

# SYNTHESIS, SPECTRAL CHARACTERIZATION, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF COPPER(II)-PABA COMPLEX

ORIGINAL SCIENTIFIC PAPER

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**ABSTRACT:** pABA (p-aminobenzoic acid or 4-aminobenzoic acid) is a chemical component of the folate molecule produced by plants and bacteria, and found in many foods. It is best known as a UV-blocking sunscreen applied to the skin, and is sometimes taken orally for certain medical conditions. Today it is known that many organic molecules in the human body can react with biometals such as copper, cobalt, manganese, iron and others. This study was performed to investigate the interaction of Cu(II) ions with p-aminobenzoic acid. Spectroscopic methods (FTIR and UV/Vis spectroscopy) were used to characterize the product obtained. The antimicrobial activity of the synthesized complex was tested by diffusion techniques. The results of spectroscopic analysis indicate the interaction of Cu(II) ions with pABA. Interaction is realized through oxygen donor atom of ligand. It was found that the Cu(II) complex has significant antimicrobial activity compared to the pABA ligand.

**KEYWORDS:** p-aminobenzoic acid, copper, FTIR, UV/Vis, antimicrobial analysis

## INTRODUCTION

Para-aminobenzoic acid (pABA), (Figure 1), is a precursor for the synthesis of folic acid (also known as vitamin B9 or folacin). Folic acid is an enzyme cofactor, and it is involved in some basic biological reactions, as nucleotide biosynthesis, DNA repair and DNA methylation [1].

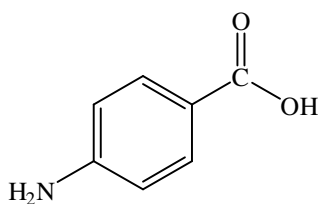


Figure 1. Structure of pABA

pABA is a chemical compound which has been of medical interest during the past decade because of its effects on bacterial metabolism and pigment metabolism [2]. It is also used as a protective drug against solar insolation and in diagnostic tests for the state of the gastrointestinal tract in medicine [3]. In normal man, pABA is rapidly absorbed and excreted, in small amount as free pABA or as acetylated pABA. Most of this drug is excreted either as a conjugation product with glycine (p-aminohippuric acid) or as the glucuronate [4]. pABA have shown a

stronger antimicrobial activity at lower pH values. It is supposed that this acid reacts in at least two mechanisms of antibacterial activity: one mechanism in common with other organic acids and the other mechanism by interfering with the synthesis of the peptidoglycan layer by an action on the dihydrofolate reductase enzyme [5].

Complexing of biologically important molecules as well as molecules introduced into the body by drugs and supplements can significantly influence their biological activity [6,7]. The analysis of the interaction of biogenic metal M(II) cations with O, N, S-donor atoms of ligands often used in the treatment of a wide spectrum of diseases is important for monitoring of distribution, pharmacokinetics, excretion, drug efficacy and adverse effects [8].

## MATERIAL AND METHODS

### CHEMICALS

All reagents used were p.a. purity and were purchased from Sinex (Bosnia and Herzegovina), Euro-Lab (Bosnia and Herzegovina), Sigma Aldrich (United States) and Fisher Scientific (United States).

### SYNTHESIS OF Cu(II)-PABA COMPLEX

The complex synthesis was performed according to a previously published procedure [6, 8]. A mixture

of ethanol and water in a volume ratio 1:1 was used as a solvent for metal salt and pABA. For the synthesis of complex, solutions of  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$  ( $0.005 \text{ mol L}^{-1}$ ) and pABA ( $0.01 \text{ mol L}^{-1}$ ) were prepared. The solutions were mixed in an equimolar volume ratio and mixed on a magnetic stirrer for 30 minutes at room temperature, with an adjustment of pH value at 5.6. After stirring, the solution was left in dark space for three days to separate the solid complex. The resulting dark green product was filtered, washed with ethanol and water and then dried in a drying oven at  $50 \text{ }^\circ\text{C}$ . The dry product is stored in a desiccator until analysis.

### FTIR 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  $4000\text{-}650 \text{ cm}^{-1}$ .

### UV CHARACTERIZATION

The aqueous solutions of pABA ( $0.12 \times 10^{-3} \text{ mol L}^{-1}$ ) and Cu(II) salt ( $0.06 \times 10^{-3} \text{ mol L}^{-1}$ ) were used for recording the UV spectra. The solutions were mixed in equimolar volume ratio, stirred for 2 hours at 300 rpm, and then the UV spectra were recorded. Absorption spectra were recorded on a UV/Vis spectrophotometer Perkin Elmer  $\lambda 25$ , in the range of wavelengths of 200-400 nm. Based on the position of the absorption maximum ( $\lambda_{\text{max}}$ ) in the tested model system, the value of the energy splitting of the central ion was calculated.

### MORPHOLOGICAL CHARACTERIZATION

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

### ANTIMICROBIAL ACTIVITY IN VITRO

Antimicrobial activities were investigated by diffusion method on reference bacterial strains *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes* and *Pseudomonas 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  $10^7\text{-}10^8 \text{ CFU/mL}$ , depending on soy).

The strains were then placed on the surface of the nutrient substrate-Mueller-Hinton agar (MH), dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 80  $\mu\text{L}$  of pABA and Cu(II) complex solutions in concentration of  $5 \text{ mg mL}^{-1}$  were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at  $37^\circ\text{C}/24 \text{ h}$ . After the incubation period, the size of the inhibitory zone was measured and the sensitivity of the microorganisms was expressed as follows: if the inhibitory zone of the microorganism growth was greater than 20 mm, it was marked with three pluses (+++), which is the highest sensitivity of microorganisms. If the inhibitory zone was in the range of 16-20 mm it was marked with two pluses (++). Very low sensitivity is indicated with one plus (+), if the inhibitory zone is 10-15 mm in diameter. The minus (-) mark is used for an inhibitory zone of less than 10 mm or if it's absolutely absent [9].

### RESULTS AND DISCUSSION

#### STRUCTURE OF THE COMPLEX

Figure 2 shows the reaction scheme and the proposed structure of the Cu(II)-pABA complex. Metal and ligand react in a 1:2 molar ratio (M:L). The oxygen atom from the carboxyl group is involved in the formation of a bond with metal. The assumption is that the metal ion can bind two molecules of water (from the metal salt).

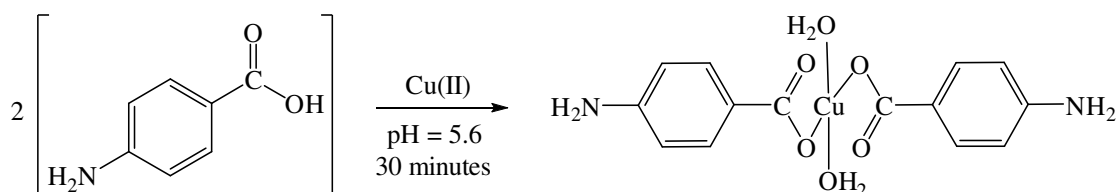


Figure 2. Reaction scheme and proposed structure of the complex

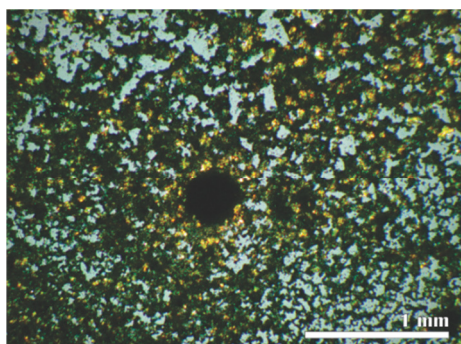
## SPECTRAL CHARACTERIZATION

On the FTIR spectrum of the ligand, a strip characteristic of the O-H stretch vibration is seen at  $3458\text{ cm}^{-1}$ , which is not visible in the spectrum of the complex. This indicates the interaction of copper(II) ions with the ligand over the oxygen atom of the carboxyl group. C=O stretch vibration is recorded as a high intensity strip at  $1657\text{ cm}^{-1}$ . For the carboxylate ion in the spectrum of the complex the characteristic band is  $1554\text{ cm}^{-1}$  ( $\nu_{\text{as}}$ ) (slight shift to smaller wave numbers compared to the ligand spectrum) and  $1380\text{ cm}^{-1}$  ( $\nu_{\text{s}}$ ). The tape characteristic of the N-H stretch vibration was recorded at  $3360\text{ cm}^{-1}$  in the ligand spectrum, or  $3248\text{ cm}^{-1}$  at the complex spectrum. Insights into the electronic spectra of the ligand, peaks at 279, 217 and 202 nm were determined. In the spectrum of the complex, two absorption peaks were recorded, with hypochromic shifts. The difference in these two spectra is that the absorbance peak at 202 nm visible in the pABA spectrum is not visible in the spectrum of complex. Based on the absorption peak for the Cu(II)-pABA model system, splitting energy of d-orbitales was determined and it was  $592\text{ kJ mol}^{-1}$ . Spectral data for pABA and  $\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$  are shown in Table 1.

**Table 1.** Spectral data for pABA and  $\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$

Infra-red spectral bands ( $\text{cm}^{-1}$ )			
Sample	Functional group		
	N-H	O-H	C=O
pABA	3360	3458	1657
$\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$	3248	-	1554
Electronic spectral bands (nm)			
pABA	279, 217, 202		
$\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$	277, 217		

## MORPHOLOGICAL CHARACTERIZATION



**Figure 3.** Morphology of  $\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$  crystals

The morphologies of  $\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$  crystals are presented in Figure 3. The images show the developed crystals, but irregular amorphous bodies.

Severe interference colors are present due to the sample thickness. The size (diameter) is up to 0.25 mm.

## ANTIMICROBIAL ACTIVITY IN VITRO

Table 2 shows the results of antimicrobial testing of pABA and Cu(II) complex. Antimicrobial screening revealed the effect of Cu(II) complexes on all tested gram positive bacteria. The largest inhibition zone was recorded with *Enterococcus faecalis* (16 mm) and the smallest in *Staphylococcus aureus* (11 mm). The synthesized complex does not act against gram negative bacteria and *Candida albicans*. In the parent ligand, antimicrobial activity is completely lacking in all bacterial strains and *Candida albicans* at a concentration of  $5\text{ mg mL}^{-1}$ . In comparison with the control antibiotic Ciprofloxacin (conc.  $1\text{ mg mL}^{-1}$ ), significantly less antimicrobial activity of  $\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$  is observed.

**Table 2.** Antimicrobial activities of pABA and  $\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$

Microorganism	Inhibition Zone [mm]	
	1	2
<i>E. coli</i>	-	-
<i>E. faecalis</i>	-	16 (++)
<i>S. aureus</i>	-	11 (+)
<i>B. subtilis</i>	-	13 (+)
<i>L. monocytogenes</i>	-	13 (+)
<i>P. aeruginosa</i>	-	-
<i>C. albicans</i>	-	-

\*Legend: (1) - pABA; (2) -  $\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$

## CONCLUSION

$\text{Cu}(\text{pABA})_2(\text{H}_2\text{O})_2$  complex is formed by interaction of Cu(II) ion with pABA in 1:2 (M:L) molar ratio. In the formation of the bond, oxygen atom of the carboxyl group are involved. There is a difference in the spectral and morphological properties of the complex and the ligand. Complexation of pABA with Cu(II) ion forms a compound that has significant antimicrobial activity on gram positive bacteria as opposed to pABA.

## REFERENCES

- [1] L. Zhonglei, K. Xiangxiang, L. Zhaoming, X. Meixiang, C. Meiyuan, Z. Liang, S. Yuemao, H. Xiangyang, S. Siyang, "Para-Aminobenzoic Acid (PABA) Synthase Enhances Thermotolerance of Mushroom *Agaricus bisporus*", *PLOS ONE*, vol. 9, no. 3, March, 2014. [Online] Available: <https://doi.org/10.1371/journal.pone.0091298>
- [2] C. J. D. Zarafonitis, "Therapeutic possibilities of para-aminobenzoic acid", *Annals of Internal Medicine*, vol. 30, no. 6, pp. 1188-1211, June 1949.

- [3] S. I. Akberova, "New Biological Properties of p-Aminobenzoic Acid", *Biology Bulletin of the Russian Academy of Sciences*, vol. 29, no. 4, pp. 390-393, July 2002.
- [4] C. W. Tabor, M. V. Freeman, J. Baily, P. K. Smith, "Studies on the metabolism of para-aminobenzoic acid", *Journal of Pharmacology and Experimental Therapeutics*, vol. 102, no. 2, pp. 98-102, June 1951.
- [5] R. M. E. Richards, D. K. L. Xing, T. P. King, "Activity of p-aminobenzoic acid compared with other organic acids against selected bacteria", *Journal of Applied Microbiology*, vol. 78, no. 3, pp. 209-215, March 1995.
- [6] E. Horozić, A. Cipurković, Z. Ademović, D. Bjelošević, A. Zukić, L. Kolarević, D. Husejnagić, S. Hodžić, "Synthesis, spectral characterization and antimicrobial activity of some M(II) complexes with Ciprofloxacin", *Journal of Engineering and Processing Management*, vol. 10, no. 2, pp. 16-22, December 2018.
- [7] E. Horozić, A. Cipurković, Z. Ademović, L. Kolarević, D. Bjelošević, A. Zukić, S. Hodžić, D. Husejnagić, M. Ibišević, "Synthesis, characterization and in vitro antimicrobial activity of the Cu(II) and Fe(III) complexes with 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid", *Bulletin of the Chemists and Technologists of Bosnia and Herzegovina*, no. 51, pp. 1-5, December 2018.
- [8] A. Cipurković, E. Horozić, N. Ljubijankić, A. Odošević, S. Galijašević, M. Saletović, "Synthesis and spectral characterization of Fe(II) and Mn(II) complexes with oral fluorouracil pro-drug Capecitabine", *RASAYAN Journal of Chemistry*, vol. 10, no. 4, pp. 1381-1390, October 2017.
- [9] L. Pirvu, C. Hlevca, I. Nicu, C. Bubueanu, "Comparative analytical, antioxidant and antimicrobial activity studies on a series of vegetal extracts prepared from eight plant species growing in Romania", *Journal of Planar Chromatography*, vol. 27, no. 5, pp. 346-356, October 2014.