 33, 35]. Nevertheless, there are many difficulties to obtain the direct electrooxidation of pyrimidine nucleobases at the conventional glassy carbon electrodes. The main reasons for that are the following: the oxidation potentials of pyrimidines are very high and the electron transfer of pyrimidines at the conventional electrode is rather slow process. Recently, some attempts were undertaken to overcome or reduce these difficulties, mainly through surface modification with use of different nanomaterials *e.g.* multiwalled carbon nanotube (MWCNT)/choline monolayer–modified glassy carbon electrode [55] or SiC nanoparticles [32].

In this study results concerning comparison of different paste type materials such as: graphite, carbon and gold, used for electrochemical detection of four nucleobasis - adenine, guanine, cytosine and thymine, are presented. The literature review point out a problem with direct determination of each individual nucleobase (A, G, T and C) in aqueous solutions. Therefore, comparative study was performed by means of electrodes made of different materials: gold, carbon and graphite paste. To our best knowledge there are no reports concerning direct electrochemical detection of nucleobases on the gold paste electrodes. The obtained results are essential for our further investigations on development of label-free DNA-based biosensors.

2. EXPERIMENTAL

2.1. Reagents

All nucleobases – adenine (A), guanine (G), thymine (T) and cytosine (C) were purchased from Sigma-Aldrich. Stock aqueous standard solutions (200 µM) of each nucleobase were prepared without further purification in 0.1 M phosphate buffer containing 0.1 M KCl solution in the case of experiments with the use of carbon and graphite electrodes or 0.1 M phosphate buffer containing 0.01 M KCl solution in the case of gold electrodes. Both phosphate buffer solutions have pH value 7.5. The stock solutions were stored at 4°C. In all experiments, purified Milli-Q water was used. All measurements were carried out by means of three-electrode system operating in a batch-mode

at temperature about 25°C. The measurements were performed by a standard addition method where initial sample volume of phosphate buffer solution was 2 mL.

2.2. Electrodes preparation

For electrochemical sensor fabrication two thick-film technologies were applied: screen–printing (SP) and direct writing (R).

The working electrodes used in experiments can be divided into two groups: 1) graphite (G_R) and gold paste (Au_R) electrodes made by direct writing method using microdosing desk–top XYZ dispensing robot (325 Ultra TT by EFD, USA) fabricated in IBIB PAN [56] and 2) carbon (C_SP) and graphite (G_SP) paste electrodes fabricated with the use of screen-printing using home–made semi–automatic screen–printer (IBIB PAN). The sensors were obtained by deposition of three layers onto polyester foil (Autostat CT7). The 1st layer for contact pads was made of silver paste (5000, DuPont, USA). Then the 2nd layer for working electrodes was formed with graphite paste (L–951, ITME, Poland), gold paste (C2041206D2, Gwent Electronic Materials Ltd, UK) or carbon paste (7102, DuPont, USA). Finally, the 3rd layer was made of insulation paste (7165, DuPont, USA). Each layer was dried at temperature 120 ÷ 130°C for 15 min.

Surface areas of working electrodes in sensors fabricated by the direct writing and screen-printing techniques were as follows: 5±0.1 mm^2 (±SD – standard deviation) and 4.5±0.1 mm^2, respectively.

In the case of carbon and graphite paste electrodes, there was no need for surface pretreatment before measurements. However, prior measurements of the gold electrodes fabricated using microdosing robot, the working electrode surface was polished with stainless steel needle and then, electrochemically cleaned in 0.5 M H_2SO_4 solution using cyclic voltammetry method under the following conditions: potential range: 0 ÷ 1.55 V, E_{step}: 0.005 V, E_{start}: 0 V, scan rate: 0.5 V/s, number of scans: 3. Afterwards the electrode surface was thoroughly rinsed with Milli–Q water.

2.3. Measurement method

Electrochemical measurements were carried out with portable potentiostat PalmSens (Palm Instruments BV, The Netherlands), with conventional three–electrode system, employing differential pulse voltammetry (DPV). Saturated calomel electrode (SCE) was used as a reference electrode, platinum wire – as an auxiliary electrode, and a sensor electrode made of different materials – as a working electrode. Two sets of parameters for DPV method were applied - set of parameters no. 1: potential range: 0 ÷ 1.4 V (in some measurements: 0 ÷ 1.35 V), E_{step}: 0.01 V, E_{pulse}: 0.008 V, scan rate: 0.005 V/s, t_{pulse}: 0.07 s, t_{cond}: 10 s, E_{cond}: 0 V, number of scans: 1 and set of parameters no. 2: 0 ÷ 1.6 V, E_{step}: 0.01 V, E_{pulse}: 0.07 V, scan rate: 0.01 V/s, t_{pulse}: 0.07 s, t_{cond}: 10 s, E_{cond}: 0 V, number of scans: 1. All experiments were performed for nucleobases by a standard addition method.

3. RESULTS

As mentioned, there is a problem with direct determination of each individual nucleobase (A, G, C, T) in aqueous solutions. To solve the problem a comparative study for different working electrode materials was applied.

3.1. Purines determination

Both, adenine and guanine oxidation reactions that involve exchange of defined electrons and protons (H^+), are irreversible. Oxidation reaction (electrooxidation) taking place at the electrode at certain potential depends on several factors, related to properties of the electrode material, such as: density of surface adsorption sites and ability to electron transfer. The adenine and guanine molecules adsorbed at the surface sites undergo oxidation reaction, which is three-step. The A and G oxidation processes involve 6 and 4 electrons and protons, respectively [44, 57]. Electrooxidation reaction of purines – adenine and guanine, are shown in Figure 1.

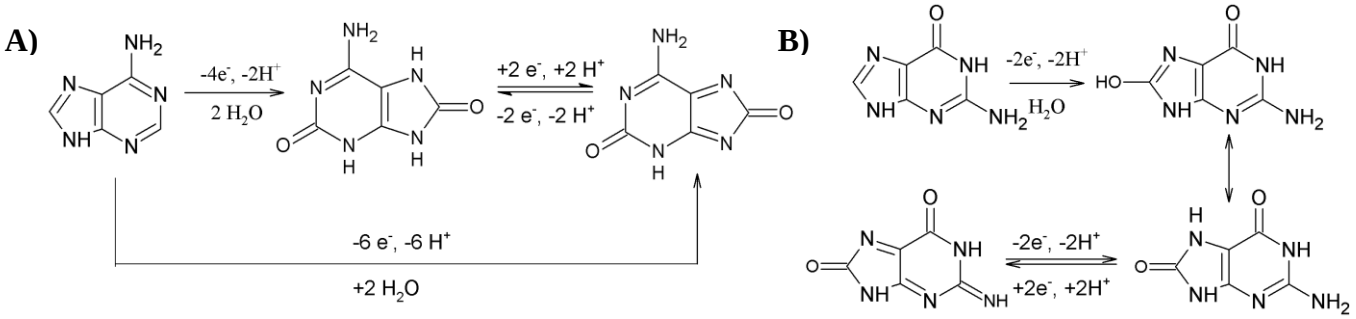

Figure 1. A) Adenine and **B)** guanine electrooxidation reactions, after [57] and [44], respectively.

Depending on the experimental conditions, in particular on the electrode material properties, at voltamperogramms obtained during electrooxidation processes one or more oxidation peaks might be visible.

Exemplary voltammograms – shown after baseline correction for adenine and without baseline correction for guanine obtained with the use of differential pulse voltammetry for set of parameters no. 2 are shown in Figure 2. Due to application of the 2nd set of parameters, it was possible to get two–step oxidation reaction of guanine received by means of the gold (two oxidation peaks visible in voltammograms – Figure 2B) and graphite paste electrodes (Table 1). In the case of both graphite and carbon screen-printed electrodes, adenine and guanine were detected using DPV method with set of parameters no. 1.

All analytical parameters obtained for purines: adenine and guanine detection with the use of four kinds of electrodes are listed in Table 1.

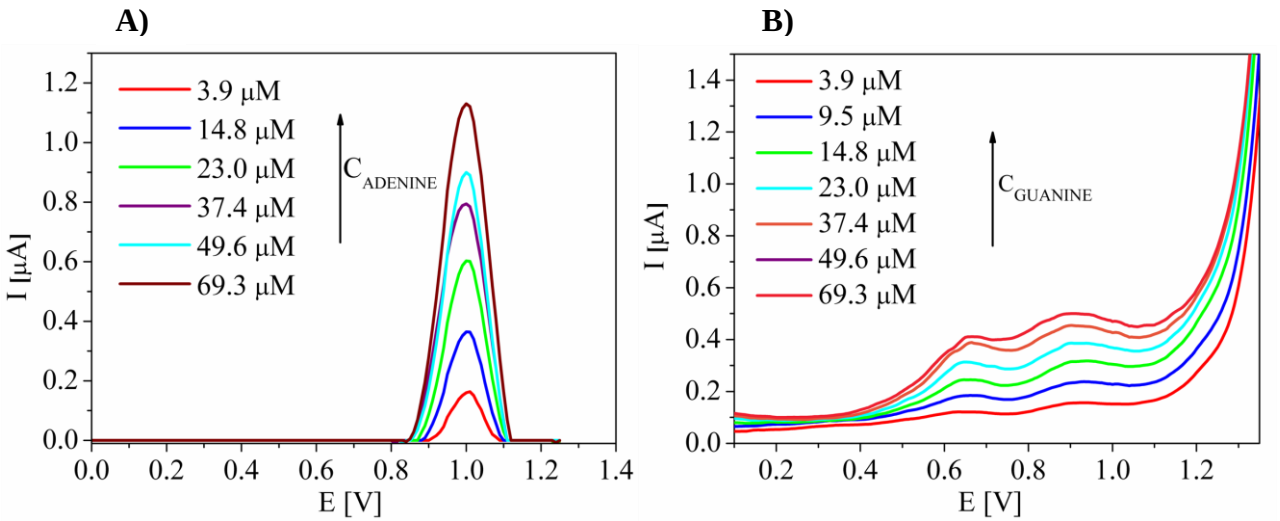

Figure 2. Exemplary voltammograms after baseline correction obtained for **A)** adenine and before baseline correction obtained for **B)** guanine with the use of gold electrodes with DPV method (set of parameters no.2).

Table 1. Sensors analytical parameters obtained for purines detection with the use of four kinds of sensors and two sets of parameters.

Sensor type → Analytical parameters ↓	G_R (par. 1)	G_SP (par. 1)	C_SP (par. 1)	Au_R (par. 2)	G_R (par. 2)
Adenine					
Mean sensitivity [nA/µM] ± RSE [%]	16.2 ±6.75	0.6 ±13.1	1.3 ±8.8	26 ±9	1.6 ±7.5
R^2	1	0.95	0.99	0.98	0.99
Linear concentration range [µM]	3.9 ÷ 37.4	3.9 ÷ 69.3	3.9 ÷ 69.3	3.9 ÷ 69.3	3.9 ÷ 69.3
Mean potential [V]	0.98	1	0.99	0.98	0.88
LOD [M]	$5.0 \cdot 10^{-8}$	$3.9 \cdot 10^{-6}$	$1.1 \cdot 10^{-6}$	$7.4 \cdot 10^{-7}$	$3.9 \cdot 10^{-6}$
Guanine					
Mean sensitivity [nA/µM] ± RSE [%] for peaks at E_{low} for peaks at E_{hi}	0.3 ±4.5	0.26 ±9.5	0.4 ±8	3 ±10.5 1.6 ±11	1.5 ±1.55 3 ±1.2
R^2 for peaks at E_{low} for peaks at E_{hi}	0.99	0.99	0.99	0.98 0.97	0.99 1
Linear concentration range [µM] for peaks at E_{low} for peaks at E_{hi}	3.9 ÷ 69.3	3.9 ÷ 49.6	3.9 ÷ 49.6	3.9 ÷ 23.0 3.9 ÷ 23.0	3.9 ÷ 37.4 3.9 ÷ 37.4
Mean ox. potential [V] for peaks at E_{low} for peaks at E_{hi}	0.67	0.67	0.67	0.65 0.9	0.67 0.88
LOD [M]	$10.2 \cdot 10^{-8}$	$3.8 \cdot 10^{-6}$	$2.1 \cdot 10^{-6}$	$1.3 \cdot 10^{-6}$ $2.6 \cdot 10^{-6}$	$3.8 \cdot 10^{-6}$ $2.1 \cdot 10^{-6}$

In the case of adenine detection, the best results, namely the highest mean sensitivity values of 16.2 and 26 nA/µM and the lowest limits of detection of $5 \cdot 10^{-8}$ M and $7.4 \cdot 10^{-7}$ M were achieved for measurements performed by means of graphite and gold paste electrodes fabricated with the use of the microdosing robot, respectively. For guanine, the best results were obtained for gold and graphite paste electrodes where in DP voltammograms 2 peaks at 0.65 V and 0.9 V, and 0.67 V and 0.88 V are visible. In this case, the mean sensitivity calculated for each peak obtained for gold and graphite electrodes was 3 nA/µM and 1.6 nA/µM, and 1.5 nA/µM and 3 nA/µM, for low (E_{low}) and high (E_{hi}) potentials of oxidation peaks, respectively. Low value of detection limit was achieved also for the screen–printed graphite electrodes – $3.8 \cdot 10^{-6}$ M with relatively low mean sensitivity.Relative standard error (RSE) for adenine detection on investigated sensors ranged from 6.75% to 13%, the lowest RSE was achieved for graphite electrodes made by microdosing robot. For guanine detection RSE was in a range - 1.2 ÷ 11%. In this case, the lowest RSE value was obtained for graphite electrodes made by microdosing robot for DPV measurements carried out using set of parameters no. 2.

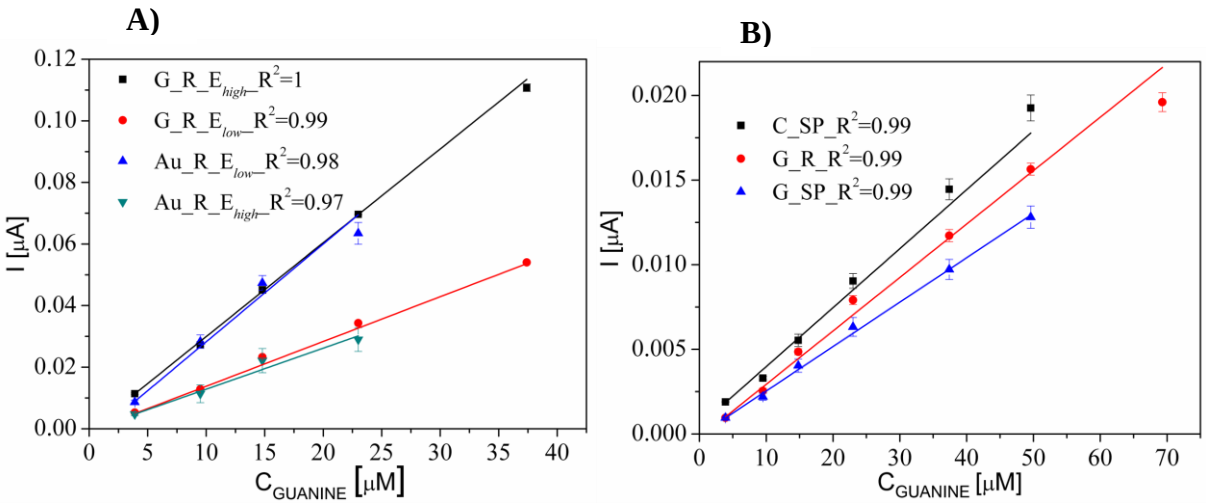

Figure 3. Calibration curves for guanine obtained by means of **A)** graphite and gold paste electrodes as well as **B)** graphite and carbon paste electrodes, presented as a mean value for peaks height (n=4, ±SE). Measurements performed in guanine solutions in concentration range from 3.9 to 69.3 µM in 0.1 M phosphate buffer (pH 7.5) with DPV method, E_{low} – lower potential and E_{hi} – higher potential of oxidation peaks.

A linear correlation coefficient calculated in the linear concentration range – calculated for each calibration curve for investigated electrode material, obtained for purines was very high – 0.99. On the other hand, for gold electrodes this parameter was lower – 0.98 and 0.98, 0.97 for adenine and guanine, respectively. The mean peak potential for adenine was about *c.a.* 1 V for graphite and carbon electrodes, and 0.98 V for gold paste ones. In the case of guanine detection, peak potential increased with the rise of guanine concentration. In Figure 4 are shown calibration curves for guanine as a purines representative. Measurements performed with the use of two sets of DPV parameters were carried out by means of four types of electrodes – graphite made by screen-printing method and with the use of microdosing robot, screen–printed carbon electrodes and gold electrodes made with microdosing robot. As it can be seen in Figure 3, the highest sensitivity

for guanine was obtained for gold paste electrodes if oxidation peaks formed at low potential are considered. In the case of graphite paste electrodes G_R type, the sensitivity calculated for the oxidation peaks obtained at higher potential was comparable to that for Au_R electrodes. Since lower oxidation potential of guanine was obtained for Au_R electrodes, this type of electrodes were selected for further experiments.

3.2. Pyrimidines determination

Since, cytosine and thymine are considered as poorly electrochemically oxidized compounds, the DPV based detection was performed with two sets of parameters. However, only measurements carried out with parameters set no. 2 allowed to get oxidation peaks of investigated nucleobases. Schemes of electrooxidation reactions for pyrimidines – cytosine and thymine, are shown in Figure 4.

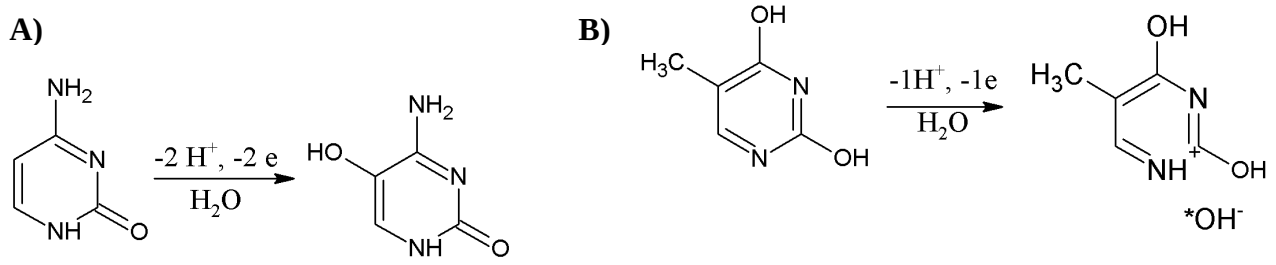

Figure 4. A) Cytosine and **B)** thymine electrooxidation reactions, after [23] and [47], respectively.

Exemplary differential pulse voltammograms obtained for two nucleobases cytosine and thymine, with the use of gold electrodes are shown in Figure 5. Depending on the experimental conditions, in particular on the electrode material properties, at voltamperogramms obtained for cytosine [23, 58] during electrooxidation processes one or more oxidation peaks might be visible, while for thymine electrooxidation reaction usually one [47, 59].

Results obtained for both cytosine and thymine nucleobases detected by means of the screen–printed carbon electrodes confirm this point with respect to measurements performed with set of parameters no. 1. Using the same set of parameters in measurements for thymine detection with use of the graphite electrodes made by microdosing robot and screen–printing technique, there was no current peaks. Positive results of cytosine detection were obtained using graphite electrodes (parameters set no. 1) with mean sensitivity 0.23 nA/μM and 0.2 nA/μM for electrodes fabricated by microdosing robot and screen–printing technique, respectively. For screen–printed graphite electrodes, relative standard error was lower (9%), linear correlation coefficient calculated for linear concentration range (37.4 ÷ 69.3 μM) for both types of graphite electrodes was equal to 1. In these cases the mean cytosine oxidation peaks occurred at the same potential - 1.26 V. Our experiments showed that change of DPV parameters (set no. 1 and 2) allowed to obtain detection of both cytosine and thymine nucleobases using graphite and carbon paste electrodes. In the case of cytosine detection, the highest mean sensitivity and the lowest limit of detection were obtained for gold electrodes made by microdosing robot – 39.8 nA/μM and $6.3 \cdot 10^{-7}$ M, respectively.

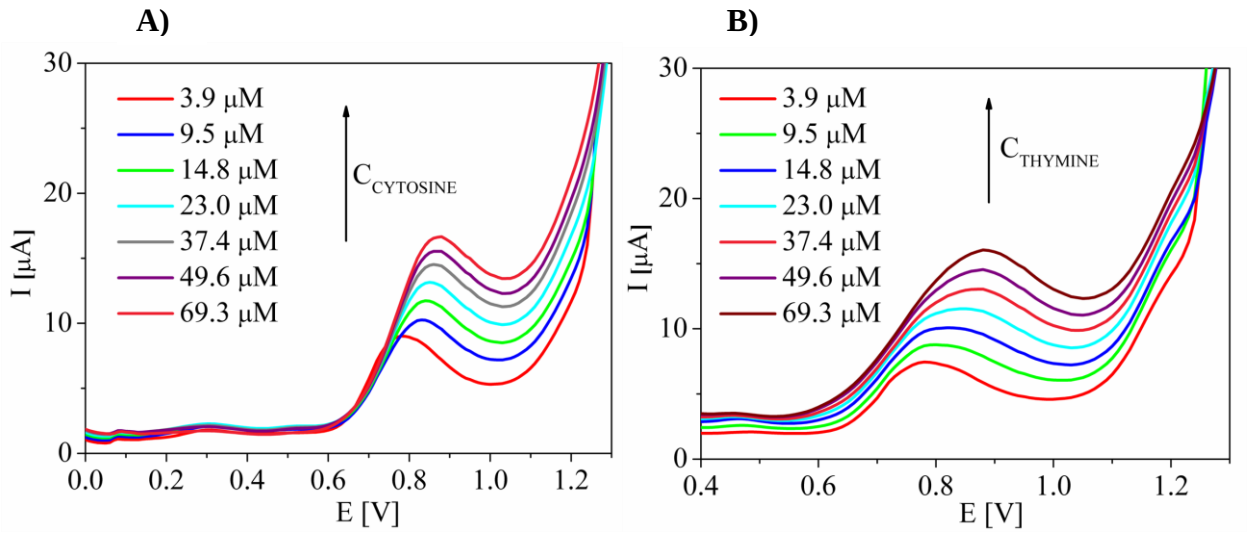

Figure 5. Exemplary voltammograms before baseline correction obtained for cytosine **A)** and for thymine **B)** with the use of gold electrodes with DPV method (set of parameters no. 2).

For thymine detection the highest sensitivity and the lowest value of detection limit were obtained also for gold paste electrodes, in particular sensitivity – 11.5 nA/µM and 34.1 nA/µM, and LOD - $1.3 \cdot 10^{-6}$ M and $6.4 \cdot 10^{-7}$ M corresponding to low and high peak potentials (E_{low} and E_{hi}) 0.45 V and 0.8 V, respectively. Relative standard errors obtained for cytosine and thymine detected with the use of gold and graphite electrodes made by microdosing robot were as follows – 6.4% and 4.5%, and 13.65% and 10.5%, respectively. As it can be seen relative standard errors for thymine were higher than for cytosine. Linear correlation coefficients obtained for cytosine were in range 0.96 ÷ 1 (the lowest for gold electrodes), and in range 0.9 ÷ 0.99 for thymine (the lowest – for gold paste electrodes). In the case of thymine detection by gold paste electrodes, two oxidation peaks were observed and linear correlation coefficients were 0.9 and 0.94. Linear concentration range for cytosine was from 37.4 up to 69.3 µM, only in the case of using second set of parameters oxidation range was wider, the best results we got for gold electrodes 14.8 ÷ 69.3 µM. Mean potential of oxidation peaks was between 0.83 ÷ 1.28 V for cytosine and 0.8 ÷ 1.1 V for thymine, both detected with graphite and carbon electrodes. In the case of pyrimidines detection with gold electrodes, potential of oxidation peaks shifted to higher values with the rise of concentration cytosine and thymine, namely for cytosine potential of oxidation peak shifted from 0.77 V to 0.85 V, and 0.75 to 0.9 V – for thymine.

As it can be seen in Tables 1 and 2, in some cases, depending on the electrode material and measurement parameters as a result one– or two–step electrooxidation reaction can be obtained. The two oxidation peaks instead of one oxidation peak of oxidized substance. Such situation was in the case of guanine and thymine oxidation on gold paste electrodes and on graphite paste electrodes made with the use of direct writing technique, but when set of parameters no. 2 was applied. Moreover, two oxidation peaks of thymine have been obtained with the use of carbon paste electrodes, when set of parameters 2 was applied, as well.

In Figure 6 A and B are shown calibration curves for cytosine – the pyrimidine representative. Measurements were carried out for two sets of DPV parameters, by means of four types of electrodes – graphite sensors made with the use of screen–printing and with the use of microdosing robot, screen–printed carbon electrodes and gold electrodes made with microdosing robot.

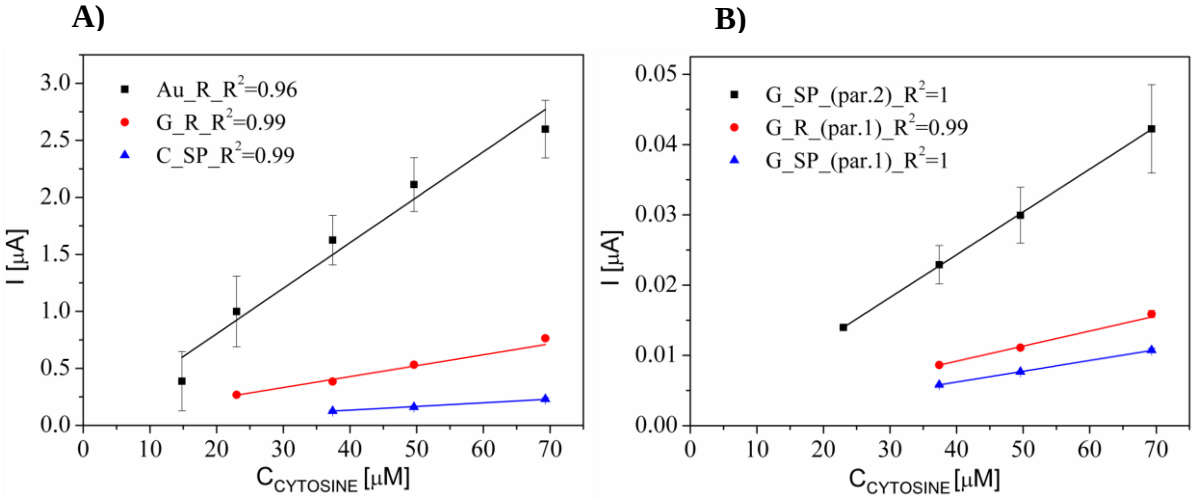

Figure 6. Calibration curves for cytosine obtained by means of **A)** graphite, carbon and gold paste electrodes with the use of parameters no. 2, **B)** graphite paste electrodes obtained using sets of parameters no. 1 and 2, presented as mean values for oxidation peaks height (n=4, ±SE). Measurements performed in cytosine solutions in concentration range from 3.9 to 69.3 µM in 0.1 M phosphate buffer (pH 7.5) with DPV method.

All analytical parameters obtained with the use of four kinds of electrodes for pyrimidines detection and two sets of parameters are listed in Table 2.

Electrochemical investigations on various electrode materials reveal mechanisms of redox reactions of nucleobases on the electrode surfaces. As it can be seen, in some cases, in particular for guanine and thymine oxidation on gold and on graphite paste electrodes made with the use of direct writing technique, when set of DPV parameters no. 2 were applied, two oxidation peaks were obtained.

Table 2. Results obtained for pyrimidines detection with the use of four kinds of sensors and two sets of parameters.

Sensor type → Analytical parameters ↓	G_R (par. 1)	G_SP (par. 1)	C _SP (par. 1)	Au_R (par.2)	G_R (par. 2)	G_SP (par.2)	C_SP (par.2)
Cytosine							
Mean sensitivity [nA/µM] ± RSE [%]	0.23 ±15.3	0.2 ±9	No peaks	39.8 ±6.4	11 ±4.5	0.6 ±26.35	3.3 ±14.6
R^2	0.99	1		0.96	0.99	1	0.99
Linear concentration range [µM]	37.4 ÷ 69.3	37.4 ÷ 69.3		14.8 ÷ 69.3	23 ÷ 69.3	23 ÷ 69.3	37.4 ÷ 69.3
Mean oxidation potential [V]	1.26	1.27		0.83	1.28	0.85	1.27

LOD [M]	$3.7 \cdot 10^{-6}$	$32.1 \cdot 10^{-6}$		$6.3 \cdot 10^{-7}$	$21.1 \cdot 10^{-6}$	$9.3 \cdot 10^{-6}$	$35.6 \cdot 10^{-6}$
Thymine							
Mean sensitivity [nA/μM] ± RSE [%] for peaks at E_{low} for peaks at E_{hi}	No peaks			11.5 ±13.65 34.1 ±7.6	3 ±10.5	0.73 ±9 2.6 ±15.85	0.8 ±9.25 2.1 ±17.6
R^2 for peaks at E_{low} for peaks at E_{hi}				0.9 0.94	0.95	0.99 0.98	0.99 0.98
Range [μM] for peaks at E_{low} for peaks at E_{hi}				3.9 ÷ 69.3 3.9 ÷ 69.3	23 ÷ 69.3	3.9 ÷ 69.3 3.9 ÷ 69.3	3.9 ÷ 69.3 9.5 ÷ 69.3
Mean ox. pot. E_{low} [V] E_{hi} [V]				0.45 0.8	1.13	0.67 1.1	0.66 1.08
LOD [M]				$1.3 \cdot 10^{-6}$ $6.4 \cdot 10^{-7}$	$3.9 \cdot 10^{-6}$	$3.9 \cdot 10^{-6}$ $2.8 \cdot 10^{-6}$	$3.8 \cdot 10^{-6}$ $8.6 \cdot 10^{-6}$

Taking into account all results obtained with the use of gold paste, carbon paste, and graphite paste electrodes with the use of parameters set number 1 and 2, it can be stated that the best results – the highest sensitivity of the sensors for all nucleobases, adenine, guanine, thymine and cytosine detection were obtained with the use of gold paste electrodes (measured with only one set of parameters – no. 2). In the case of this material oxidation peaks for the four nucleobases occurred, with high sensitivity and good linear correlation coefficient. Moreover, investigated gold paste electrodes have important advantage over the other types of electrodes, namely they can be used for controlled immobilization of oligonucleotides, *via* thiol terminal groups, which are able to form covalent Au–S bonds. This kind of immobilization method is more efficient than physical adsorption on an electrode surface, which is often used for carbon–based electrodes.

4. DISCUSSION

In the case of adenine electrochemical determination with the use of electrodes made of different materials and in some cases with the use of multistage electrode modifications: graphene-NF, graphene-COOH and cyclodextrin - poly(N-acetylaniline) (PNAANI) described in publications [36, 38, 44], very low values of detection limits – $9 \cdot 10^{-7}$ M, $2.5 \cdot 10^{-8}$ M and below $5 \cdot 10^{-8}$ M were obtained, respectively (Table 3). Sensitivity of these sensors for adenine detection was very high, especially for the carbon paste electrodes modified with graphene/New–Fuchsin film and cyclodextrin poly(N–acetylaniline), in particular it was equal to 1401.2 nA/μM and 598.8 nA/μM, respectively.

Due to the fact, that majority of the presented sensors were chemically modified, the results obtained with our sensors can only be directly compared with commercially available bare glassy carbon electrodes [30, 60]. The highest sensitivity for thymine determination was obtained with the use of sensor described in [36] – 153.7 nA/μM, but with quite high value of detection limit – $9.9 \cdot 10^{-6}$ M. Comparing results obtained by means of the developed in our laboratory gold paste electrodes with

commercially available glassy carbon ones [30], it can be stated that analytical parameters for both are similar. For the gold paste electrodes fabricated by direct writing, sensitivity calculated for the second oxidation peak formed at potential of 0.8 V and low detection limit were 34.1 nA/μM and $6.4 \cdot 10^{-7}$ M, respectively.

Table 3. Parameters of electrochemical detection of nucleobases: adenine, guanine, cytosine and thymine by means of electrodes made of different materials.

Electrode material, Electrochem. method, Electrode surface area	Nucleobase	Mean potential [V]	Mean sensitivity [nA/μM]	LOD [M]	Ref
glassy carbon DPV, 1.8 mm^2	Adenine Guanine Cytosine Thymine	0.96 0.70 1.13 1.17	33.67 34.46 3.35 5.04	$7.0 \cdot 10^{-8}$ $6.0 \cdot 10^{-8}$ $1.8 \cdot 10^{-8}$ $8.9 \cdot 10^{-7}$	[30]
glassy carbon (graphene-COOH/GCE) DPV, 7.1 mm^2	Adenine Guanine	1.30 0.90	6.9 10.3	$2.5 \cdot 10^{-8}$ $5.0 \cdot 10^{-8}$	[38]
carbon paste modified with cyclodextrin - poly(N-acetylaniline) (PNAANI) DPV, 3.1 mm^2	Adenine Guanine	0.90 0.65	598.8 598.0	$<5 \cdot 10^{-8}$ $<5 \cdot 10^{-8}$	[44]
CV, 3.1 mm^2	Adenine Guanine	1.00 0.70	36.5 25.4	$<1 \cdot 10^{-6}$ $<1 \cdot 10^{-6}$	
electrochemically activated glassy carbon LSV, 7.9 mm^2	Adenine Guanine Thymine	1.04 0.74 1.23	97.6 18.3 14.5	$0.1 \cdot 10^{-6}$ $1.01 \cdot 10^{-6}$ $1.1 \cdot 10^{-7}$	[60]
glassy carbon (GCE/graphene/NF) DPV, 7.0 mm^2	Adenine Guanine Cytosine Thymine	0.95 0.71 1.37 1.11	1401.2 29.7 176.1 153.7	$9.0 \cdot 10^{-7}$ $1.9 \cdot 10^{-5}$ $9.9 \cdot 10^{-6}$ $9.9 \cdot 10^{-6}$	[36]
gold paste deposited by direct writing DPV, 5.0 mm^2	Adenine Guanine Cytosine Thymine	0.98 (A) 0.65 (G) 0.90 (G) 0.83 (C) 0.45 (T) 0.80 (T)	26.0 (A) 3.0 (G) 1.6 (G) 39.8 (C) 11.5 (T) 34.1 (T)	$7.4 \cdot 10^{-7}$ (A) $1.3 \cdot 10^{-6}$ (G) $2.6 \cdot 10^{-6}$ (G) $6.3 \cdot 10^{-7}$ (C) $1.3 \cdot 10^{-6}$ (T) $6.4 \cdot 10^{-7}$ (T)	This paper
graphite paste deposited by direct writing DPV, 5.0 mm^2	Adenine Guanine Cytosine	0.98 0.67 1.26	16.2 0.3 0.23	$5.0 \cdot 10^{-8}$ $1.0 \cdot 10^{-7}$ $3.7 \cdot 10^{-6}$	This paper

Summarizing, the results obtained for the sensors fabricated by relatively cheap, flexible and simple technique such as direct writing, are similar to the results obtained for commercially available GC electrodes. Results obtained for measurements performed with the use of gold paste electrodes reveal possibility of each individual nucleobase detection (A, G, T and C). Moreover, it is worth

to say, that oxidation peaks of nucleobases obtained for measurements performed with the use of gold paste electrodes developed in our laboratory do not exceed potential of 1 V. This is particularly important for electrochemical oxidation of pyrimidines, which usually exhibit very high oxidation potentials exceeding 1.1 V [30, 36, 60]. According to these results it can be stated that usage of the gold paste electrodes allows to decrease oxidation peak potentials of nucleobases, especially pyrimidines and in some cases purines [38, 60].

5. CONCLUSIONS

Four types of electrochemical sensors were applied for determination of four nucleobases – adenine, guanine, cytosine and thymine. In the case of differential pulse voltammetry (DPV) for pyrimidines determination, graphite paste electrodes made with the use of microdosing robot as well as screen–printed graphite and carbon electrodes, two sets of parameters were used. In the case of parameters no. 2, for gold paste electrodes and graphite paste electrodes (Au_R and G_R), oxidation peaks for the four nucleobases were obtained. Owning to chemical modifications of the electrodes surfaces, only results for glassy carbon ones can be compared with our sensors [30, 60].

The best results and oxidation peaks for the four nucleobases were obtained for gold paste electrodes. For purines limits of detection (LOD) and sensitivities obtained with the use of the developed gold paste electrodes were as follows: for adenine – $7.4 \cdot 10^{-7}$ M with mean sensitivity of 26 nA/μM, for guanine for two oxidation peaks – $1.3 \cdot 10^{-6}$ M and $2.6 \cdot 10^{-6}$ M with mean sensitivities 3 nA/μM and 1.6 nA/μM, respectively. Whereas, for pyrimidines, LOD and sensitivities obtained with the use of the same type of electrodes were as follows: for cytosine – $6.3 \cdot 10^{-7}$ M with sensitivity of 39.8 nA/μM, for thymine for two oxidation peaks – $1.3 \cdot 10^{-6}$ M and $6.4 \cdot 10^{-7}$ M with mean sensitivities 11.5 nA/μM and 34.1 nA/μM, respectively. Therefore, the highest sensitivities were obtained for adenine and cytosine, while the lowest values of LOD were for cytosine and thymine determination.

Presented results reveal possibility of use of gold paste electrochemical sensors for adenine, guanine, cytosine and thymine individual determination. Moreover, in comparison to results described in literature [30, 36, 38, 60], it can be stated that the usage of gold paste electrodes allows to decrease oxidation peak potentials for all nucleobases, especially for pyrimidines.

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