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Synthesis, Characterization, Antioxidant and Antimicrobial Activity of Copper(II) Complex with Schiff Base Derived from 2,2-dihydroxyindane-1,3-dione and Tryptophan

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Abstract Schiff bases and complexes are today the subject of many studies because of the established biological, inhibitor and catalytic properties. The aim of this paper is to investigate the interaction of Schiff base obtained by reaction of 2,2-dihydroxyindane-1,3-dione (ninhydrin) and the essential amino acid tryptophan with copper(II) ion. Spectral characterization and examination of the potential antimicrobial and antioxidant activity of the synthesized complex were performed. The imine Cu(II) complex is characterized by FTIR and UV/Vis spectroscopy. Stoichiometric M:L ratio was determined by Joe and Yones method. Antioxidant activity was tested by DPPH and FRAP method. Antibacterial and antifungal activity was determined by diffusion technique on reference strains from the ATCC collection. The results showed that the synthesized Schiff base coordinates the Cu(II) ion as a tridentate ligand, in a molar ratio of 1:2 (M:L). The synthesized complex showed significant antioxidant activity. The antimicrobial effect of the Cu(II) complex in the case of *S. aureus*, *E. faecalis*, *L. monocytogenes*, *B. subtilis* and *C. albicans* was obtained, with inhibition zones of 11-20 mm.

Keywords Copper, Schiff base, FTIR, UV/Vis, Antimicrobial activity

1. Introduction

Schiff bases are synthetically accessible and structurally diverse compounds, typically obtained by condensation between an aldehyde or a ketone with primary amines [1]. Schiff base ligands are essential in the field of coordination chemistry, especially in the development of complexes of Schiff bases because these compounds are potentially capable of forming stable complexes with metal ions [2].

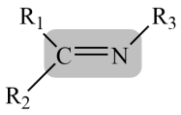

Figure 1. General structure of Schiff base

Schiff bases and complexes are significant industrial agents that show a wide range of biological activities such as antimicrobial, antitumor, antioxidant, antipyretic, and others [3-6]. The synthesis of new organic ligands is a key step for the construction of metal-organic complexes with desired structures. Schiff bases have played an important role in the development of coordination chemistry because of their numerous biological applications [7].

A large number of Schiff bases and their complexes have been investigated for their interesting and important properties, such as their ability to reversibly bind oxygen, catalytic activity in the hydrogenation of olefines, photochromic properties and complexing ability towards some toxic metals [8].

Tryptophan is an essential plant-derived amino acid that is needed for the in vivo biosynthesis of proteins. After consumption, it is metabolically transformed to bioactive metabolites, including serotonin, melatonin and the vitamin niacin (nicotinamide) [9].

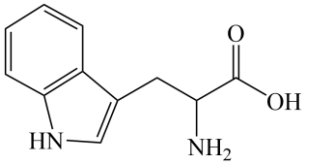

Figure 2. Structure of Tryptophan

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2. Experimental

All chemicals were of reagent grade, where possible, purchased from Aldrich and used without further purification.

2.1. Synthesis of the Complex

Synthesis of the complex was carried out according to the previously published procedure [10]. 0.01 mole of 2,2-dihydroxyindane-1,3-dione (**L_1**) was dissolved in 30 mL of ethanol and 0.01 mole of $CuCl_2$ x $2H_2O$ salt was added to the resulting solution, which was shaken and heated until all of it dissolved. This mixture was refluxed for about half an hour, followed by addition of 0.01 mole tryptophan (**L_2**) to the hot mixture and the mixture was refluxed for a two hours. The resulting brown colored precipitate was then filtered off. The product was then dried and stored in a desiccator. Yield: 65%. Melting point of product is 220.5°C.

2.2. Spectral Characterization

In order to determine structure of the complex, samples were recorded on Nicolet iS10 FT-IR spectrophotometer - Thermo Fisher Scientific. The ATR technique was used for sample analysis. Samples were recorded in the range of 4000-650 cm^{-1}.

Aqueous Cu(II) solutions at concentration of 0.6 x 10^{-3} mol L^{-1} were used to recording UV spectra. The metal salt, 2,2-dihydroxyindane-1,3-dione and tryptophan were added in the same volume ratio, mixed for 2 hours at 300 rpm, after which UV spectra were recorded. Absorption spectra were recorded on a double-bean UV/Vis spectrophotometer Perkin Elmer λ25, in the wavelength range of 200-400 nm [11].

2.3. Determination of Stoichiometric Ratio

Stoichiometric ratio M:L was tested using Joe and Yones method [12]. A series of solutions with a constant concentration of Cu(II) ions of 1.35 x 10^{-4} mol L^{-1} were prepared, while ligand concentration was changed. Based on the obtained results, the stoichiometric composition of the complex and the calculated stability value of the K_{ML} were determined.

2.4. Morphological Characterization

Prior to morphological characterization, solid complexes were treated with DMSO. The color, size and shape of Cu(II) crystal complex were determined by microscopic analysis, performed with the binocular microscope, the Leica DM 2500P mark.

2.5. Determination of Antioxidant Capacity

2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was performed according to earlier described method [13]. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation:

$$[(A_{control}-A_{sample})/A_{control}] \times 100$$

where A_{sample} is the absorbance of the solution containing the sample at 517 nm and $A_{control}$ is the absorbance of the DPPH solution. The results are expressed as the IC_{50} value (mg mL^{-1}) or the concentration of extract that caused 50% neutralization of DPPH radicals.

The determination of ferric reducing antioxidant power or ferric reducing ability (FRAP assay) was performed as described earlier [14]. To prepare the calibration curve, solutions of $FeSO_4$ x $7H_2O$ were prepared in the concentration range of 200-1000 μmol L^{-1} ($y = 0.001x + 0.0615$; $R^2 = 0.9907$). In each tube, 0.1 mL of Cu(II) complex and 3 mL of FRAP reagent were added. The samples were incubated in an aqueous bath for 30 minutes at 37°C, and the absorbance was measured at 593 nm.

2.6. Antimicrobial Activity *in vitro*

Antimicrobial activity was analyzed following the published procedures [15,16]. Antibacterial activity were investigated by diffusion method on reference bacterial strains *E. coli*, *E. faecalis*, *S. aureus*, *B. subtilis*, *L. monocytogenes* and *P. aeruginosa*. Antifungal activity of the complex was tested on *Candida albicans*. From the microorganisms strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared (density 10^7-10^8 CFU mL^{-1}). The strains were then placed on the surface of the nutrient substrate-Mueller-Hinton agar, dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 80 μL of Cu(II) complex solutions in concentration of 5 mg mL^{-1} were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at 37°C/24 h. After the incubation period, the size of the inhibitory zone was measured, and the sensitivity of the microorganisms was expressed in the manner described above [17].

3. Results and Discussion

3.1. Reaction Scheme and Structure of Complex

The reaction scheme and the proposed structure of the Cu(II) complex is shown at Figure 3. It is assumed that the synthesized Schiff base coordinates the copper ion as the tridentate ONO ligand. The formation of the bond with metal involves the oxygen atom of the carbonyl group of the indane part of the molecule and the other from the deprotonated carboxyl group of tryptophan. A third bond is formed between the metal ion and the nitrogen atom of the imine group.

3.2. Spectral Characteristics

Spectral characteristics of reactants and Cu(II) complex were described at Table 1.

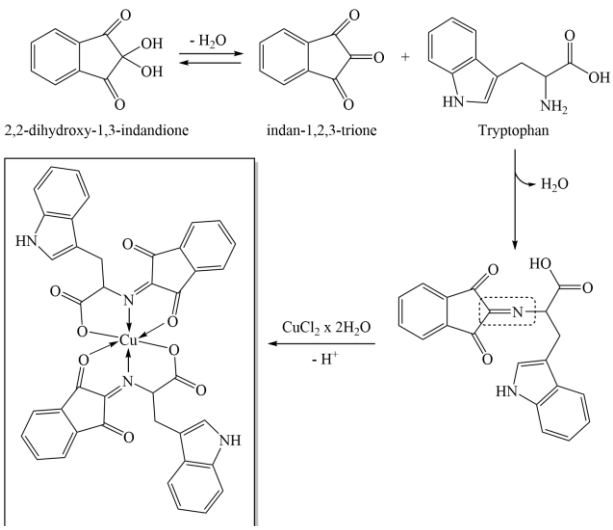

Figure 3. Reaction scheme and proposed structure of the complex

Table 1. Spectral data of reactants and Cu(II) complex

Infra-red spectral bands (cm^{-1})					
Sample	v(C=O)	v(O-H)	v(N-H)	v(COO^-)	v(C=N)
L_1	1746	3228	-	-	-
L_2	-	3009	3400	1655	-
Complex	1710	-	3393	1652	1511
Electronic spectral bands (nm)					
Sample			λ_{max} / A		
L_1			231 / 2.94		
L_2			219 / 2.40		
Complex			225 / 1.52		

FTIR data spectrum of the synthesized complex differs significantly from the reactant spectra. IR absorption frequency of O-H group was recorded at 3228 cm^{-1} on the 2,2-dihydroxyindane-1,3-dione spectrum. The specified band is absent on the spectrum of the complex. IR absorption frequency of C=O group in the complex spectrum was displaced at lower frequencies (1710 cm^{-1}) compared to the 2,2-dihydroxyindane-1,3-dione spectrum where it was recorded at 1746 cm^{-1}. The newly formed C=N bond in the complex was recorded at 1511 cm^{-1} as an intense, wide band.

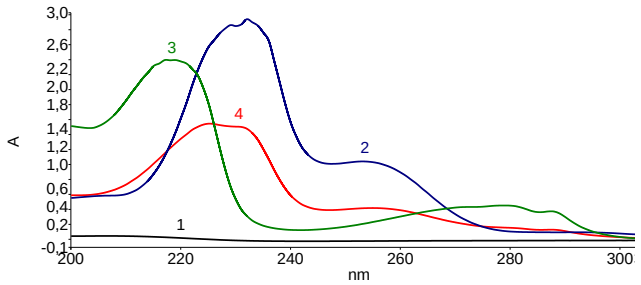

Figure 4. UV spectra: (1) Cu(II) ion; (2) Ninhydrin; (3) Triptophan; (4) Cu(II) complex

The UV/Vis spectrum of 2,2-dihydroxyindane-1,3-dione showed the absorption maximum at 231 nm corresponding to the $\pi \rightarrow \pi^*$ transition. Tryptophan spectrum showed two bands, one at 219 nm (λ_{max}) corresponding to $\pi \rightarrow \pi^*$ transition and the other at 280 nm indicating the $n \rightarrow \pi^*$ transition. The spectrum of the Cu(II) complex is similar to the 2,2-dihydroxyindane-1,3-dione spectrum, with expressed hypochromic shift.

3.3. Stoichiometric Ratio

The solutions of metal salt and Schiff base were prepared by dissolving them in an ethanol-water mixture in volume ratio 3/1. The spectra of the complex used to determine the stoichiometric ratio are shown in Figure 5. Absorbance vs. molar ratio (M/L) graph is shown in Figure 6. From the obtained data, the calculated stability constant of the complex K_{ML_2} is 2.83 x 10^7.

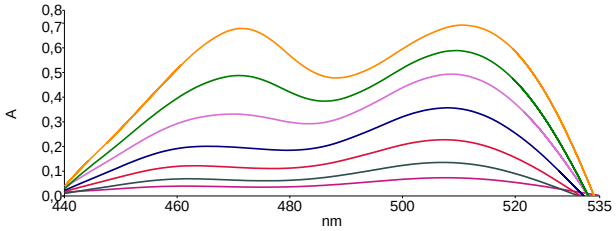

Figure 5. Absorption spectra of the Cu(II) complex

Absorption spectrum of the complex is characterized by a wide absorption band at 510 nm as a result of the $d \rightarrow d$ transition. Based on the position of the absorption maximum, the cleavage energy of the d-subunit was calculated and a value of 234 kJ mol^{-1} was obtained.

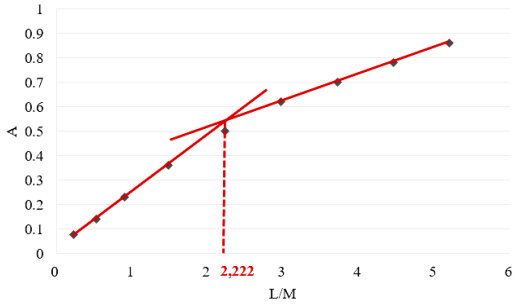

Figure 6. Absorbance vs. molar ratio (M/L) diagram

3.4. Morphological Characteristics

The crystals of the synthesized Cu(II) complex (Figure 7) are round-shaped forms, with a characteristic radial-air aggregates (from a common central part, aggregates are circularly matched) and diameter of up to 0.15 mm. Interferential colors are live of the first order.

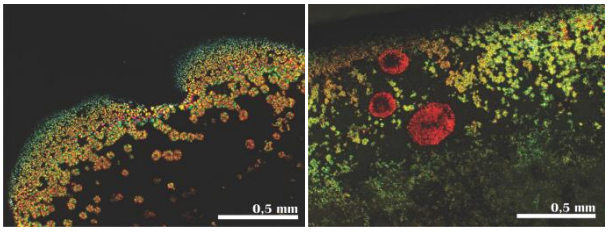

Figure 7. Morphology of Cu(II) complex crystals

3.5. Antioxidant Activity *in vitro*

The calibration curves obtained by the DPPH method for complex and vitamin C are shown in Figures 8 and 9. Based on the IC_{50} value, it has been established that the Cu(II) complex has a high antioxidant capacity. Vitamin C has a slightly better antioxidant effect (IC_{50} = 0.035 mg mL^{-1}).

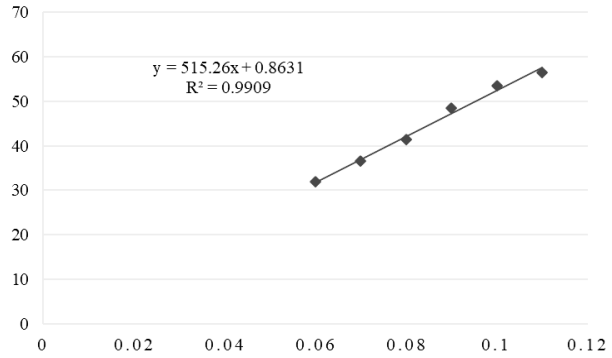

Figure 8. Calibration curve of Cu(II) complex obtained by DPPH method

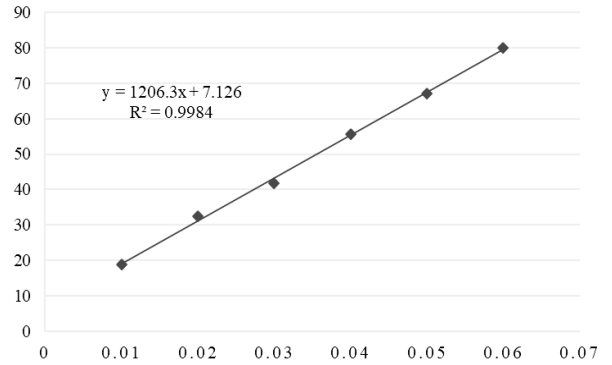

Figure 9. Calibration curve of vitamin C obtained by DPPH method

Figure 10. shows the calibration curve of the standard $FeSO_4$ x $7H_2O$ solutions used to determine the FRAP value. For concentrations of a solution of 1 mg mL^{-1}, the FRAP values listed below are calculated.

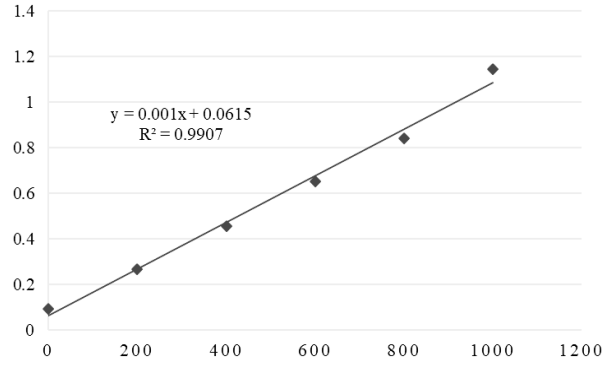

Figure 10. Calibration curve for $FeSO_4$ x $7H_2O$

The FRAP value for the synthesized complex is 587.5 µmol L^{-1}, which is significantly less than the calculated FRAP value for vitamin C at the same concentration (14 250 µmol L^{-1}). Summarized results of antioxidant capacity are shown in Table 2.

Table 2. Summarized results of antioxidant capacity

Sample	DPPH value [mg mL^{-1}]	FRAP value [µmol L^{-1}]
Cu(II) complex	0.095	587.5
Vitamin C	0.035	14 250

3.6. Antimicrobial Activity *in vitro*

The results of antimicrobial activity are shown in Table 3. Ciprofloxacin and Nystatin were used as controls. The Cu(II) complex has no antibacterial action against gram-negative bacteria (*E. coli* and *P. aeruginosa*). Poor activity was recorded in *E. faecalis* and *B. subtilis*. Significant action was observed in other strains, with inhibition zones of 16 to 20 mm. However, Ciprofloxacin showed significant antibacterial activity in relation to the complex, at a lower concentration (1 mg/mL). The complex showed similar activity as to Nystatin in the case of *C. albicans*, with an inhibition zone of 20 mm.

Table 3. Summarized results of antimicrobial activity

Sample	1	2	3	4	5	6	7
Cu(II) complex	-	++	+	++	+	-	++
Control	+++	+++	+++	+++	+++	+++	++

Legend: **1** - E. coli; **2** - S. aureus; **3** - E. faecalis; **4** - L. monocytogenes; **5** - B. subtilis; **6** - P. aeruginosa; **7** - C. albicans

4. Conclusions

Schiff base derived from 2,2-dihydroxyindane-1,3-dione and Tryptophan coordinates Cu(II) ion as a tridentate ONO donor ligand, in a stoichiometric ratio of 1:2 (M:L). Crystals of complexes are round-shaped forms. The synthesized complex has a significant antioxidant activity. Significant antimicrobial activity was found in the case of *C. albicans*, *L. monocytogenes* and *S. aureus*, while lower antimicrobial activities were found for other tested microorganisms.

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