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Research Article

Synthesis, physicochemical characterization and antimicrobial activity of Co(III) complexes with diamine chelate ligands

A. Chylewska[1*], K. Turecka[2], A. Dąbrowska[1], W. Werel[2] and L. Chmurzyński[1]

[1]Department of Chemistry, University of Gdańsk, Sobieskiego 18/19, 80-952 Gdańsk, Poland.

[2]Department of Pharmacy, Medical University of Gdańsk, Al. Hallera 107, 80-416, Gdańsk, Poland.

ABSTRACT

Four coordination compounds of *trans*-[Co(*N,N*)$_2$(H_2O)$_2$](ClO_4)$_3$ and *trans*-[Co(*N,N*)$_2$$Cl_2$]Cl type where *N,N* denotes ethylenediamine or 1,3-diaminopropane as chelating moieties were prepared and characterized by spectroscopic, potentiometric and other auxiliary analytical methods. It had been assumed that these compounds are potential models for several novel antimicrobial agents. The results of chemical and biological investigations showed a direct effect of the type of ligand on microbiological activity of the analogues compounds studied. UV-Vis absorption titration spectra of chlorates of the Co(III) coordination compounds were recorded over a wide pH range in the constant temperature (25 °C). Based onabove investigations the relationships between absorbance *vs.* pH were plotted and deprotonation equilibrium constants in the form of pK's were determined. Furthermore, it was found that the pKs determined using a potentiometric titration methodin the same temperature are in a very good agreement with those obtained using spectrophotometric data. Calculations of these constants were performed using the CVEQUID computer program. The reduction potential values of selected Co(III) complexes with aqua ligands were determined by using a cyclic voltamperometry method. Moreover, the *in vitro* antimicrobial activities of the synthesized complexes were examined against some antimicrobial strains of Gram-positive bacteria *viz. Enterococcus hirae*, *Staphylococcus aureus*, Gram-negative bacteria *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and fungus, *Candida albicans*.

Keywords: Co(III) complexes, Deprotonation and reducion processes, Antimicrobial activity.

INTRODUCTION

The scientific literature has described in detail the antiviral, antibacterial, antitumor and antifungal activities of coordination compounds of Co(II) and Co(III)[1-11]. However, only the cobalt(III) complexes enjoy growing interest due to these properties. The Dwyer's study concerned the assessment of toxicity of optical isomers of [Co(en)$_3$](NO_3)$_3$[12]. It was found that these complexes show the highest antimicrobial activity among different classes of chemotherapeutics. Mishra *et al.*[13] studied the coordination compounds of cobalt(III) with pyridine-amide as bidentate and tridentate ligands. These complexes demonstrate strong anti-bacterial properties only against *Pseudomonas, Escherichia Coli, Shigella flexneri* and *Klebsiella planticola*. Presently, the complexes of cobalt(III) are studied to confirm their potential use as anticancer drugs. Measurements involving substances used in anticancer therapies consist of finding cytostatic factors that strongly interact with cancer cells and very weakly influence the body's healthy cells[14,15]. It was confirmed that the values of the electrochemical redox potential of complex[16,17] and the structure of the ligand have a significant impact on the effectiveness and the stability of compounds used. These features allow to apply the cobalt(III) complexes as components of drugs (Co(III) complexes show higher stability than Co(II) complexes). The main aim of the presented investigations is to characterize physicochemical properties of compounds studied and then to apply them in pharmacological tests against microorganisms. We focused on obtaining complexes of Co(III), in which the structure features a

short *N,N*-donor organic ligands and simple monodentate inorganic ligands (en - ethylenediamine and dap – 1,3-diaminopropane). The future goal of our studies is to test much higher number of complexes of the same type to find a compound with specific bactericidal, fungicidal properties and its application as a drug.

MATERIAL AND METHODS

Synthesis of *trans*-$[Co(N,N)_2(H_2O)_2](ClO_4)_3$

In 25 cm^3 of double distilled water was dissolved 29.1 g of $Co(NO_3)_2 \cdot 6H_2O$. Then was added saturated $NaHCO_3$ hot solution until precipitation of a pink precipitate $CoCO_3$. The resulting mixture was introduced to 11.35 cm^3 of ethylenediamine (*N,N*=en) or to 16.74 $cm^3$of 1,3-diaminopropane (*N,N*=dap) and 10 cm^3 hydrogen peroxide. This solution was heated for two hours in a water bath. After cooling, the red-purple crystals were precipitate, which was then filtered off. The solution was concentrated by an hour in a water bath and then obtained residue was stored for a few days in the refrigerator. The precipitated dark red crystals were filtered off and washed with double distilled water and 96% ethanol. The resulting carbonate complexes type of $[Co(N,N)_2CO_3]NO_3 \cdot 1{,}5 \cdot H_2O$ were dissolved again in 25 cm^3 of double distilled water. Next, the $HClO_4$ (60 cm^3, 0.001 M) was slowly poured to the above solution and resulting mixture was acidified to pH = 1. After removal of carbon dioxide, the obtained product was concentrated, until the dry pink crystals were precipitated. Visible absorption spectra, molar activity coefficients for *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ agreed (±3%) with published values. Elemental analyses (C, H, N) were within 0.3% of the calculated values. The blood-red powder of *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$, obtained in 49% yield, was soluble in water, methanol, ethanol and DMSO. *Anal. Calcd* for $CoC_6H_{24}N_4O_{14}Cl_3$: C, 13.30; H, 4.43; N, 10.35. *Found*: C, 13.28; H, 4.38; N, 10.21%. Selected IR bands (cm^{-1}): 3340 (*ν*(N-H)), 3435 (*ν*(O-H)), 904 (*ν*(Co-O)), 420 (*ν*(Co-N)).

Synthesis of *trans*-$[Co(N,N)_2Cl_2]Cl$

The chloride salts were prepared by the methods of Broomhead and Kane-Maguire[18,19] and were recrystallized from 1 M HCl before use. Visible absorption spectra, molar activity coefficients for *trans*-$[Co(en)_2Cl_2]Cl$ agreed (±3%) with published values. Elemental analyses (C, H, N) were within 0.2% of the calculated values. The blue powder of *trans*-$[Co(dap)_2Cl_2]Cl$, obtained in 41.2% yield, was soluble in water, methanol, ethanol and DMSO. *Anal. Calcd* for $CoC_6H_{20}N_4Cl_3$: C, 22.98; H, 6.43; N, 17.87. *Found:* C, 22.91; H, 6.44; N, 17.80%. Selected IR bands (cm^{-1}): 3350 (*ν*(N-H)), 920 (*ν*(Co-Cl)), 450 (*ν*(Co-N)).

Measurements

The measurements were carried out by means of potentiometric titrations at constant ionic strength using an automated system and applying the *Microtitator* program. All probes, which were used in titrations, were prepared in nitrogen atmosphere to avoid CO_2 contamination and the temperature was kept at 25 ± 0.1 °C. Constant ionic strength of 0.1M was maintained with $NaClO_4$. The titration system consisted of a titration cell, a magnetic stirrer and an automatic titrator with Hamilton's syringe (0.5 mL). The pH-combined electrode was bought from *Mettler Toledo* firm. The electrode was calibrated using pH standard buffers. The CVEQUID computer program was used to get the acid-base constants from potentiometric method.

Perkin Elmer Lambda 800 UV-Vis double beam spectrophotometer, with automatic stirrer, was used for absorbance measurements. 1 cm quartz microcells were utilized, at 25 °C and 0.1M ionic strength ($NaClO_4$). For each pH point a known aliquot of solution was extracted and the absorption spectrum was recorded. All the titrations were performed under complete computer control.

Electrochemical measurements were made at scan speed 100 mV/s using Gamry potentiostat Reference 600. The measuring system consisted of a vessel in which there was a test solution with a concentration $c = 2 \cdot 10^{-3}$ M and immersed in the electrodes: platinum (working), calomel (reference) and an auxiliary (platinum wire). Measurements were carried out in DMSO and before registering CV curves the solution was deoxidized by using argon. Tetrabuthylammonium perchlorate was used as an electrolyte core.

Chemical procedures

A stock solution of each complex was prepared as water solution (the same procedures for potentiometry and spectrophotometry). Aqueous solutions were prepared just before taking the spectra by adding equal volumes of stock solution and appropriate buffer and placed in UV-Vis cells. After this, the spectra were recorded between 300 and 650 nm at pH range from 2.05 to 13.56. The absorbance data in the same range wavelength were used for calculations of values of pK constants. The absorbance readings were then entered on to a computer spreadsheet that solved for the pK's and absorptivity values of 1. Each absorbance reading is an average value of three measurements at a given pH value. The absorbance of the samples was measured at 25 °C.

Calculations

The pK_1 and pK_2 values obtained from spectrophotometry and potentiometry hybride method were computed by using an Origin 8.5 program, based on absorbance variations at a selected wavelength and by using a nonlinear least squares method according to the Eq. (1)[20]:

$$A = \frac{A_{[BH_2^{3+}]} + A_{[BH^{2+}]} \cdot 10^{(pH - pK_1)}}{10^{(pH - pK_1)} + 1} + \frac{A_{[BH^{2+}]} + A_{[B^{+}]} \cdot 10^{(pH - pK_2)}}{10^{(pH - pK_2)} + 1} \quad (1)$$

The values of dissociation constants of complexes of Co(III) were determined from potentiometric measurements using a CVEQUID computer program by Liwo and Kostrowicki[20-24]. The program is based on an algorithm that matches the assumed equilibrium model to measurement data, to fit the data obtained from the program as precise as possible with the data obtained from experiments. For this purpose, using the methods of Gauss-Newton-Marquart, the iterative method used to solve nonlinear problems. It allows you to determine the equilibrium constant values regardless of their degree of dependence.

Microbiological procedures

Determination of antimicrobial activity of coordination compounds studied was performed *in vitro* on selected strains of bacteria: *Enterococcushirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* 8739, *Proteus vulgaris* 4635, *Pseudomonas aeruginosa* 9077 and fungus, *Candida albicans* ATCC 10231 using a quantitative method[25]. Control strains are derived from the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wroclaw, Poland.

The minimal inhibitory concentration (MIC) and the minimal bactericidal and fungicidal concentration (MBC)

Into a sterile 96-well microplates were introduced 100 µl BHI broth (for *Enterococcus hirae*), 100 µl Muller-Hinton broth (for other bacteria) or 100 µl Sabouraud broth (for *Candida albicans*). Dilutions of tested compounds were performed in exponentially[26]. For the prepared dilutions of compounds were added 100 µl of night bouillon cultures of bacteria or fungi with a density of 10^5 CFU/ml (colony forming units). After 24 hours of incubation at 35-37 °C for bacteria and 48 h at 25 °C for fungi, microbial growth was visually observed and the values of MIC were determined. The MIC (minimal inhibitory concentration) is taken as the lowest antimicrobial substance concentration at which observable growth is inhibited. To determine the values of MBC, 100 µl of appropriate dilutions of samples without visible growth were taken from each tube and spread on agar plates. Mueller-Hinton agar plates were incubated for 24 hours at 35-37 °C for the bacterial strains and Sabouraud agar for 48 h at 25 °C for *Candida albicans* and the values of MBC were determined[27]. MBC was defined as the lowest drug concentration at which 99,9% of the inoculums was killed. All the tests were repeated up to three times.

Disc diffusion method

Discs measuring 6.25 mm in diameter punched from Whatmanno. 1 filter paper was used[28]. Discs were sterilized in capped bottles by dry heat at 140 °C for one hour. The fresh solutions of the compounds were prepared with different concentrations using sterile distilled water and added to each bottles with discs in appropriate volume, that each disc contained approximately 0.01 ml of solution. Overnight bacterial and fungal cultures were diluted with Mueller-Hinton and Sabouraud broth respectively to the density of 10^6 CFU/ml. The discs of each concentrations were placed on the inoculated appropriate nutrient agar plates and incubated at 37 °C for 24 h (bacteria) and 25 °C for 72 h (fungi). Cefoperazon, gentamycin and chloramphenicol for bacteria and nystatin for funges were used as standards drugs. In each case triplicate tests were performed. After incubation the antimicrobial inhibition zone values (mm) for the testing compounds and standards were measured.

RESULTS AND DISCUSSION

The four synthesized complexes (Fig. 1) have been studied by using potentiometry and electron spectroscopy to take them fully characterized. These compounds were tested microbiologically in the next step of the study.

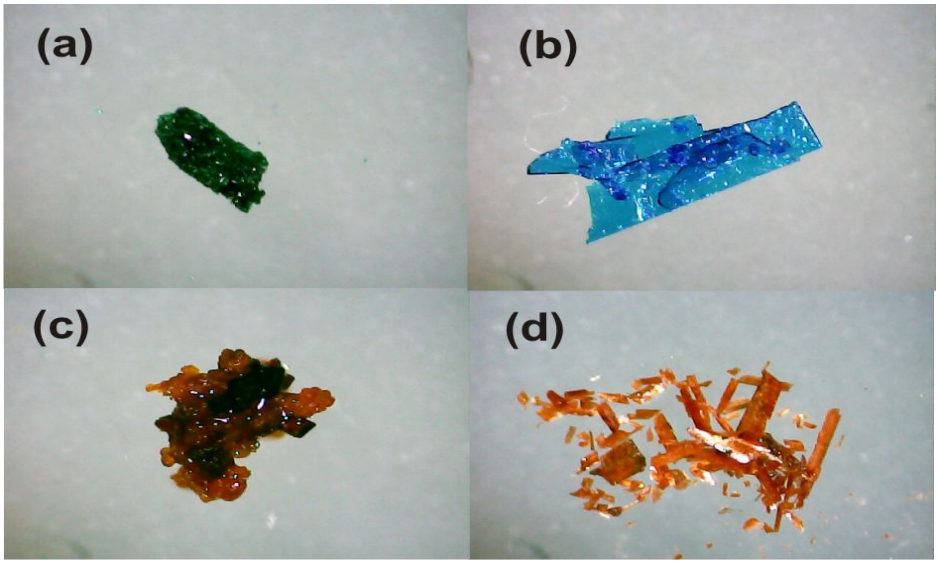

Fig. 1: Microscopic images of compounds studied: *trans*-$[Co(en)_2Cl_2]Cl$ (a), *trans*-$[Co(dap)_2Cl_2]Cl$ (b), *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ (c), *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ (d), twenty times zoom

Spectrophotometric study

Overlap plays an important role in the investigation of two-step acid-base equilibrium systems. That is, such systems are likely to be characterized by certain pH regions within which it is necessary to take into account the concentrations of all species. The UV-Vis spectrum of *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$, made in the acidic environment, has two absorption maxima. One maximum in the 483 nm (λ_1), the second in the 342 nm(λ_2). The pH increases during the titration with sodium hydroxide solution, which affects the growth of the intensity λ_1 and λ_2 absorption bands. This leads to the absorption maximum with hipsochromic shifts λ_1 of about 12 nm and the disappearance of the band λ_2. Fig. 2 shows the titration spectra, which intersect in complicated ways. No isosbestic points (or even quasi-isosbestic points) are observed, so a determination of rank is impossible on the basis of spectra alone. Each spectrum is recorded at a specific pH, and it is easy to ascertain at what wavelengths the absorbance varies most.

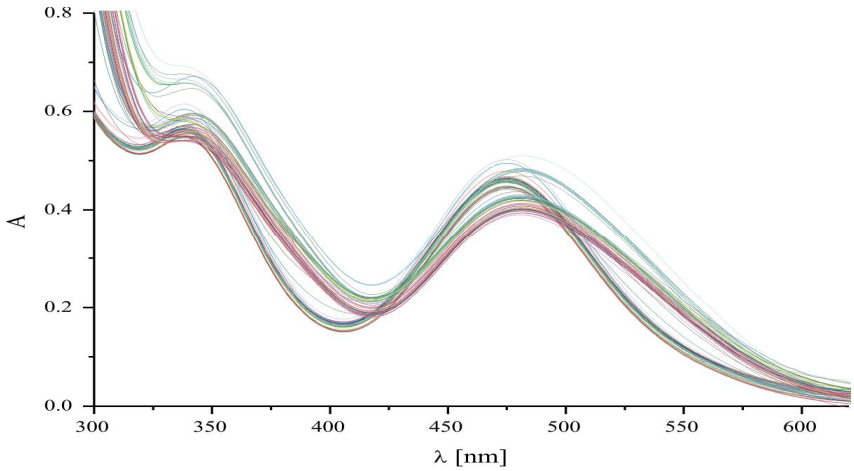

Fig. 2: Spectrophotometric titration curves for *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ (0.025 M in 0.075M HCl) by using 0.494 M aqueous solution of NaOH (25 °C)

The absorbance at 474 nmas dependence of absorbance at 416 nmwas plottedto determine the exact number of equilibria. The A-diagram obtained is shown in Fig. 3. Two straight sections are visible, which indicates the presence of two equilibria of the system studied.

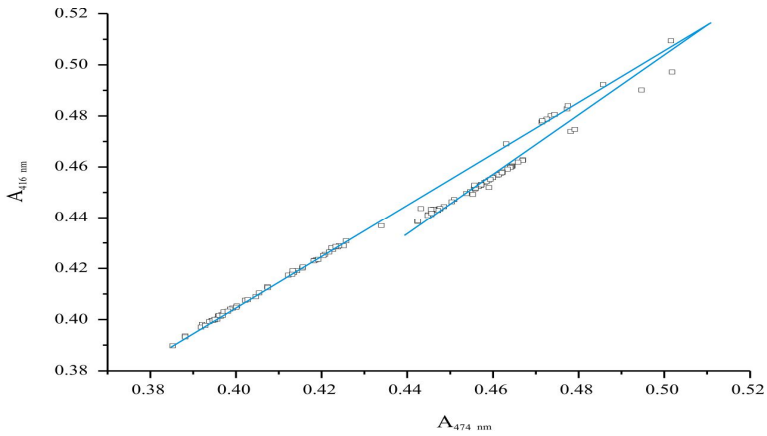

Fig. 3: The plot of A-diagram for trans-$[Co(en)_2(H_2O)_2](ClO_4)_3$

The values of deprotonation constants were calculated using the equation of Henderson-Hasselbalch. The value of pK_1 was calculated from the dependence of absorbance at different wavelengths. Fig. 4 represents the results of calculations for *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ as function of absorbance at 528 nm *vs.* pH.

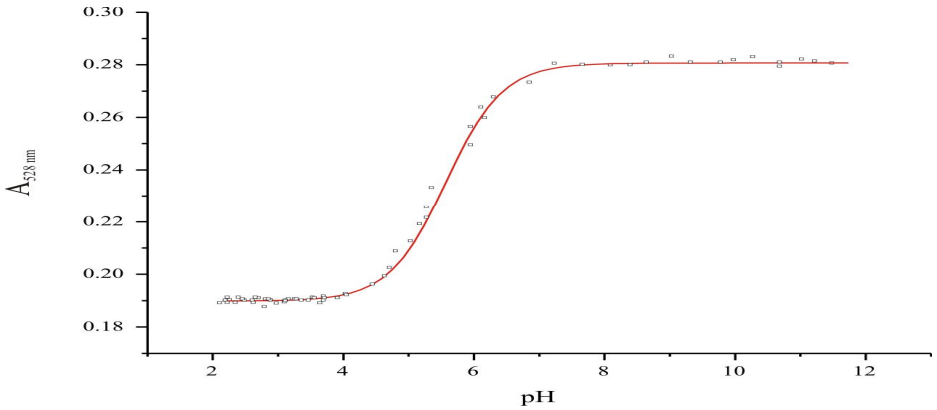

Fig. 4: The dependence of absorbance at 528 nm vs. pH and the curve-fitting of the spectrophotometric titration calculations during titration of trans-$[Co(en)_2(H_2O)_2](ClO_4)_3$ (0.025 M in 0.075 M HCl) by using solution of 0.494 M NaOH

The values of the second acidic dissociation constant were calculated from the dependence of absorbance at 474 nm as a function of pH (Fig. 5).

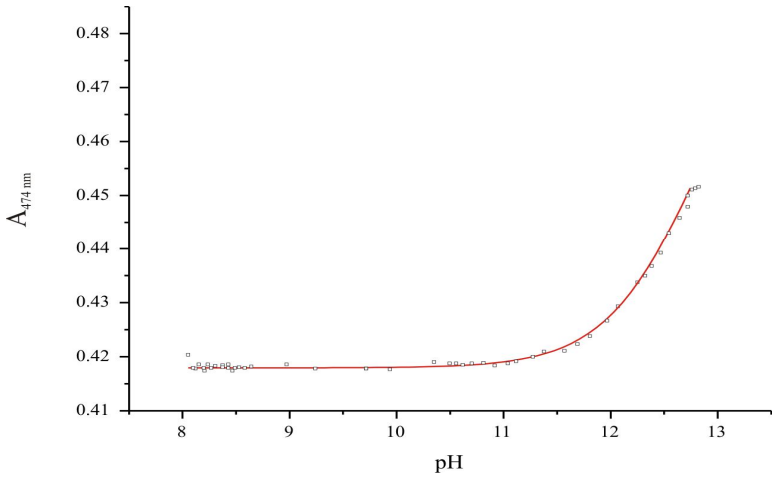

Fig. 5: The dependence of absorbance at 474 nm vs. pH and the curve-fitting of the spectrophotometric titration calculations during titration of trans-$[Co(en)_2(H_2O)_2](ClO_4)_3$ (0.025 M in 0.075MHCl) by 0.494 M NaOH as titrant

The spectrum of *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ in an acidic solution has two absorption maxima. One maximum in 503 nm, the second in 349 nm. The pH increasing during the titration with sodium hydroxide solution (Fig. 6), affects the growth of the intensity spectra obtained consecutively. The absorbance maximum in the shorter wave disappears.

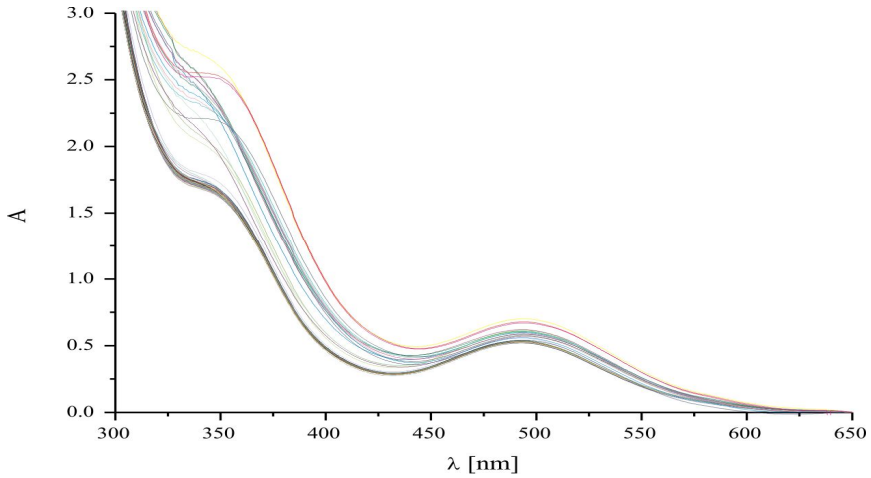

Fig. 6: Spectrophotometric titration curves for trans-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ (0.05 M in 0.15 MHCl) by using 1.02 M aqueous solution of NaOH (25 °C)

Fig. 6 presents the spectrophotometric titration spectra, which intersect in complicated ways too. No isosbestic points (or even quasi-isosbestic points) are observed, so a determination of rank is impossible on the basis of spectra alone. The A-diagram was plotted to determine the exact number of equilibria occurring in this system (Fig. 7), with the same result like for complex of Co(III) with *en*. Two protolytic equilibria of the system studied were observed.

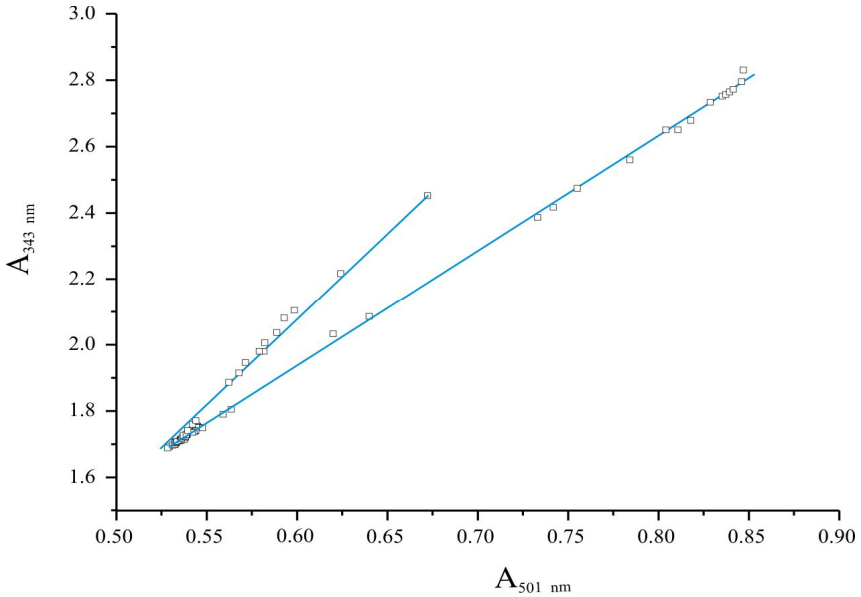

Fig. 7: The plot of A-diagram for trans-$[Co(dap)_2(H_2O)_2](ClO_4)_3$

The values of deprotonation constants (pK_1 and pK_2) were calculated using the Henderson-Hasselbalch's equation. Fig. 8 presents the results of measurements and calculations for pK_1 (492 nm) and Fig. 9 shows the results for pK_2 (468 nm).

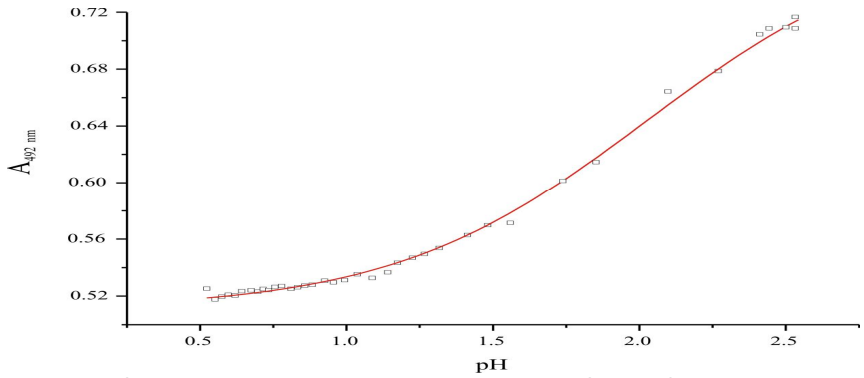

Fig. 8: The dependence of absorbance at 492 nm *vs.* pH and the curve-fitting of the spectrophotometric titration calculations during titration of *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ (0.05 M in 0.15MHCl) by 1.02 M NaOH as titrant

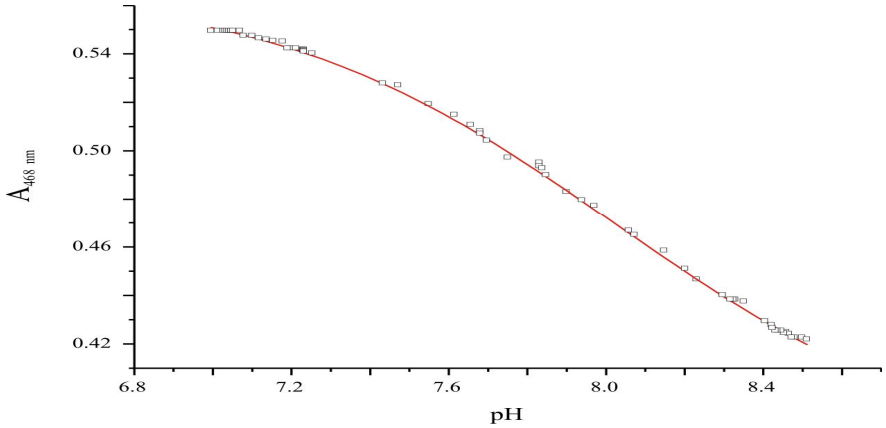

Fig. 9: The dependence of absorbance at 468 nm *vs.*pH and the curve-fitting of the spectrophotometric titration calculations during titration of *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$(c = 0.05 M in 0.15 MHCl) by 1.02 M NaOH as titrant

Potentiometric study

In this study, a reverse titration method was used. On the basis of potentiometric and spectrophotometric data from titration curves for the complexes of type *trans*-$[Co(N,N)_2(H_2O)_2](ClO_4)_3$ are shown in Figs 10 (where *N*,*N*=en) and 11 (where *N*,*N*=dap). Following acid-base equilibrium model was proposed:

$$[Co(N,N)_2(H_2O)_2]^{3+} + OH^- \rightleftarrows [Co(N,N)_2(H_2O)(OH)]^{2+} + H_2O \quad (2)$$

$$[Co(N,N)_2(H_2O)(OH)]^{2+} + OH^- \rightleftarrows [Co(N,N)_2(OH)_2]^{+} + H_2O \quad (3)$$

The model proposed was used to prepare the stoichiometric matrix and to determine the values of pK. Figs 10 and 12 present the potentiometric titrations curves of the complexes of type *trans*-$[Co(N,N)_2(H_2O)_2](ClO_4)_3$, together with the fitting line obtained from calculations. Additionally, Figs 10 and 12 show the reaction of neutralization the pure strong acid with a strong base, which were used during the measurements.

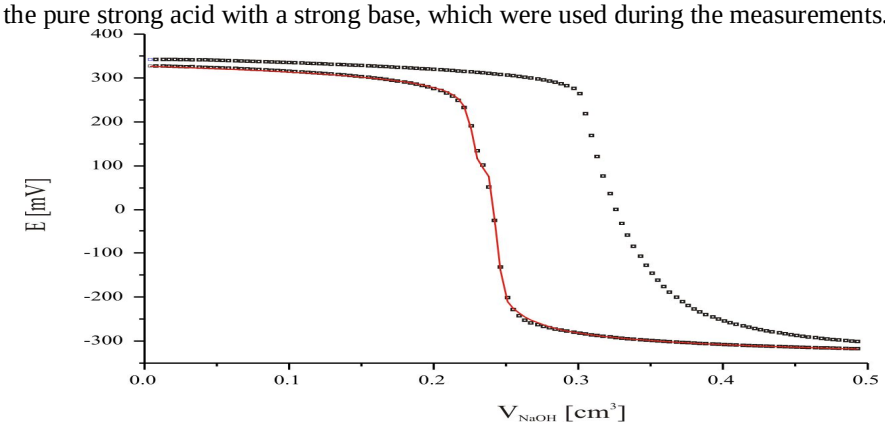

Fig. 10: Potentiometric titration curves of *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$(0.025 M in 0.075 M HCl) by 0.494 M NaOH (squares with solid line obtained from CVEQUID) and the second of pure HCl by NaOH (points without fitting), 25 °C

The curves of concentration for every form of complex *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ are plotted and shown in Fig. 11. Points of intersection of these curves correspond to the calculated value of the constantspK's.

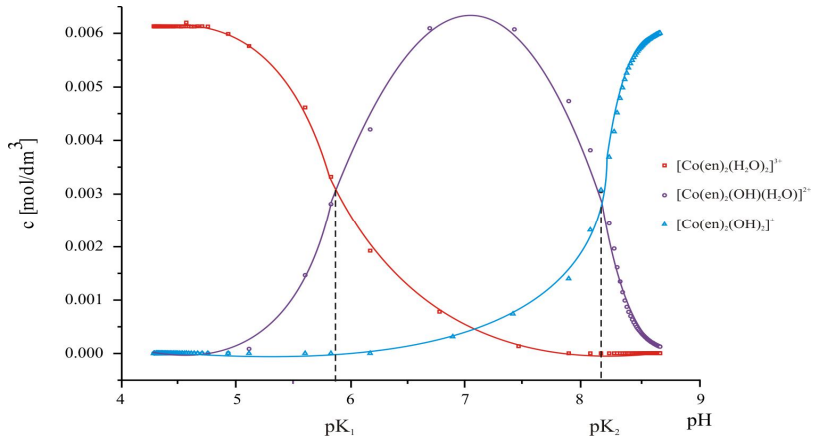

Fig. 11: Concentration diagrams of *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ obtained from results of potentiometric titration

During the titrationan acidic solution of *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ by using sodium hydroxide, the green precipitate of cobalt(III) hydroxide was formed. These experimental points were not taken into account during calculations (rejected value of potentialin Fig. 12).

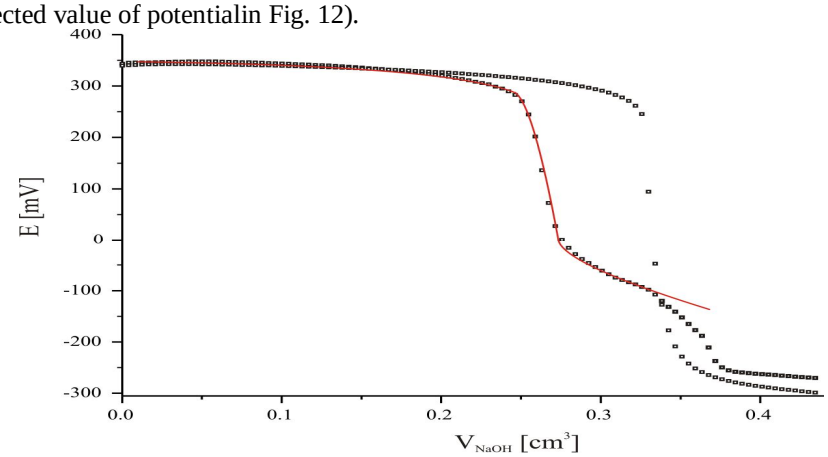

Fig. 12: Potentiometric titration curves of *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$(0.05 M in 0.101 M HCl) by 0.152 M NaOH (squares with solid line obtained from CVEQUID) and the second of pure HCl by NaOH (points without fitting), 25 °C

The concentration diagrams for ionic form of Co(III) complex with 1,3-diaminopropane were plotted on the basis of the calculation (Fig. 13). The curves are intersecting at points corresponding to the values of the consecutive deprotonation constants pK's obtained from calculations.

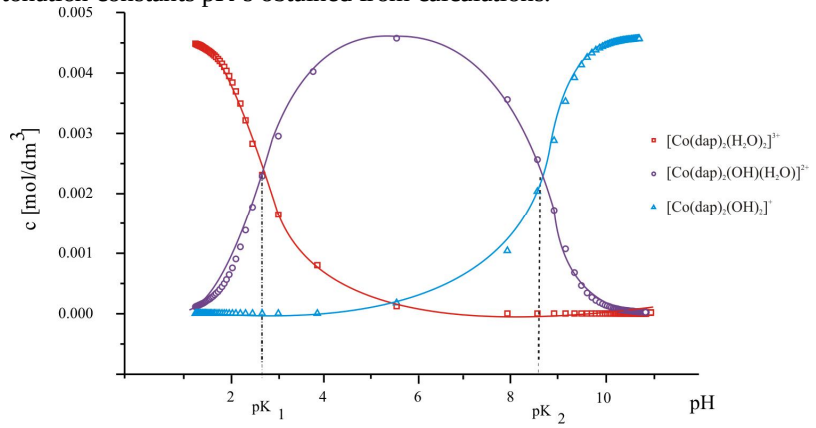

Fig. 13: Concentration diagrams of *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ obtained from results of potentiometric titration

On the basis of potentiometric and spectrophotometric titration measurements, the number of protolytic equlibria has been determined and the values of dissociation constants for two coordination compounds of Co(III) have been calculated. The data obtained are involved in Table 1, which shows results obtained using two independent methods.The comparable values of constants were obtained. Additional titration (for *N*,*N*=dap) was carried out also using potassium hydroxide as a titrant to eliminate the error of sodium. The results of titration allow the determination only of the pK_1 (the calculation results of titrations with KOH were summarized in the last row of the Table 1).

Table 1: Acidity constants of the Co(III) complexes, obtained by the spectroscopic and potentiometric titration methods

Complex	Spectrophotometric titration		Potentiometric titration	
	pK_1	pK_2	pK_1	pK_2
trans-$[Co(en)_2(H_2O)_2](ClO_4)_3$	5.74 (± 0.11)	8.19 (± 0.35)	5.98 (± 0.23)	8.08 (± 0.34)
trans-$[Co(dap)_2(H_2O)_2](ClO_4)_3$	2.42 (± 0.14)	8.02 (± 0.17)	2.65 (± 0.45)	8.51 (± 0.21)
	2.57 (± 0.03)*	not observed*	2.47 (± 0.21)*	not observed*

*results obtained from titration with KOH as a titrant

Moreover, the values of the reduction potentials were determined by using cyclic voltamperometry technique for two selected coordination compounds. The results showed that the reduction potential for *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ is -0.997 V and for *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ is -0.611 V. Co(III) complex containing 1,3-diaminopropane is reduced much easier to Co(II) complex, which is shown the number of peaks obtained on cathode, anode and the occurrence of peak values of their potentials.

Microbiological measurements

The synthesized Co(III) complexes with diamino chelate ligands were tested against control microbial strains: Gram-positive bacteria *Enterococcus hirae* ATCC 10541, *Staphylococcus aureus* ATCC 6538, Gram-negative bacteria *Escherichia coli* ATCC 8739, *Proteus vulgaris* 4635, *Pseudomonas aeruginosa* ATCC 9077 and fungus *Candida albicans* ATCC 10231. Also $CoCl_2 \cdot 6H_2O$ salt and diamino chelate ligands separately were investigated. Investigations of *in vitro* antimicrobial activity of compounds included experiments of MIC (minimal inhibitory concentration) using microbroth dilution method and MBC (minimal bactericidal or fungicidal concentration) determination. The MIC and MBC of the tested compounds are collected in Table 1 and Table 2, respectively. All of types of Co(III) complexes like *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$, *trans*-$[Co(dap)_2Cl_2]Cl$, *trans*-$[Co(en)_2Cl_2]Cl$ showed similar activity with tested ligands. Comparison of antibacterial activities as values of MIC and MBC obtained for $CoCl_2 \cdot 6H_2O$ salt and complexes revealed that complexes *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$, *trans*-$[Co(dap)_2Cl_2]Cl$ and *trans*-$[Co(en)_2Cl_2]Cl$ presented similar or smaller activity to tested Co(II) salt. The strongest effect of compounds {except complex *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$} was observed against *Candida albicans.* Three above complexes of Co(III) showed approximately 4-10 fold greater activity than ligands, and similar activity to $CoCl_2 \cdot 6H_2O$. Complex *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$ appeared to have very poor effectiveness against all tested species.

Table 2: The results of the minimal inhibitory concentration studies (MIC)

No.	Compound	MIC (mg/ml)					
		Enterococcus hirae	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Candida albicans*
1	$CoCl_2 \cdot 6H_2O$	1	0.5	1	0.5	1	0.125
2	en	2	1	1	1	1	2
3	dap	2	0.5	1	1	1	2
4	*trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$	4	2	4	2	4	0.5
5	*trans*-$[Co(dap)_2Cl_2]Cl$	2	1	1	1	2	0.13
6	*trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$	>32	16	32	16	>32	>32
7	*trans*-$[Co(en)_2Cl_2]Cl$	2	2	2	1	2	0.25

Table 3: The results of the minimal bactericidal concentration method (MBC)

No.	Compound	MBC (mg/ml)					
		Enterococcus hirae	*Staphylococcus aureus*	*Escherichia coli*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Candida albicans*
1	$CoCl_2 \cdot 6H_2O$	1	1	1	1	2	0.125
2	en	4	2	1	2	2	4
3	dap	2	2	1	1	1	4
4	*trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$	4	4	4	4	4	1
5	*trans*-$[Co(dap)_2Cl_2]Cl$	2	4	1	1	2	0.25
6	*trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$	>32	>32	32	>32	>32	>32
7	*trans*-$[Co(en)_2Cl_2]Cl$	4	2	4	2	2	0.5

CONCLUSION

Differences in the values of molar absorption coefficient and the positions of absorption bands in different pH indicate the presence of various protolytic forms in aqueous solution and enable to determine the values of deprotonation constants.

Based on potentiometric and spectrophotometric measurements, it was found that the investigated complexes undergo two-step reaction of dissociation in water. In the reversed-titration method each coordination compound had to be dissolved in a strong acid to show the maximum protonated state or to expand the scale of pH during titration. The values of equilibrium constants of these reactions indicate that the investigated complexes exhibit weak acidic properties. Moreover, the values of acidity constants determined by two independent methods are compatible.

Analysis of the values of deprotonation constantsfor coordination compounds of Co(III) with diamine ligands, showed that the pK_1 and $pK_2$for the complex with 1,3-diaminopropane are much lower than for complex with ethylenediamine. This means that the *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ exhibits stronger acidic properties than *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$. It can be concluded that the shorter carbon chain in diamine ligandin complex the less their acidic properties.

Based on the values of the reduction potentials it can be concluded that the compound *trans*-$[Co(dap)_2(H_2O)_2](ClO_4)_3$ is a stronger oxidant than *trans*-$[Co(en)_2(H_2O)_2](ClO_4)_3$. Co(III) complex containing 1,3-diaminopropane is reduced much easier to Co(II) complex. The increase in carbon chain length of the diamine ligand affects its powerful oxidizing properties.

All biological tests with coordination compounds of Co(III) showed antibacterial and antifungal activity. Relatives compounds containing in the coordination sphere of the chloride anions were characterized by approximately twice lower minimum concentration inhibiting growth of bacteria and fungi than their counter parts containing aqualigands. The same dependence was found for the lowest concentration at which 99.9% of germs were killed. Considering the compounds as homologues, such as pair *trans*-$[Co(en)_2Cl_2]Cl$ - *trans*-$[Co(dap)_2Cl_2]Cl$, it can be concluded that the use of about twice smaller amount of Co(III) complex with dap causes both inhibition of microbial growth and death. Compound with the highest antifungal activity among the tested turned out to be *trans*-$[Co(dap)_2Cl_2]Cl$. It inhibited most strongly the growth of fungi of the genus *Candida*, bacterial growth of Gram-negative bacilli *Escherichia coli* and colon freak vulgaris *Proteus vulgaris*. Comparison of results for *trans*-$[Co(en)_2Cl_2]Cl$ and *trans*-$[Co(dap)_2Cl_2]Cl$ suggests the dependence: the longer carbon chain of the organic *N,N*-donor ligand in the Co(III) complex, the compound is more effective bactericidal and fungicidal. Moreover, the results concerning antimicrobial activities of testing complexes studied by disc diffusion method revealed medium sensitivity of *trans*-$[Co(dap)_2Cl_2]Cl$ against *Proteus vulgaris* and *Candida albicans* (results not shown).

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