European Journal of Chemistry 3 (4) (2012) 411-415

European Journal of Chemistry

Journal homepage: www.eurjchem.com

Spectrophotometric investigation of DL-tryptophan in the presence of Ni(II) or Co(II) ions

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ARTICLE INFORMATION

Received: 04 July 2011
Received in revised form: 27 July 2011
Accepted: 27 July 2011
Online: 31 December 2012

KEYWORDS

DL-Tryptophan
Biological activity
Gibbs free energy
Spectrophotometry
Dissociation constant
Potentiometric titration

ABSTRACT

In the present study, synthesis of transition metal complexes of DL-tryptophan with metal precursors such as nickel (II) and cobalt (II) ions in water under refluxing conditions and optimization of the reactions to obtain the composition of complexes in water solutions has been reported. The preparation and structural elucidation of the complexes was undertaken by using physico-chemical, potentiometric titration and spectroscopic methods (UV/Vis, FT-IR and XRD). Comparisons of the spectral measurements of DL-tryptophan with those of the nickel (II) and cobalt (II) complexes are useful in determining the atoms of the ligand that are coordinated to the metal ion. In addition, K (dissociation constant) and ΔG (Gibbs free energy) values were calculated using the Babko and Stanley & Turners methods. Antibacterial and antifungal activities of the complexes were studied screened against bacteria and fungi. The activity data shows that and cobalt complexes of DL-tryptophan are more potent than the DL-tryptophan.

1. Introduction

DL-Tryptophan (Abbreviated as Trp or W, $C_{11}H_{12}N_2O_2$, MW: 204.23 g/mol, melting point: 295 °C [1]), one of the 20 standard and an essential amino acid, is encoded in genetic code as the codon UGG. It is a heterocyclic compound that is found in small amounts in most proteins. Only the L-stereoisomer of tryptophan is commonly found in proteins, however the D-stereoisomer is occasionally noted in naturally occurring peptides (for example, the marine venom peptide contryphan) [2]. The distinguishing structural characteristic of tryptophan is that it contains an indole functional group. The isolation of tryptophan was first reported by Hopkins in 1901 through hydrolysis of casein [3].

DL-Tryptophan plays an important role in the growth and development of infants and in the biosynthesis of serotonin [4] and niacin [5] (deficiency of niacin or tryptophan can cause pellagra). Its occurrence in milk has been suggested as the reason that drinking milk before bed time helps sleeping. It is used in medicinal [6-9] and nutritional research, in enriched foods, and as a dietary supplement [10,11].

Computational [12], gravimetric [13,14], titrimetric [15], electrochemical [16], spectrophotometric and kinetic methods [17-36] were used to determine tryptophan in the presence of various metals. For example, in the previous study of DL-tryptophan [33], polarographic studies of nickel (II) + DL-tryptophan solutions were reported.

In this work, the polarography of nickel in the presence of DL-tryptophan has been studied in KNO_3 supporting electrolyte. Nickel yielded two irreversible steps which are due to the formation of two varieties of the complex in sluggish equilibrium with each other. The reduction is an irreversible, kinetically controlled process as evidenced from cyclic voltametry and *i-t* curves.

From the literature studies, there are no experiments on the synthesis, the determination of optimum conditions, the spectral analyses and the assignment of dissociation constant. Therefore, in the present study, as a contribution for the literature, nickel (II) and cobalt (II) complexes of racemic DL-tryptophan were synthesized and analyzed by using physico-chemical, spectroscopic and potentiometric titration techniques. Optimum conditions and the molar ratio for the reaction were determined by spectral measurements. In addition, their antibacterial and antifungal activities were studied.

2. Experimental

2.1. Material and measurements

All reagents were purchased from commercial sources and used as supplied. Elemental analyses for C, H and N were performed with a Costech elemental analyzer. Conductivity measurements were obtained in DMSO using an Inolab Thermal 740P. The magnetic susceptibilities were measured MK-1 Sherwood scientific magnetic susceptibility balance. UV/Vis spectra were measured with a Shimadzu UV-1700 Pharma spectrophotometer in the 200-800 nm range. IR spectra were recorded with a Shimadzu IR-470 spectrophotometer as KBr pellets in the frequency range 400-4000 cm^{-1}. Shimadzu XRD-6000 was employed for the XRD analyses. All pH measurements were obtained using a calibrated Metrohm 654 digital pH meter with a Sensorex combination pH glass electrode assembly. The pH meter was calibrated daily before use with pH = 4 and 7 Metrohm AG CH 9100 Hersau buffers. Metrohm Multi-Burette E-845 was used as the burette.

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
DOI:10.5155/eurjchem.3.4.411-415.482

2.2. Synthesis of nickel (II) and cobalt (II) complexes of DL-tryptophan

A solution of DL-tryptophan (0.102 g, 0.5 mmol) in water was added drop-wise to a solution of $NiCl_2.6H_2O$ or $CoCl_2.6H_2O$ in water at the mole ratio of 1:1. The pH of the solution was adjusted to ~7 by adding 0.1 M NaOH in water and the system was refluxed for two hours. The obtained solid materials were washed with water, and dried. The complexes were completely soluble only in DMSO.

Nickel complex of DL-tryptophan: Colour: Very light green. Yield: 82%. M.p.: 264.6 °C. Anal. calcd. for $[C_{11}H_{12}N_2O_2.NiCl_2(H_2O)_2].4\ H_2O$: C, 64.70; H, 5.88; N, 13.72. Found: C, 63.88; H, 6.04; N, 14.07%. Magnetic moment (μeff, BM): 3.88. UV/Vis bands (nm): 290, 511. FT-IR (KBr, ν, cm^{-1}): 3422 (ν_{O-H}), 3235 (ν_{N-H}), 1766 ($\nu_{C=O}$), 1667 (ν_{C-N}), 1472 (ν_{CH}), 1350 (ν_{CH2}), 1130 (ν_{C-O}).

Cobalt complex of DL-tryptophan: Colour: Very light pink. Yield: 83%. M.p.: 264.4 °C. Anal. calcd. for $[C_{11}H_{12}N_2O_2.CoCl_2(H_2O)_2].4\ H_2O$: C, 64.70; H, 5.88; N, 13.72. Found: C, 64.22; H, 6.04; N, 14.04%. Magnetic moment (μeff, BM): 4.80. UV/Vis bands (nm): 287, 486. FT-IR (KBr, ν, cm^{-1}): 3424 (ν_{O-H}), 3237 (ν_{N-H}), 1769 ($\nu_{C=O}$), 1667 (ν_{C-N}), 1475 (ν_{CH}), 1353 (ν_{CH2}), 1132 (ν_{C-O}).

2.3. General procedure

In the spectroscopic studies, in order to determine the wavelength which would be used in the reaction, various solutions including DL-tryptophan in presence nickel (II) or cobalt (II) between pH = 1-10 were prepared in the proportion of 1:1 and the spectra were measured after waiting for 10 min. As a result of the investigation at various the pH range, the nickel and cobalt complexes of DL-tryptophan showed absorption peaks at λ = 511 and 486 nm, respectively. We observed that the wavelength values changed very little in the pH range 6~7 and a pH value of 7 was chosen for all experiments at room temperature.

In the potentiometric titration studies, the solutions including $HClO_4$, $HClO_4$ + DL-tryptophan and $HClO_4$ + DL-tryptophan + Ni(II) or Co(II) solutions were titrated potentiometrically by using NaOH (0.1 N). The results are demonstrated in Figures 1a and 1b.

3. Result and discussion

3.1. Optimization of reactions

In order to determine the optimum conditions which would be used in the subsequent reaction, the effect of pH, temperature, time and concentrations on the reactions was studied.

The effect of pH was studied in the range 1-10 by adjusting the pH with $HClO_4$ and NaOH solutions. The general procedure was applied and the absorbance was measured by UV-VIS spectrometer. The results are shown in Figure 2. A pH value of 7 was chosen as the optimum in two reactions.

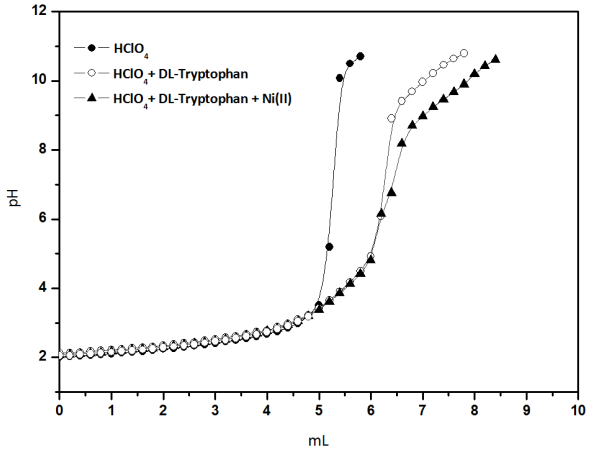
The effect of temperature on the reaction was observed for eight different temperatures under the general procedure. The highest absorbance was determined at room temperature as shown Figure 3.

The effect of time on the reaction was observed from 5 min to 50 min and the general procedure was applied. Optimum results were obtained at 40 min. The absorbance was found to be stable for at least 40 min as shown in Figure 4. According to these results reactions are completed after 40 min.

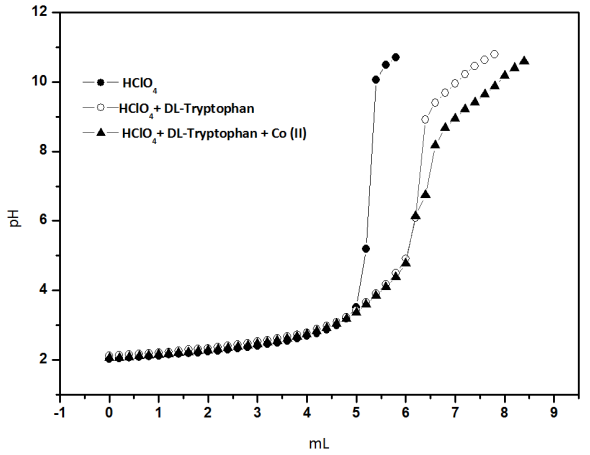
The effect of the concentration of DL-tryptophan in the presence of nickel (II) or cobalt (II) was investigated in the range $1x10^{-3}$-$1x10^{-2}$ M under the general procedure. Concentrations of $1x10^{-2}$ M were chosen as the optimum (Figure 5). According to this Figure 5, the reactions are in favour of products.

3.2. Composition and dissociation constants of complexes

Job's method of continuous variations [36] and the methods of Babko [37] and Turner & Anderson [38] were used in the investigation of the composition and stability constants of the complex during the reaction.



(a)



(b)

Figure 1. DL-Tryptophan + Ni (II) (**a**); DL-Tryptophan + Co (II) (**b**).

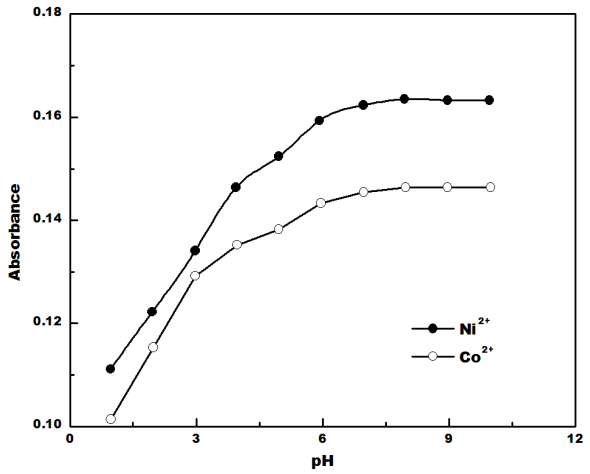


Figure 2. Effect of pH.

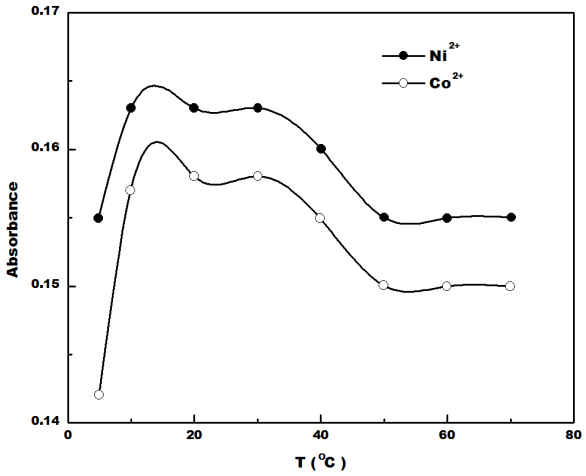


Figure 3. Effect of temperature.

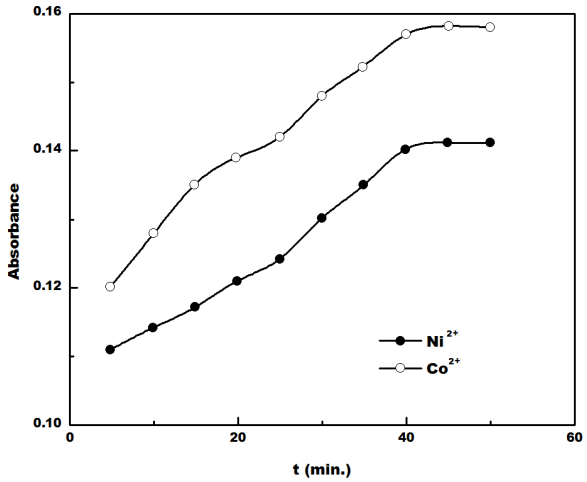


Figure 4. Effect of time.

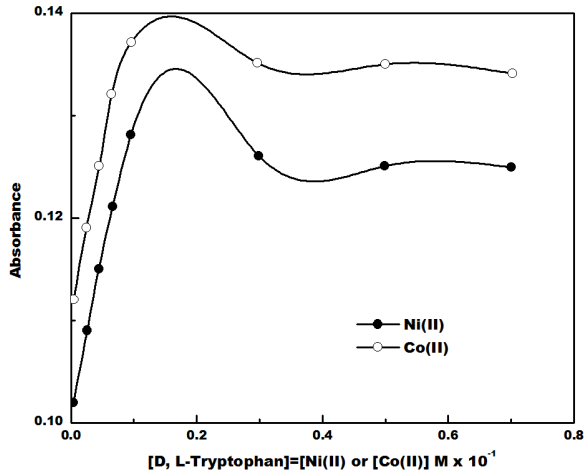


Figure 5. Effect of concentration.

Various solutions were prepared at total concentrations of 1 x 10^{-3} M, under the general procedure, including DL-tryptophan and nickel (II) or cobalt (II). Firstly, the perpetual change graph was plotted after necessary absorbance adjustment was made and demonstrated Figure 6.

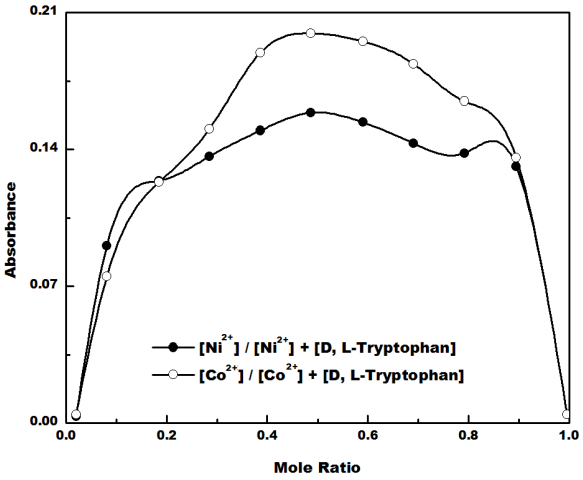


Figure 6. Determination of mole ratio.

Secondary, dilution curves were drawn fort the determination of stability constants with help solutions that give equal absorption [37-39]. The measured absorbance values of DL-tryptophan with nickel (II) and cobalt (II) solutions at different concentrations were shown in Table 1. The plots of Absorbance (nm) against [Ni(II)) or Co(II)] = [DL-tryptophan] are linear.

Table 1. The values of dilution curves.

$[Ni^{2+}]/[Ni^{2+}]$ + [DL-tryptophan] (M)	A_{511} (nm)	$[Co^{2+}]/[Co^{2+}]$ + [D,L-tryptophan] (M)	A_{486} (nm)
$8.0x10^{-5}$	0.070	$8.0x10^{-5}$	0.058
$1.6x10^{-4}$	0.140	$1.6x10^{-4}$	0.128
$2.4x10^{-4}$	0.222	$2.4x10^{-4}$	0.210
$3.3x10^{-4}$	0.300	$3.3x10^{-4}$	0.288
$4.2x10^{-4}$	0.380	$4.2x10^{-4}$	0.368

Job's method of continuous variations [37] demonstrated one maximum for $[Ni^{2+}]/\{[Ni^{2+}]$ + [D,L-tryptophan] }= 0.5 and one maximum for $[Co^{2+}]/\{[Co^{2+}]$ + [D,L-tryptophan]} = 0.5 in Figure 6, one molecule of DL-tryptophane reacts with one molecule nickel(II) ion andone molecule of DL-tryptophan reacts with one molecule cobalt(II) ion.

In the 1:1 stoichiometric reactions, the stability constant formula [38,39] are

$$K= x/(a_1-x))\ (b_1-x)) = x/(a_2-x)(b_2-x) \text{ and } x = a_2 b_2 - a_1 b_1 / a_2 - a_1 + b_2 - a_1 \quad (1)$$

where the a's and b's denote initial concentration of nickel (II) or cobalt (II) and DL-tryptophan, respectively, Where a_1 is the concentration of nickel or cobalt and is calculated from $[M^{2+}]$ /$[M^{2+}]$ + [DL-tryptophan], b_1 is the concentration of DL-tryptophan and is computed from $[M^{2+}]$+[DL-tryptophan] = $1x10^{-3}$ M. Further, a_2 andb_2 are $[M^{2+}]$ and [DL-tryptophan], respectively. They are calculated from dilution curves (M^{+2} = Ni^{2+} or Co^{2+}), and x denotes the concentration of the complex formed. K values calculated by this method are of the same order of magnitude as those obtained by the first method. The values of K (dissociation constant) for the reactions of DL-tryptophan with nickel (II) or cobalt (II) were calculated by means of the above equations.

The Gibbs free energy of formation of the complex may be calculated readily from the relation

$$\Delta G = -R.T.\ln K \quad (2)$$

where R is gas constant (1.987 cal/mol.K), T is temperature (273.16 + 20 = 293.16 K) and K is stability constant. The values of K (Stability Constant) and ΔG (Gibbs free energy) for the

reactions DL-tryptophan with nickel or cobalt were calculated by means of above equations.

3.3. Synthesis and analyses

Nickel (II) and cobalt (II) metal complexes of DL-tryptophan were synthesized in water under refluxing conditions. High yields were obtained (over 80 %). Both complexes are air stable and soluble only in DMSO.

When the elemental analyses of the complexes were investigated, the calculated and found ratios were found to be 1:1 in the complexes of DL-tryptophan in the presence of nickel (II) or cobalt (II). The elemental analyses indicate that all the metal complexes have 1:1 stoichiometry. The magnetic moment values for nickel (II) and cobalt (II) complexes are in the range of 3.88 and 4.80 BM, respectively, indicating that the nickel (II) and cobalt (II) complexes are typically high spin complexes and having octahedral structure. According to specific and equivalent conductivity values (Table 2) obtained for these complexes, while specific values of DL-tryptophan in the presence of nickel (II) and cobalt (II) increase with concentration, the equivalent conductivity values decrease. These results show the presence of ions belonging to the system. In addition, when Figures 1a and 1b is investigated, DL-tryptophan and DL-tryptophan + M^{2+} (M^{2+} = Ni^{2+} or Co^{2+}) curves appeared different places and complexation had occurred.

Table 2. Specific and equivalent conductivity values of DL-tryptophan with M (II) (M: Ni or Co).

C (M)	Specific conductivity, L (s/cm) x 10^{-6}	Equivalent conductivity, Λ
0.01	1027 (1789)	102.7 (178.9)
0.02	1440 (2205)	72.0 (110.2)
0.03	1870 (2636)	62.0 (87.8)
0.04	2300 (3065)	57.5 (76.6)
0.05	2720 (3485)	54.4 (69.7)

The UV/Vis spectra of free DL-tryptophan molecule displays an absorption band at 302 nm, whereas the complexes display a number of distinct bands at 290 and 511 nm for DL-tryptophan + nickel (II), and 287 and 486 nm for DL-tryptophan + cobalt (II). The absorption bands at 290 and 287 nm are attributed to π-π* transitions. The high energy bands at 511 and 486 nm originated from the *d-d* transitions.

The IR spectra of the free ligand (DL-tryptophan) and its metal complexes are interpreted by comparing the spectra of the complexes with that of the free ligand. The infrared spectrum of DL-tryptophan results are listed in Table 3. The comparison of the band positions of various vibrations are ascertained with good evidence. In the infrared spectrum of complexes, two bands centered at 3422 and 3235 cm^{-1} for nickel (II) complex and 3424 and 3237 cm^{-1} for cobalt (II) complex are attributed to the $\nu_{(OH)}$ and $\nu_{(NH)}$ vibrations of the lattice water molecule and the amine group, respectively. The broadness of these bands indicates hydrogen bonding. The sharp bands at about 1760 cm^{-1} are assigned to the absorption of the carbonyl group. The $\nu_{(C-N)}$ and $\nu_{(C-O)}$ stretchings of the complexes are found in the frequency range about 1660 and 1160 cm^{-1}, respectively, other vibrations are observed for nickel (II) at 1472 $\nu_{(CH)}$ and 1350 $\nu_{(CH2)}$, and for cobalt (II) at 1475 $\nu_{(CH)}$ and 1353 $\nu_{(CH2)}$, respectively.

Table 3. Selected FT-IR data for DL-tryptophan.

Assignment	DL-tryptophan, frequencies, cm^{-1}
$\nu_{(OH)}$	3408
$\nu_{(NH)}$	3220
$\nu_{(COOH)}$	1756
$\nu_{(C-N)}$	1655
$\nu_{(CH)}$	1411
$\nu_{(CH2)}$	1315
$\nu_{(C-O)}$	1142

The XRD (Powder Pattern) spectra of the complexes are indexed by an X-Ray diffractometer (Figure 7 and 8). Three peaks are determined for both complexes at 5.2300, 14.9200 and 19.5600 (2θ) and the interplanar spacing (d) has been calculated as 16.97784, 5.93296 and 4.53476, respectively. All the important peaks have been indexed and the observed values of the interplanar distance are compared with the calculated ones. The presence of the forbidden number confirms the tetragonal system. This implies that nickel (II) and cobalt (II) complexes are distorted octahedral in structure.

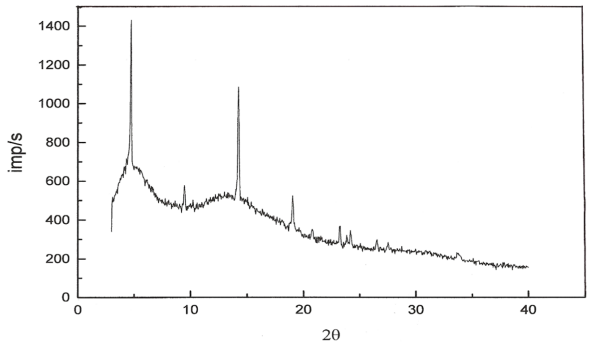


Figure 7. The XRD Spectrum of DL-tryptophan in the presence of Ni (II).

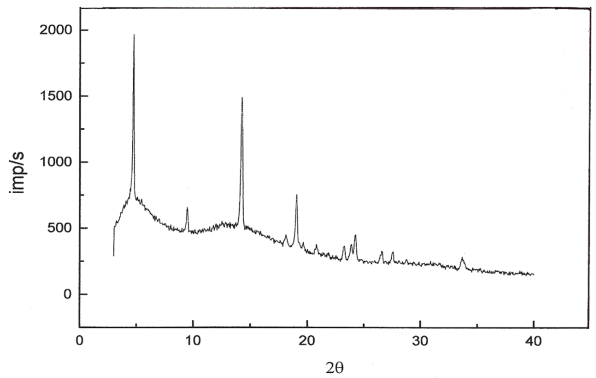


Figure 8. The XRD Spectrum of DL-tryptophan in the presence of Co (II).

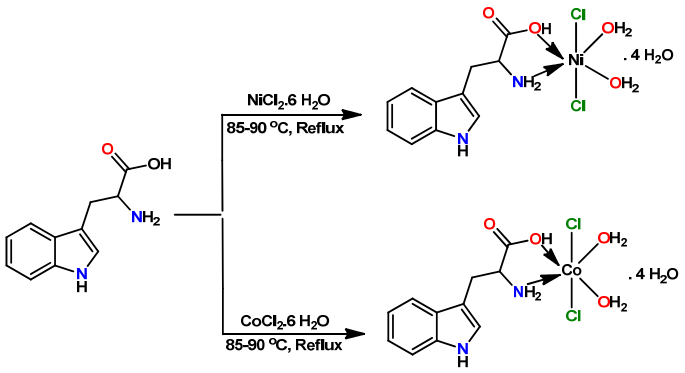
The synthesized complexes are estimated for their microbial toxicity against a set of bacterial and fungal strains. The antibacterial activity of the complexes is tested using the agar well-diffusion method [40], and the results are given in Table 4. The results show that the complex has significant antibacterial activity.

Table 4. The antibacterial activities of the complexes and standard *.

Bacteria	Zone of inhibition, mm		
	Nickel (II) complex	Cobalt (II) complex	Standard drug (Imipenem, 10 µg/disc)
Gram positive bacteria			
Bacillus subtilis	25	25	30
Staphylococcus aureus	25	25	33
Gram negative bacteria			
Escherichia coli	20	20	32
Pseudomonas aeruginosa	25	22	30

* Concentration of sample is 3 mg/mL of DMSO.

The antifungal test is caried out by using the agar tube dilution protocol method [41]. The antifungal results of the synthesized complexes are given in Table 5. According to Table 5, complex shows significant antifungal activity.



Scheme 1

Table 5. The antifungal activities of the complexes and standard [a].

Fungus	Inhibition, %			
	Nickel (II) complex	Cobalt (II) complex	Standard drug [a]	MIC [b], µg/mL
Candida albicans	75	75	Miconazole	110
Candida glaberata	40	50	Miconazole	73
Fusarium solani	70	75	Miconazole	110

[a] Incubation temperature: 27 °C.
[b] Concentration of sample 200 µg/mL of DMSO.

4. Conclusion

According to spectrophotometric and potentiometric titration results, the reactions of DL-tryptophan with nickel (II) or cobalt (II) are complexation reactions. One molecule of DL-tryptophan reacts with one molecule nickel (II) or cobalt (II) ion. K (dissociation constant) values are calculated from perpetual change and dilution curves as 112201 and -6773.41 cal/mol for nickel(II) + DL-tryptophan and 30549 and -6015.57 cal/mol for cobalt(II) + DL-tryptophan, respectively. The negative values of ΔG mean that the kinetic process is a spontaneous one. The DL-tryptophan and nickel (II) and cobalt (II) complexes are found to be active against some of the representative bacterial and fungal strains. The possible reaction mechanism is given in Scheme 1.

Acknowledgement

We would like to thank Prof.Dr. Cindy Samet from Dickinson College, Carlisle, PA, USA, Prof.Dr. Stella Christoskova and Prof.Dr. Vassil Delchev from Paissii Hilendarski University, Plovdiv, Bulgaria. Prof.Dr. Ômer Zaim from Trakya University, Chemistry Department, Edirne, Turkey and dietician Figen Esen from Osman Gazi University, Faculty of Medicine, Eskisehir, Turkey for their helps.

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