D. N. Khomenko[1]*, R. A. Doroshchuk[1], V. S. Starova[1], I. V. Raspertova[1], O. V. Severinovskaya[2], R. D. Lampeka[1]

SYNTHESIS AND STUDY OF PROPERTIES OF ZINC COMPLEX WITH 3-(2-PYRIDYL)-5-(3,4,5-TRIMETOXYPHENYL)-1,2,4-TRIAZOLE

[1] *Taras Shevchenko National University of Kyiv, 60 Volodymyrska Str., Kyiv, 01033, Ukraine*
[2] *Chuiko Institute of Surface Chemistry, National Academy of Sciences of Ukraine, 17 General Naumov Str., Kyiv, 03164, Ukraine*
*e-mail: dkhomenko@ukr.net

A procedure has been developed for the synthesis of a zinc complex with chelating ligand 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole. Within the framework of the density functional method, with the B3LYP functional in the SBKJC basis, the equilibrium geometry of the ground electronic state of the ligand molecule and zinc complex was determined. The physicochemical properties of the ligand were also characterized: $\log P = 4.1\pm0.1$, $pK^f_1 = 3.31\pm0.05$, $pK^f_2 = 10.2\pm0.1$, $\varepsilon^{275} \approx 1\cdot10^5 \ \text{l·mol}^{-1}\text{·cm}^{-1}$. It was shown that complexation is accompanied by an increase in fluorescence intensity, for the complex $\lambda^{em}_{max} \approx 458$ nm. The absorption spectrum of the complex is characterized by two bands with maxima at 278 nm ($\varepsilon^{278} \approx 1.3\cdot10^4 \ \text{l·mol}^{-1}\text{·cm}^{-1}$) and 322 nm ($\varepsilon^{322} \approx 1.2\cdot10^4 \ \text{l·mol}^{-1}\text{·cm}^{-1}$).

K e y w o r d s: 1,2,4-triazole, zinc, fluorescence, DFT.

INTRODUCTION. The study of coordination compounds of zinc with chelating ligands is relevant in the context of the development of fluorophores — compounds for measuring the distribution and concentration of metal ions in biological systems [1]. Of the variety of currently available organic reagents that are used for fluorescence determination of microquantities of Zn^{2+} ion, hydrophobic reagents low sensitive to changes in the acidity of the medium over a wide pH range with high values of molar extinction coefficient and quantum yield are the most interesting [2–4].

In our opinion, the previously obtained [5] 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole (**HL**) seems to be a promising fluorophore reagent for the quantitative determination of zinc ions, because it can meet all of the above requirements. The fluorescence properties of this ligand, like most natural compounds that are derivatives of pyrogallol, are due to the rigidity of the structure and the presence of three electron-

donor groups in the phenyl substituent [6, 7]. Additionally, the use of the 3-(2-pyridyl)-1,2,4-triazole system as the chelating part of the ligand molecule makes it possible for its complexes to be in the deprotonated form, which increases the stability of the latter [8].

This paper presents the synthesis and study of the physicochemical properties of a zinc complex compound based on 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole.

EXPERIMENT AND DISCUSSION OF THE RESULTS. For the synthesis of complexes, $Zn(CH_3COO)_2$ (Merck) was used. 3-(2-pyridyl)-5-(3,4,5-tri-methoxyphenyl)-1,2,4-triazole was prepared according to the previously described procedure [5] from 2-cyanopyridine and 3,4,5-trimethoxybenzoic acid hydrazide .

$Zn_2(L)_4 \cdot 6CH_3OH$. To a solution of $Zn(CH_3COO)_2$ (0.091 g, 0.5 mmol) in 10 ml of CH_3OH a solution of HL (0.312 g, 1 mmol) in 50 ml of CH_3OH was added. With slow crystallization for two days at room temperature, colorless crystals precipitated from this solution. They were filtered off, washed with methanol and dried in air. The yield of $Zn_2(L)_4 \cdot 6CH_3OH$ was 76 %. Calculated for $C_{70}H_{84}N_{16}O_{18}Zn_2$ (%): C 53.6; H 5.4; N 14.3. Found: C 53.8; H 5.8; N 14.2.

NMR spectra were recorded on a Varian Mercury 400 instrument (400 MHz). To measure the chemical shift in the PMR spectra, the central signal of the residual protons DMSO-d_6 (δ = 2.503 ppm) was used as a secondary standard in DMSO-d_6.

When studying the properties of HL and the zinc complex based on it, their working solutions were prepared by dissolving the corresponding exact weights in dimethyl sulfoxide (DMSO). This organic solvent was of spectroscopic purity (Merck).

The study of the acid-base properties of the ligand was carried out by potentiometric titration using a pH meter (pH-340) with a glass electrode ESL-43-07. Titration of the protonated form of 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole was carried out with a standardized NaOH solution in a mixture of water-DMSO (ω_{H2O}:ω_{DMSO} = 10:90). Based on the pH metric titration curves in Hyperquad 2000, the values of the formal dissociation constants of this ligand were calculated.

The ligand distribution coefficient in the ($\log D_{7.4}$) water-octanol extraction system was determined at pH 7.4 (phosphate buffer) using the formula $\log D_{7.4} = C_o/C_w$. The ligand concentration in the aqueous phase (C_w) was determined spectrophotometrically by addition technique, registering the optical density at the maximum of the absorption band; and the ligand concentration in the octanol phase was determined by the difference in the substance content in the solution before extraction and the C_w. Distribution constant was calculated by the formula: $\log P = \log D_{7.4} + \log?_{10}(1 + 10^{(pKa-pH)})$.

The complexation of the ligand with Zn^{2+} ions was studied spectrophotometrically. The ligand and complex spectra were recorded using a 2800 UV/Vis spectrophotometer (Unico, USA). The comparison solution was DMSO-water (ω_{H2O}:ω_{DMSO} = 10:90), the thickness of the cuvette was 1 cm.

The fluorescence spectra of HL solutions and its complex were recorded using LS 55 luminescent spectrophotometer (Perkin–Elmer, UK) at optimal wavelengths selected for each compound minus the background of the solvent. Spectra were recorded in the wavelength range from 200 to 800 nm.

The frequency of data collection was 0.5 nm. The error in determining the wavelength of the maximum fluorescence intensity did not exceed 1 nm. The sizes of the cuvette, excitation and emission gaps were 1 cm, 10 and 20 nm respectively.

MALDI mass spectra were obtained at the CCDU of Chuiko Institute of Surface Chemistry of the National Academy of Sciences of Ukraine by the method of matrixless laser desorption/ionization using the "Autoflex II" device (Bruker Daltonics, Germany). The experiments were carried out in the reflex mode of positive and negative ions registration in the mass range 0–1500 m/z. For mass spectroscopic experiments, aliquots of 1 µl of methanol solutions of the studied compounds with a concentration of 1 mg/ml were applied to a standard steel substrate and left to dry completely, after which they were placed in the working area of the device. Data was processed using Flex Analysis software (Bruker Daltonics, Germany).

The equilibrium geometry of the studied compounds in the singlet electronic state was optimized by the density functional method (DFT) using the B3LYP hybrid functional [9, 10] in the SBKJC basis [11]. All calculations at the DFT theory level were performed within the framework of the Firefly v.8.1.1 software package [12].

3-(2-Pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole is characterized by high hydrophobicity ($\log P = 4.1 \pm 0.1$) and is practically insoluble in water, therefore, further study of its physicochemical properties were carried out in water–DMSO medium (ω_{H2O}: $\omega_{DMSO} = 10:90$). Based on the obtained titration curves of this ligand in a water–

DMSO solution, it was found that the exponent of its first formal dissociation constant (pK^f_1) is 3.31 ± 0.05. This value is responsible for the dissociation of protonated nitrogen in the pyridine ring. Dissociation of the N–H in triazole fragment occurs in a strongly alkaline medium at pH > 10 (the found value of $pK^f_2 = 10.2 \pm 0.1$).

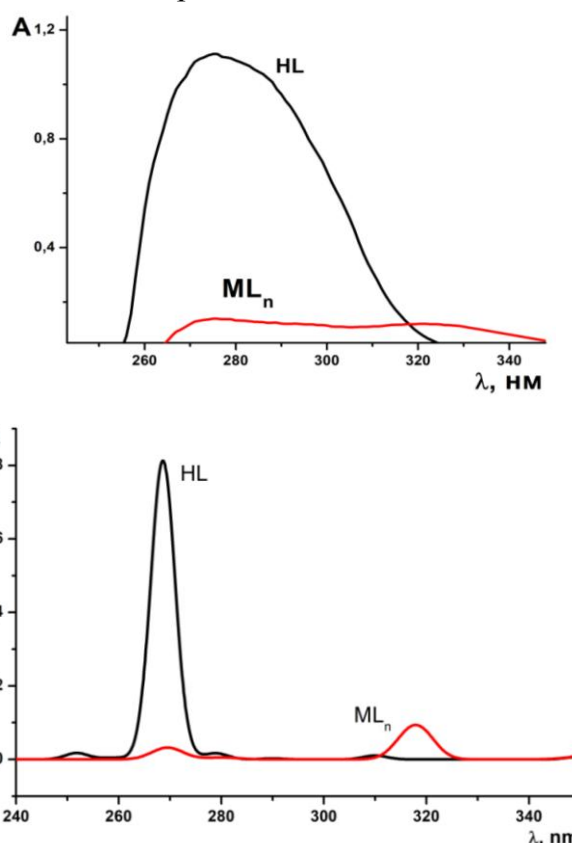

Fig. 1. Absorption spectrum of 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole and its complex with Zn^{2+}. $C = 1 \cdot 10^{s5}$ M, pH 2.5. (top – experimental, bottom - calculated).

The absorption spectrum of 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole is characterized by one intense band with a maximum at 275 nm, which corresponds to π-π* transitions inside the ligand chromophore system (fig. 1). The molar ex-

tinction coefficient of the ligand is ε^{275} $\approx 1 \cdot 10^5$ l·mol^{-1}·cm^{-1}. The complexation of
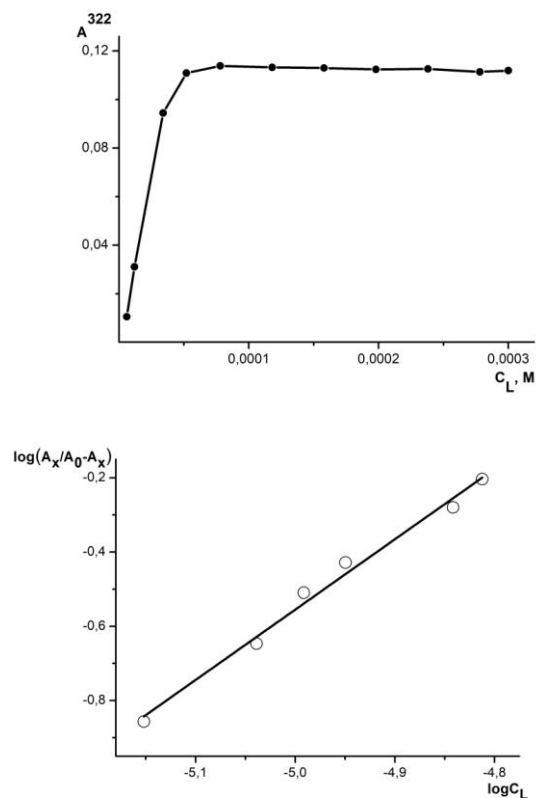

Fig. 2. Graphical determination of the composition and stability of the complex 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole with Zn (II) by the equilibrium shift method. $C_{Zn2+} = 5 \cdot 10^{-6}$ M, $C_{HL} = 0$–$3 \cdot 10^{-4}$ M, pH 2.5. The linear regression equation: $\log(A_x/A_0 - A_x) = (8.9 \pm 0.5) + (1.9 \pm 0.1) \cdot \log C_L$; $r^2 = 0.987$, where A_x is the optical density at a given point of the saturation curve, A_0 is the optical density at which full binding of the central ion to the complex is achieved, C_L is the total concentration of the ligand.

3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1, 2,4-triazole with Zn^{2+} ions is accompanied by a slight bathochromic shift of this band ($\Delta\lambda \approx 4$ nm) and the appearance of another absorption band in the long-wa-

velength region of the spectrum $\lambda_{max} = 322$ nm (fig. 1), which appears due to electron transfer from the ligand to the central ion. The molar absorption coefficients of these bands are: $\varepsilon^{278} \approx 1.3 \cdot$ l·mol^{-1}·cm^{-1} and $\varepsilon^{322} \approx 1.2 \cdot 10^4$ l·mol^{-1}·c^{-1}, respectively.

In the absorption spectrum of the ligand, calculated in the vacuum approximation, there is one absorption band with a maximum at 268 nm. In the calculated absorption spectrum of the zinc complex, two bands are observed at 270 and 318 nm. It should be noted that the calculated curves of the absorption spectra in the form of intensity agree well with the experimental data.

Using the equilibrium shift method, it was shown that in the presence of an excess amount of ligand in a DMSO–water solution, a coordination compound with M:L ratio of 1:2 is formed (fig. 2). The logarithm of the stability constant of this complex is approximately 8.9±0.5.

The fluorescence spectra of the HL solution in DMSO show an intense band with a maximum at 454 nm, and in the excitation spectrum — at 294 nm (fig. 3).
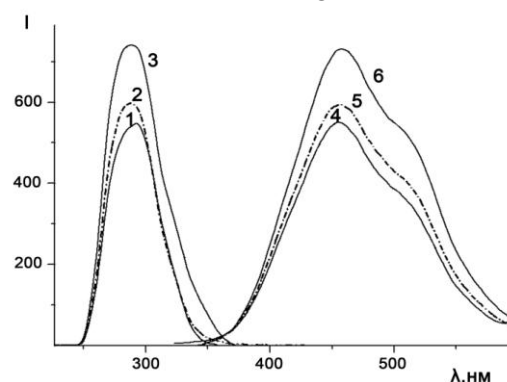

Fig. 3. Excitation (*1–3*) and fluorescence (*4–6*) spectra of solutions of ligand (*1, 4*) and complex (*2, 3, 5, 6*) in DMSO–water. $C_{ligand} = 3 \cdot 10^{-7}$ M (*1, 4*), $C_{complex}$ (M) = $1.5 \cdot 10^{-7}$ (*2, 5*), $3 \cdot 10^{-7}$ (*3, 6*).
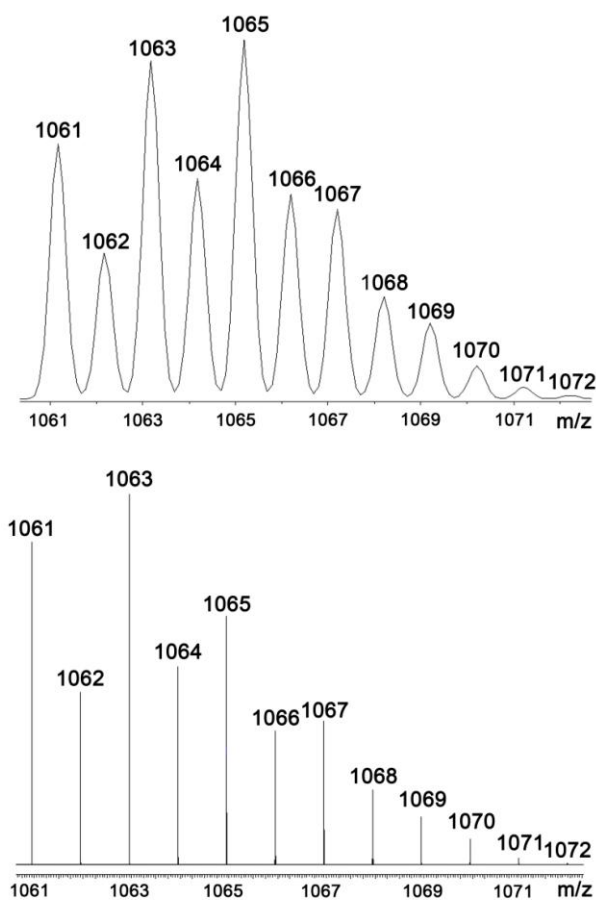
Fig. 4. The mass spectrum registered in MALDI (a) and the calculated (b) isotopic pattern of the molecular ion $[Zn_2(L)_3]^+$

Also, a slight bathochromic shift of the fluorescence spectrum of the complex with respect to the ligand fluorescence spectrum ($\Delta\lambda = 4$ nm) is observed. The maximum fluorescence intensity of the complex in the excitation spectra is observed at 287 nm, in the emission spectra — at 458 nm.

In the mass spectrum of the zinc complex, a small number of low-intensity peaks with masses of 218, 334, and 396 Da are observed. The peak at 334 Da corresponds to the formation of a quasimolecular ion for-med by a ligand molecule and a sodium cation $[HL+Na]^+$. In addition, in the mass spectrum there is a more intense peak with a mass of 1061 Da (fig. 4). The isotopic pattern corresponding to this peak indicates the presence of two zinc atoms and three ligands in the molecular ion — $[Zn_2(L)_3]^+$. The presence of such a molecular ion in the mass spectrum indicates that the complex contains a binuclear fragment, which with a most likely has the composition $Zn_2(L)_4$.

The ^{1}H NMR spectrum of the stu-died coordination compound in the DMSO-d_6 solution has a number of features that distinguish it from the ligand spectrum (fig. 5). First, in the spectrum of the complex there is no signal of the triazole proton, as well as signals of the methyl group protons from the acetate ion.
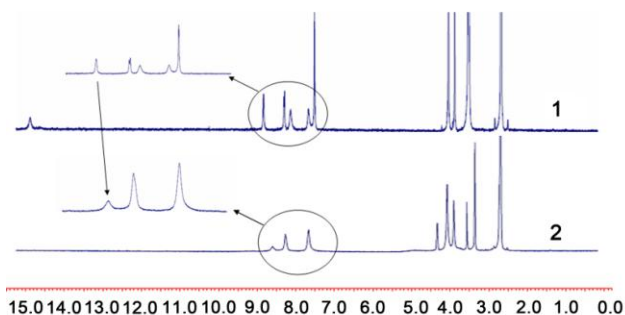

Fig. 5. ^{1}H NMR spectrum of HL (*1*) and $Zn_2(L)_4 \cdot 6CH_3OH$ (*2*) (spectra measured in DMSO-d_6). The arrow indicates the signal of the α-pyridine proton

This indicates that the ligand is included in the complex in deprotonated form, resulting in the formation of a molecular complex. The second difference is a noticeable

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broadening of proton signals in the ^{1}H NMR spectrum of the complex, compared with the spectrum of an uncoordinated ligand, which is typical for zinc complexes with derivatives of 3-(2-pyridyl)-1,2,4-triazole and, most likely, is due to complex lability [13]. Thirdly, the signal of the α-pyridine proton in $Zn_2(L)_4 \cdot 6CH_3OH$ is at 8.4 ppm, while in HL the signal of this proton is at 8.7 ppm. The difference in 0.3 ppm means the coordination of the ligand through the nitrogen of the pyridine ring. Based on the foregoing, we can make an unambiguous conclusion about the bidentate-chelate coordination of the ligand by zinc ion.
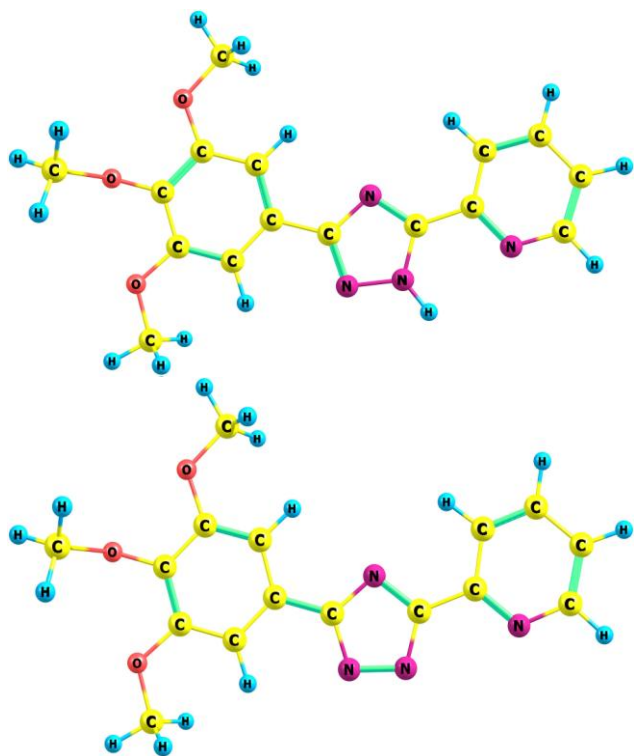

Fig. 6. Equilibrium geometry of 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole (*1*) molecule and anion (*2*) calculated using the DFT method with B3LYP functional in SBKJC basis

It should be noted that methanol proton signals are also present in the ^{1}H NMR spectrum. Based on their integrated intensity, the results of CHN analysis and the results of mass-spectrometric studies for the resulting complex, we can propose the following composition — $Zn_2(L)_4 \cdot 6CH_3OH$.

The calculation of the ligand ground state structure by the DFT method showed that the calculated geometry agrees well with the experimental data of X-ray diffraction analysis [14]. Fig. 6 shows the calculated equilibrium configurations of HL and L^-. For the ligand and its anion, the total energies of the molecules were calculated, which amounted to −187.1293 a.u. and −186.5783 a.u. Based on these data, the energy of deprotonation of the ligand is 0.551 a.u (1446.65 kJ/mol).

Based on the assumption of binuclear structure of the zinc complex, the equilibrium configurations and total energies for particles $(Zn(HL)_2)^{2+}$ ($E = 600.0003$ a.u), ZnL_2 ($E = −599.2812$ a.u) and Zn_2L_4 ($E = −1198.5947$ a.u) were calculated (fig. 7). Dimerization energy according to the formula $\Delta E = \sum E_{full(reaction\ products)} - \sum E_{full(reagents)}$ is −0.0323 a.u (−84.79 kJ/mol).

It is known that the HOMO energy describes electron-donor properties, while the LUMO characterizes electron-acceptor properties. The HOMO–LUMO orbital schemes for the ligand and inc complex are presented in fig. 8. In the ligand, the electron density of the HOMO is localized on 3,4,5-trimethoxybenzene and partially triazole fragments. At the same time, LUMO is localized on pyridine and partially triazole fragments. A similar picture is observed for the complex. The difference is that the
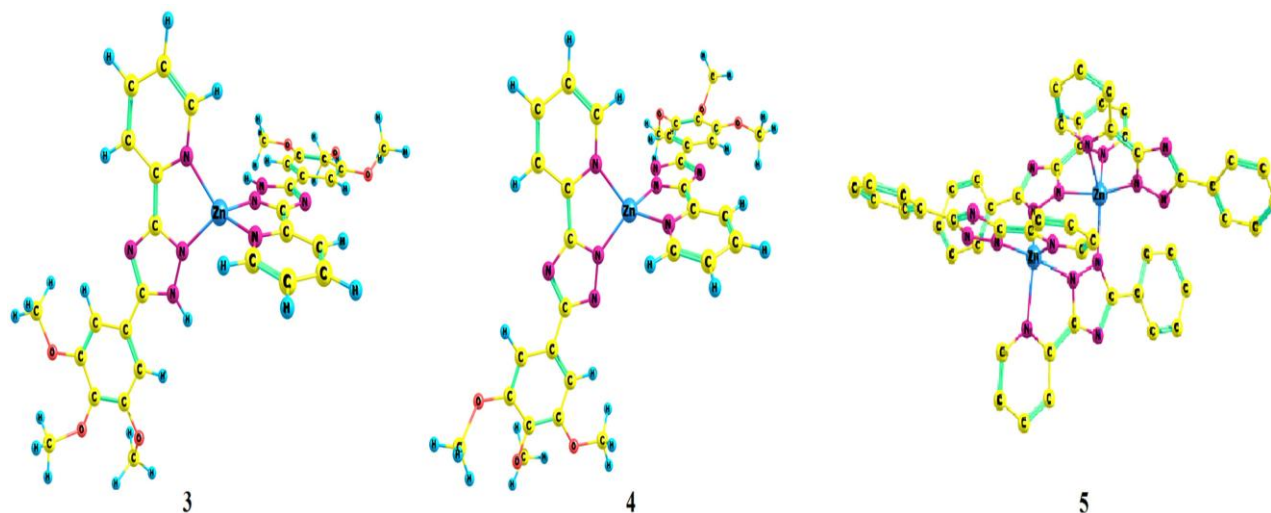
Fig. 7. Equilibrium geometry $(Zn(HL)_2)^{2+}$ (*3*), ZnL_2 (*4*) and Zn_2L_4 (*5*) calculated using the DFT method with the B3LYP functional in the SBKJC basis. (Hydrogen atoms and methoxy groups for *5* are not shown to simplify the figure)

localization of electron density is observed in two "separate" ligand molecules. The widths of the energy gaps for the ligand and complex are 3.73 and 3.31 eV, respectively.
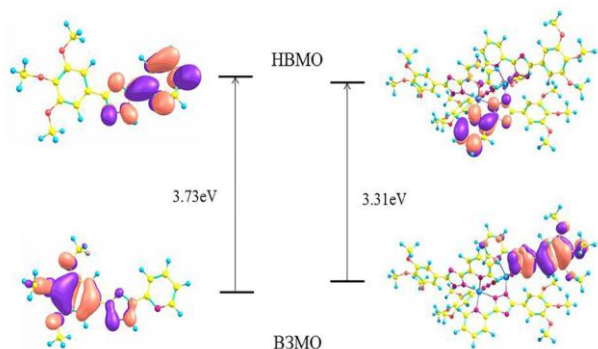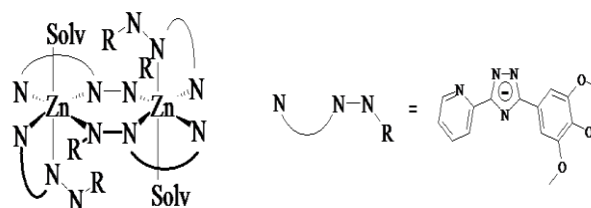

Fig. 8. Diagram of the boundary molecular orbitals for L and Zn_2L_4

Based on the totality of the obtained data, we proposed the structure of the $Zn_2(L)_4$·6CH$_3$OH complex in which the ligands are in bidentate-chelate coordination and only two of them are bridged:

CONCLUSION. It was found that 3-(2-pyridyl-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole is a highly hydrophobic ligand, which in a wide pH range (4–10) is in molecular form, characterized by high molar absorption coefficient and intense fluorescence. As a result of the complexation of this ligand with zinc ions, a bidentate chelate complex $Zn_2(L)_4$·6CH$_3$OH is formed, which also exhibits intense fluorescence at $\lambda^{em}_{max} \approx$ ≈ 458 nm. Based on the density functional method, the equilibrium geometry of the ground electronic state of both the ligand and the zinc complex is determined. Such physicochemical properties of the ligand and

its complex with zinc confirm the prospects of using 3-(2-pyridyl)-5-(3,4,5-trimethoxyphenyl)-1,2,4-triazole as an analytical reagent for determining the trace amounts of zinc ions.

СИНТЕЗ ТА ДОСЛІДЖЕННЯ ВЛАСТИВОСТЕЙ КОМПЛЕКСУ ЦИНКУ З 3-(2-ПІРИДИЛ)-5-(3,4,5-ТРИМЕТОКСИФЕНІЛ)-1,2,4-ТРИАЗОЛОМ

Д. М. Хоменко[1]*, Р. О. Дорощук[1], В. С. Старова[1], І. В. Распертова[1], О. В. Севериновська[2], Р. Д. Лампека[1]

[1] Київський національний університет імені Тараса Шевченка, вул.°Володимирська, 60, Київ, 01033, Україна
[2] Інститут хімії поверхні ім. О. О. Чуйка НАН України, вул. Генерала Наумова, 17, Київ, 03164, Україна
*e-mail: dkhomenko@ukr.net

Розроблено методику синтезу комплексу цинку з хелатуючим лігандом 3-(2-піридил)-5-(3,4,5-триметоксифеніл)-1,2,4-триазолом. Методом функціоналу густини з фунціоналом B3LYP в базисі SBKJC визначено рівноважну геометрію основного електронного стану молекули ліганду та комплексу цинку. Також охарактеризовано фізико-хімічні властивості ліганду: $\log P = 4.1\pm0.1$, $pK^f_1 = 3.31\pm0.05$, $pK^f_2 = 10.2\pm0.1$, $\varepsilon^{275} \approx 1\cdot10^5$ л·моль$^{-1}$·см$^{-1}$. Показано, що комплексоутворення супроводжується збільшенням інтенсивності флуоресценції, для комплексу $\lambda^{em}_{max} \approx 458$нм. Спектр поглинання комплексу характеризується двома смугами з максимумами при 278 нм ($\varepsilon^{278}\approx1.3\cdot10^4$ л·моль$^{-1}$·см$^{-1}$) та 322 нм ($\varepsilon^{322}\approx 1,2\cdot10^4$ л·моль$^{-1}$·см$^{-1}$).

К л ю ч о в і с л о в а: 1,2,4-триазол, цинк, флуоресценція, теорія функціоналу.

СИНТЕЗ И ИССЛЕДОВАНИЕ СВОЙСТВ КОМПЛЕКСА ЦИНКА С 3-(2-ПИРИДИЛ)-5-(3,4,5-ТРИМЕТОКСИФЕНИЛ)-1,2,4-ТРИАЗОЛОМ

Д.Н. Хоменко[1]*, Р.А. Дорощук[1], В.С. Старова[1], И.В. Распертова[1], О.В. Севериновская[2], Р.Д. Лампека[1]

[1] Киевский национальный университет имени Тараса Шевченка, ул. Владимирская, 60, Киев, 01033, Украина
[2] Институт химии поверхности им. А.А. Чуйко НАН Украины, ул. Генерала Наумова, 17, Киев, 03164, Украина
*e-mail: dkhomenko@ukr.net

Разработана методика синтеза комплекса цинка с хелатирующим лигандом 3-(2-пиридил)-5-(3,4,5-триметоксифенил)-1,2,4-триазолом. В рамках метода функционала плотности, с функционалом B3LYP в базисе SBKJC, определена равновесная геометрия основного электронного состояния молекулы лиганда и комплекса цинка. Также охарактеризованы физико-химические свойства лиганда: $\log P=4.1\pm0.1$, $pK^f_1= 3.31\pm0.05$, $pK^f_2= 10.2\pm0.1$, $\varepsilon^{275}\approx 1\cdot10^5$ л·моль$^{-1}$·см$^{-1}$. Показано, что комплексообразование сопровождается увеличением интенсивности флуоресценции, для комплекса $\lambda^{em}_{max}\approx 458$ нм. Спектр поглощения комплекса характеризуется двумя полосами с максимумами при 278 нм ($\varepsilon^{278}\approx 1,3\cdot10^4$ л·моль$^{-1}$·см$^{-1}$) и 322 нм ($\varepsilon^{322}\approx 1,2\cdot10^4$ л·моль$^{-1}$·см$^{-1}$).

К л ю ч е в ы е с л о в а: 1,2,4-триазол, цинк, флуоресценция, теория функционала плотности.

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