Fast Track

Marketa Ryvolova[1,2]
Kristyna Smerkova[1]
Jana Chomoucka[2,3]
Jaromir Hubalek[2,3]
Vojtech Adam[1,2]
Rene Kizek[1,2]

[1]Department of Chemistry and Biochemistry, Faculty of Agronomy, Mendel University in Brno, Brno, Czech Republic
[2]Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic
[3]Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Brno, Czech Republic

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Research Article

Glutathione modified CdTe quantum dots as a label for studying DNA interactions with platinum based cytostatics

Cisplatin, carboplatin, and oxaliplatin represent three generations of platinum based drugs applied successfully for cancer treatment. As a consequence of the employment of platinum based cytostatics in the cancer treatment, it became necessary to study the mechanism of their action. Current accepted opinion is the formation of Pt-DNA adducts, but the mechanism of their formation is still unclear. Nanomaterials, as a progressively developing branch, can offer a tool for studying the interactions of these drugs with DNA. In this study, fluorescent CdTe quantum dots (QDs, $\lambda_{em} = 525$ nm) were employed to investigate the interactions of platinum cytostatics (cisplatin, carboplatin, and oxaliplatin) with DNA fragment (500 bp, $c = 25$ μg/mL). Primarily, the fluorescent behavior of QDs in the presence of platinum cytostatics was monitored and major differences in the interaction of QDs with tested drugs were observed. It was found that the presence of carboplatin ($c = 0.25$ mg/mL) had no significant influence on QDs fluorescence; however cisplatin and oxaliplatin quenched the fluorescence significantly (average decrease of 20%) at the same concentration. Subsequently, the amount of platinum incorporated in DNA was determined by QDs fluorescence quenching. Best results were reached using oxaliplatin (9.4% quenching). Linear trend ($R^2 = 0.9811$) was observed for DNA platinated by three different concentrations of oxaliplatin (0.250, 0.125, and 0.063 mg/mL). Correlation with differential pulse voltammetric measurements provided linear trend ($R^2 = 0.9511$). As a conclusion, especially in the case of oxaliplatin-DNA adducts, the quenching was the most significant compared to cisplatin and nonquenching carboplatin.

Keywords:
Capillary electrophoresis / DNA / Gel electrophoresis / Platinum cytostatic drugs / Quantum dots
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1 Introduction

Cancer, a plague of 21st century, is a leading cause of a death in developed countries [1]. The biological activity of the first platinum based cytostatic drug called cisplatin (*cis*-diamminedichloroplatinum(II)), which is still one of the most frequently used cytotoxic agent, was discovered in 1965 by Rosenborg [2]. The "second generation" Pt drug-carboplatin (*cis*-diammine-1,1-cyclobutane dicarboxylate) was developed in the 1980s as a less toxic alternative to cisplatin, providing less severe side effects [3]. Cisplatin and carboplatin are widely used in chemotherapy, and are particularly effective in the treatment of testicular, ovarian, head, neck, and nonsmall cell lung cancer. However, both cisplatin and carboplatin have significant toxicity and are mutagenic in cell culture and animal model systems [4, 5]. They form the same Pt-DNA adducts *in vivo* and are generally not effective in cell lines or tumors that have developed resistance to either agent. Therefore, oxaliplatin ((*trans-R,R*)1,2-diaminocyclohexaneoxalatoplatinum(II)) was developed in the 1990s as a novel ("third generation") Pt drug to overcome cisplatin and carboplatin resistance [6] and moreover it appears to be less mutagenic [7]. Oxaliplatin has recently been approved for the treatment of colon cancer in the United States [8].

As a consequence of the employment of platinum based cytostatics in tumor diseases treatment, it became necessary to study the mechanism of their main activity as formation

Correspondence: Dr. Rene Kizek, Department of Chemistry and Biochemistry, Mendel University in Brno, Zemedelska 1, CZ-613 00 Brno, Czech Republic
E-mail: kizek@sci.muni.cz
Fax: +420-5-4521-2044

Abbreviations: HMDE, Hanging Mercury Drop Electrode; **QDs**, Quantum dots; **TEM**, transmission electron microscope

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of Pt-DNA adducts. Their formation is still unclear, however it has been found out that both cisplatin and oxaliplatin form about 60–65% intrastrand adducts between two adjacent guanine bases (PtGG), 25–30% intrastrand adducts between adjacent adenine and guanine bases (PtAG), 5–10% intrastrand adducts of the type PtGNG, where *N* represent any of the four DNA bases, and 1–3% interstrand adducts (G-Pt-G) [9].

Numerous methods are suitable for study of Pt-DNA adducts including nuclear magnetic resonance [10, 11], atomic absorption spectrometry [12], mass spectrometry [9, 13], electrochemical biosensors [14, 15], and/or atomic force microscopy [16]. On the other hand, these methods are often destructive, expensive, laborious, and/or cannot be used for in vivo monitoring of drug–DNA interactions. Therefore, an easy-to-use method for study of drug–DNA interaction, which will be able to determine not only the presence of DNA adducts but also the strength of the bound and the effectiveness of the tested drug is searched for. Nanomaterials including quantum dots (QDs) as progressively developing branch, can offer a way how to investigate interactions of drugs with DNA in real time. Numerous studies investigating the fluorescence quenching caused by metal ions have been carried out [17–27], which could be used for sensing a metal-containing compounds. Based on this fact the aim of this study was to determine binding of platinum based cytostatics (cisplatin, carboplatin and oxaliplatin) by CdTe QDs.

2 Materials and methods

2.1 Chemicals

Cisplatin and oxaliplatin were purchased from Ebewe Pharma (Austria) and carboplatin from Teva Pharmaceuticals (Czech Republic). All other chemicals were purchased by Sigma Aldrich (Czech Republic) in ACS purity unless stated otherwise. Solutions were made using MilliQ water. Deionized water underwent demineralization by reverse osmosis using the instrument Aqua Osmotic 02 (Aqua Osmotic, Czech Republic) and then subsequently purified using Millipore RG (Merck Millipore, Billerica, MA, USA, 18 MΩ)—MilliQ water.

2.2 Synthesis of QDs

The procedure for synthesis of these dots was adapted from the work of Duan et al. [28]. Briefly, the synthesis of CdTe QDs and their subsequent coating were as follows: 330 μL of the $CdCl_2$ solution (0.04 M) was diluted with 2.5 mL of water. During constant stirring, 8 mg of sodium citrate, 330 μL of Na_2TeO_3 solution (0.01 M), 15 mg of reduced glutathione (GSH), and 3.3 mg of $NaBH_4$ were added into water–cadmium(II) solution. The mixture was kept at 95°C under the reflux cooling for 2.5 h. As a result, yellow solution of the GSH-QDs was obtained.

2.3 Characterization of QDs using transmission electron microscope

Morphology studies were carried out with the transmission electron microscope (TEM) Philips CM 12 (tungsten cathode, using a 120 kV electron beam). Samples for TEM measurements were prepared by placing drops of the solution (sample and water) on coated Cu grids (holey carbon and holey SiO_2/SiO) and subsequently drying in air.

2.4 DNA amplification and isolation

Taq PCR kit and DNA isolated from bacteriophage λ (48 502 bp) were purchased from New England BioLabs (USA). Primers for PCR were synthesized by Sigma–Aldrich. The sequence of a forward primer was 5′-CCTGCTCTGCCGCTTCACGC-3′ and the sequence of a reverse primer was 5′-TCCGGATAAAAA CGTCGATGACATTTGC-3′. The 50 μL reaction mixture was composed of 5 μL 10× standard *Taq* reaction buffer, 1 μL of 10 μM deoxynucleotide solution mix, 1 μL of each primer (10 μM), 0.25 μL of 5 U/μL *Taq* DNA polymerase, 1 μL of 0.5 μg/μL λ DNA and 40.75 μL H_2O (sterile). The PCR tubes with mixture were placed into the cycler (Eppendorf, Germany) and cycling conditions were as follows: initial denaturation at 95°C for 120 s; 25 cycles of denaturation at 95°C for 15 s, annealing at 64°C 15 s, extension at 72°C 45 s and a final extension at 72°C for 5 min. 100 μL of PCR product (500 bp) was purified by MinElute PCR Purification Kit (Qiagen, Germany) according to manufacturer's instruction and DNA was concentrated to 10 μL of water solution. The DNA concentration was determined by spectrophotometric analysis at 260 nm using spectrophotometer Specord 210 (Analytik Jena, Germany).

2.5 Formation of DNA-cytostatic adducts

The solution of 500 bp DNA fragment (25 μg/mL) was mixed with the cytostatic drug as cisplatin, carboplatin, and/or oxaliplatin of various concentrations (0.125, 0.25, and 0.5 mg/mL) in ratio 1:1 (v/v). The mixture was incubated for 24 h at 37°C. Subsequently the mixture underwent the dialysis using centricons Amicon Ultra 50K (Millipore) to remove the excess of cytostatics. The centrifugal filter with sample was centrifuged at 14 000 × g for 30 min.

2.6 Fluorimetric analysis

Platinum cytostatics (concentration range from 4 to 500 μg/mL) were mixed with GSH-QDs in ratio 1:1 (*v*/*v*). Fluorimetric analysis was performed using multifunctional microplate reader Tecan Infinite 200 PRO (TECAN, Switzerland). 350 nm was used as an excitation wavelength and the fluorescence scan within the range from 400 to 850 nm was measured with 5 nm steps. Each intensity value is an average

of five measurements. The detector gain was set to 100. The sample (60 μL) was placed in transparent 96 well microplate with flat bottom by Nunc (Thermo Scientific, USA).

2.7 Gel electrophoresis

Agarose gel (1%) was prepared by boiling of 1× TAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA, Bio-Rad, USA) for 2 min in microwave. Then the gel was cooled to 60°C and ethidium bromide was added (5 μL per 100 mL of the gel). The gel was transferred into electrophoretic bath containing TAE buffer. Samples, prepared with 5% (v/v) bromophenol blue and 3% (v/v) glycerol, were loaded into the gel in 10 μL aliquots. DNA ladder in the size range 0.5–1.5 kbp was used to monitor the size of analyzed fragment. The electrophoresis (Bio-Rad) was running at 100 V and 6°C for 45 min. The bands were visualized by UV transilluminator at 312 nm (Vilber-Lourmant, France).

2.8 Electrochemical determination of platinum

Differential pulse voltammetric measurements were performed with 747 VA Stand instrument connected to 746 VA Trace Analyzer and 695 Autosampler (Metrohm, Switzerland), using a standard cell with three electrodes and cooled sample holder (4°C). A hanging mercury drop electrode (HMDE) with a drop area of 0.4 mm^2 was the working electrode. An Ag/AgCl/3M KCl electrode was the reference and glassy carbon electrode was auxiliary electrode. GPES 4.9 supplied by EcoChemie was employed. The analyzed samples were deoxygenated prior to measurements by purging with argon (99.999%) for 200 s.

Platinum was determined by differential pulse adsorptive stripping voltammetry in the presence of 1.98 mL of 17.822 M sulfuric acid, 0.24 mL of 0.015 M hydrazine and 0.01 mL of 2 M formaldehyde. Sample volume was 20 μL and total volume in the measuring vessel was 2 mL. Pt-formazan complex were formed in electrochemical cell during 200 s long purging and was accumulated for 15 second on HMDE. The scan was measured from −0.5 to −1.2 V with scan rate of 10 mV/s. The other experimental parameters were as follows: modulation time 0.057 s, time interval 0.2 s, potential step of 1.95 mV, modulation amplitude 49.95 mV, deposition potential of −0.7 V, deposition time of 0 s. Characteristic peak for platinum was detected at −0.9 V.

2.9 Electrochemical determination of DNA

Electrochemical manual measurements were performed with AUTOLAB PGS30 Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm) using a standard cell with three electrodes. A HMDE with a drop area of 0.4 mm^2 was employees the working electrode. An Ag/AgCl/3M KCl electrode served as the reference electrode. Pt electrode was used as the auxiliary electrode.

Adsorptive transfer technique was used for the electrochemical DNA determination. The adsorptive transfer technique is based on the sample accumulation (120 s) onto the working electrode surface and consequently on the electrode washing and square wave voltammetric (SWV) measurement. All experiments were carried out at room temperature (21°C). SWV measurements were carried out in the presence of 0.2 M acetate buffer pH 5.0. SWV parameters: start potential 0 V, end potential −1.5 V, potential step 5 mV, frequency 280 Hz, and amplitude 25.05 mV. For smoothing and baseline correction the software GPES 4.9 supplied by EcoChemie was employed.

3 Results and discussion

A lot of papers have shown the influence of metal ions on fluorescence of QDs [29–31]. Based on the published papers, it can be concluded that QDs are suitable for sensing of metal ions, which predetermine them to sense metal containing biologically active compounds including drugs. Platinum based drugs are one of the most intensively investigated cytostatics, which mechanism of action is not fully understood. Using of QDs could cast the light on the mechanism of action of platinum based cytostatics, because one may expect that the cytostatics would have different action mechanism.

To investigate the behavior of QDs in the presence of platinum ions, glutathione capped CdTe QDs were synthesized. The TEM analysis showed that dried droplets consisted of a fine grain powder of a typical size of particles below 10 nm. These QDs exhibit also a strong emission of green light with emission maximum at 525 nm as shown in Fig. 1 and the fluorescence intensity is concentration dependent with the coefficient of determination $R^2 = 0.9818$ (inset in Fig. 1). A photograph of QDs solution under UV light illumination is also shown in the inset in Fig. 1.

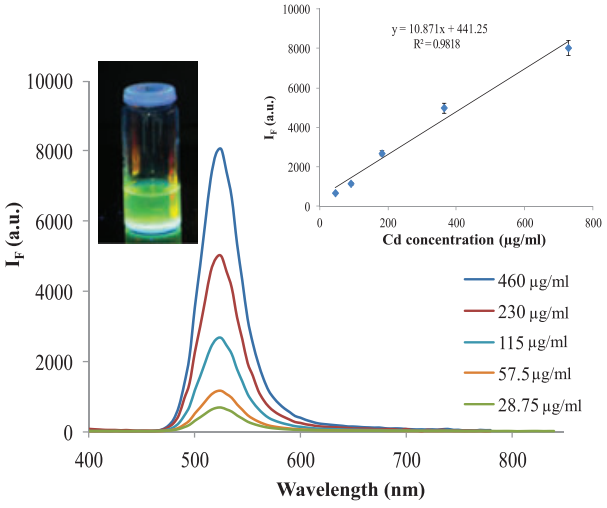

Figure 1. Emission spectra of CdTe QDs, excitation 480 nm (inset: calibration curve of CdTe QDs and photograph of CdTe QDs solution under UV light illumination).

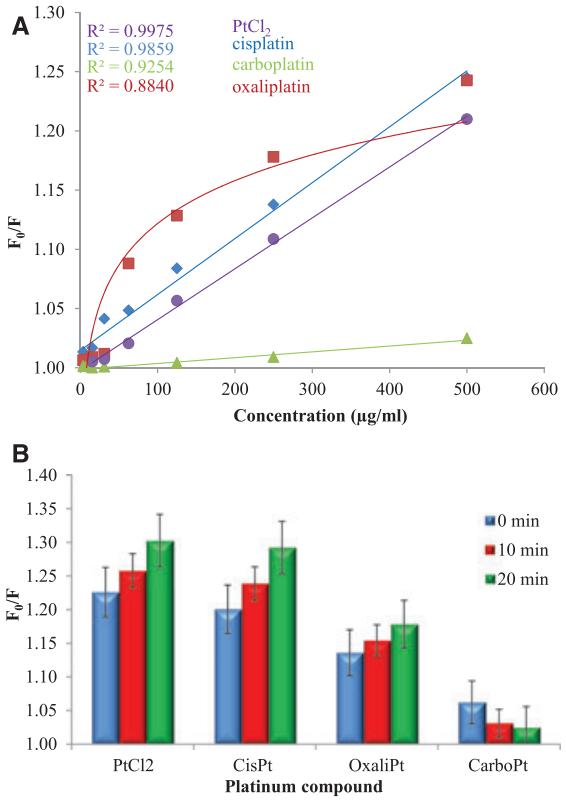

Figure 2. (A) Stern–Volmer plots of CdTe QDs fluorescence quenching effect caused by platinum containing compounds. (B) Time dependence of the fluorescence quenching effect caused by platinum containing compounds.

3.1 The interactions of QDs with platinum based cytostatics

Subsequently the investigation of in vitro interactions of the dots with platinum chloride and cisplatin, oxaliplatin, and carboplatin was carried out. The impact of platinum containing compounds such of interest was investigated and the quenching effect of these compounds on the fluorescence of QDs was observed. The concentration dependent quenching effect is expressed as Stern–Volmer plot. The slope of the curve represents the Stern–Volmer quenching constant (K_{SV}) and the higher K_{SV}, the higher the quenching effect. In the case of $PtCl_2$, this dependence exhibited a very good linearity with coefficient of determination $R^2 = 0.9975$ (Fig. 2A). Based on these results, the quenching effect of platinum based cytostatic drugs (cisplatin, oxaliplatin, and carboplatin) was investigated and the results are shown in Fig. 2A. The Stern–Volmer plot of cisplatin quenching properties is shown exhibiting linear trend with coefficient of determination $R^2 = 0.9859$. This behavior suggests that only dynamic quenching is taking place. K_{SV} of 142.2 L/mol was determined for this drug. In the case of oxaliplatin the curve was not linear and the shape suggests that the dynamic quenching occurring between QDs and oxaliplatin is influenced by some steric barriers. This might be explained by the structure of oxaliplatin, which is more complex compared to cisplatin and therefore the interaction with QDs and its quenching abilities are influenced. The carboplatin behavior is linear with coefficient of determination $R^2 = 0.9254$, however the quenching abilities expressed as K_{SV} (18.18 L/mol) is significantly lower compared to cisplatin.

In addition, the time dependence of interaction of QDs with platinum containing compounds was monitored. It clearly follows from results in Fig. 2B that the fluorescence signal of QDs was changing with time. Using $PtCl_2$, cisplatin and oxaliplatin, the fluorescence intensity was decreasing with time; however using carboplatin the time dependence was exhibiting the opposite trend. During 20 min of interaction, the signal of QDs interacting with cisplatin decreased for 5.9% and the signal of oxaliplatin decreased for 3.2%, however the signal of carboplatin increased for 3.4%. This suggests that the structure of the drug plays an important role in the interaction.

3.2 The interactions of QDs with DNA

Based on the obtained results it can be concluded that tested platinum compounds do not interact with QDs according to similar mechanism. This could be clearly associated with the various structures of cytostatics itself, but, all of them cause changes in fluorescence signal. This feature can be used for studying of intercalation of cytostatics into DNA duplex, which is one of the most accepted mechanisms of action of platinum based cytostatics [32, 33]. Therefore, the impact of DNA fragment (500 bp) on QDs fluorescence was monitored and expressed as Stern-Volmer plot. Although it is obvious that the curves are not linear, we applied linear correlation to demonstrate the time dependent trend. The blue curve in Fig. 3 represents the concentration dependent fluorescence quenching obtained immediately after mixing of QDs and DNA expressed by $R^2 = 0.7384$. However, the coefficients of determination increased to 0.8508 and/or 0.9898 after 10 and 20 min long interaction between QDs and DNA, respectively. This led to the conclusion that the steric effects occurred during the interaction. In inset in Fig. 3, the dependence of the slopes of Stern–Volmer curves on the interaction

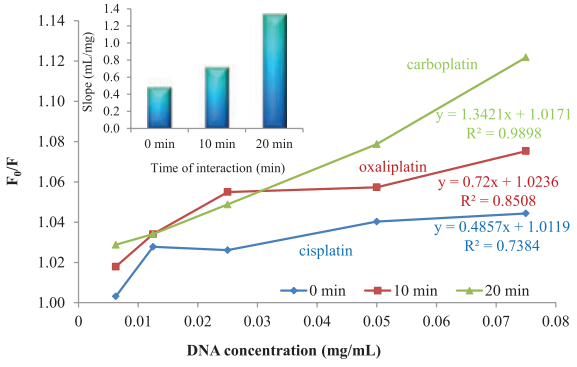

Figure 3. Stern–Volmer plot of CdTe QDs fluorescence quenching effect caused by DNA fragment (500 bp) and its dependence on time.

time is shown. It follows from the results obtained that the quenching was time dependent and the during first 10 min of interaction the quenching increased for 48% and during 20 min long interaction the quenching increased for 176%.

3.3 The sensing of platinum drugs-DNA interactions using QDs

Subsequently, adducts of DNA and platinum cytostatic drugs, with exception of $PtCl_2$, were prepared and their abilities of QDs fluorescence quenching were investigated. DNA concentration of 25 μg/mL was mixed with three concentrations of platinum cytostatic drugs to reach final concentrations of 62.5, 125, and 250 μg/mL of each drug and 12.5 μg/mL of DNA. After 24 hours long incubation at 37°C and dialysis to remove excess of platinum drugs, QDs were added in ratio 1:1 (*v*/*v*) and the fluorescence was monitored in three reaction times (0, 10, and 20 min). The Stern–Volmer plots of these samples are shown in Fig. 4. In the case of cisplatinated DNA (Fig. 4A) all curves exhibited linear trend depending on concentration (Stern–Volmer curves) with coefficients of determination $R^2 = 0.9048$, 0.9414, and 0.9400 for 0, 10, and 20 min interaction time, respectively. In the case of oxaliplatinated DNA the concentration dependence exhibited significantly different behavior suggesting that oxalipaltinated DNA is more effective fluorescence quencher than cisplatinated DNA (Fig. 4B). The linearity of the Stern–Volmer curves expressed as coefficients of determination $R^2 = 0.9598$, 0.9883, and 0.9627 for 0, 10, and 20 min interaction time, respectively was very good.

Finally, the effect of carboplatinated DNA on QDs fluorescence is shown in Fig. 4C. The linearity of the Stern-Volmer curves exhibited the highest coefficients of determination—0.9766, 1, and 0.9765 for 0, 10, and 20 min interaction time, respectively.

Comparing the results showed in Fig. 4, it can be concluded that the quenching abilities increase in the row carboplatinated DNA < cispaltinated DNA < oxaliplatinated DNA (20 min of interaction). Moreover, the interaction time

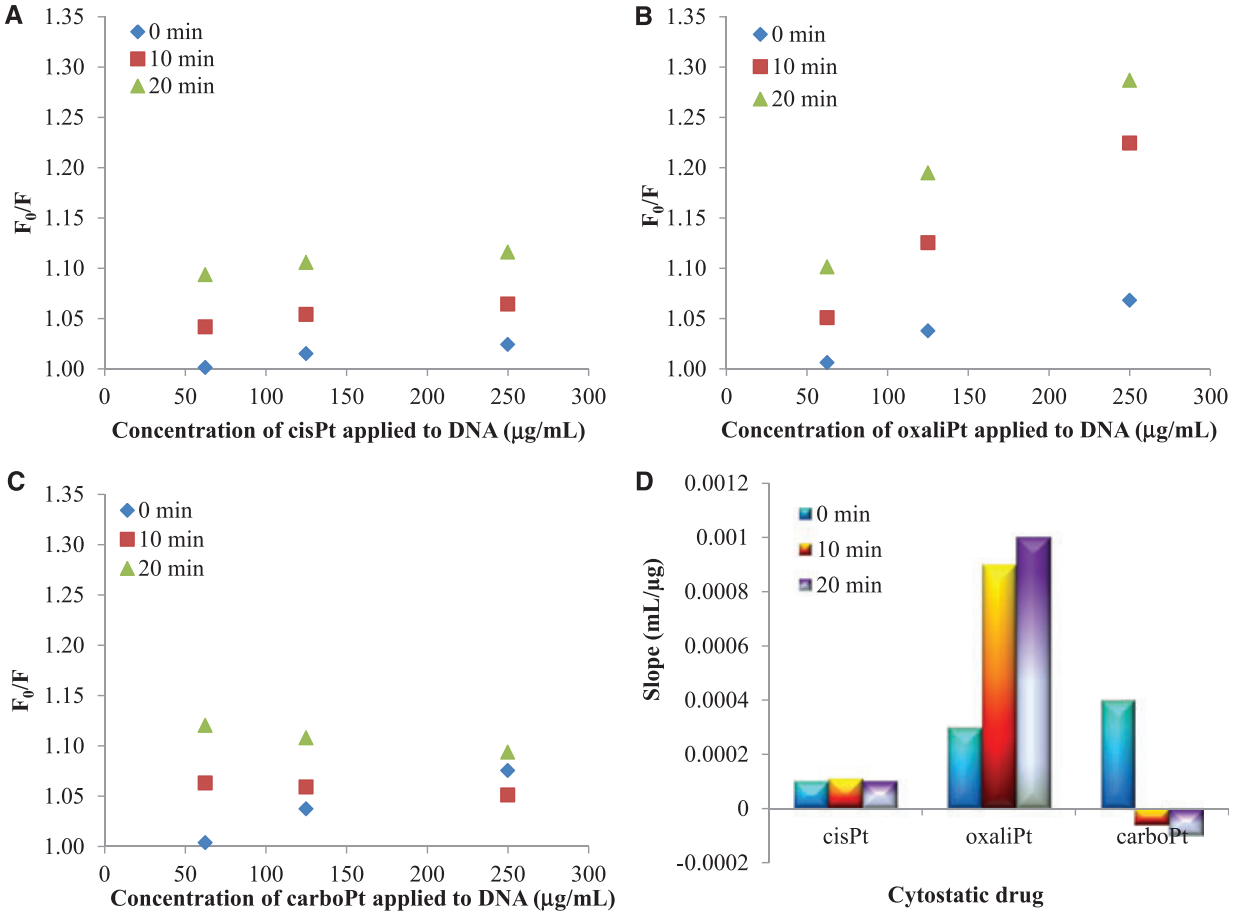

Figure 4. CdTe QDs fluorescence quenching caused by platinated DNA fragment. (A) Stern–Volmer plot of CdTe QDs fluorescence quenching effect caused by cisplatin and its dependence on time. (B) Stern–Volmer plot of CdTe QDs fluorescence quenching effect caused by oxaliplatin and its dependence on time. (C) Stern–Volmer plot of CdTe QDs fluorescence quenching effect caused by cisplatin and its dependence on time. (D) Comparison of slopes of the Stern–Volmer curves for each platinum based cytostatics in different times of interaction.

between the fluorophore and quencher plays an important role for structurally complex fluorescence quencher, such as adduct of DNA and platinum based cytostatic drug. The summary of time dependent behavior is presented in Fig. 4D. In this plot, the slopes of the Stern–Volmer curves (shown in Figs. 4A, B, and C) changes over the interaction time are plotted for each cytostatic drug–DNA complex. In the case of cisplatinated DNA, the quenching effect on QDs is time-independent because the slopes of the Stern–Volmer curves do not change significantly in time. We believe that this may be related with size of the cisplatin molecule interacting fast with QDs. However, in the case of oxaliplatinated DNA, the trend is increasing, which suggests that some more complex process is taking place in the mixture requiring longer interaction time. Finally, in the case of carboplatinated DNA, completely opposite trend is observed. This is in a good agreement with data shown in Fig. 2D, where quenching effect of pure cytostatic drugs is shown. Based on these results it can be concluded that the mechanism of carboplatin quenching of QDs fluorescence differs from the mechanism of cisplatin and oxaliplatin.

3.4 Studying of adducts by gel electrophoresis

Agarose gel electrophoretic analysis of DNA adducts with cytostatic drugs is shown in Fig. 5A. Lane "L" contains DNA ladder with size range from 0.1 to 1.5 kbp. In the lane 1, DNA fragment, which underwent the process of formation of DNA-cytostatic adduct (incubation and dialysis procedure), however without addition of cytostatic drug (blank), was injected. In the lane 2, cisplatinated DNA fragment was injected. The shift in the molecular weight from 500 to 600 bp is observed, which is caused by the binding of the cytostatic drug molecule into DNA increasing its molecular weight. Moreover, the intensity of ethidium bromide labeling decreased significantly. In the lane 3, the sample of oxaliplatinated DNA was injected and surprisingly no signal is observed. Lane 4 was injected by carboplatinated DNA and similarly to lane 2 a signal with shifted molecular weight was observed. Even though the fluorescence intensity of this band was lower than blank DNA it was higher compared to cisplatinated DNA. The changes in the electrophoretic behavior are caused probably by intercalation of the cytostatic drugs into DNA structure. This intercalation influences the intercalation of the ethidium bromide, which serves as a fluorescent label for DNA visualization. As the DNA is distorted by the presence of the drug, the labeling is precluded or significantly reduced. Based on this it can be concluded that interaction of oxaliplatin with DNA caused the largest deformation of DNA structure. The second most reduced signal is caused by cisplatin followed by carboplatin.

To verify the hypothesis that QD interact with platinated DNA creating some kind of bioconjugates gel electrophoretic analysis of the mixture of platinated DNA with QDs (lanes 5–8) was performed. We believe that if the bioconjugate would be created, a band with the increased molecular mass would

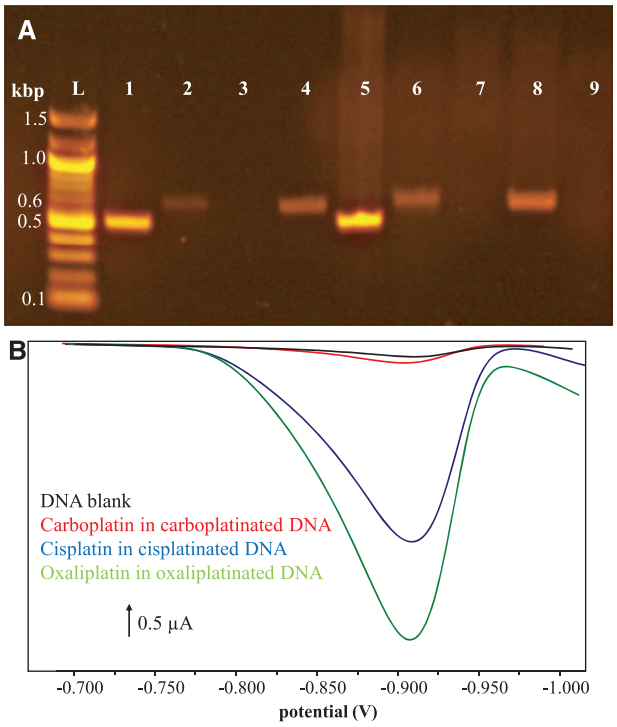

Figure 5. (A) Agarose gel analysis of DNA and DNA-cytostatic drug adducts (L – DNA ladder, 1 – DNA after incubation at 37°C for 24 h without platinum cytostatic drugs, 2 – DNA incubated with cisplatin, 3 – DNA after incubation with oxaliplatin, 4 – DNA after incubation with carboplatin, 5–8 – samples in lanes 1–4 with addition of GSH-QDs, 9 – GSH-QDs). (B) Determination of platinum-based cytostatic drugs in DNA-drug adducts.

be observed otherwise unspecific signal would be present. In the lane 5, the blank DNA mixed with QDs was injected providing a strong signal with molecular size of 0.5 kbp. In the lanes 6, 7, and 8, there are injected cisplatinated, oxaliplatinated and carbopaltinated DNA mixed with QDs. Similarly to lanes 2–4, the signals of DNA were shifted to higher number of base pairs and the signal intensities decreased in the same order (carboplatinated < cisplanated < oxaliplatinated). The only difference compared to lanes 1–4 was the presence of the background signal caused probably due to QDs. This suggests that QDs are not bound to DNA–drug adducts by any stronger interaction. The background signal was observed also in the lane 9, where QDs were injected only.

3.5 Electrochemical quantification of platinum in adducts

To be able to judge whether the fluorescent signal reduction in gel electrophoresis is caused by the cytostatic drug structure (the type of the drug) or its amount, electrochemical analysis was carried out. The amount of platinum in DNA–drug adduct (the same solution used for gel electrophoretic analysis) was determined (Fig. 5B). The highest amount of platinum was determined in the oxalipaltinated DNA solution

followed by cisplatinated and finally carboplatinated DNA solution.

3.6 Correlation

The hypothesis of applicability of the QDs fluorescence quenching for determination of the amount of cytostatic drug intercalated into the DNA was investigated. The electrochemical determination of platinum in the DNA adducts with cytostatic drugs was compared to the amount of cytostatic drug obtained by quenching of the QDs fluorescence (Fig. 6). It has to be noted that quenching effect of the DNA has been subtracted from the total quenching effect provided by DNA–drug adducts to determine the contribution of the cytostatic drug bound in the DNA structure. The best agreement between electrochemical and fluorimetric determination was found in the case of oxaliplatin (Fig. 6A). In the case of cisplatinated DNA (Fig. 6B), the fluorimetricaly determined platinum-based drug content was significantly lower.

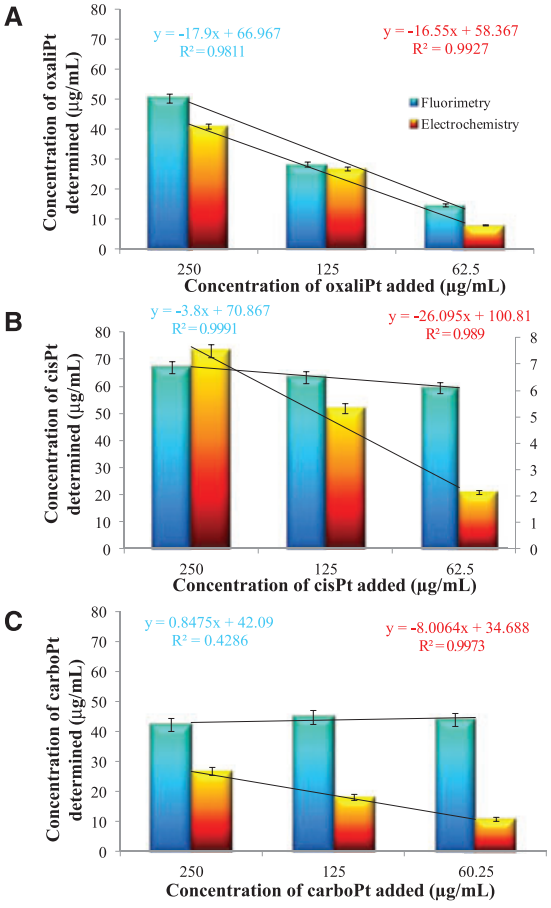

Figure 6. Correlation between amount of platinum-based cytostatic drug (A) oxaliplatin, (B) cisplatin (left-hand side *y*-axis—electrochemistry, right-hand side *y*-axis—fluorimetry) and (C) carboplatin incubated with DNA and amount determined fluorimetrically and electrochemically.

The amount of platinum determined by electrochemistry was almost one order of magnitude higher than the amount determined fluorimetricaly using quenching of QDs (two *y*-axes in Fig. 6B). Even though it as shown in Figs. 2A and B that cisplatin and oxalipaltin are similarly strong quenchers, there is probably a significant contribution of the structure of the drug, not only the metal ion, responsible for quenching effect when bound to the DNA. In the case of carbopaltin (Fig. 6C), the fluorimetric method is not applicable for determination of the amount of the drug bound in DNA structure. This may be caused by the fact that the quenching effect of DNA itself is stronger that the quenching effect of the carboplatin. This is in the agreement with the results shown in Fig. 2C.

4 Concluding remarks

It can be concluded that especially in the case of oxaliplatin-DNA adducts, the quenching was the most significant compared to cisplatin and nonquenching carboplatin. Considering the fact that the tested drugs have different way of the effect, the quenching of QDs intensity could be a new option how to study their action with DNA.

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The authors have declared no conflict of interest.

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