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Voltammetric Characterization of Lawsone-Copper(II) Ternary Complexes and Their Interactions with dsDNA

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Metal complexes of fungal and plant secondary metabolites are in the centre of interest, especially due to their biological properties including cytotoxicity. In our work, we focused on electrochemical behaviour of seven newly prepared ternary copper(II) complexes of lawsone with additional *O*-donor (water) and *N*-donor ligands (pyridine, 2-, 3-, and 4-aminopyridine, 3-hydroxypyridine, and 3,5-dimethylpyrazole) using the methods of cyclic voltammetry and differential pulse voltammetry. In addition, we tested ability of these complexes to interact with DNA. Our results indicate that the most simple complex of lawsone - $Cu(lawsone)_2(H_2O)_2 \cdot 0.5H_2O$ proved significant prooxidant properties, which may contribute to its cytotoxicity that has been demonstrated in recent literature. In addition, all complexes evidenced ability to interact with dsDNA. In article, possible mechanisms of these interactions are discussed.

Keywords: Medicinal Chemistry; Nucleic Acid Interaction; Electrochemistry; Cyclic Voltammetry, Differential Pulse Voltammetry; Copper; Lawsone

1. INTRODUCTION

Naphthoquinones are less common secondary metabolites of some fungi, plants, but also bacteria [1-6]. Their structure is based on 1,4-naphthoquinone skeleton, which is usually substituted by hydroxyl groups, or by side chain derived from isoprene unit(s) [7]. The most important naphthoquinones-containing plant families are *Bignoniaceae, Droseraceae, Ebenaceae, Juglandaceae*, and *Plumbaginaceae* [2,8]. Naphthoquinones have many physiological roles, because some members of this group as ubiquinone, plastoquinone and K vitamins are key functional constituents of biochemical systems. In addition, role of vitamin K in some pathological processes, such as atherosclerosis, is widely discussed [9,10]. It was also shown that vitamin K demonstrated possibility to induce differentiation and apoptosis in several types of cancer cell lines [11,12]. Generally, naphthoquinones are very interesting compounds with wide range of biological actions, including antibiotic, antiviral, antifungal, antiparasital, anti-inflammatory, antiproliferative and cytotoxic effects [2,13]. Cytotoxicity is one of the most important properties of naphthoquinones, which is based on generation of reactive oxygen species, disruption of mitochondrial functions, inhibition of thymidine incorporation into DNA and DNA intercalation [14-16]. Recently, it was determined that naphthoquinone plumbagin is able to inhibit invasion and migration of malignant cells, especially by inhibition of metalloproteinase-2 and urokinase-plasminogen activator [17,18].

Besides naphthoquinones itself, complexes of secondary metabolites may represent very interesting possibility of modification of their biological properties. Ruthenium(II) complexes with derivatives of chalcone and flavone showed interesting cytotoxic properties [19]. Quercetin nickel(II) complexes demonstrated DNA binding and cleavage properties *in vitro*. In addition, significant cytotoxicity on three tumour cell lines HepG2 (liver hepatocellular carcinoma), SMMC7721 (human hepatoma) and A549 (human lung adenocarcinoma epithelial cell line) was found [20]. Also naphthoquinones-based complexes have been prepared. Rhodium(III), platinum(II) and iridium(III) complexes were investigated due to their cytotoxic activities and transition metal complexes of plumbagin and its azo- derivatives were studied due to potent antimicrobial properties [21-23]. It is clear that complexes of naphthoquinones with metal ions are in the centre of interest. It seems that these complexes have higher cytotoxic properties in comparison with naphthoquinone itself or with metal ions. Five lanthanide(III)-plumbagin complexes had significantly higher cytotoxic properties on tumour cell line BEL7404 in comparison with plumbagin itself [24]. Phytotoxicity of lawsone copper(II) complexes was also investigated [25]. However, mechanism of cytotoxic action of newly synthesized naphthoquinones-based complexes remains still unknown.

Electrochemistry represents suitable tool for detection and quantification of different compounds from metal ions to high molecular mass organic compounds [26-32]. However, the number of papers focused on electrochemistry of naphthoquinones is relatively limited compared to other compounds and applications. Electrochemical methods were used for characterization of 1,4-naphthoquinone complexes [33], rhodium(III) 1,2-naphthoquinone-1-oxime complexes [34] or palladium-1,4-naphthoquinone complexes containing bis(pyrazol-1-yl)methane ligands [35]. Babula et al. demonstrates using electrochemical techniques for detection and quantification of naphthoquinones lawsone, juglone and plumbagin in plant tissues [4]. Besides possibility to quantify naphthoquinones,

electrochemistry is suitable for studying of interactions between drugs and their possible targets, most of all, with DNA due to outstanding sensitivity [36-42]. DNA biosensor technologies are in the focus of interest of many scientists due to their application in recognition of mechanism of interaction of compounds with DNA [43-50]. In this study, we aimed at studying of interactions of newly prepared complexes of lawsone (2-hydroxy-1,4-naphthoquinone) with copper(II) ions with DNA. Possible mechanisms of DNA-ternary lawsone-copper(II) complexes interactions were suggested.

2. EXPERIMENTAL PART

2.1. Ternary lawsone–copper(II) complexes

Lawsone (Law, 2-hydroxy-1,4-naphthoquinone), copper(II) acetate monohydrate (Cu $(CH_3COO)_2 \cdot H_2O$), and other chemicals of p.a. purity, which were used for synthesis of complexes, were purchased from Sigma-Aldrich (Sigma-Aldrich, St. Louis, USA). Ternary lawsone-copper(II) complexes were prepared by slightly modified methods, reported previously by Yamada et al. [51] and Hernández-Molina et al. [51,52]. Chemical composition of the complexes was confirmed by elemental analysis and spectral methods (UV-Visible, FTIR, ESI-MS). The results of spectral analyses (*unpublished data*) support the assumption that the molecular structure of complexes 1 and 5-8 is similar to the structures of $Cu(lawsone)_2(H_2O)_2$ and $Cu(lapachol)_2(py)_2$, which are characterized by the octahedral *trans*-arrangement of coordination polyhedron with two 2-oxido-naphthoquinone ligands occupying the basal plane and two apical positions occupied by additional ligands (water molecules or nitrogen-donor ligands). On the other hand, complexes 2 and 4 showed different composition and we assume that their molecular structure is quite different. We assume that the central ion in these complexes holds the square-pyramidal polyhedron with two 2-oxido-naphthoquinone ligands occupying the basal plane and nitrogen-donor additional ligand occupying the apical position. The specific composition and basic properties of the studied complexes are summarized in Table 1. The proposed molecular structure of complexes 1-8 and ligands used for synthesis of complexes are introduced in Fig. 1.

Table 1. Chemical composition of tested complexes; py- pyridine, 2-,3-,4-apy- 2-,3-,4-aminopyridine, 3-OH-py- 3-hydroxypyridine, 3,5diMepz- 3,5-dimethylpyrazole, EtOH- ethanol, DMSO- dimethyl sulphoxide.

Designation	Complex structure	Summary formula	Mr	Solubility
Complex 1	$Cu(lawsone)_2(H_2O)_2 \cdot 0.5H_2O$	$C_{20}H_{15}CuO_{8,5}$	454.89	H_2O, EtOH, DMSO
Complex 2	$Cu(lawsone)_2(py) \cdot H_2O$	$C_{25}H_{17}CuNO_7$	506.96	H_2O, EtOH, DMSO
Complex 4	$Cu(lawsone)_2(2\text{-}apy) \cdot 1.5H_2O$	$C_{25}H_{19}CuN_2O_{7,5}$	530.98	H_2O, EtOH, DMSO
Complex 5	$Cu(lawsone)_2(3\text{-}apy)_2 \cdot 0.5H_2O$	$C_{30}H_{23}CuN_4O_{6,5}$	607.08	H_2O, EtOH, DMSO
Complex 6	$Cu(lawsone)_2(4\text{-}apy)_2 \cdot 0.5H_2O$	$C_{30}H_{23}CuN_4O_{6,5}$	607.08	H_2O, EtOH, DMSO
Complex 7	$Cu(lawsone)_2(3\text{-}OH\text{-}py)_2$	$C_{30}H_{20}CuN_2O_8$	600.04	H_2O, EtOH, DMSO
Complex 8	$Cu(lawsone)_2(3,5\text{-}diMepz)_2 \cdot 0.5H_2O$	$C_{30}H_{27}CuN_4O_{6,5}$	611.12	H_2O, EtOH, DMSO

2.2 Chemicals and material

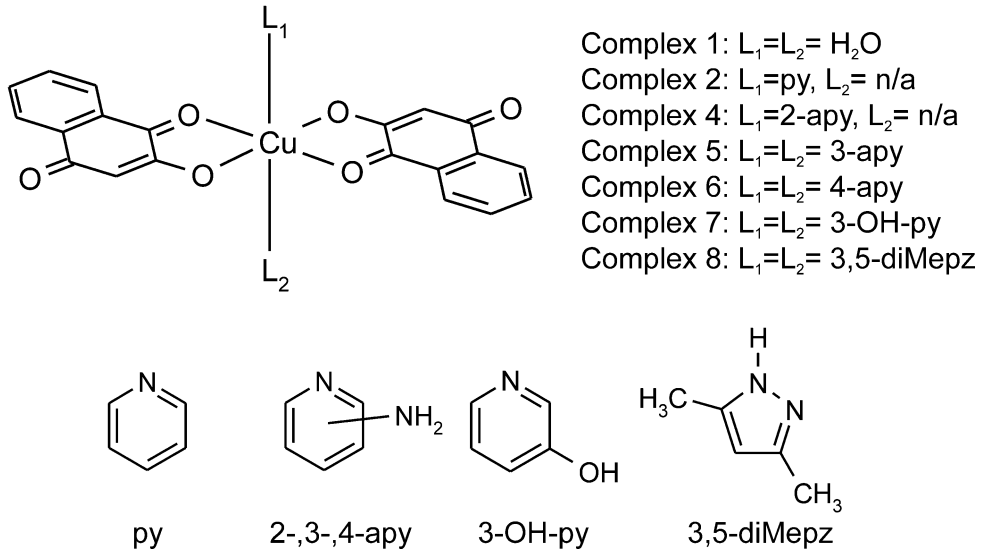

Figure 1. The proposed molecular structure of ternary lawsone-copper(II)complexes (1-8) and the ligands used in their synthesis, n/a = not present.L_1=L_2=H_2O (complex 1), L_1=py, L_2=n/a (complex 2), L_1=2-apy, L_2=n/a (complex 4), L_1=L_2=3-apy (complex 5), L_1=L_2=4-apy (complex 6), L_1=L_2=3-OH-py (complex 7), L_1=L_2=3,5-diMepz (complex 8). Py= pyridine, 2-,3-,4-apy= 2-,3-,4-aminopyridine, 3-OH-py = 3-hydroxypyridine, 3,5diMepz= 3,5-dimethylpyrazole.

Dimethyl sulphoxide of p.a. purity, copper(II) nitrate trihydrate, mineral oil, carbon (carbon, glassy, spherical powder, 2-12 micron, 99,5%), sodium acetate and acetic acid (ACS purity) were purchased from (Sigma-Aldrich, USA). dsDNA isolated from chicken blood was obtained from Reanal (Reanal, Hungary) and 3 M solution of potassium chloride from Metrohm (Metrohm, Switzerland). The deionised water was further purified by using apparatus MiliQ Direct QUV equipped with the UV lamp. The resistance was 18 MΩ. The pH was measured using pH meter WTW inoLab (Weilheim, Germany).

2.3. Cyclic voltammetry of ternary lawsone-copper(II) complexes

Electrochemical analyser (Metrohm AG, Switzerland) was used for determination of copper(II) ions. The analyser (757 VA Computrace from Metrohm, Herisau, Switzerland) employed a conventional three-electrode configuration with a hanging mercury drop electrode (HMDE) working electrode: 0.4 mm^2, Ag/AgCl/3MKCl as reference electrode, and a platinum auxiliary electrode. The following setup assembled of automated voltammetric analysis was supplied by Metrohm. A sample changer (Metrohm 813 Compact Autosampler) performed the sequential analysis of up to 18 samples in plastic test tubes. For the addition of standard solutions and reagents, two automatic dispensers (Metrohm 765 Dosimat) were used, while two peristaltic pumps (Metrohm 772 Pump Unit, controlled by Metrohm 731 Relay Box) were employed for transferring the rinsing solution in the cell and for removing solutions from the voltammetric cell. Method of cyclic voltammetry was used for measurement of electrochemical behaviour of ternary lawsone-copper(II) complexes. Following

parameters were applied for measurement itself: deoxygenating with argon 120 s, start potential 0 V; vertex potential -1.5 V, end potential 0 V, step potential -2.0 mV, scan rate -0.2 V/s. All measurements were carried out in five repetitions.

2.4. Differential pulse voltammetry of copper(II) ions, lawsone and its complexes

Preparation of carbon paste electrode. The carbon paste (about 0.5 g) was made of 70% graphite powder (Sigma-Aldrich) and 30% mineral oil (*m*/*w*) (Sigma-Aldrich; free of DNase, RNase, and protease). This paste was housed in a Teflon body having a 2.5-mm-diameter disk surface. Prior to measurements, the electrode surface was renewed by polishing with a soft filter paper in preparation for measurement of a sample volume of 5 µl. Electrochemical measurements were performed using a CH-instruments (CH-Instruments, USA) using a plastic cell with three electrodes. The three electrode system consisted of carbon working electrode, an Ag/AgCl/3 M KCl reference electrode and a platinum counter electrode.

Determination of copper(II) ions. Copper(II) nitrate trihydrate (Sigma-Aldrich, USA) was used for preparation of the stock solution (10,000 µg/ml, MiliQ water). Next concentrations (10.0, 19.5, 39.1, 78.1, 156.3, 312.5, 625.0, 1250.0, 2500.0 and 5000.0 µµg/ml) were prepared by subsequent dilution of the stock solution with MiliQ water. These solutions were used for measurement immediately after dilution. Differential pulse voltammetric measurements were carried out under the following parameters: deoxygenating with argon for 60 s; deposition potential -0.6 V; time of deposition 240 s; start potential 0.3 V; end potential -1.2 V; pulse amplitude 0.05 V; pulse time 0.05 s; step potential 4.0 mV; time of step potential 0.2 s. Time of accumulation was 5 s. All measurements were carried out in five repetitions.

Determination of lawsone. Stock solution of lawsone was prepared by dissolving of lawsone in dimethyl sulphoxide (1,000 µg/ml). Working solutions (3.95, 7.90, 15.70, 31.30, 62.50 and 125.00 µg/ml) were prepared immediately by subsequent dilutions of the lawsone stock solution with 0.2 M acetate buffer (pH 5.0). Differential pulse voltammetric measurements were carried out under the following parameters: deoxygenating with argon 60 s; deposition potential 0.3 V; time of deposition 240 s; start potential 0.3 V; end potential -1.2 V; pulse amplitude 0.05 V; pulse time 0.05 s; step potential 4.0 mV; time of step potential 0.2 s. Time of accumulation was 5 s. All measurements were carried out in five repetitions.

Determination of ternary lawsone-copper(II) complexes. Stock solutions of ternary lawsone-copper(II) complexes were prepared immediately before measurement in dimethyl suphoxide to the final concentration of 1,000 µg/ml. Working solutions (15.70, 31.30, 62.50 and 125.00 µg/ml) were prepared immediately by subsequent dilutions of the stock solution with 0.2 M acetate buffer (pH = 5.0). Differential pulse voltammetric measurements were carried out under the following parameters: deoxygenating with argon for 60 s; deposition potential 0.3 V; time of deposition 240 s; start potential 0.3 V; end potential -1.0 V; pulse amplitude 0.05 V; pulse time 0.05 s; step potential 4.0 mV; time of step potential 0.2 s. Time of accumulation was 5 s. All measurements were carried out in five repetitions.

2.5. Differential pulse voltammetry for studying the interactions of ternary lawsone-copper(II) complexes with dsDNA

Carbon paste electrode prepared according to the protocol mentioned above and modified with dsDNA (stock solution 1,000 μg/ml, applied volume 10 μl, time of accumulation 2, 4, 6, 10 and 20 min.) was used for the studying the interactions between dsDNA and ternary lawsone-copper(II) complexes. Prior dsDNA carbon paste electrode incubation, solution of ternary lawsone-copper(II) complex (stock solution 1,000 μg/ml, applied volume 10 μl, time of interaction 2 min.) was applied for electrode modification. Then, the modified electrode was carefully washed with miliQ water to remove excess complex. In the next step, the electrode was incubated with dsDNA (stock solution 1,000 μg/ml, applied volume 10 μl, time of accumulation 2, 4, 6, 10 and 20 min.). Further, the electrode surface was washed with MiliQ water. Measurements were carried out in the presence of 0.2 M acetate buffer (pH = 5.0, volume = 2,000 μl). All measurements were carried out in triplicates. The conditions of measurement were as follows: time of accumulation 60 s, modulation time 0.057 s, interval time 0.2 s, initial potential 0.3 V, end potential -1.2 V, step potential 10 mV, modulation amplitude 25 mV.

2.6. Mathematical treatment of data and estimation of detection limits

Results are expressed as mean ± standard deviation (S.D.) unless noted otherwise (EXCEL®). The detection limits (3 signal/noise, S/N) and quantification limits (10 S/N) were calculated according to Long and Winefordner [53], whereas N was expressed as standard deviation of noise determined in the signal domain unless stated otherwise.

3. RESULTS AND DISCUSSION

3.1. Oxidation-reduction properties of ternary lawsone-copper(II) complexes

Primarily, we aimed our attention on studying of electrochemical behaviour of ternary lawsone-copper(II) complexes at HMDE. Firstly, oxidation-reduction properties of complexes were determined by cyclic voltammetry. Using cyclic voltammetry method, oxidation/reduction signals of ternary lawsone-copper(II) complexes were determined. The differences between position and height of signals were determined. The highest number of signals was determined in the complex 4 and the lowest in the case of the aquacomplex ($Cu(lawsone)_2(H_2O)_2.0.5H_2O$, Complex 1). It clearly follows from the obtained results that the four main peaks, observed in the cyclic voltammograms correspond to the two one-electron metal centered oxidation/reduction process, involving the changes of the oxidation states of the central ion (Cu(II)/Cu(I) and Cu(I)/Cu(0)) in the studied complexes. The participation of ligands in the electrochemical reactions is less evident and depends markedly on the type of the additional ligand. Signals and their positions are introduced in Tab. 2 and cyclic voltammograms for individual complexes are shown in Fig. 2.

Table 2. Positions and heights of signals for individual ternary lawsone-copper(II) complexes determined by CV method, nd= not present.

Complex	Signal 1		Signal 2		Signal 3		Signal 4		Signal 5		Signal 6	
	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)
1	-0.022	154.0	-0.183	339.0	nd	nd	nd	nd	nd	nd	nd	nd
2	-0.020	143.0	-0.181	255.0	nd	nd	-0.583	0.9	nd	nd	-0.764	11.0
4	-0.022	94.3	-0.193	68.8	-0.449	5.4	-0.593	8.4	nd	nd	-0.768	13.3
5	-0.014	91.9	-0.179	140.0	nd	nd	-0.582	0.6	-0.703	1.3	-0.770	1.2
6	-0.012	107.0	-0.151	37.9	-0.395	14.9	-0.599	13.4	nd	nd	-0.766	13.7
7	-0.020	101.0	-0.177	152.0	nd	nd	-0.589	1.6	nd	nd	-0.764	13.8
8	-0.038	18.7	-0.179	198.0	nd	nd	nd	nd	nd	nd	-0.758	2.6

Complex	Signal 7		Signal 8		Signal 9		Signal 10		Signal 11	
	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)	Position (V)	Signal height (nA)
1	nd	nd	-1.077	10.1	-0.143	254.0	nd	nd	0.033	540.0
2	nd	nd	-1.079	25.7	-0.145	193.0	nd	nd	0.031	557.0
4	-0.877	31.4	-1.044	1.7	-0.145	34.0	-0.052	6.3	0.031	364.0
5	nd	nd	-1.034	36.4	-0.135	107.0	nd	nd	0.029	400.0
6	-0.871	17.0	-1.064	78.4	-0.125	35.5	nd	nd	0.029	414.0
7	-0.869	0.8	-1.083	29.1	-0.139	114.0	nd	nd	0.031	397.0
8	-0.867	1.0	-1.083	6.8	-0.145	159.0	nd	nd	0.033	328.0

According the observed positions and intensities of the main signals can be assumed, that the electrochemical process is quasi-reversible. On the other hand, however, the cathodic-to-anodic peak current ratios observed for all tested complexes are higher than the one, suggesting either adsorption of the re-oxidized complex to the electrode surface, or the diffusion disturbance on the electrode-surface [52].

The number of reduction signals and summation of their heights is introduced in Tab. 3. The highest reduction potential was determined for the aquacomplex $Cu(lawsone)_2(H_2O)_2.0.5H_2O$. The summation of all reduction signals revealed the following order of ternary lawsone-copper(II) complexes: 1 > 2 >> 7 > 6 > 5 > 8 > 4. It is obvious that oxidation-reduction properties of complexes depend mainly on the central ion. However, in case of the studied complexes with naphthoquinone lawsone and additional oxygen-donor or nitrogen-donor ligands, several reduction peaks were observed, probably connected with the reduction of additional ligands without being re-oxidised as no other anodic peaks (besides the main ones) were observed. There is just one exception for complex 4, where the splitting of the first anodic peak (i.e. See Signal 10 in Tab.3) was observed. The differences between complexes 4, 5 and 6 are not only in the number of additional ligands, but also in their substitution (i.e. the position of amino substitution at pyridine), which has notable effect on their electrochemical properties. However, oxidation/reduction properties and generally electrochemical behaviour of aminopyridine, its derivatives and complexes with aminopyridine as an additional ligand

are only poorly understood. Only few papers focused on the possibility of aminopyridine and its derivatives to form transition metal complexes have been published [54-58].

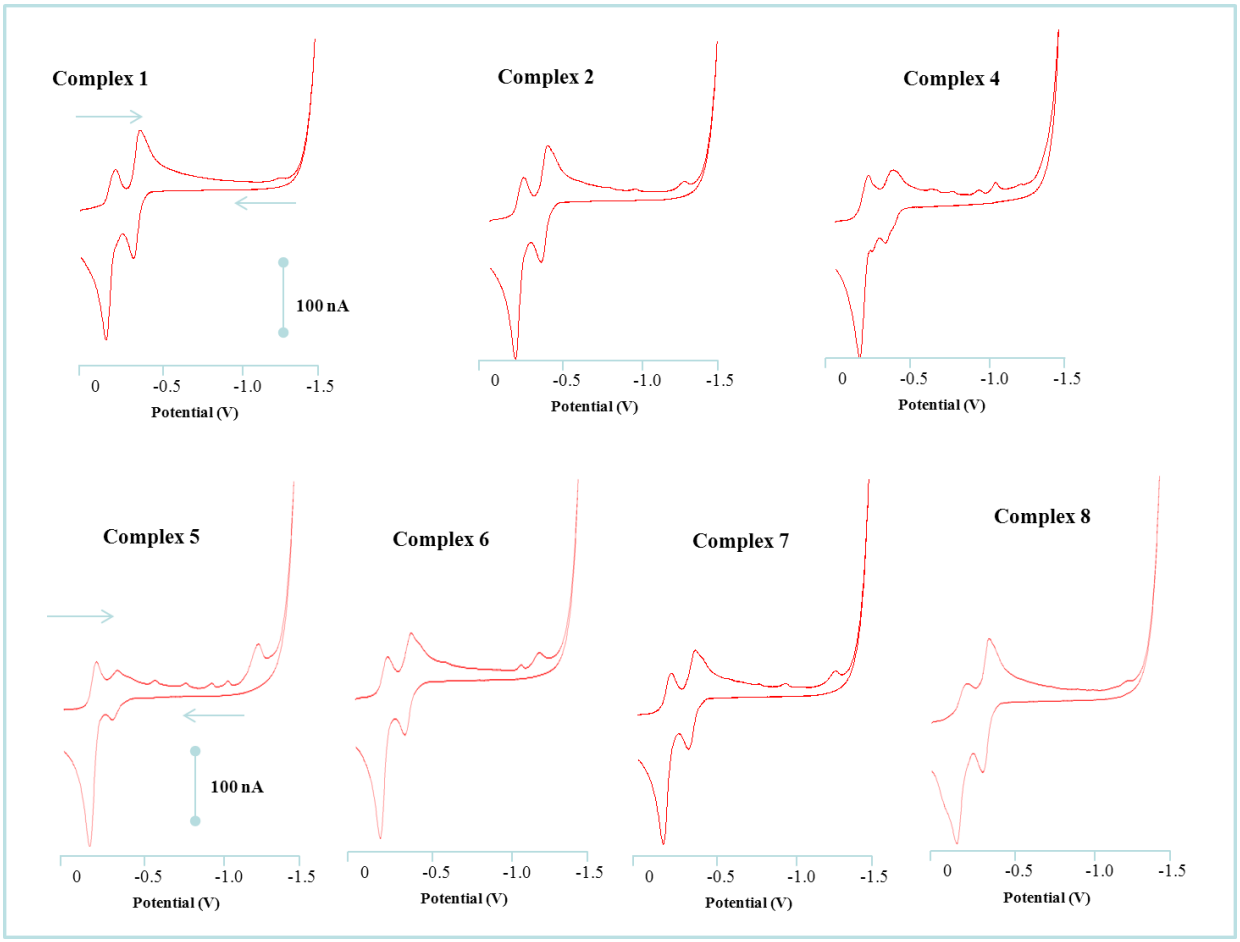

Figure 2. Cyclic voltammograms of studied complexes. Following parameters were applied for measurement itself: deoxygenating with argon for 120 s, start potential 0 V; vertex potential -1.5 V, end potential 0 V, step potential -2.0 mV, scan rate 0.2 V/s. All measurements were carried out in five repetitions.

Table 3. Number of reduction signals and summation of their heights determined by CV method.

Sample	Number of reduction signals	Summation of heights of all reduction signals (nA)
Complex 1	3	503.1
Complex 2	5	435.6
Complex 4	7	223.3
Complex 5	6	271.4
Complex 6	7	282.3
Complex 7	6	298.4
Complex 8	5	227.1

It is obvious that substitution of the additional ligand, pyridine, decrease the reduction properties of complex in dependence on the rate and especially position of substitution. In conclusion, all tested complexes demonstrate interesting oxidation-reduction properties, however, the effect of their structure on these properties has to be further investigated.

3.2. Differential pulse voltammetric determination of copper(II) ions and lawsone

Primarily, we focused on position of copper(II) ions. Signal of copper(II) ions appeared at the potential of – 0.05 V. The second step consisted in construction of calibration curve for Cu(II) ions within the concentration range from 10.0 to 5000.0 µg/ml. This curve was of logarithmic character with the following parameters: y = 3.478ln(x) – 8.0041, R^2 = 0.9816 (Fig. 3A), LOD = 30 ng/ml and LOQ = 100 ng/ml. However, this dependence had linear course within concentration range from 10.0 – 156.3 µg/ml (y = 0.0546x + 1.26320, R^2 = 9937, in inset in Fig. 3A). Further, we characterized lawsone. Signal of lawsone was determined at -0.3 V. Dependence of signal height on lawsone concentration proved polynomial course (y = -5– $0.5x^2$ + 0.038x – 0.0817, R^2 = 0.9908, LOD = 20 ng/ml, LOQ = 70 ng/ml, Fig. 3B). Linear course of this curve was determined within the concentration range from 3.95 to 125.00 µg/ml (y = 0.0378x – 0.1644, R^2 = 0.9968, in inset in Fig. 3B).

3.3. Differential pulse voltammetry of the studied complexes

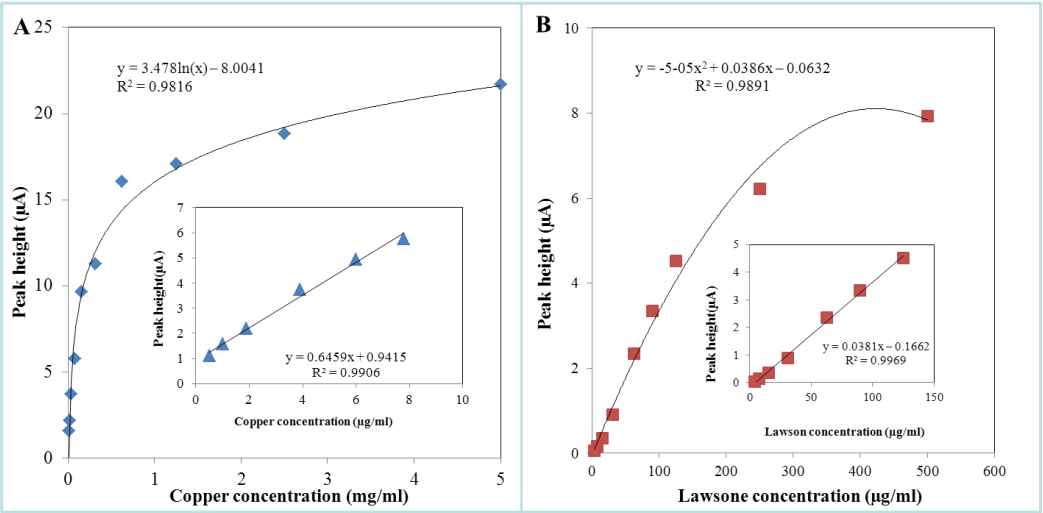

Figure 3. Calibration curves for (**A**) copper(II) ions and (**B**) lawsone. (**A**) Calibration curve of copper(II) ions with logarithmic dependence within the concentration range from 10.0 to 5000.0 µg/ml); **inset**: linear course of dependence of copper(II) ions concentration on signal height in the concentration range from 10.0 to 156.3 µg/ml. (**B**) Calibration curve of lawsone with polynomic course in the concentration range from 3.95 to 500.00 µg/ml; **inset**: linear course of dependence of lawsone concentration on signal height in the concentration range from 3.95 to 125.0 µg/ml.

Table 4. Characterization of copper(II) complexes of lawsone in the whole concentration range (15.7; 31.3; 62.5; 125.0; 250.0 and 500.0;0 μg/ml), regression equations for both signal 1 (copper(II), linear) and 2 (lawsone, polynomial), coefficients of determination (R^2), limits of detection (LOD) and limits of quantification (LOQ) for individual ternary lawsone-copper(II) complexes.

complex	signal	concentration range [μg/ml]	regression equation	R^2	LOD [ng/ml]	LOQ [ng/ml]
1	1	15.7 – 500.0	y = 0.0086x + 0.2642	0.9602	20	70
	2	15.7 – 500.0	y = -4 – 5x² + 0.04x - 0.1353	0.9997	15	50
2	1	15.7 – 500.0	y = 0.0074x + 0.5653	0.9542	30	100
	2	15.7 – 500.0	y = -4 – 5x² + 0.0381x – 0.4848	0.9976	20	70
4	1	15.7 – 500.0	y = 0.013x + 0.0816	0.9607	30	100
	2	15.7 – 500.0	y = -7 - 5x² + 0.0549x – 0.8615	0.9975	5.00	20.00
5	1	15.7 – 500.0	y = 0.0036x + 0.2808	0.8907	20	70
	2	15.7 – 500.0	y = -3 - 5x² + 0.0227x + 0.5452	0.9943	3	10
6	1	15.7 – 500.0	y = 0.0046x + 0.0348	0.9861	10	35
	2	15.7 – 500.0	y = -3 - 5x² + 0.0261x – 0.3938	0.9984	10	35
7	1	15.7 – 500.0	y = 0.0059x + 0.2181	0.9237	5	20
	2	15.7 – 500.0	y = -3 - 5x² + 0.028x – 0.0522	0.9988	10	35
8	1	15.7 – 500.0	y = 0.0012x + 0.0703	0.9929	30	100
	2	15.7 – 500.0	y = -2 - 5x² + 0.0208x – 0.1251	0.9996	15	50

In the following part of this study, we focused on studying of the electrochemical behaviour of ternary lawsone-copper(II) complexes of interest using differential pulse voltammetry at carbon paste electrode. All complexes were studied within the concentration range from 15.7 to 500.0 μg/ml with the focus on the signals of copper(II) ions (-0.05 V, signal 1) and lawsone (-0.4 V, signal 2). All studied complexes demonstrated appearance of both signals and their changes with the increasing concentration of the complexes (Fig. 4).

Table 5. Concentration ranges of linear dependences of both signals 1 and 2 on concentration of individual complexes. Coefficients of determination (R^2) are also introduced.

complex	signal	concentration range [μg/ml]	regression equation	R^2
1	1	15.7 – 250.0	y = 0.0117x + 0.0153	0.9876
	2	15.7 – 250.0	y = 0.0279x + 0.2961	0.9854
2	1	15.7 – 250.0	y = 0.0103x + 0.3336	0.9868
	2	15.7 – 250.0	y = 0.0266x – 0.0947	0.9971
4	1	15.7 – 250.0	y = 0.018x – 0.3125	0.9962
	2	15.7 – 250.0	y = 0.0378x – 0.2802	0.9972
5	1	15.7 – 250.0	y = 0.006x + 0.0924	0.9914
	2	31.3 – 250.0	y = 0.0136x + 1.0332	0.9863
6	1	15.7 – 250.0	y = 0.0046x + 0.0348	0.9861
	2	15.7 – 250.0	y = 0.0184x – 0.1238	0.9896
7	1	15.7 – 250.0	y = 0.0091x – 0.0382	0.9998
	2	31.3 – 250.0	y = 0.0185x + 0.4047	0.9860
8	1	15.7 – 250.0	y = 0.0014x + 0.0555	0.9963
	2	15.7 – 250.0	y = 0.0156x + 0.061	0.9899

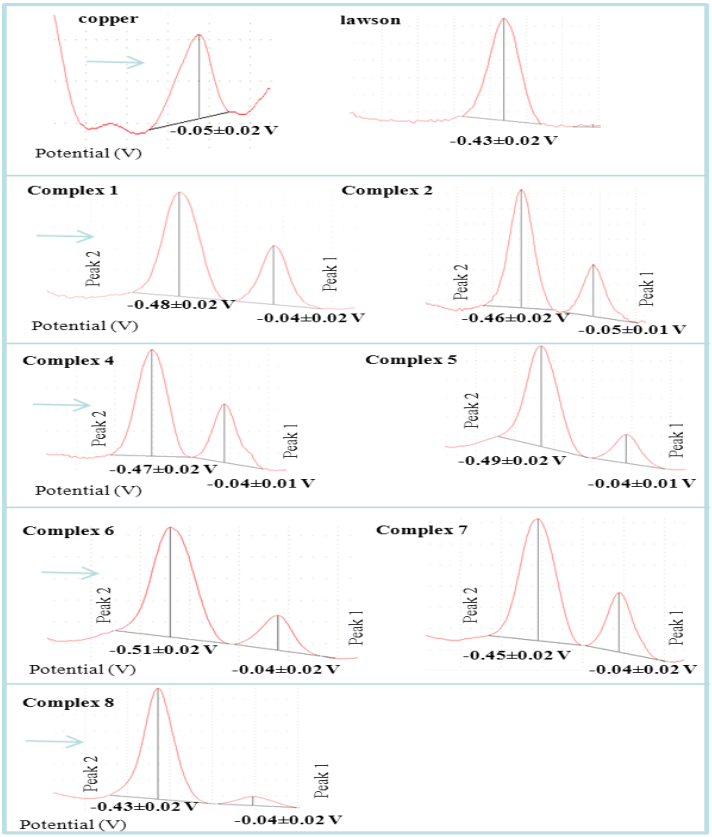

Figure 4. Differential pulse voltammograms of copper(II) ions, lawsone and individual studied lawsone-copper(II) complexes. Individual figures show positions of detected signals. Differential pulse voltammetric measurements were carried out under the following parameters: deoxygenating with argon for 60 s; deposition potential 0.3 V; time of deposition 240 s; start potential 0.3 V; end potential -1.0 V; pulse amplitude 0.05 V; pulse time 0.05 s; step potential 4.0 mV; time of step potential 0.2 s. Time of accumulation was 5 s. All measurements were carried out in five repetitions.

In the whole concentrations range, dependences of heights of these signals on the concentration of the complexes were linear and/or polynomial. All data including concentration range, equations of dependences, coefficients of determination (R^2), limits of detection (LOD) and limits of quantification (LOQ) for individual complexes are introduced in Tab. 4. In the case of all studied complexes, linear dependence appeared within the concentration range, which is summarized in Tab. 5. Concentration dependences for both signal 1 and signal 2 are shown in Fig. 5.

The obtained experimental data allowed us to compare both the heights of signal 1 (copper(II) ions) and signal 2 (lawsone). These data partially elucidate structure of complexes in the direction of their electrochemical behaviour and possible interactions with biomolecules. The highest values of both signal 1 and signal 2 were determined in the case of complex 4, structurally $Cu(lawsone)_2(2\text{-}apy)(H_2O)\cdot 0.5H_2O$ and the lowest in the case of complex 8 for signal 1 and in the case of complex 5 for signal 2. The highest values were taken as 100 %, other values were recalculated to this value: complex 1 - signal 1 69.21 %, signal 2 85.31 %; complex 2 - signal 1 64.87 %, signal 2 74.22 %;

complex 5 - signal 1 30.37 %, signal 2 44.14 %; complex 6 - signal 1 36.84 %, signal 2 52.96 %; complex 7 - signal 1 46.39 %, signal 2 57.38 %; complex 8 - signal 1 11.01 %, signal 2 53.83 %.

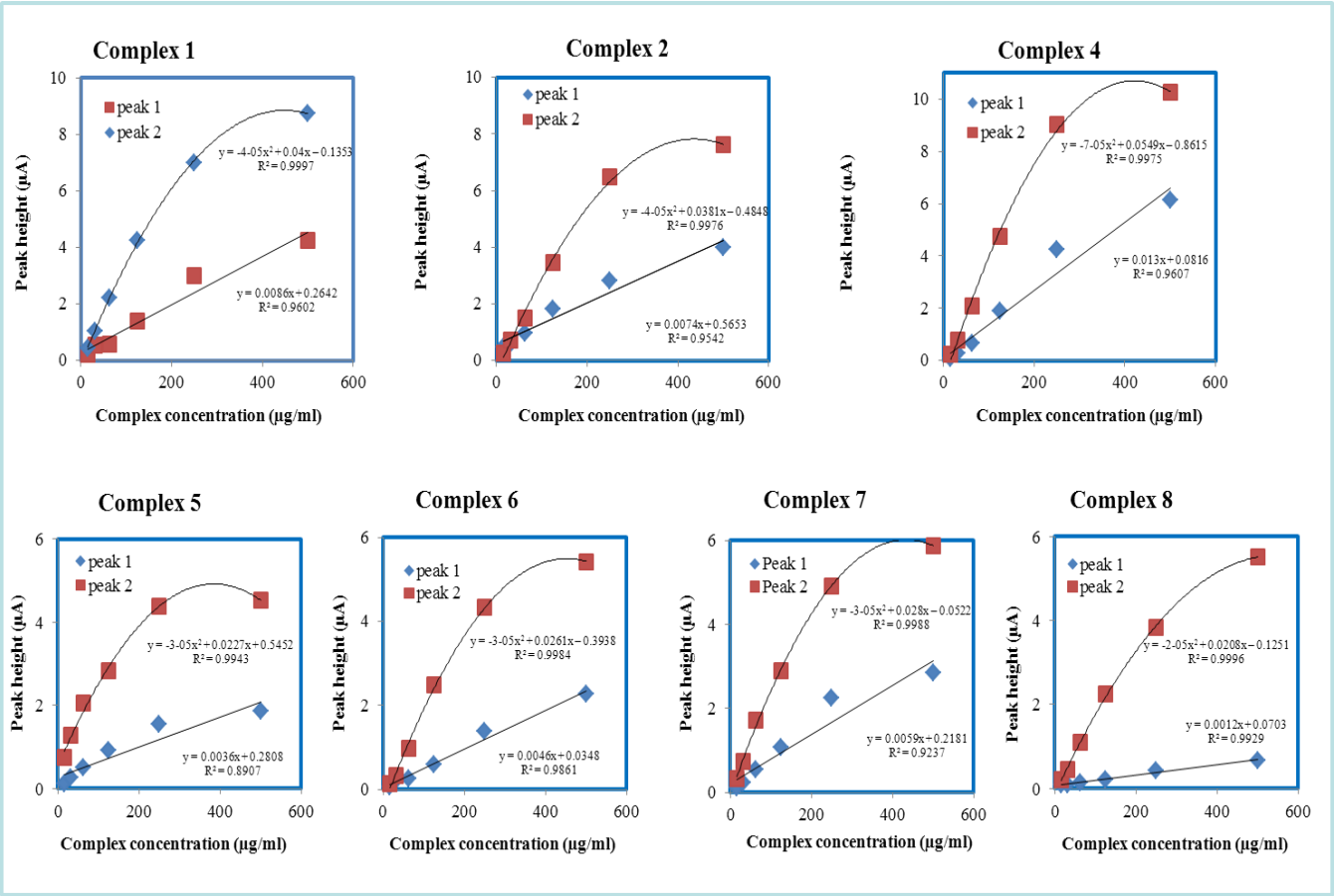

Figure 5. Calibration dependences of individual lawsone-copper(II) complexes within the whole studied concentration range from 15.7 to 500.0 µg/ml for signal 1 corresponding to copper(II) ions and signal 2 corresponding no lawsone. Regression equations with corresponding coefficients of determination are introduced.

3.4. Interactions of ternary lawsone-copper(II) complexes with dsDNA

dsDNA-modified carbon paste electrode was used for determination of dsDNA-ternary lawsone-copper(II) complexes interactions. DNA immobilized on the surface of carbon paste electrode brings interesting possibilities in the determination of interactions between DNA and studied compounds. These techniques elucidated mechanism of interactions between important pharmaceuticals, such as antibiotics like levofloxacin [59] and rifampicin [60], compounds with DNA intercalating potential, such as acridine orange [61], anticancer agent daunomycin [62] and mitomycin [63] or compounds, which are commonly used as stains or eventually as DNA stains [64-66]. In this study, we focused on the decrease of signal 1 (copper(II) ions) and signal 2 (lawsone) after accumulation on dsDNA modified carbon paste electrode.

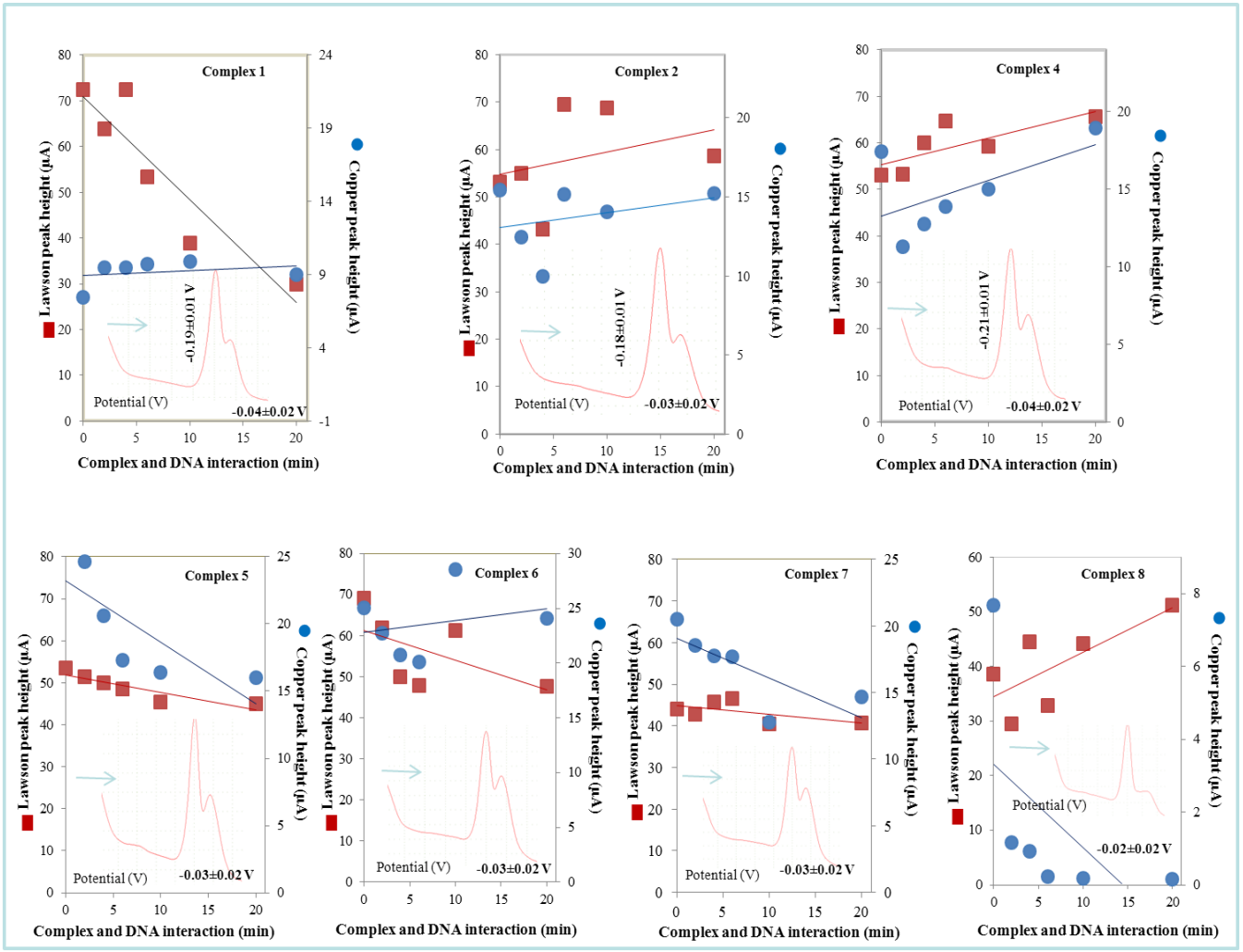

Figure 6. Dependences of heights of copper(II) ions and naphthoquinone lawsone signals of individual studied complexes on the time of dsDNA interaction within the range from 0 to 20 min. Voltammograms for individual complexes with marked potentials are introduced in insets.

In this step, we obtained first voltammogram for comparison with further voltammograms; after removing the excess of complex with miliQ water, electrode was incubated with 10 µl of dsDNA stock solution (1000 µg/ml) for strictly defined time intervals of 2, 4, 6, 10 and 20 min. For each time of accumulation, independent voltammogram was obtained. Finally, we evaluated changes of heights of both signal 1 and signal 2, which inform us about possible interactions with dsDNA. Based on the obtained results, Figure 6 was prepared, The figure compares changes in signal 1 and signal 2 of complexes after interaction with dsDNA.

The highest value of signal 1 (copper(II) ions) was detected for complex 6 followed by complexes 5, 4, 7, 2, 1 and 8. In the case of signal 2 (lawsone), its highest value was determined for the aquacomplex 1, followed by complexes 2, 4, 6, 5, 7 and 8. Based on these results, we compared also individual signals. In this case, all values were recalculated to the value determined for complex 6 with the highest signal (represents 100 %). The subsequent values for signal 1 in individual complexes were as it follows: 86.15 % (complex 5), 66.84 % (complex 4), 64.78 % (complex 7), 53.31 % (complex 2),

46.94 % (complex 1) and 4.10 % (complex 8). Also in the case of comparison of heights of signal 2, the highest obtained value (complex 1) was taken as 100 % and values detected for remaining complexes were recalculated to this value. The values for other complexes were as it follows: 95.58 % (complex 2), 90.60 % (complex 4), 85.55 % (complex 6), (77.69 % (complex 5), and 64.23 % (complex 7). The lowest value of signal 2 height was determined for complex 8 (61.11 %).

Nucleic acids are the most important target of action of many compounds, including drugs. Cytostatic drugs, which effect is based on DNA intercalation and other DNA interactions, such as groove-binding interactions and cross-linking interactions, represent the most suitable example [67]. Anthracycline antibiotics as doxorubicin and daunorubicin originally isolated from actinomycete *Streptomyces peuticeus* serving as a template for synthesis of more effective derivatives belonging to the most often studied drugs [68-72]. In this study, we studied possible dsDNA interactions between newly prepared ternary lawsone-copper(II) complexes. Lawsone (2-hydroxy-1,4-naphthoquinone) represents derivate of 1,4-napthoquinone with significant cytotoxic properties, which were demonstrated on different cell lines [2,73-75]. Despite the fact that mechanism remains still unclear, lawsone itself is able to induce production of reactive oxygen species, which are responsible for cytotoxic effect and toxicity of lawsone-containing plants to animals including human [76,77]. Oxidation/reduction properties of lawsone contribute to its toxicity too [78]. Copper(II) ions are well known inductors of reactive oxygen species [79,80]. In addition, they can interact with proteins and, in addition, they can modify antioxidant properties of protective compounds [81-84]. Due to this fact, ternary lawsone-copper(II) complexes represent an interesting group of compounds. Lawsone itself has been used for preparation of complexes with lanthanum(III) [85], mercury(II) [86,87], manganese(II) [88], iron(II) [89] and copper(II) [90] ions; however, data about biological properties of these complexes are almost missing. It clearly follows from our experimental data that studied complexes were able to interact with dsDNA, which was observed as a decrease of both signal 1 and signal 2. However, we studied only change of response of signal 1 and signal 2. So, it is very difficult to determine the possible mechanism of ternary lawsone-copper(II) complexes interactions with dsDNA. The contribution of the second ligand to dsDNA interaction was inconclusive. There are many metal complexes based on secondary metabolite as the first ligand and other additional ligands, which modify physical-chemical properties of complex. Flavonoid- and anthraquinone-based complexes with transition metals represent the best example [91-95]. Rutin and quercetin metal complexes have been verified to interact with DNA. This interaction is based on three-dimensional conformation of complex [20,96-102]. In our case, we tested ternary complexes, containing two different ligands. Nevertheless, the second ligand together with the first ligand (lawsone, which is planar) determines the final three-dimensional arrangement of the molecule of complex. There are papers, which connect three-dimensional arrangement of studied compounds with possibility of DNA intercalation. The ability of planar compounds (complexes) to intercalate DNA has been established [103,104]. We can assume that structure of all studied complexes has a planar part, thus, DNA intercalation is very probable mechanism of interaction between studied complexes and dsDNA. The positive effect may also have the coordination availability of interaction sites in the apical positions of coordination polyhedron, freed after the cleavage of nitrogen-donor ligands.

4. CONCLUSIONS

All studied complexes were in our previous study tested for cytotoxicity and all of them demonstrated significant cytotoxicity, which was higher compared to cytotoxicity of only lawsone, copper(II) ions and eventually for the second ligand, in addition, complexes proved also significant phytotoxicity [25]. Possible mechanism of their cytotoxicity may be based on induction of formation of reactive oxygen species (ROS) [25]. Literature describes the preparation of lawsone-trace metal complexes with significant prooxidant properties [105]. This fact must be carefully considered in direction to biomolecules including nucleic acids and their possible damage by free radicals. Possibility of compounds to induce ROS is closely associated with their oxidation/reduction properties. This fact must be further investigated in the light of the results introduced in the first part of experimental work. Moreover, determined oxidation/reduction properties of studied compounds may be involved in the cytotoxicity of complexes, where the most simple complex based only on copper(II) ions and lawsone proved the most significant cytotoxicity. This complex proved the highest reduction potential in our experiment. In conclusion, experimental work brings new knowledge not about oxidation/reduction properties of newly prepared copper(II)-lawsone based complexes, but also knowledge about possibility of these complexes to interact with DNA. This knowledge can be used in further experiments focused on determination of mechanisms of cytotoxicity of studied complexes.

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