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Article

Quantum Dots for Electrochemical Labelling of Neuramidinase Genes of H5N1, H1N1 and H3N2 Influenza

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Influenza is a highly contagious respiratory infectious disease causing a respiratory tract infection with significant morbidity and mortality in high-risk group**s.** The most important tool in the fight against influenza pandemic is timely and right diagnosis. The aim of this study was determine QDs-ODN complexes. Electrochemical methods are suitable not only for the detection of metal parts of the QDs, but also for detection of target oligonucleotide. Therefore, we determined metal peaks as well as CA and G peaks. Primarily, we aimed at studying of electrochemical behaviour of Zn(II), Cd(II), Pb(II) and Cu(II) ions by differential pulse anodic stripping voltammetry (DPASV). Further, we compared the results with behaviour of CdS, ZnS, PbS and CuS quantum dots (QDs). Finally, we analysed complexes between oligonucleotides (ODNs) derived from influenza and the synthesized QDs not only by DPASV but also by square wave voltammetry to detect peaks of nucleic bases. We found that ODN-QDs complexes could be easily recognized by voltammetries used in this study.

Keywords: Electrochemical Detection; Quantum Dots; Influenza; Voltammetry; Oligonucleotide; Adsorptive Transfer Technique; Differential Pulse Voltammetry; Stripping Voltammetry

1. INTRODUCTION

Influenza is a common and highly contagious respiratory infectious disease causing a respiratory tract infection with significant morbidity and mortality in high-risk group as elderly, new-borns and immunosuppressed [1-7]. Influenza viruses of varying pathogenicity cause not only annual epidemics but also continue to cause unpredictable and perilous worldwide pandemics [8-11]. The

greatest pandemic was the Spanish flu in 1918 with nearly 50 million deaths [12-14]. There are two major antigenic glycoproteins on the surface of influenza virion, which are responsible for influenza virulence as hemagglutinin (HA) and neuraminidase (NA) [5,15]. Mutational changes of influenza surface antigens pose a risk of generation of new and more dangerous subtype as highly pathogenic avian influenza (HPAI), which have morbidity app. 60 % [16,17], and can be transmissible in human population. These changes can also pose a risk of origin of resistance to antiviral drugs [18-22]. The most important tool in the fight against influenza pandemic is timely and right diagnosis. Developing of accurate, rapid and more sensitive method for detection of disease is critical for global health [23-25]. Standard methods such as immunoassays, cultivation and polymerase chain reaction (PCR) have few drawbacks such as cultivation is time consuming, PCR is very sensitive to contamination and sometime give together with immunoassays false positive results [6,26,27].

The unique chemical and physical properties of nanoparticles make them extremely suitable for designing new sensing devices, especially electrochemical sensors and biosensors [1,28-30]. Various types of nanoparticles, such as metal, oxide and semiconductors have been used for designing and constructing electrochemical sensors and biosensors [31-40]. The important functions provided by nanoparticles are the immobilization of biomolecules, the catalysis of electrochemical reactions, the enhancement of electron transfer between electrode surfaces and biologically active compounds, labelling of biomolecules and even acting as reactant [34]. Due to mentioned facts, the application of biosensing methods for influenza detection is topic [41]. Target ODN is usually detected by hybridization process using different materials such as biotin-avidin, protein (substrate) -enzyme, antigen-antibody and fluorescent active substances as QDs [42,43]. Recent advantages in using of QDs lead to a wide range of application area [44-48].

Electrochemical tools are suitable to be used in biosensing technologies to determine DNA [49-61]. Electrochemical DNA analysis is based on direct determination of DNA, or on determination of electroactive label in DNA [62]. Label-free detection of DNA is the easiest and low cost variant of detection and from these reasons it is widespread [63]. During the direct detection of DNA, there are adenine and cytosine reduced at the surface of mercury electrode (at -1.4 V) giving CA peak. Moreover, we can observe the oxidation of product of guanine reduction, which is not noticeable at mercury electrode, at -0.3 V [64]. The most sensitive voltammetric method for the direct determination of DNA is square wave voltammetry connected with adsorptive transfer technique (AdT SWV) [49].

Label-based methods can be used for recognition of modified and unmodified DNA [1]. It can be labelled only one nucleotide, all nucleotides or just target sequence [65]. Ability to determine the target (modified) DNA from other (non-modified) one is advantageous for hybridization assays and, therefore, for diagnosis of pathogen presence [66]. Another advantage is the lower detection limit compared to direct DNA detection [1]. Some of the widely used labels include methylene blue [67], Meldola's blue [68], ferrocene and organic ligand complexes with osmium, ruthenium and copper [69-71]. In addition to the mentioned labels, quantum dots (QDs) become more intensively used tags for DNA labelling that have favourable electroactive and optical properties [72,73]. QDs are nearly spherical semiconductor particles with diameters from 2 to 10 nm, comprising 200 to 10,000 atoms [74-76]. QDs have size-controlled luminescence functions, which means that the same material with variable size can exhibit different colours under the excitation of an appropriate wavelength; broad

absorption spectra; and narrow emission spectra, which means simultaneous excitation of different coloured QDs by a single wavelength [75,77]. Moreover, they have good electroactivity, which can be used for sensing of various biologically active compounds [24,78]. The aim of this study was determine QDs-ODN complexes. Electrochemical methods are suitable not only for the detection of metal parts of the QDs, but also for detection of target oligonucleotide. Therefore, we determined metal peaks as well as CA and G peaks.

2. MATERIAL AND METHODS

2.1 Chemicals

All chemicals used in this study were purchased from Sigma Aldrich (Sigma-Aldrich, USA) in ACS purity unless noted otherwise. Stock solutions were prepared with ACS water. pH value was measured using inoLab Level 3 (Wissenschaftlich-Technische Werkstatten GmbH; Weilheim, Germany). Deionised water underwent demineralization by reverse osmosis using Aqua Osmotic 02 (Aqua Osmotic, Tisnov, Czech Republic) and was subsequently purified using Millipore RG (MiliQ water, 18 MΩ, Millipore Corp., USA). Deionised water was used for rinsing, washing and buffer preparation.

2.2 Preparation of QDs (CdS, ZnS, PbS and CuS)

All chemicals were purchased from Sigma-Aldrich and used without further purification. CdS QDs were prepared according to previously published paper [79] with a few modifications. Briefly, cadmium nitrate tetrahydrate $Cd(NO_3)_2 \cdot 4H_2O$ (0.0309 g, 0.1 mM) was dissolved in ACS water (25 ml). 3-mercaptopropionic acid (35 µl, 0.4 mM) was slowly added to stirred solution. Afterwards, pH was adjusted to 9.11 with 1 M NH_3 (1.5 ml). Sodium sulphide nanohydrate $Na_2S \cdot 9H_2O$ (0.0240 g, 0.1 mM) in 23 ml of ACS water was poured into the first solution with vigorous stirring. Obtained yellow solution was stirred for 1 h. ZnS QDs were prepared similarly to CdS QDs; zinc nitrate hexahydrate $Zn(NO_3)_2 \cdot 6H_2O$ (0.0298 g, 0.1 mM) was used in the preparation. Colourless solution was obtained.

PbS QDs were prepared according to Hennequin et al. [80]. Lead acetate trihydrate $Pb(OAc)_2 \cdot 3H_2O$ (0.0379 g, 0.1 mM) was dissolved in ACS water (25 ml). 3-mercaptopropionic acid (60 µl, 0.69 mM) was slowly added to stirred solution. White precipitate was formed, which disappeared after addition of 3.8 ml of 1 M NH_4OH (pH = 9.88). Sodium sulphide nonahydrate $Na_2S \cdot 9H_2O$ (0.0120 g, 0.05 mM) in 21.2 ml of ACS water was added with vigorous stirring. Colour of solution was brown. CuS QDs were prepared by reaction of copper acetate monohydrate $Cu(OAc)_2 \cdot H_2O$ (0.0200 g, 0.1 mM) dissolved in ACS water (25 ml) with mercaptosuccinic acid (0.0800 g, 0.53 mM). 0.5 ml of 1M NH_4OH was added with stirring to yellow solution, followed by sodium sulfide nonahydrate $Na_2S \cdot 9H_2O$ (0.0120 g, 0.05 mM) in 24.5 ml of ACS water. Yellow turned to light brown. All QDs solutions were stored in dark at 4 ºC.

2.3 Labelling of influenzas' derived oligonucleotides with QDs (CdS, CuS, PbS, ZnS)

Influenza oligonucleotides of the sequence shown in Table 1 were synthesised by Sigma-Aldrich. 100 µl of each ODN-SH (100 µg/ml) was mixed with a solution of one of four different QDs (100 µl). This mixture was shaken for 24 h at room temperature (Vortex Genie2, Scientific Industries, USA). Subsequently, solution was dialysed against 2000 ml of miliQ water (24 h, 4 ºC) using a Millipore membrane filter 0.025 µm VSWP (Millipore, USA). During dialysis the sample was diluted to approximately 800 µl. Diluted sample was concentrated to the final volume of 500 µl on a centrifuge filter device Amicon Ultra 3k (Millipore, USA). Centrifuge 5417R (Eppendorf, Germany) and the following parameters as 15 min, 4,500 rpm and 15 ºC were used.

Table 1. Probes and targets oligonucleotide (ODN) H1N1 and H3N2 by [18], H5N1 by [81] and H3N8 by [82].

Probes	Target oligonucleotide
H1N1- 5′ (AAAAA) CCA TTG GTT C 3′ +	H1N1- 5′ (Th) GAA CCA ATG G 3′
H3N2- 5′ (AAAAA) CCC GTT ACA C 3′ #	H3N2- 5′ (Th) GTG TAA CGG G 3′
H5N1- 5′ (AAAAA) CCT CAA GGA G 3′ *	H5N1- 5′ (Th) CTC CTT GAG G 3′
H3N8- 5′ (AAAAA) TTC TAA CCG A 3′ /	H5N1- 5′ (Th) TCG GTT AGA A 3′

+ 26 is number of the starting position of the primer according to GenBank accession number GQ377078
# 606 is number of the starting position of the primer according to GenBank accession number GQ293082
* according to EpiFlu Database Acc No EPI156789
/ A/equine/Sydney/2007

2.4 Detection of ODNs labelled by QDs

Electrochemical analysis was used for detection for metal and oligonucleotide part of ODN-QDs complexes. Peaks of metal part of QDs (CdS, CuS, PbS and ZnS) were determined by differential pulse anodic stripping voltammetry (DPASV). Oligonucleotides were analysed by adsorptive transfer technique square wave voltammetry (AdT SWV) and/or square wave voltammetry only.

2.5 AdT SWV determination of CA peak from ODN-SH-QDs

For detection of CA peak, AdT SWV was used. Measurements were carried out with an Autolab analyser in connection with VA-Stand 663, 800 Dosino and 846 Dosing Interface (Metrohm, Switzerland) in standard electrochemical cell with three electrodes. Hanging mercury drop electrode (HMDE) was used as a working electrode, Ag/AgCl/3 M KCl electrode served as reference electrode and glassy carbon electrode was auxiliary. All measurements were performed in the presence of acetate buffer 0.2 M CH_3COOH + 0.2 M CH_3COONa (pH 5.0) at 25 °C. The analysed samples were deoxygenated prior to measurements by purging with argon (99.999%). GPES 4.9 software was employed for data processing. The parameters of electrochemical determination were as follows: initial potential 0 V; end potential -1.85 V; frequency 10 Hz; potential step 0.005 V; amplitude 0.025 V [83-85].

2.6 Electrochemical determination of G peak from ODN-SH-QDs

Square wave voltammetry (SWV) only was applied for determination of G peak. Measurements were carried out with an Autolab analyser in connection with VA-Stand 663, 800 Dosino and 846 Dosing Interface (Metrohm, Switzerland) in standard electrochemical cell with three electrodes. Hanging mercury drop electrode (HMDE) was used as a working electrode, Ag/AgCl/3 M KCl electrode served as reference electrode and glassy carbon electrode was auxiliary. All measurements were performed in the presence of phosphate buffer (50 mM, pH 6.9) and 0.3 M ammonium formate at 25 °C. The analysed samples were deoxygenated prior to measurements by purging with argon (99.999%). GPES 4.9 software was employed for data processing. The parameters of electrochemical determination were as follows: purge time 20 s, initial potential -1.85 V; end potential 0 V; conditioning potential -1.85 V; deposition potential -0.6 V; equilibration time 5 s; frequency 280 Hz; potential step 0.005 V; amplitude 0.025 V [86-89]. Conditioning time and time of accumulation were optimized.

2.7 Electrochemical determination of metal part of QDs

For electrochemical detection of metals, differential pulse voltammetry (DPV) and differential pulse anodic stripping voltammetry (DPASV) were employed. Measurements were carried out with an Autolab analyser in connection with VA-Stand 663, 800 Dosino and 846 Dosing Interface (Metrohm, Switzerland) in standard electrochemical cell with three electrodes. Hanging mercury drop electrode (HMDE) was used as a working electrode, Ag/AgCl/3 M KCl electrode served as reference electrode and glassy carbon electrode was auxiliary. All measurements were performed in the presence of the acetate buffer (0.2 M, pH 5.0) at 25 °C. The analysed samples were deoxygenated prior to measurements by purging with argon (99.999%). GPES 4.9 software was employed for data processing. The parameters of DPV were as it follows: initial potential 0.15 V; end potential -1.3 V; deposition potential 0.15 V; duration 240 s; equilibration time 5 s; modulation time 0.06 s; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025 V. The parameters of DPASV were as it follows: initial potential -1.3 V; end potential 0.15 V; deposition potential -1.3 V; duration 240 s; equilibration time 5 s; modulation time 0.06 s; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025 V. Other experimental details are indicated in the following papers [90-95].

2.8 Fully automated pipetting station

There was used an automatic pipetting system epMotion 5075 (Eppendorf, Germany) for preparation of samples. This system was equipped with original devices: tips holders (for tips 1000, 300, 50 μl), tips (1000, 300, 50 μl), sample rack with 96 position (in the 21 position eppendorf test tubes was placed), reservoir holder (where reservoir with ACS water and acetate buffer, sample (QDs or ionic solution) was placed) and container for the used tips. The sample rack was placed in thermo adapter 2.0 ml. 20 point calibration series for and QDs solutions of all four metals were prepared. Preparation of calibration curve was made by epMotion following these steps. There were 20 positions in sample rack in epMotion, where tubes were placed. In the sample rack 21 test tubes was placed and

after that 100 µl of the initial concentration of sample (QDs or ionic solution) was pipetted in the first test tube. Further, 50 µl of ACS water was pipetted into 19 test tube, after that half dilution steps were done (in the position 1-20). Final volume of 20 test tubes was added to 1000 µl with acetate buffer. Other experimental conditions are the same as in Huska et al. [96].

2.9 Mathematical treatment of data and estimation of detection limits

Mathematical analysis of the data and their graphical interpretation was realized by MICROSOFT EXCEL® (USA). Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®). The detection limits (3 signal/noise, S/N) were calculated according to Long and Winefordner [97], whereas N was expressed as standard deviation of noise determined in the signal domain unless stated otherwise.

3. RESULTS AND DISCUSSION

3.1 Determination of metal ions

In the previously published paper, we presented a way how to modify oligonucleotides with metal QDs. The results obtained were promising and enabled us to suggest a sensor for detection of influenza nucleic acid [24]. In this study, we attempted to optimize detection of metal ions. Electrochemical quantification of metal ions is well known, sensitive and robust enough to be used routinely for analysis of various types of real samples [61,92]. In our study, four metal ions were detected as zinc(II), cadmium(II), lead(II) and copper(II). For detection of these metal ions, differential pulse voltammetry and/or differential pulse anodic stripping voltammetry. The parameters were adopted from our previously published papers [1,24]. Automatic pipetting station epMotion was used for preparing of 20 points calibration series within the range from 0.0001 to 100.0000 µg/ml for Zn(II), Cd(II), Pb(II) and Cu(II). The obtained calibration curves are shown in Figs. 1A, B, C and D, respectively. The curves were strictly linear, however, DPASV gave app. 10 times higher peaks compared to standard DPV. It is not surprising that detection limits (3 S/N) were lower in the case of the using of DPASV compared to DPV.

3.2 Determination of metal parts of QDs

As it was mentioned above, quantum dots (ZnS, CdS, PbS, CuS) could be electrochemically determined due to theirs metal part. For this purpose, DPV and DPASV were employed. Parameters for these measurements were the same as for metal ions determination mentioned in the previous paragraph. Peak potential of pure metal ions did not change compared to metal analysed in the form of QDs. Potentials of individual peaks were as follows: zinc -1.03 ± 0.005 V, cadmium -0.63 ± 0.005 V, lead -0.46 ± 0.005 V and copper 0.04 ± 0.005 V. Calibration curves of ZnS, CdS, PbS and CuS measured by DPV and DPASV are shown in Figs. 2A, B, C and D, respectively. Curves, which were measured by DPV, had polynomial character for all QDs. On the other hand, dependences measured by DPASV had polynomial character for lead and copper in the whole measured concentration range. PbS calibration curve measured by DPASV had polynomial character as it follows: $y = -0.0046x^2 +$

6.3133x + 63.034; R^2 = 0.994; n = 5; R.S.D = 2.35 % (Fig. 2C). CuS calibration curve measured by DPASV had polynomial character as it follows: y = -0.0227x^2 + 6.0961x + 15.287; R^2 = 0.978; n = 5; R.S.D = 2.51 % (Fig. 2D). DPASV dependences measured for zinc and cadmium quantum dots were divided in two parts. For ZnS, it is linear within concentration range from 16.4 to 131 µg/ml according to the following equation: y = 0.590x + 262; R^2 = 0.950, n = 5, R.S.D = 2.54 % (Fig. 2A). For the lower concentrations (under 16.4 µg/ml), this dependence had polynomial character as it follows: y = -3.5197x^2 + 56.441x + 3.5916; n = 5; R.S.D = 1.57 %. Cadmium quantum dots had linear concentration dependence within concentration range from 28 to 225 µg/ml as it follows: y = 0.0622x + 104.5; R^2 = 0.983; n = 5; R.S.D = 2.13 % (Fig. 2B).

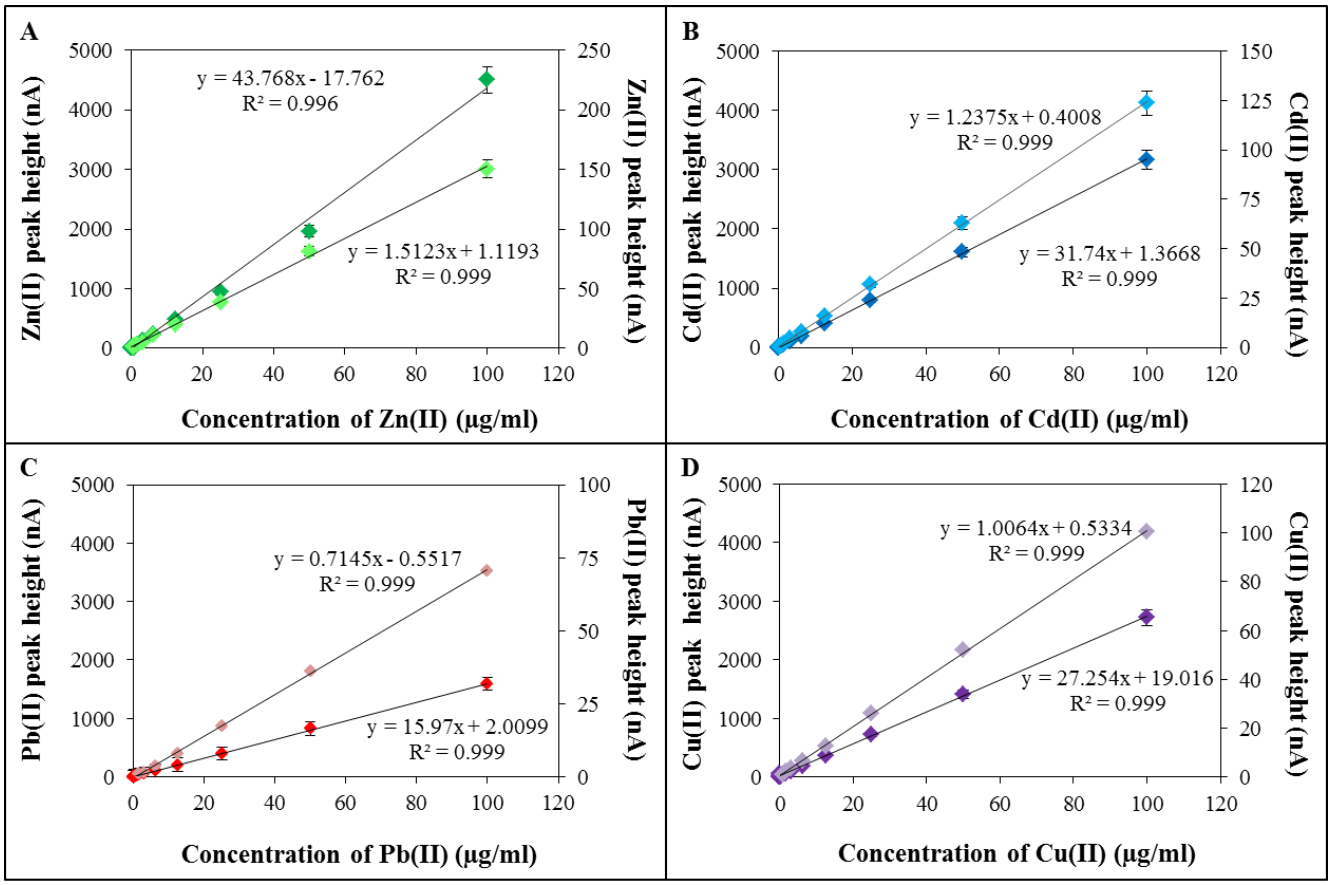

Figure 1. Linear calibration curves of individual metal ions. (**A**) Zn(II); (**B**) Cd(II); (**C**) Pb(II); (**D**) Cu(II). Dependences of ions peak height on concentration of ions were measured by differential pulse voltammetry (DPV) (light colour ♦) and by differential pulse anodic stripping voltammetry (DPASV) (dark colour ♦). The parameters of DPV were as follows: initial potential 0.15 V; end potential -1.3 V; deposition potential 0.15 V; duration 240 s; equilibration time 5 s; modulation time 0.06 s; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025 V. The parameters of DPASV were as follows: initial potential -1.3 V; end potential 0.15 V; deposition potential -1.3 V; duration 240 s; equilibration time 5 s; modulation time 0.06 s; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025 V.

For the lower concentrations (under 28 µg/ml), this dependence exponential character as it follows: y = 49.323$x^{0.1943;}$ n=5; R.S.D = 2.61 %. It clearly follows form the results obtained that ZnS gives ten times lower signals compared to Zn(II). CdS peaks were 30 times lower compared to Cd(II)

and PbS up to 5 times lower compared to pure ions. CuS gave ten times lower peaks compared to Cu(II). The changes can associated with structure of nanoparticles. The obtained results clearly show that DPASV is more suitable method for QDs determination than DPV. Therefore, DPASV was selected for further study.

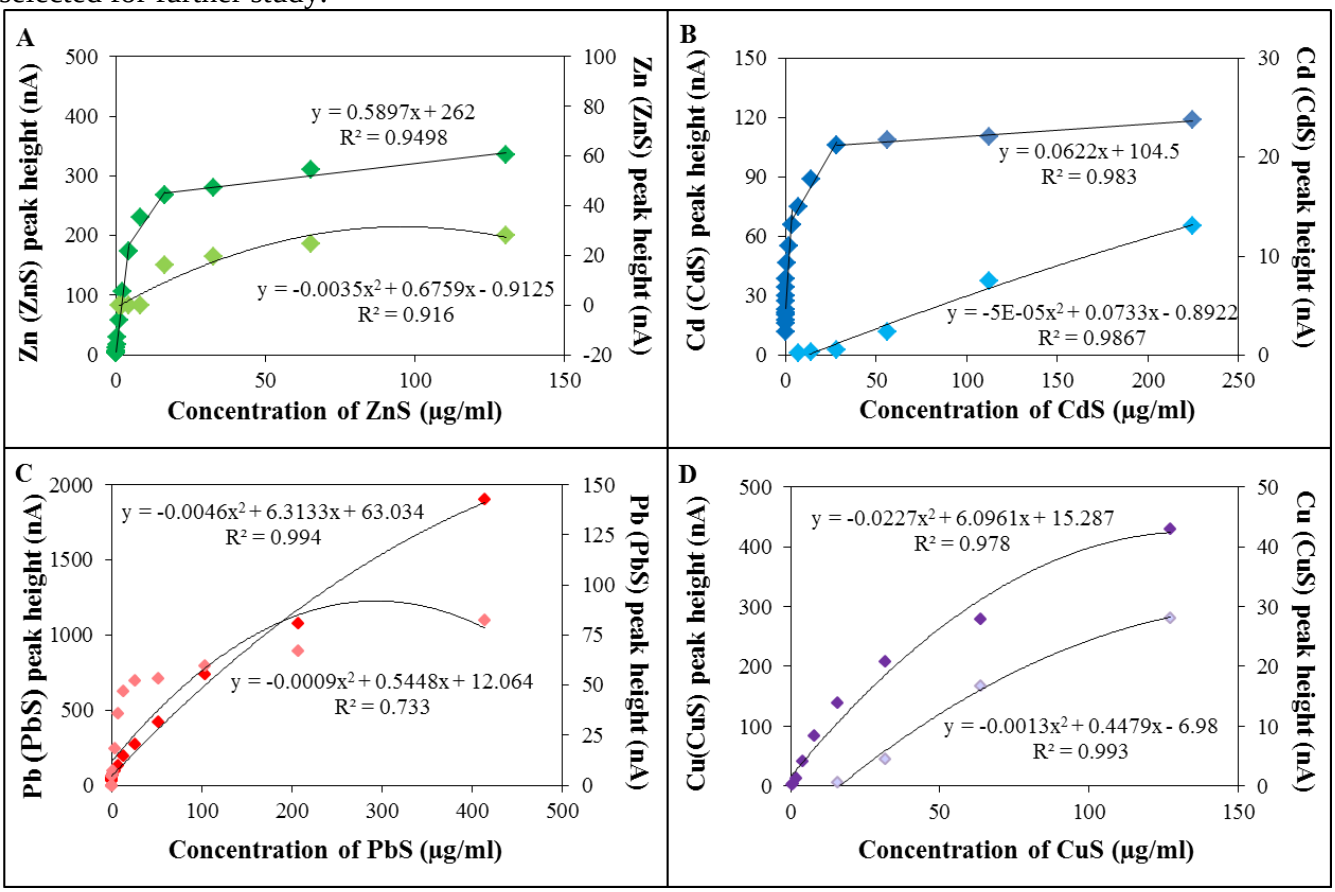

Figure 2. Calibration curves of individual metal part of quantum dots. (**A**) CdS; (**B**) ZnS; (**C**) PbS; (**D**) CuS. Dependences were measured by DPV on right axis (light colour ♦) and by DPASV on left axis (dark colour ♦). The parameters of DPV were as follows: initial potential 0.15 V; end potential -1.3 V; deposition potential 0.15 V; duration 240 s; equilibration time 5 s; modulation time 0.06 s; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025 V. The parameters of DPASV were as follows: initial potential -1.3 V; end potential 0.15 V; deposition potential -1.3 V; duration 240 s; equilibration time 5 s; modulation time 0.06 s; time interval 0.2 s; potential step 0.002 V; modulation amplitude 0.025 V.

3.3 Optimization of DPASV QDs determination in ODN-QDs

Electrochemical study of ODN-QDs complex needs to investigate behaviour of individual parts of this complex. Therefore, QDs bounded to ODN were detected and the experimental parameters were optimized. As the first parameter, deposition potential was investigated. The obtained results are shown in Fig. 3A. The maximum signal for ODN-ZnS QDs was determined at -1.2 V. This parameter was -0.8 V for CdS, -0.6 V for PbS and -0.1 V for CuS. Changes in the second optimized parameter, time of accumulation, are shown in Fig. 3B. In this case, there were selected values related to the break point in presented curves at 360 s for all four metals. The third optimized parameter was pH of electrolyte (acetate buffer). Dependences of measured signal on pH value are shown in Fig. 3C. ODN

Zinc, cadmium and lead quantum dots had optimal pH value of electrolyte at pH 5. Only copper had pH optimum at point 3, where other three QDs did not give any signal. The effect of all optimized parameters on peak potentials was negligible as it is shown in Figs. 3E, and F and G. Under the optimized parameters (time of accumulation 360 s and acetate buffer pH 5.0), simultaneous determination of various QDs complexes with ODN was done. DPAS voltammograms are shown in Fig. 4A. The obtained signals were well developed and separated. Changes of peaks heights on accumulation time of individual ODN-QDs complexes are shown in Fig. 4B. Change of signal intensity of zinc complex with changing accumulation time is described by equation y = 0.2511x + 12.08 with R^2 0.993. Characteristic for the other metals complexes are as it follows: cadmium y = 0.022x + 0.2624 with R^2 0.989; lead y = 0.0024x + 0.1199 with R^2 0.992; copper y = 0.0016x + 0.0839 with R^2 0.992. After the optimization of QDs determination, we aimed our attention at ODN analysis.

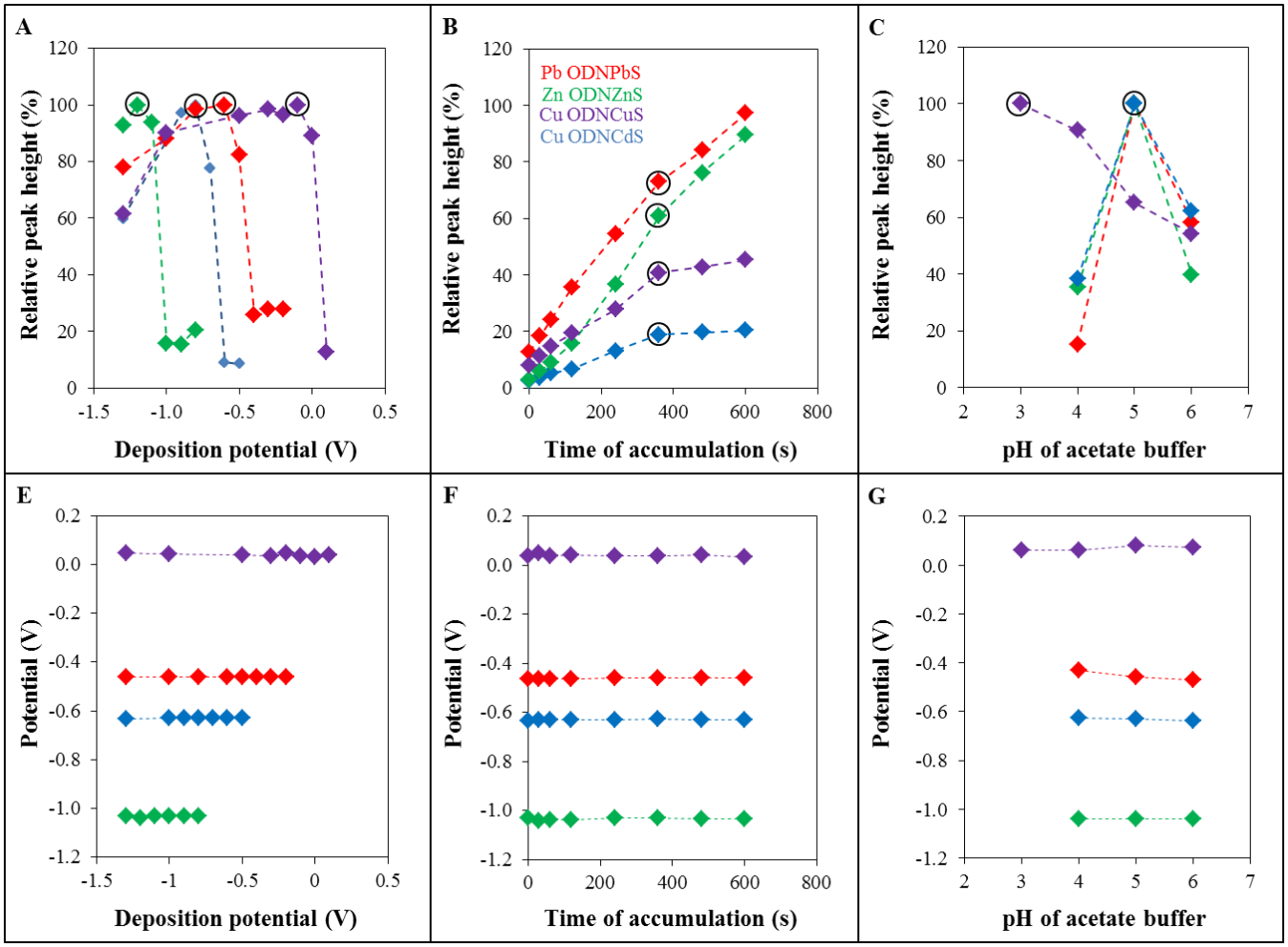

Figure 3. Dependences of metal peak height of ODN-QDs created by Cd; Zn; Pb; Cu on (**A**) deposition potential and (**B**) time of accumulation. (**C**) Dependences of metal peak height from quantum dots created by Cd; Zn; Pb; Cu on pH of acetate buffer. Dependence of metal peak potential on (**E**) deposition potential, (**F**) time of accumulation and (**G**) pH of acetate buffer. All dependences were measured by DPASV method.

3.4 Electrochemical determination of ODN in ODN-QDs

3.4.1 CA peak detection

For detection of CA peak, square wave voltammetry coupled with adsorptive transfer technique (AdT SWV), which is considered as a one of the most sensitive technique for ODN detection [49,98], was employed. CA peak was determined at -1.4 ± 0.02 V (Fig. 4C). The highest peak was obtained for ODN-ZnS followed by ODN-CdS, ODN-PbS and ODN-CuS. In addition, accumulation time (30, 60, 120, 240 and 360 s) was optimized. All QDs labelled oligonucleotides showed the optimum at time of accumulation at 120 s (Fig. 4D).

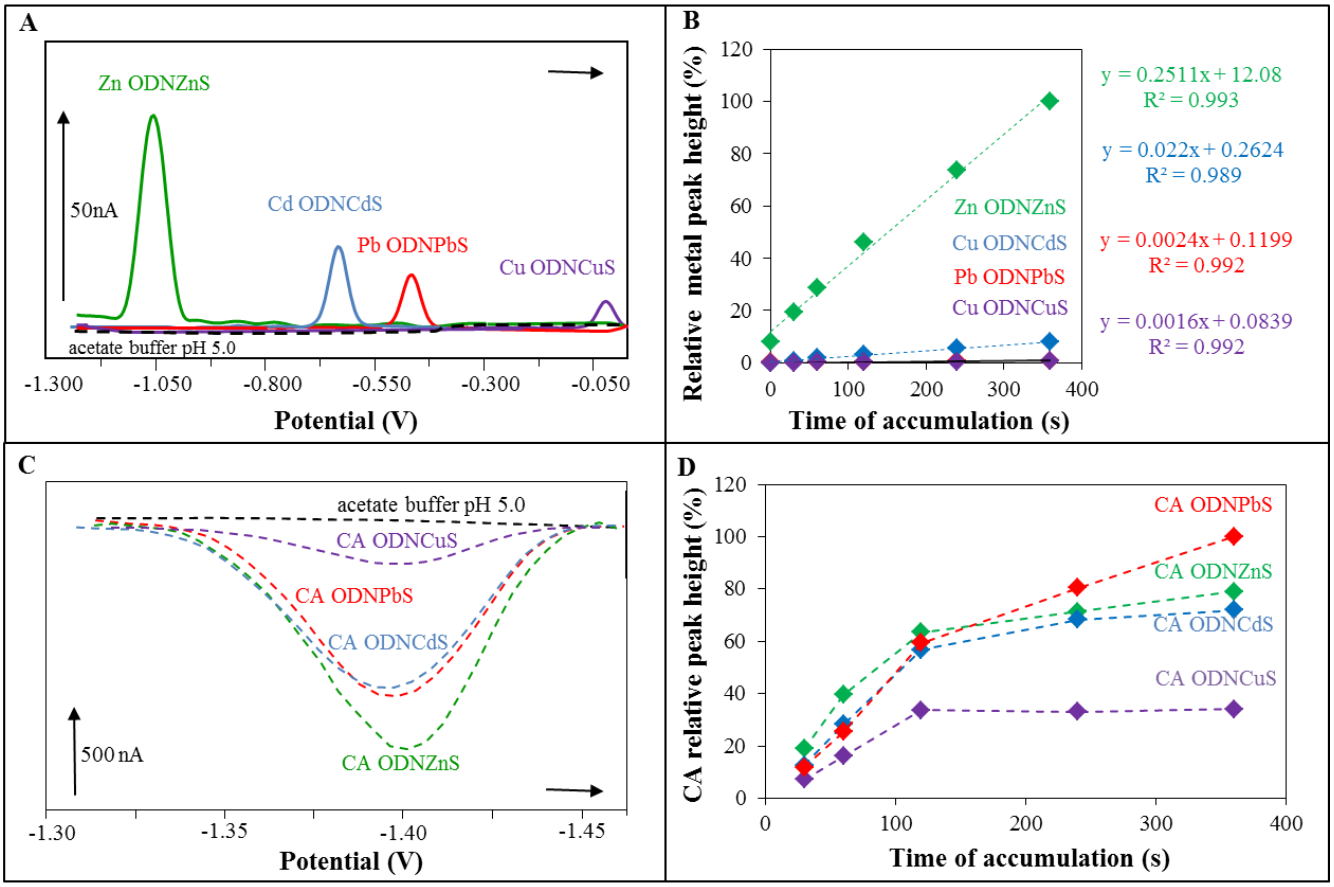

Figure 4. Electrochemical characterization of complex QD-ODN by: square wave voltammetry connected with adsorptive transfer technique (AdTSWV) and differential pulse anodic stripping voltammetry (DPASV). (**A**) DP voltammograms of metal peaks - CdS ; ZnS; PbS; CuS. (**B**) Dependence of metal peak height (from QDs part of the complex) on time of accumulation. (**C**) SW voltammograms of CA peaks labelled with quantum dots. (**D**) Dependence of CA peak height (from ODN part of the complex) on time of accumulation. Metal part of QDs is consisted from Cd ; Zn; Pb; Cu. As a supporting electrolyte acetate buffer with pH 5.0 was used, only for measurements of CuS by DPASV pH of acetate buffer was adjusted on pH 3.0. Parameters of AdTSWV method were as follows: initial potential 0 V; end potential - 1.85 V; frequency 100 Hz; potential step 0.005 V; amplitude 0.025 V and parameters of DPASV method were as follows: initial potential -1.3 V; end potential 0.15 V; deposition potential -1.3 V; equilibration time 5 s; modulation time 0.06; time interval 0.2 s; potential step 0.002 V, modulation amplitude 0.025, deposition potential -0.1 V (CuS), -0.6 V (PbS), -0.8 V (CdS) and -1.2 V (ZnS).

3.4.2 G peak detection

Besides adenine and cytosine peak, G peak was also detected. For detection G peak we used SWV, but, as a background electrolyte phosphate buffer of pH 6.9 with 0.3 M ammonium formate was used. Instead of scanning the potential to negative values (to obtain the cathodic peaks) we made the potential jump to -1.85 V and after waiting at -1.85 V (to generate the reduction product of the DNA G residues), the potential was scanned to positive values. The anodic peak G, which appeared at -0.3 V, is shown in Fig. 5A. This method was used for all ODN-SH-QDs, as a control ODN-SH was used. According to our assumption, ODN-SH gave lower G peak than ODN-SH-QDs. The interactions of ONDs with QDs enhanced G peak, whereas the most intensive increase was detected in ODN-ZnS.

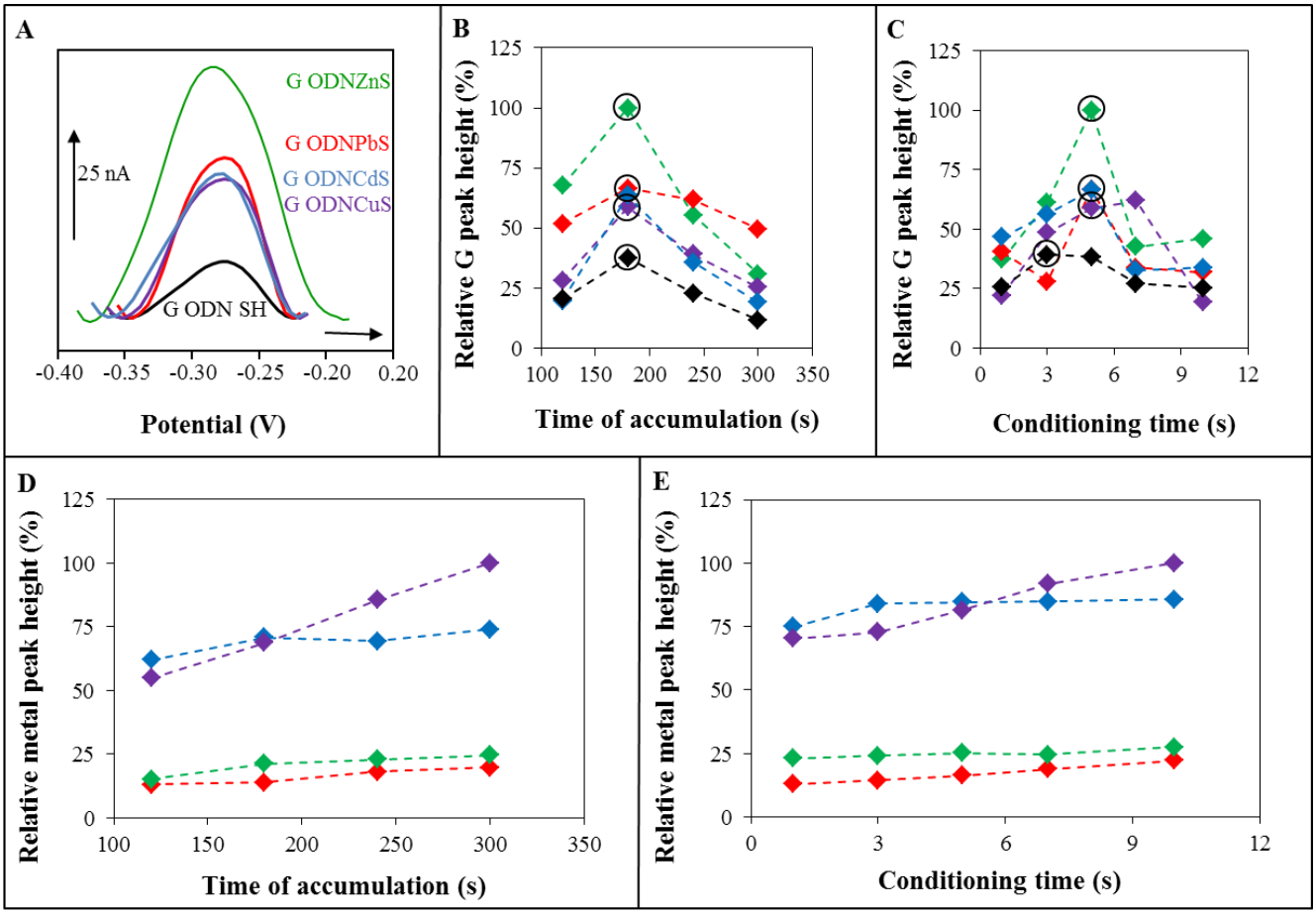

Figure 5. Dependences of G peaks height from complex QD-ODN are presented. (**A**) Voltammograms of G peaks of ODN labeled with quantum dots (CdS ; ZnS; PbS; CuS). (**B**) Influence of relative G peak height on accumulation time. (**C**) Influence of relative G peak height on conditioning time. (**D**) Influence of relative metal peak height on accumulation time. (**E**) Influence of metal peak height on conditioning time. Electrochemical characterization (G peak method) of complex QD-ODN by square wave voltammetry (SWV). As a supporting electrolyte phosphate buffer (50 mM, pH 6.9) with 0.3 M amonium formiate was used. Parameters of method were as it follows: purge time 20s, initial potential -1.85 V; end potential 0 V; conditioning potential -1.85 V; deposition potential -0.6 V; equilibration time 5 s; frequency 280 Hz; potential step 0.005 V; amplitude 0.025 V.

As was mentioned above, the scan begun at -1.85 V and accumulation of sample was made at -0.6 V. The optimization of electrochemical detection method was done, where we focused on time of accumulation and conditioning time. The influence of the first parameter on the relative G peak height is shown in Fig. 5B. It clearly follows from the results obtained that the optimal accumulation time for all four ODN-QDs was 180 s. Dependence of relative G peak height on conditioning time (at -0.6 V) is shown in Fig. 5C. These dependences had optimum at 5 s and therefore this time was selected as optimal.

Method applied for the G peak detection gave the metal signal too. The voltammograms are shown in Fig. 5D. Each QDs gave individual metal peak signals, which were detected at potentials as it follows: zinc -1.0 ± 0.05 V, cadmium about - 0.65 ± 0.03 V, lead about -0.45 ± 0.03 V and copper about 0.05 ± 0.03 V. The influence of accumulation time and conditioning time on individual metal peaks were investigated too. Dependence of accumulation time on individual metal peak heights is shown in Fig. 5E. Peak heights increased within accumulation range from 120 to 300 s. All dependences increased linearly, except cadmium where decrease after 180 s signal was detected. The highest signals were detected for copper and cadmium. Similar results were obtained for dependence of metal peak height on conditioning time (Fig. 5F). Conditioning time was changed from 1 to 10 s and the measured signals increased within the whole range.

4. CONCLUSION

The detection of a pathogens is useful for therapeutic and control of infectious disease as well as clinical diagnosis. Labelling of target molecules using tags (especially metal QDs) has a wide range of application and detection possibilities. Our study was focused on electrochemical detection of such labels and labelled oligonucleotide too. Anodic stripping voltammetry was shown as a more sensitive tool for detection of quantum dots metal part. Other electrochemical methods were applied for detection of complex ODN-QD, where four various QDs were used. Detection of CA peak was applicable only for ODN detection. On the other hand method for G peak detection gave more information, because metal part of quantum dots was detected by this method too. The study provides the first information on the electrochemical behaviour of nucleic acids labelled with quantum dots without releasing of metal ions by acid [99,100], which greatly affect the electrochemical signals.

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