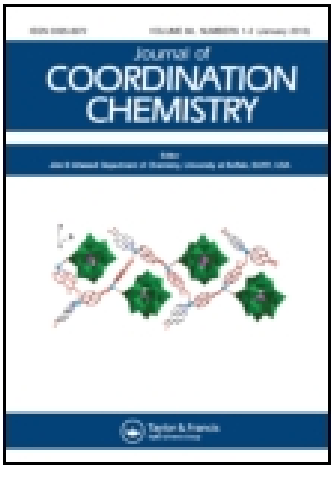

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Coordination mode and reactivity of nickel(II) with vitamin B_6

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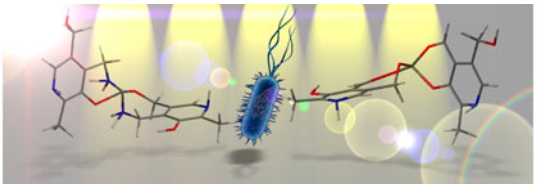

This contribution presents a selection of results obtained using spectrophotometric and potentiometric titrations. For several reasons, the investigated equilibria present particular challenges to traditional analysis techniques. Equilibrium constants and UV–vis absorption spectra for different ligands in the complexation process of Ni(II) with pyridoxamine (pm), pyridoxal (pl) and pyridoxine are reported. The gradual and cumulative stability constants occurring in aqueous solution are presented for all complexes studied. Additionally, crystal-field parameters were calculated for two nickel(II) complexes synthesized, $[Ni(pm)_2]Cl_2$ and $[Ni(pl)_2]Cl_2$, respectively. The minimum inhibitory concentration and minimal bactericidal/fungicidal concentration values for Ni(II) complexes studied were obtained at 25 °C for 24–48 h. The activity data show that the complexes are more potent antimicrobials than the parent ligands.

Keywords: Ni(II) complexes; Vitamin B_6; Spectroscopy; Potentiometry; Antimicrobial activity

1. Introduction

The d^8 Ni(II) ion coordinates with chelate ligands due to the vacancy in its e_g d-orbital subshell. Nickel(II) complexes with N- and O-donor ligands are stable and, in general, inert to substitution of other ligands [1, 2]. Complexes of Ni(II) can be in various stereochemical forms and nickel(II) is one of the most spectroscopically studied metal ions. Due to physicochemical properties and biological activities [3, 4], Ni(II) complexes are interesting to study.

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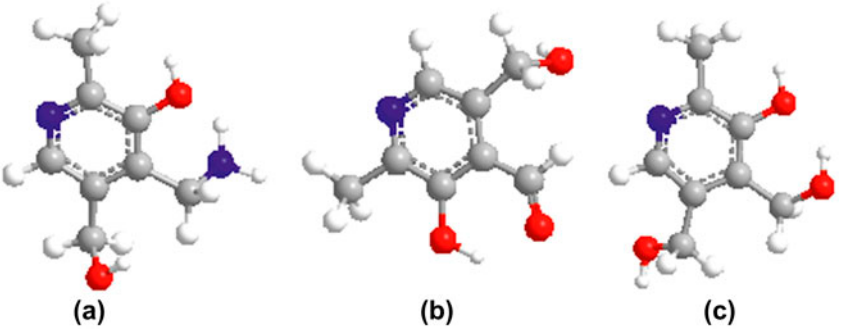

Figure 1. The three natural forms of vitamin B_6: (a) pyridoxamine, (b) pyridoxal, and (c) pyridoxine.

Pyridoxal (pl), pyridoxine (pn), and pyridoxamine (pm) are naturally occurring free forms of vitamin B_6 (figure 1). Vitamin B_6 is a universal coenzyme involved in many aspects of metabolism, such as transamination, decarboxylation, and dehydration [5–7]. Complexes of vitamin B_6 with various transition metal ions have been reported [8]. The mechanism of coordination of these forms of vitamin B_6 with Ni(II) is important as a model to understand the biosynthetic role of vitamin B_6 *in vivo* as well as to develop novel bioactive compounds.

Spectrophotometric and potentiometric titrations are very useful methods in research to determine stability constants in solution. Based on spectrophotometric measurements, it is possible to show a number of equilibria existing in a particular solution and provide complete information about species formed during the titration. In general, the potentiometric method presents information about proton migration. Despite disadvantages of potentiometry, it is advisable that at least two methods be used together for a detailed description of metal ion–ligand interactions.

The complexation equilibrium of vitamin B_6 with Ni(II) has been reported for only one of these spectrophotometric or potentiometric techniques. Most previous research was carried out for only binary complexes [9]. Despite the similarity of measurement conditions [10, 11], the values of formation constants for these complexes were different. Due to these differences, our research team has decided to determinate stability constants of complexes of vitamin B_6 with nickel(II) using the two methods.

This article reports results of our spectrophotometric and potentiometric studies on coordination compound formation between three forms of vitamin B_6 and Ni(II) ion. The tetrahedral coordination compounds $[Ni(pm)_2]Cl_2$ and $[Ni(pl)_2]Cl_2$ were synthesized and studied to check the strength of metal–ligand bonds. Moreover, the complexes studied were examined in microbiological tests to get antimicrobial activities.

2. Experimental

2.1. *Materials and solutions*

All chemicals used were of analytical grade and purchased from Sigma-Aldrich Co. Ltd. All solutions were prepared with Hydrolab-Reference-purified water. Solutions of the compound studied and metal cation were prepared directly before measurements.

The solutions used in potentiometric studies were as follows. A stock solution of nickel (II) chloride containing 1 mM L^{-1} was made fresh right before using and dissolving in standard stock solution of the selected ligand. The standard stock solutions of pm, pl, and pn with 2 mM L^{-1} were prepared daily by dissolving in 10 mL of 7 mM L^{-1} HCl in volumetric flasks. Stock solutions of NaOH titrant contained 36 mM L^{-1}.

The titration with the spectroscopic methods was performed by adding to solution of ligand in a cell (about 5×10^{-5} M L^{-1} for pm, 4×10^{-5} M L^{-1} for pl and pn) a solution of nickel(II) chloride (concentration was 15–30 times higher than that of ligand).

2.2. *Syntheses of the complexes*

2.2.1. $[Ni(pm)_2]Cl_2$ and $[Ni(pl)_2]Cl_2$. Nickel(II) chloride hexahydrate (1.42 g) and lithium chloride (1.49 g) were dissolved in 30 mL of dimethylformamide. The mixture was stirred at 50 °C for about 20 min, until complete dissolution of the reagents. To this dark blue solution, 3 g of pyridoxamine hydrochloride (2.44 g of pyridoxal hydrochloride) was added and heated again. A change of color from blue to yellow for pm (light brown for pl) was observed when 10 mL of ethanol was added to the solution after 30 min. The solution was cooled on ice and allowed to crystallize. Thin crystals of $[Ni(pm)_2]Cl_2$ or $[Ni(pl)_2]Cl_2$ were then collected after about a month. The filtrate was then left standing in the refrigerator for several days and a small amount of ethanol was added to obtain a yellow solid. The re-crystallization of this solid from warm water by cooling on ice with a new portion of ethanol resulted in the final complexes studied. The syntheses were repeated six times. Elemental analyses for all complexes studied (C, H, and N) were within 0.3% of the calculated values. Anal. Calcd for $NiC_{16}H_{24}N_4O_4Cl_2$(%): C, 41.24; H, 5.19; N, 12.02. Found: C, 41.28; H, 5.20; N, 11.99, and those for $NiC_{16}H_{18}N_2O_6Cl_2$(%): C, 41.42; H, 3.91; N, 6.04; Found: C, 41.38; H, 4.01; N, 5.91. Selected IR bands (cm^{-1}) $[Ni(pm)_2]Cl_2$ and $[Ni(pl)_2]Cl_2$ are: 3011 (ν(N–H)), 3323 (ν(O–H)), 1356 (ν(C–N)), 1630 (C=C), and 2661 (ν(N–H)), 3340 (ν(O–H)), 1322 (ν(C–N)), 1641 (C=C), respectively. The light yellow crystals of $[Ni(pm)_2]Cl_2$ and $[Ni(pl)_2]Cl_2$ were obtained in 54% and 52% yields, respectively, and were soluble in water and DMSO.

2.3. *Procedures and apparatus*

The stability constants of vitamin B_6 and Ni^{2+} complexes in the presence of 0.1 M L^{-1} KNO_3 were determined at 25 °C using pH-metric titrations in the pH range 2–12, 4–10.7 and 4–12 for the Ni(II)-pm, Ni(II)-pl, and Ni(II)-pn, respectively. Changes in pH were monitored using a combined glass/calomel electrode (Mettler Toledo) calibrated in pH standard buffers [12] on a CerkoLab automatic titrator using 2 mL samples. The titration systems with 1 : 1 and 1 : 2 nickel-to-ligand molar ratios consisted of a titration cell, a magnetic stirrer and an automatic titrator with Hamilton's syringe (0.5 mL). The relations between activity and concentration were calculated daily by titration of HNO_3 [13]. The data obtained were then analyzed using the CVEQUID program [14], based on an algorithm that matches the assumed equilibria model to measurement data, to fit the calculated data as precisely as possible with the experimental one. For this purpose, the iterative method of Gauss–Newton–Marquardt is used to solve nonlinear problems [15, 16]. It allows determination of the equilibrium constant values regardless of their degree of dependence. Standard deviations reported were calculated on the assumption of error randomness. To obtain the

complex stabilities, it is necessary to take into account the values of deprotonation constants for each vitamin species. The preliminary potentiometric studies with vitamin B_6 included the determination of pKa values for the various ligand species [11].

Absorption spectra were recorded with a Perkin Elmer Lambda 650 spectrophotometer from 220 to 500 nm. All titration solutions were maintained at a constant temperature of 25 °C by circulating thermostated water through spectrophotometer cuvette holders and, for external titrations, through the external titration vessel. The stability constants of the complexes were determined using the EQUID software [15], by minimization of the differences between the theoretical model and the experimental data.

Molar conductances of the complexes (10^{-3} M L^{-1}) in water were measured at room temperature using an ELMETRON CC-401 conductivity meter.

2.4. *Determination of minimal inhibitory concentration and minimal bactericidal/fungicidal concentration*

Determination of antimicrobial activities of tested compounds was performed *in vitro* on selected bacterial strains in appropriate broths: *Enterococcus hirae* ATCC 10541 (Brain Heart Infusion, BHI), *Staphylococcus aureus* ATCC 6538, *Escherichia coli* 8739, *Proteus vulgaris* 4635, *Pseudomonas aeruginosa* 9077 (Mueller-Hinton, MH) and yeasts, and *Candida albicans* ATCC 10231 (Sabouraud), using standard microbroth dilution assay. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined in three independent measurements [17, 18]. The tested compounds were diluted with proper medium in geometric progression in 96-well flat-bottomed microculture plates, so that the final volume was 100 μL. Each well was inoculated with 100 μL of overnight bacteria cultures containing 10^5 cells mL^{-1} diluted with Mueller–Hinton or BHI broth, and yeast cultures containing 10^4 cells mL^{-1} diluted with Sabouraud broth. The plates were incubated at 35–37 °C for 18 h (bacteria) or 25 °C for 36 h (yeasts). After an incubation time, the growth of micro-organisms was examined to determine the MIC value, which was taken as a lowest concentration of tested complexes that inhibits visible growth of bacteria. In addition, 100 μL of suspension from each tube without growth was inoculated in proper agar plate to control bacterial viability. The MBC was defined as the lowest concentration at which antimicrobial compounds will kill a particular micro-organism in the medium after 24 h incubation [19].

3. Results and discussion

3.1. *Spectrophotometric titration results*

The overlap of consecutive shifts in the spectra plays an important role in investigation of multi-step complexation equilibrium systems. It is necessary to take into account the concentrations of all species formed.

During titration of pyridoxamine by titrant solution ($NiCl_2$ with pyridoxamine), a slight bathochromic shift to a longer wavelength occurs (figure 2). At the same time, an isosbestic point appears at 274 nm, which may indicate that an equilibrium of complexation occurs.

A-diagrams are plotted to demonstrate determination of a specific quantity of equilibria in solution studied. The A-diagram for Ni(II) and pyridoxamine complexation process (see figure 3) indicates one straight section, suggesting the presence in only one equilibrium of the system studied.

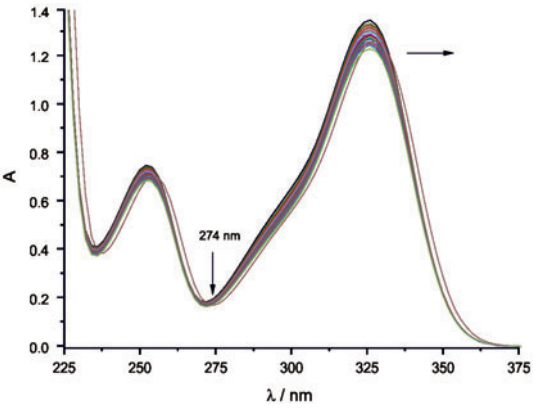

Figure 2. Spectrophotometric titration curves of pyridoxamine hydrochloride (0.05 mM L^{-1}) using a mixture of pyridoxamine at the same concentration and $NiCl_2$ (0.82 mM L^{-1}).

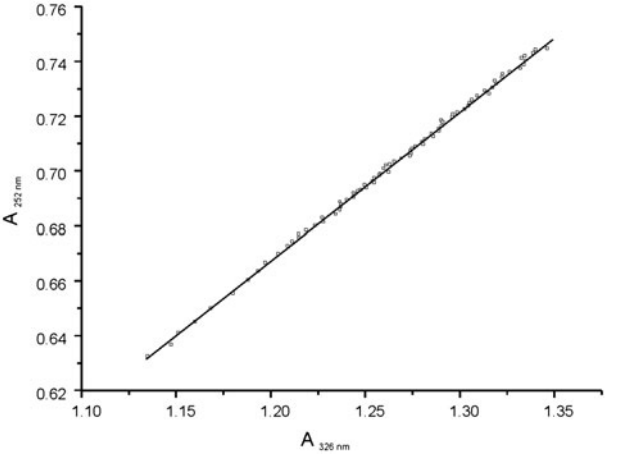

Figure 3. The plot of A-diagram for Ni(II) ion with pyridoxamine complexation process, major stoichiometry 1 : 1.

The results of spectrophotometric titrations for Ni(II)-pyridoxal system studied have been presented in figure 4. The presence of two isosbestic points can be observed at 266 and 304 nm. The intensities of absorption bands gradually increase; an indication of a hyperchromic effect. Changes in absorbance describing the reaction of nickel ion–pyridoxal are small as well as for other species of vitamin B_6 reacting with Ni(II).

To define the exact number of equilibria present in the studied system, the A-diagrams were analyzed. The absorbance at 290 nm as function of absorbance at 316 nm was plotted for Ni(II) with pyridoxal solution (see figure 5). Two straight sections are visible indicating the presence of two equilibria in the system studied.

An increase in absorption (or extinction) is observed at a particular wavelength of light by a solution due to structural changes. The most interesting changes in absorbance occur for nickel(II) with pyridoxine complex ion (figure 6). Two isosbestic points at 264 and

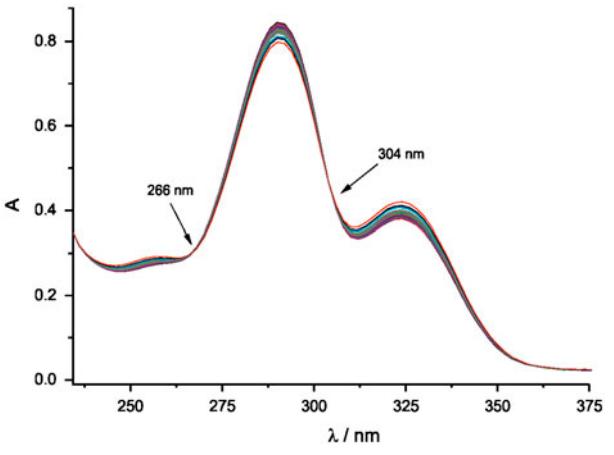

Figure 4. Spectrophotometric titration curves of pyridoxal hydrochloride (0.04 mM L^{-1}) using a mixture of pyridoxal (0.04 mM L^{-1}) and $NiCl_2$ (0.85 mM L^{-1}).

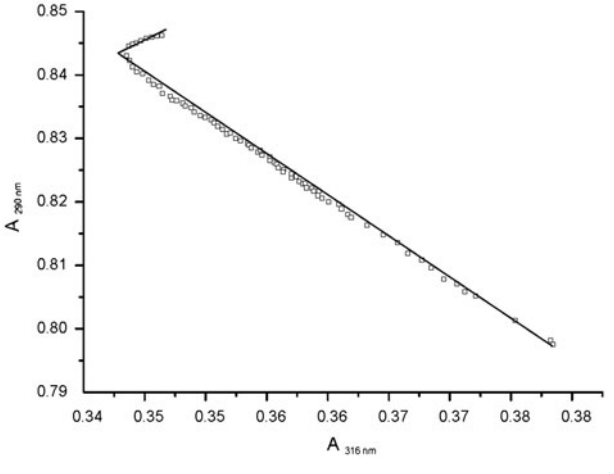

Figure 5. The plot of A-diagram for Ni(II) ion with pyridoxal complexation process, major stoichiometry 1 : 2.

305 nm, as well as a bathochromic shift, and hypochromic, and finally hyperchromic effects are present.

A-diagram for Ni(II) ion with pyridoxine complexation process (figure 7) indicates only one straight section. This suggests the presence of only one equilibrium in the system studied which is associated with the formation of complex.

3.2. *Potentiometric titration results*

Potentiometric methods do not require formation of a colored product. They are based on the potential or pH change measurements of the examined solution. In a potentiometric

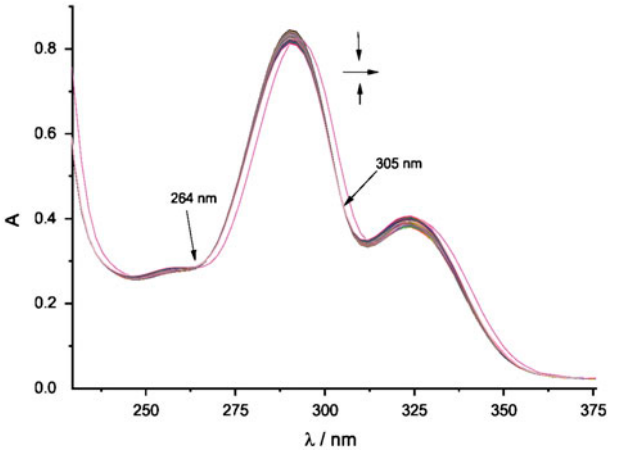

Figure 6. Spectrophotometric titration curves of pyridoxine hydrochloride (0.04 mM L^{-1}) using a mixture of pyridoxine (0.04 mM L^{-1}) and $NiCl_2$ (1.18 mM L^{-1}).

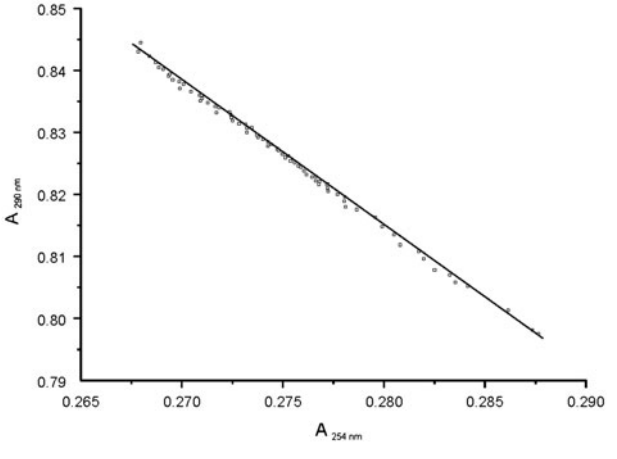

Figure 7. The plot of A-diagram for Ni(II) ion with pyridoxine complexation process, major stoichiometry 1 : 1.

titration, the protonated form of the ligand is used. The protonation is obtained by flooding the ligand with strong acid solution and adding a known amount of metal to the titration sample. At the beginning of the titration, the ligand has inactive donor sites. During titration of the acid–metal solution with a strong base, deprotonation of the ligand occurs (activation of donors) allowing the formation of the metal complex. Equations (1) and (2) describe qualitatively the above-mentioned process:

$$HL + OH^- \leftrightarrows L^- + H_2O \tag{1}$$

$$M^+ + L^- \leftrightarrows ML \tag{2}$$

The potentiometric measurements require maintaining a constant ionic strength to ensure that activity coefficients remain constant for all species during the experiment. In our case, the ionic strength was 0.012 M L^{-1}.

The titration curves obtained for $[Ni(pm)(OH_2)_2]Cl_2$ and $[Ni(pl)_2]Cl_2$ (figures 8 and 9, respectively) show three potential jumps and for $[Ni(pn)(OH_2)_2]Cl_2$ (figure 10) only two. Figures 8–10 introduce experimental data (black squares) and those fitted with the use of CVEQUID program (red solid line). Comparison of these data leads to the conclusion that there is a good correlation between experimental and calculated results for each of the complexes studied. Moreover, it can be also concluded that spectrophotometric and potentiometric methods are accurate, easy to apply, and suitable for stability constant determination of coordination compounds.

Three forms of Ni(II) complexes with vitamin B_6 were formed during the complexation process in aqueous solution, $[Ni(pm)(OH_2)_2]^{2+}$, $[Ni(pl)_2]^{2+}$, and $[Ni(pn)(OH_2)_2]^{2+}$, respectively. By taking into consideration the calculation in EQUID, some assignments can be made regarding the observed bands in the electronic spectra of the four-coordinate complexes in solution.

3.3. *Complexation of Ni(II) with vitamin B_6*

Based on experimental measurements, the values of gradual and cumulative stability constants for the three complexes were determined. Only one for $[Ni(pm)(OH_2)_2]Cl_2$, $[Ni(pn)(OH_2)_2]Cl_2$, and two for $[Ni(pl)_2]Cl_2$ formation constants can be obtained on the basis of the results of spectrophotometric and potentiometric titrations. The values of stability constants of systems studied are shown in table 1.

Results obtained by two independent experimental methods have been compared. The values of gradual and cumulative stability constants of the compounds studied are similar, which suggests that these values are correct. Difference in the values of stability constants was in case of $[Ni(pn)(OH_2)_2]^{2+}$, related to the fact that the interaction between pyridoxine and nickel(II) was very weak; that is why the results from potentiometry show greater error.

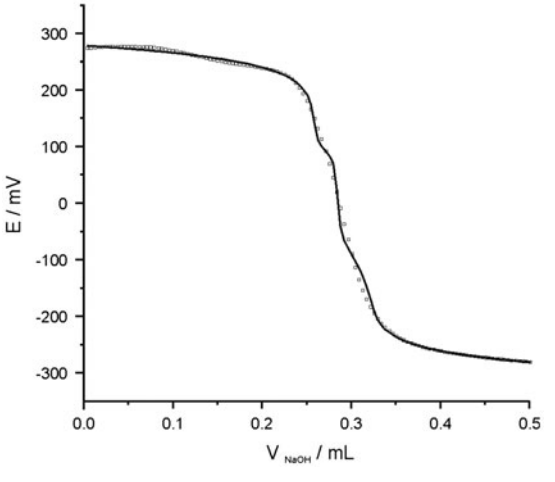

Figure 8. Potentiometric titration curve of a Ni(II) and pyridoxamine hydrochloride solution using NaOH (36 mM L^{-1}); experimental points and fitting line – the result of calculations, $R^2 = 0.997$.

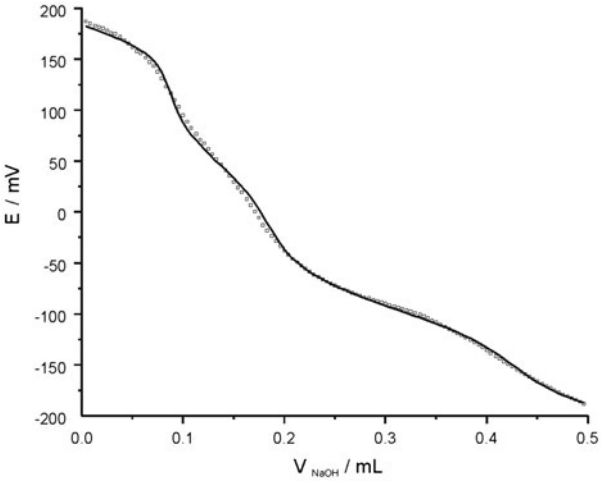

Figure 9. Potentiometric titration curve of a Ni(II) and pyridoxal hydrochloride solution using NaOH (36 mM L^{-1}); experimental points and fitting line – the result of calculations, $R^2 = 0.991$.

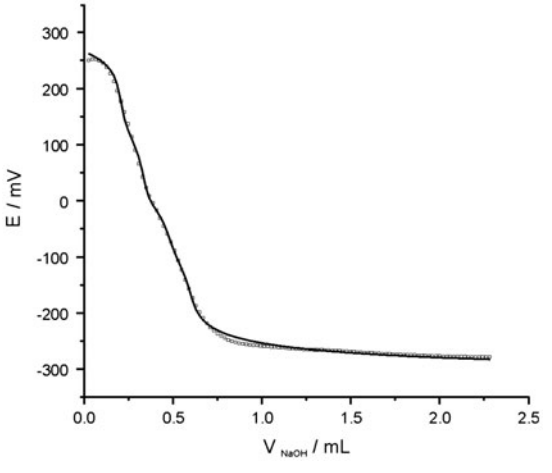

Figure 10. Potentiometric titration curve of a Ni(II) and pyridoxine hydrochloride solution using NaOH (36 mM L^{-1}); experimental points and fitting line – the result of calculations, $R^2 = 0.983$.

Table 1. Values of parameters describing the stability of Ni(II) complexes with vitamin B_6 formed in aqueous solution. All results were determined at $T = 298$ K.

		Potentiometric method			Spectrophotometric technique		
Complex cation	Molar ratio	log K_1	log K_2	log β	log K_1	log K_2	log β
$[Ni(pm)(OH_2)_2]^{2+}$	1 : 1	2.76 (±0.01)	–	2.76	2.75 (±0.02)	–	2.75
$[Ni(pl)_2]^{2+}$	1 : 2	3.83 (±0.12)	5.25 (±0.21)	9.08	3.86 (±0.10)	5.29 (±0.08)	9.15
$[Ni(pn)(OH_2)_2]^{2+}$	1 : 1	4.43 (±0.14)	–	4.43	2.68 (±0.05)	–	2.68

3.4. *Electronic spectra of complexes synthesized*

Absorption spectra of Ni(II) complexes with pyridoxamine, pyridoxine, and pyridoxal in water solution were studied. The ligand-field parameters are calculated with allowance for d^8 electronic configuration directly from the positions of the bands found in the spectrum.

Electronic spectra data of the complexes are given in table 2. The bands are assigned according to the Tanabe–Sugano diagram for tetrahedral d^8 configuration. The ground state of nickel(II) is $^3T_1(F)$. First excited triplet levels in order of increasing energy are $^3T_2$ and $^3T_1$ (P). In electronic absorption spectra of $[Ni(pm)_2]Cl_2$ (figure 11), appearance of two broad bands with intensities about 7000 and 10,600 cm^{-1} is observed. In the spectra of $[Ni(pl)_2]Cl_2$, three bands with intensities 7200, 9200, and 10,200 cm^{-1} are observed, respectively. The peak at 10,200–10,600 cm^{-1} is assigned to $^3T_1(F) \rightarrow {}^3A_2$. The compounds studied (figure 11) have a rather broad band at 7000–7200 cm^{-1}. But only a sharp band at 9200 cm^{-1} is observed in the $[Ni(pl)_2]Cl_2$ spectra. The relative intensity of this band suggests that the absorption at 9200 may be the highest component of the transition $^3T_1(F) \rightarrow {}^3T_2$.

Table 2. Electronic absorption spectra and ligand-field parameters (cm^{-1}) of Ni(II)–vitamin B_6 complexes.

Complex formula	Observed bands/cm^{-1}	ε [L M^{-1} cm^{-1}]	Assignments	v_2/v_1	B	β	Δ_0 = Dq
$[Ni(pm)_2]^{2+}$	7000	5760					
	10,600	9800		1.514	139.86	0.134	6098
			$^3T_1$ (F) → $^3T_2$				
			$^3T_1$ (F) → $^3T_1$ (P)				
$[Ni(pl)_2]^{2+}$	7200	10,750	$^3T_1(F) \rightarrow {}^3A_2$				
	9200	14,375		1.417	133.47	0.128	6122
	10,200	16,000					

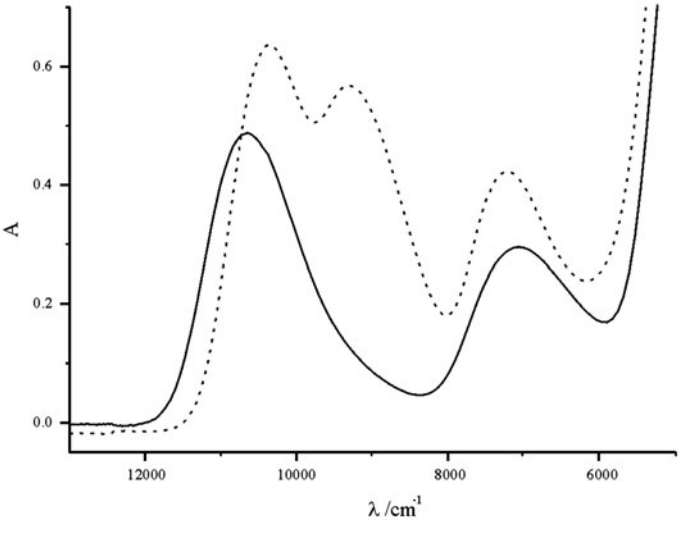

Figure 11. Electronic spectra for Ni(II) with vitamin B_6 complexes; solid line – $[Ni(pm)_2]Cl_2$; dashed line – $[Ni(pl)_2]Cl_2$.

The Racah parameters are lower for complexes than for the free ions. This considerable decrease in the inter-electronic repulsion of parameter B indicates the presence of less covalent metal–ligand bond. The Δ_0-values for $[Ni(pm)_2]Cl_2$ and $[Ni(pl)_2]Cl_2$ are 6098 and 6122 cm^{-1}, respectively, usually suggesting electron delocalization. The nephelauxetic parameter β is in the range 0.128–0.134 and depends strongly on electronegativity of the donor atoms and the ligand structure.

3.5. *Conductance*

Conductivity in water showed that the complexes were ionic (244 μS cm^{-1} for $[Ni(pm)_2]Cl_2$ and 144 μS cm^{-1} for $[Ni(pl)_2]Cl_2$.

3.6. *Antimicrobial activity*

The ligands and new metal complexes were evaluated for *in vitro* antibacterial activity against *P. vulgaris*, *E. coli*, and *S. aureus* and *in vitro* antifungal activity against *C. albicans* by using microbroth dilution assay. The complexes are more toxic than the ligands. Vitamin B_6 showed multiplying properties against bacteria and fungi. MIC and MBC concentrations, which were obtained by standard microbroth dilution, are presented in tables 3 and 4, respectively. The results show weaker activity of the $[Ni(pm)_2]Cl_2$ complex than in the pm·HCl one against the tested strains of bacteria and yeast, and present MIC and MBC values above 32 mg mL^{-1}. The pm·HCl presents higher activity determined by MIC and better killing properties against all tested micro-organisms, except *E. hirae* and *C. albicans* than the $[Ni(pm)_2]Cl_2$ complex. $[Ni(pl)_2]Cl_2$ gives quite good results of MIC concentration for tested Gram-negative bacteria: *P. vulgaris*, *P. aeruginosa*, and *E. coli* (0.720; 5.730; 2.870 mg mL^{-1}, respectively), and for Gram-positive bacteria only for *S. aureus* (1.440 mg mL^{-1}), but it is still less active than pl·HCl. $[Ni(pl)_2]Cl_2$ shows bactericidal activity only against *S. aureus* and *P. aeruginosa* (1.910 and 5.730 mg mL^{-1}, respectively), while the pl·HCl is not active only against *E. hirae* and *C. albicans*. The $NiCl_2{\cdot}6H_2O$, from among all the tested compounds, gives higher MIC values against all tested bacteria and presents killing activity against *E. hirae* (1.450 mg mg mL^{-1}), *S. aureus* (0.360 mg mL^{-1}), *P. vulgaris* (1.450 mg mL^{-1}), and *C. albicans* (0.090 mg mL^{-1}).

Table 3. Antimicrobial activity of nickel chloride, ligands, and nickel–vitamin B_6 complexes using MIC method [20].

	MIC (mg mL^{-1})					
	Bacteria Gram-positive		Bacteria Gram-negative			Yeast
Compound	*Enterococcus hirae*	*Staphylococcus aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
$NiCl_2{\cdot}6H_2O$	0.750	0.360	0.725	1.450	0.725	0.045
pm·HCl	5.730	1.450	2.900	2.900	2.900	5.730
pl·HCl	No inhibition effect	0.725	0.725	1.450	0.725	No inhibition effect
$[Ni(pm)_2]Cl_2$	>32	>32	>32	>32	>32	>32
$[Ni(pl)_2]Cl_2$	No inhibition effect	1.440	0.720	5.730	2.870	No inhibition effect

Table 4. Antimicrobial activity of nickel chloride, ligands, and nickel–vitamin B_6 complexes using MBC method [21].

	MBC (mg mL^{-1})					
	Bacteria Gram-positive		Bacteria Gram-negative			Yeast
Compound	*Enterococcus hirae*	*Staphylococcus aureus*	*Proteus vulgaris*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
$NiCl_2 \cdot 6H_2O$	1.450	0.360	1.450	No inhibition effect	No inhibition effect	0.090
pm·HCl	No inhibition effect	1.450	2.900	5.730	5.730	No inhibition effect
pl·HCl	No inhibition effect	1.450	1.450	2.900	1.450	No inhibition effect
$[Ni(pm)_2]Cl_2$	>32	>32	>32	>32	>32	>32
$[Ni(pl)_2]Cl_2$	No inhibition effect	1.910	No inhibition effect	5.730	No inhibition effect	No inhibition effect

4. Conclusion

The synthesis of Ni(II) with pyridoxal and pyridoxamine has been described in this article. The structures of these compounds correspond to four-coordinate monomeric Ni(II)-complexes. The binding set of $[Ni(pm)_2]Cl_2$ included phenolato oxygens and 4-hydroxyamino nitrogens. Ni(II) is coordinated by phenolato oxygens and carbonyl oxygens in $[Ni(pl)_2]Cl_2$. The geometry of synthesized complexes is distorted tetrahedral. Under the same conditions of synthesis, it was impossible to obtain the complex of Ni(II) with pyridoxine because it decomposed in time. Our results confirmed the observed phenomenon, that the Ni(II)-pn bond is characterized by the lowest value of the stability constant.

In this article, we present also two independent and different experimental techniques, which are useful for determination of stability constants of complexes studied. Results obtained from the experimental measurements enable us to conclude that among other complexes of metal ions, complexes studied in this article have low stability, ordered by increasing stability:

$$[Ni(pl)_2]^{2+} > [Ni(pm)(OH_2)_2]^{2+} > [Ni(pn)(OH_2)_2]^{2+}$$

In this ranking, nickel(II) forms the most stable coordination compound with pyridoxal, while Ni(II) forms the weakest one with pyridoxine. Pyridoxine, as a ligand, causes larger crystal-field splitting but lower Racah parameter than pyridoxamine. The *B*-values suggest less covalency in the metal–ligand bond, and Δ_0-values suggest a weak ligand field. In this case, during confluent aqueous solutions, we can rather talk about metal ion–ligand interactions than typical chemical coordination bonds. These conclusions are based on the values of gradual and cumulative stability constants and analysis of synthesis products.

Antimicrobial activities of tested compounds show higher activity of $[Ni(pl)_2]Cl_2$ complex against tested micro-organisms than the $[Ni(pm)_2]Cl_2$, values of MIC concentrations and killing properties are better. This suggests that enhanced antimicrobial activity of $[Ni(pl)_2]Cl_2$ complex may be due to higher stability constant of this complex.

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