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COMPLEXES OF Nd(III) AND 3d-METALS BASED ON ETHYLENEDIAMINEDISUCCINIC ACID AS POTENTIAL ANTIFUNGAL AGENTS.

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Neodymium heterometallic complexes $[(NdM^{II}EDDS)(H_2O)_6]\cdot n2H_2O$ (M^{II}=Zn, Co; n = 3; 2) were synthesized by the «block» synthesis method using protonated ethylenediaminedisuccinate of the 3-d metal and Nd^{III} nitrate. The complexes were characterized by spectroscopic methods (UV-VIS electronic absorption spectroscopy and FT-IR) and elemental analysis. It is shown that the f-d-complexes belong to the «folded» type complexes, in which the ligand-EDDS realizes the maximum dentateness to Nd^{III}, and the coordination sphere of the 3-d cation is formed by chain carboxyl groups of EDDS and intraspherical water molecules. At the same time, the cations of 3d metals are in a distorted octahedral environment, and the coordination polyhedron of the neodymium ion corresponds to a square antiprism (C_{4v}) with the coordination number Nd^{III} = 8. In solutions and in the solid state, the complexes have the same type of structure. The sensitivity of various morphological forms of *Candida albicans* in Spider and Saburo media to neodymium complexes Nd^{III} with ethylene diamine disuccinate: NdEDDS (**I**), NdEDDSZn (**II**) NdEDDSCo (**III**) in the range of concentrations of the studied compounds 1; 10 and 100 μM was studied. It is shown that the antifungal properties of the complexes vary in the range NdEDDSCo> NdEDDSZn ≥ NdEDDS. The inhibition index of *C. albicans* in the composition of the biofilm in the Saburo medium under the action of the complexes was 20–25% of the control value, and in the Spider medium the complexes led to 95% of cell death.

Keywords: ethylenediaminedisuccinates complexes, neodymium, cobalt (II), zinc (II), antifungal properties.

INTRODUCTION. In recent decades, studies of lanthanide (Ln) complexes have shown that they play an important role in several biochemical reactions [1, 2], and are widely used in medical diagnostics as fluorescent labels for biomolecular sensing, for the diagnosis and therapy of cancer, and also as biosensors [3–7]. Cerium group lanthanides (La, Ce, Pr, Nd), primarily used in biology, perform chemical actions similar to other biologically useful metals and do so more efficiently due to their higher Lewis acidity. Ln have a high coordination ability, allowing selective absorption, transport and incorporation into enzymes similar to biogenic metals, especially Ca^{II} and Fe^{III} [8].

Luminescent ions of some lanthanides are used as labels that help to study the distribution of drugs and metabolites *in vivo* [9]. Although lanthanides are not classified as bimetals, having great similarity with calcium, they can replace it in biological systems. For example, on the basis of Ln complexes, drugs are created that regulate calcium metabolism in the body, for example, blood anticoagulants [10, 11]. Also, based on Ln, preparations have already been developed that can affect calcium metabolism in the body and inhibit the development of tumor cells. The ionic radii of lanthanides are close to the ionic radius of Ca^{2+}, but due to their higher charge (Ln^{3+}), lanthanide ions are characterized by a high affinity for Ca^{2+} sites in biological molecules, which determines their ability to block the corresponding channels. Thus, although Ln^{3+} ions alone cannot penetrate cell membranes, they can block the outer side of the calcium channel [12, 13]. Complex compounds of polyaminocarboxylic acids with lanthanides and iron can be used in the therapy of oncological diseases, immunodeficiencies and blood diseases. Nitrates $[LnL(NO_3)_2(H_2O)_2](NO_3)$, (Ln = La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Er) with polydentate Schiff bases (L = N,N-bis(2-hydroxy-1-naphthylidene)-1,6-hexadimine), as well as hydrazine complexes of 3,3'-thiodipropanoates $[M(N_2H_5)(tdp)_2]$ (M = La, Ce, Sm, Pr, Nd) by *in vitro* evaluation demonstrated high antimicrobial activity against six bacteria (*Bacillus cereus, Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Escherichia coli, Serratia species*) and four fungi (*Candida albicans, Aspergillus niger, Aspergillus fumigatus, Penicillium variance*) [14–16].

The review [17] analyzed a large number of studies devoted to the antimicrobial activity of Ln^{III} complexes. A fairly wide range of free organic ligands were found to be inactive against various types of bacteria (except *E. coli*), while complexes La^{III}, Pr^{III}, Sm^{III}, $Tb^{III}$and Yb^{III}- showed some inhibitory activity against them. It was concluded that the increase in the activity of the complexes compared to free ligands can be due to the effect of metal ions on the cell. Moreover, most Ln complexes are more effective against gram-positive microorganisms, in particular *Staphylococcus aureus*, than against gram-negative ones, primarily *Escherichia coli*. Comparing the effect on gram-positive microorganisms of free ligands and corresponding complexes with lanthanides, it was determined that the activity of the latter is 15–27% higher. Based on the analysis, the authors concluded that Ln complexes represent a possible alternative to antimicrobial agents currently used. The use of nanomaterials based on lanthanides in biomedicine has increased the likelihood of their use *in vivo* [18].

Biocoordination compounds of metals as medical and biological agents form the basis of modern socially and technologically sig-

nificant innovative developments. Combining metal ions with an organic compound enables the production of supramolecular associates, which are inherently close to the endogenous coordination compounds that exist in the living organism and in general in biosystems. They are less toxic than their constituents and usually have a wide range of biological activity. The most promising chelating ligands are aminopolycarboxylic acids containing carboxyl (–C(O)O-) and amino (NH-) groups, which makes it possible to obtain metal-containing compounds in which metal ions are bound to polydentate ligands using electron-donating atoms (oxygen and nitrogen) with the formation of heterocyclic rings (metal chelate rings). Among the wide variety of aminopolycarboxylic acids, a special place is occupied by ethylenediamine-N, N-disuccinic acid (EDDS), which contains fragments of aspartic and succinic acids in the molecule, known as adaptogens. In a living organism, it performs not only the function of delivering microelements but also carries a biologically active load. Under the action of enzymes in a living organism, the organic part of the metal complex MEDDS breaks down into essential amino acids (arginine, leucine, isoleucine, valine, histidine, asparagine, alanine), which are components of the metabolic chain [19].

For example, complexes of metals with ethylenediaminedisuccinic acid has antimicrobial activity against fungi and bacteria [20] and antiviral activity against cytomegalovirus [21]. *In vitro* ethylenediaminedisuccinic acid inhibited viral replication in mice (EC_{50} 8.6 mg/ml) and reduced their mortality. The introduction of EDDS may be an important immunopathological factor in terms of modulating the response to cytomegalovirus infections. Ethylenediaminesuccinic acid is one of the effective zincophores because it has a relatively high affinity for removing zinc ions. Zincophores are distinct from ionophores that transport zinc across the membrane, such as pyrithione, which forms a neutral ZnL_2 complex. Zincophores are commonly found in bacteria that need to acquire Zn^{2+} in environments with low zinc concentrations or high competition for zinc [22.] A promising group of antimicrobial compounds are metal pharmaceuticals based on aminocarboxylate complexes of 3-d metals, which are included in biomolecules (amino acids, proteins, oligonucleotides or DNA) and can interact with them [23]. Recently, interest in cobalt coordination complexes has increased due to their antimicrobial and antitumor properties [24].

Thus, taking into account that complexes of metals with ethylenediaminedisuccinic acid have antimicrobial activity against fungi and bacteria [19], the aim of this study was the synthesis of new homo- and heterometallic ethylenediaminedisuccinate complexes of Nd^{III} and 3-d metals (Co^{II}, Zn^{II}) and the study of their antimicrobial activity by in relation to bacterial strains of *Candida albicans* cultures.

EXPERIMENT AND DISCUSSION OF THE RESULTS.

Neodymium nitrates $Nd(NO_3)_3 \cdot 6H_2O$ and zinc $Zn(NO_3)_2 \cdot 6H_2O$ or cobalt $Co(NO_3)_2 \cdot 6H_2O$ were used as starting compounds for the synthesis of homo- and heterometallic complexes of Nd^{III} with ethylenediaminesuccinic acid. Ethylenediaminediasuccinic acid was obtained by the condensation reaction of maleic acid with ethylenediamine [25]. All reagents were of analytical grade and were used without further purification. The strain *Candida albicans* ATCC 18804 was obtained from the Museum

of Cultures of Microorganisms, Department of Microbiology, Virology and Biotechnology, Odesa National University named after I.I. Mechnikov (Ukraine). Solutions of salts and H_4EDDS of the required concentration (0.01 mol/dm^{-3}) were obtained by dissolving the appropriate samples of reagents in bidistilled water. Solutions were prepared immediately before synthesis. The concentration of metals in the solutions was determined by complexometric titration with the indicators murexide (for 3-d metal ions) and arsenazo 1 (for Nd^{3+} ions).

Neodymium ethylenediaminedisuccinate homonuclear complex was obtained according to the method [26]. The synthesis of heterometallic complexes (HMC) in the Nd^{III}-M^{II}EDDS (M^{II}=Zn, Co) systems was carried out by the «block» method, which consists in sequential complexation first with one and then with the second metal. Homonuclear complexes of 3-d metals obtained by the interaction of aqueous solutions of EDDS and d-metal salts in an acidic medium were used as a «building block». Heterometallic complexes were obtained without separating the «building block» from the solution [26, 27].

The study of complexes in aqueous solutions was carried out by the methods of UV-VIS electronic absorption spectroscopy (EAS) at an equimolar ratio of components and $C_M = 1 \cdot 10^{-2}$mol/dm^{-3}. Electronic spectra were recorded on a spectrophotometer Specord M·40 in the range of 50,000–10,000 cm^{-1}. The pH value was monitored using a pH meter pH – 150 MA.

In fig. 1, as an example, the UV-VIS spectra of hono- and heterometallic ethylenediaminedisuccinate complexes of Nd^{III} is given, and in Table. 1 position of the main absorption bands of the Nd^{III} ion in the spectra of the complexes.

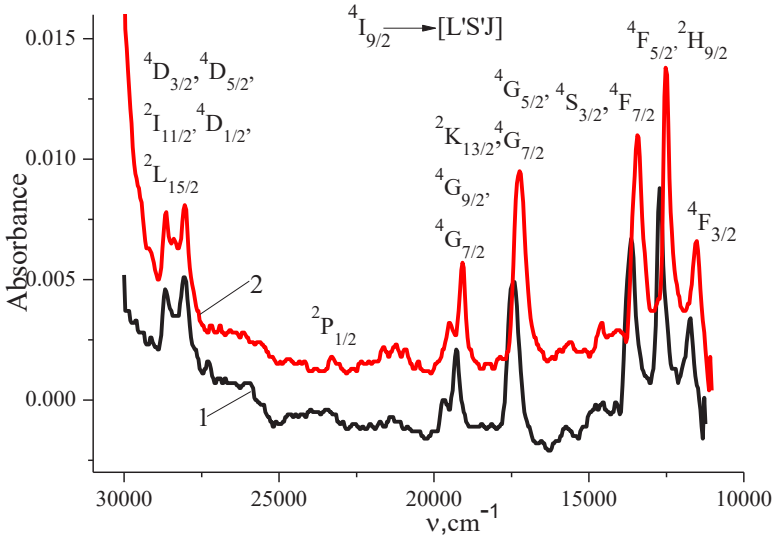


Fig. 1. – UV-VIS spectra of homo- (1) and heterometallic (2) complexes of Nd^{III} and Zn^{II} with H_4EDDS (pH 7; $C_{Nd} = C_{Zn} = C_{EDDS} = 1 \cdot 10^{-2}$ mol/dm^3).

The UV-VIS spectra of heterometallic complexes of Nd^{III} with H_4EDDS contain a set of bands corresponding to *f-f* transitions of the Nd^{3+} ion from the ground state $^4I_{9/2}$ to multiplets of excited levels [L'S'J] (Table 1). The absorption band maxima of HMC are shifted to longer wavelengths compared to the monometallic complex ($\Delta\nu_{max} \approx$ 90–250 cm^{-1} for NdZnEDDS and ≈ 60–190 cm^{-1} for NdCoEDDS). In this case, the absorption intensity increases significantly. This indicates both the formation of heterometallic metal complexes and a different coordination environment of complex-forming ions (in particular, 3-d metal ions) in a heteronuclear complex compared to mononuclear ones. In this case, the absorption intensity increases significantly. This indicates both the formation of heterometallic metal complexes and a different coordination environment of complex-forming ions (in particular, 3-d metal ions) in a heteronuclear complex compared to mononuclear ones. For both heteronuclear neodymium complexes, the positions of the supersensitive transitions $^4I_{9/2} \rightarrow {}^2P_{1/2}$ and $^4I_{9/2} \rightarrow {}^4G_{5/2,}\ {}^2G_{7/2}$ are in a lower frequency range compared to the corresponding bands in the spectrum of the NdEDDS complex.

Table 1.

Transition energies (cm^{-1}) in UV-VIS spectra of Nd^{III} and M^{II} (M^{II}=Co, Zn) complexes with ethylenediaminedisuccinic acid.

Transition	NdZnEDDS	ν_{NdL}-ν_{NdZnL}	NdEDDS	NdCoEDDS	ν_{NdL}-ν_{NdCoL}
$^4I_{9/2}\rightarrow{}^2P_{1/2}$	23254	216	23470	23358	112
$^4I_{9/2}\rightarrow{}^4G_{9/2}$	19502	186	19688	19537	151
$^4I_{9/2}\rightarrow{}^4G_{7/2}$	19017	247	19264	19113	151
$^4I_{9/2}\rightarrow{}^4G_{5/2}$	17196	255	17451	17261	190
$^4I_{9/2}\rightarrow{}^4F_{9/2}$	14621	91	14781	14712	69
$^4I_{9/2}\rightarrow{}^4F_{7/2}$	13388	242	13630	13440	190
$^4I_{9/2}\rightarrow{}^4F_{5/2}$	12475	268	12743	12588	155
$^4I_{9/2}\rightarrow{}^4F_{3/2}$	11567	159	11726	11549	177

The $^4I_{9/2}\rightarrow{}^2P_{1/2}$ transition is a singlet, which indicates the existence of one type of connection. However, the bathochromic shift of the maximum of this band in the spectrum of HMC (Fig. 2) relative to the monometallic complex ($\Delta\nu_{NdZnEDDS}$ = 216 cm^{-1}, $\Delta\nu_{NdCoZnEDDS}$ = 112 cm^{-1}) indicates a decrease in the coordination number of Nd^{III} in hetero-complexes.

The general pattern of shifts of the main maxima of the f-f-transitions of the Nd^{3+} ion in the synthesized heterometallic complexes compared to monometallic ones is as follows (Fig. 2).

The absorption bands of the Nd^{III} ion at ≈.17200 cm^{-1} and 191200 cm^{-1} belong to the transitions $^4I_{9/2} \rightarrow {}^4G_{5/2,}\ {}^2G_{7/2}$, respectively, and from their shape and position one can determine the symmetry of the coordination environment of the central atom. In the absorption spectra of NdM^{II}EDDS, the half-width of the supersensitive transitions band $^4I_{9/2} \rightarrow {}^4G_{5/2,}\ {}^2G_{7/2}$ is +172 (+174) cm^{-1}, and the band itself does not undergo splitting, which is typical for compounds with low symmetry [28, 29]. Since ethylenediaminedisuccinic acid is a fairly bulky ligand,

no significant splitting of the spectral lines is observed. It should be noted that the position of the absorption bands of the *f-f* transitions of the Nd^{3+} ion, as well as the shape of the spectra, indicate the formation of an eight-coordinated complex of C_{4v} symmetry, and the Nd^{III} coordination polyhedron corresponds to a square antiprism.

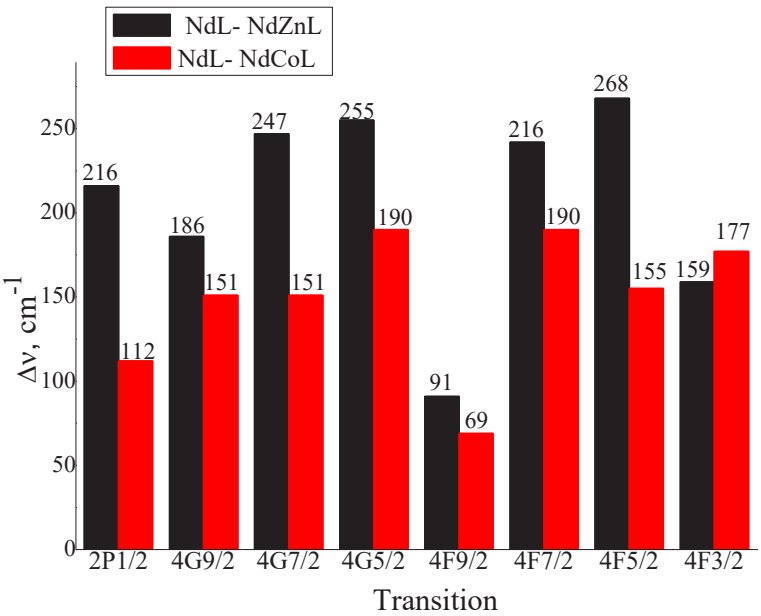


Fig. 2 – Diagram of shifts (cm^{-1}) in the UV-VIS spectra of heterometallic complexes Nd^{III} with ethylenediaminedisuccinic acid compared to NdEDDS.

In both heterometallic complexes, the Nd^{3+} ion coordinates two nitrogen atoms, three oxygen atoms of the EDDS molecule and three water molecules. The 3-d metal ions have a distorted octahedral $M^{II}O_6$ environment formed by three oxygen atoms of the bridging β-carboxyl groups of EDDS and three water molecules. That is, the NdM^{II}EDDS complex is a «folded» type complex, in which 3d-metal complexes play the role of exo-coordinated ligands during the formation of heterometallic complexes. It should be noted that the larger bathochromic shift of the *f-f* transition maxima in the NdZnEDDS complex compared to the NdCoEDDS complex is associated with less distortion of the octahedron due to the filled $3d^{10}$ zinc shell, which determines its resistance to various deformations.

The synthesized NdM^{II}EDDS heterometallic complexes were isolated from solutions using the isothermal method (salting out with ethanol from aqueous solutions) at pH 7.5, which corresponds to the maximum accumulation of deprotonated complexes according to the distribution diagrams [26, 27]. Identification and purity of HMC was established by elemental analysis and IR spectroscopy. The elemental analyzes for Carbon, Hydrogen and Nitrogen were obtained on microanalyses Perkins – Elmer 2400. FT-IR spectra were recorded on a spectrophotometer Specord M-80 in the region of 4000–400 cm^{-1} in tablets with KBr.

NdZnEDDS: $NO_3[NdZn(C_{10}N_2O_8H_{12})\cdot 6H_2O]\cdot 3H_2O$, found/calculated (%): Nd – 19,84/19,86; Zn – 8,95/8,96; C – 16,57/16,55; N – 5,81/5,79; H – 4,18/4,14. FT-IR (KBr, cm^{-1}): 1635 [ν_{as}(COO)]; 1410, 1339, 1321 [ν_s(COO)]; 2952 [ν(C-H)]; 3264, 3424 [ν(H_2O)]; 680, 612, 557, 500 [ν(MO)], 460[ν(MN)].

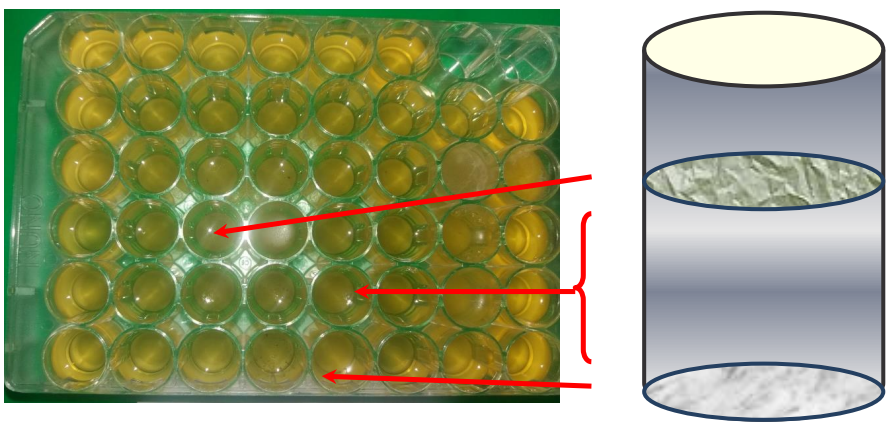
NdCoEDDS: $NO_3[NdCo(C_{10}N_2O_8H_{12})\cdot 6H_2O]\cdot 2H_2O$, found/calculated (%): Nd – 19,84/19,86; Co – 8,95/8,96; C – 16,57/16,55; N – 5,81/5,79; H – 4,18/4,14. FT-IR (KBr, cm^{-1}): 1636,1620, 1548 [ν_{as}(COO)]; 1410, 1329, 1312 [ν_s(COO)]; 2943. 2362 [ν(C-H)]; 3242, 3454 [ν(H_2O)]; 687, 620, 576, 510 [ν(MO)], 456[ν(MN)].

The analysis of the given data proves that the neodymium complexes have the same type of structure in solutions and in the solid state.

Preliminary preparation and cultivation of *Candida albicans* ATCC 18804 strain to test the antifungal properties of synthesized neodymium ethylene diamine disuccinate complexes was carried out using Sabouraud dextrose agar according to the standard method according to [30]. The concentration range of the studied compounds was 1, 10 and 100 μM. The starting solutions were prepared using a water-ethanol mixture (2–4% ethanol), after which they were autoclaved at 0.5 atm.

To obtain a suspension of microorganisms, they were washed with a sterile physiological solution and standardized to 0.5 units according to the McFarland standard [31]. To stimulate changes in morphogenesis and the formation of specific cell forms of *C. albisaps*, in particular hyphae, in parallel with the liquid version of Sabouraud's medium, Spider medium containing mannitol was used. Sabouraud liquid nutrient medium contained peptone 10 g/l, glucose 40 g/l, yeast extract 5 g/l. The composition of the Spider medium with the addition of mannitol corresponded to the following: meat-peptone broth 20 g, mannitol 20 g, K_2HPO_4 4 g, distilled water 1 l.

0.05 ml of the culture of the microorganism was introduced into the wells of a sterile tablet containing 1.0 ml each of Saburo and Spyder liquid nutrient mediums, as well as corresponding solutions of the studied compounds (Fig. 3).



a b

Fig. 3 – General view of the tablet in which *Candida albicans* was cultivated (Sabureau or Spider medium): a – polystyrene tablet; b – schematic arrangement of cells during cultivation.

Microorganisms were cultivated for 24–48 hours at a temperature of 37^0C, after which the culture liquid containing the «suspension» culture of *C. albicans* was carefully removed from the wells. Determination of the number of cells in the liquid medium was carried out using a spectrophotometer «μQuant» BioTek at a wavelength of 540 nm, calculating the value of the optical density in CFU/ml (CFU – colony-forming units)) using the appropriate calibration curve. Cultures of the microorganism under study, grown in similar conditions with the addition of H_4EDDS of the appropriate concentration, served as controls After that, the wells of the tablets were washed with physiological solution and filled with 96% ethyl alcohol, which fixed the biofilm formed by S. albisaps. After 10 minutes, the alcohol was removed and the tablet was left to dry in the air. An aqueous solution of crystal violet (0.1%) was used to stain the cells that make up the biofilm, which was poured into the wells of the tablet and left for 10–20 minutes. For the quantitative analysis of the intensity of biofilm formation, a dye was isolated that absorbed the cells, and the optical density of the resulting solution was determined. An aqueous solution containing dodecyl sulfate and sodium hydroxide was used for cell lysis. Cell lysis was performed for 60 min. The results were recorded using a BioTek μQuant spectrophotometer at a wavelength of 592 nm. To obtain reliable results, all experiments were performed in 5 repetitions. The Student's t-test [32] was used in the comparative analysis of research results. A difference of $p \leq 0.05$ was considered significant.

The sensitivity of various morphological forms of *Candida albicans* to neodymium complexes with ethylenediaminedisuccinate: NdEDDS (**I**), NdEDDSZn (**II**) NdEDDSCo (**III**) was studied. The development of *Candida albicans in vivo* can take place in the form of several morphological forms: in the form of budding yeast, pseudohyphae, and true hyphae [30]. The switch between the yeast-like form and hyphae depends on various factors, but primarily on the composition of the nutrient medium. Despite the fact that recent studies have shown that the «yeast-hyphae» transition does not always occur during the development of systemic candidiasis, such dimorphism is still considered as one of the factors of pathogenicity of *C. albicans* [33]. In addition, the formation of hyphae is necessary for *C. albicans* to avoid phagocytosis, penetration into body tissues, and also to colonize medical devices by forming a biofilm [34]. The search for new compounds, as well as elucidation of the mechanisms of resistance development of S. albicans during biofilm formation, is one of the most urgent areas of research that will significantly increase the success of antibacterial therapy [35].

At the first stage of the work, the antifungal activity of the studied compounds was determined in relation to the planktonic culture of *C. albicans*, which contains cells of the same size and physiological state (Fig. 4, 5). Comparing the inhibitory effect of the «Control» (C=H_4EDDS) and neodymium complexes based on it, it was established that the latter were more effective against *C. albicans* cells at all stages of cultivation in Saburo nutrient medium (Fig. 4).

Neodymium-cobalt heterometallic complex (**III**) at a concentration of 1 μM was the most active, inhibiting the growth of *C. albicans* cells in suspension culture by four times compared to the control value. Lower concentrations of

this complex had a less pronounced antimycotic effect. Complexes **I** and **II** were characterized by an antifungal effect, which was about 50–55% compared to the Control. When the term of cultivation of the microorganism was extended, the degree of inhibitory activity of all studied complexes decreased slightly, accompanied by an increase in the number of *C. albicans* cells in the suspension.

In the Spider nutrient medium, which stimulated the formation of the hyphal form of a yeast-like fungus, the effect of complex compounds was also quite pronounced (Fig. 5). At the same time, for complexes **I** and **II**, the level of antifungal activity turned out to be higher, by approximately 9–23%, compared to the corresponding values in the Saburo medium.

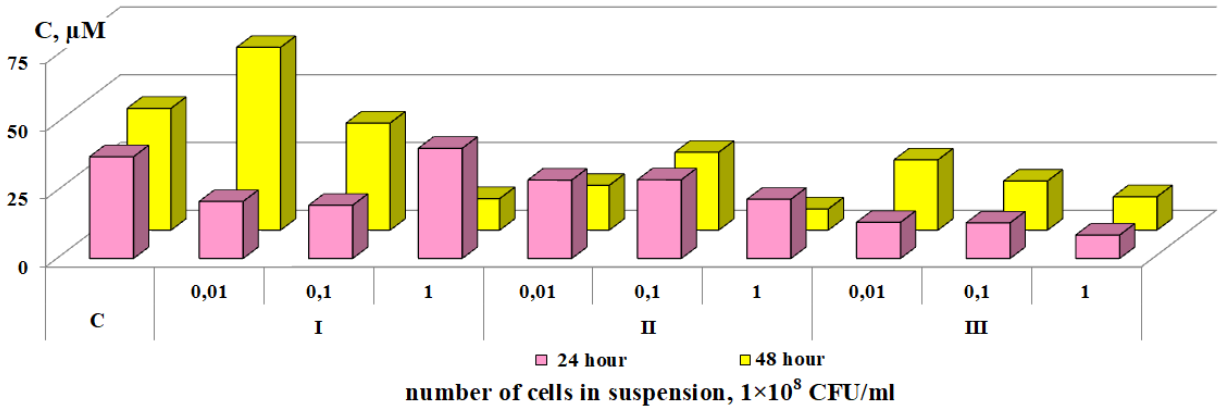


Fig. 4 – Activity of Nd^{III} complexes with H_4EDDS against the planktonic culture of *Candida albicans* cultivated in Saburo nutrient medium.

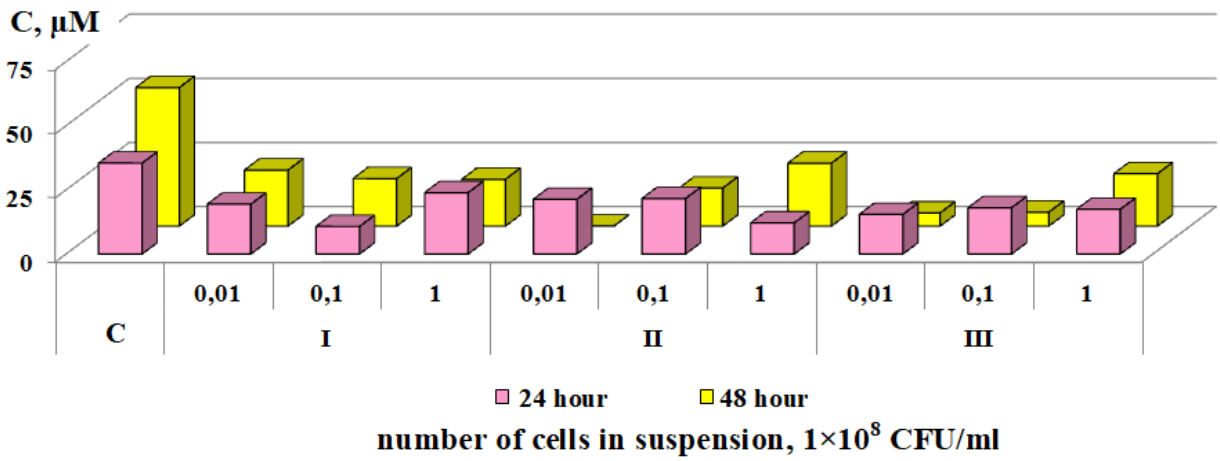


Fig. 5 – Activity of Nd^{III} complexes against the planktonic culture of *Candida albicans* cultivated in the Spider nutrient medium.

With the continuation of cultivation of *C. albicans* in the Spider medium for up to 48 hours, the activity of the studied complexes practically did not change. For complex **III**, on the contrary, it increased: on average, it was about 35–50% of the control value. It was also recorded that complexes **II** and **III** at 48 hours of cultivation of *C. albicans* were more active at the lowest concentration of 0.01 μM.

Therefore, in the course of the research, it was found that the sensitivity of the cells, which are part of the suspension culture, is quite pronounced and turns out to be greater to the minimum concentrations of the compounds. Obviously, this is due to the nature of the interaction of complexonate molecules with *C. albicans* cells. It was also determined that the variant of the environment and, accordingly, the predominant form of development of the microorganism do not have a significant effect on the effectiveness of the studied compounds.

According to numerous studies, *C. albicans* as part of a biofilm is a less sensitive microorganism to the action of antimycotic drugs compared to plankton culture cells. In the work at the second stage, the sensitivity of the cells of the biofilm, which was formed in the Saburo and Spyder media, to the action of the studied complexes was determined (Fig. 6, a, b).

As for ethylenediaminedisuccinic acid, i.e. Control, this compound practically did not show fungicidal activity in any version of biofilm cultivation.

In the medium of Sabouro cells, the biofilm consisted mainly of yeast-like cells, the part of hyphal elements did not exceed 15–20% of the total mass of the formation. In the experiment, it was established that the studied complexes practically did not affect the development of this version of the biofilm. In this case, the reduction of microorganisms in the composition of the association did not exceed 20–25% of the control value. Another feature of the action of the studied complexes was the preservation of the level of antifungal activity, or its increase on the second day of biofilm cultivation.

The biofilm that was formed in Saburo nutrient medium contained mostly cells in the form of hyphae: almost 80% of the total number of structural elements (Fig. 6, b).

In contrast to the variant of the biofilm in the Saburo environment, as well as the Control, the studied synthetic complexes in the Spider environment led to 95% of the cell death in the biofilm composition. At the same time, compounds **II** and **III** turned out to be the most active: at lower concentrations, the inhibitory activity against the *C. albicans*sass ociation was higher. It was also determined that in most concentrations the exposure level of the compounds was maintained for 48 hours.

Compound **I** was less effective: the level of its antifungal activity was approximately 50% compared to the control. Only in the case of 0.1 μM and 1 μM during 24 h of cultivation, a more pronounced inhibition of biofilm formation in the Spider medium was noted.

In general, the investigated complexes were characterized by a significantly greater effect on the cells of the biofilm formed in the Spider nutrient medium, that is, on the hyphal form of *C. albicans* development. Therefore, the hyphal elements were more sensitive to the action of the studied compounds than the yeast-like elements of *C. albicans*. When using almost all complexes, both in the early stages of association cultivation and later stages, inhibition of the biofilm formation process was observed. As for yeast-like elements, more pronounced antifungal activity of the studied compounds was observed at later stages of biofilm formation.

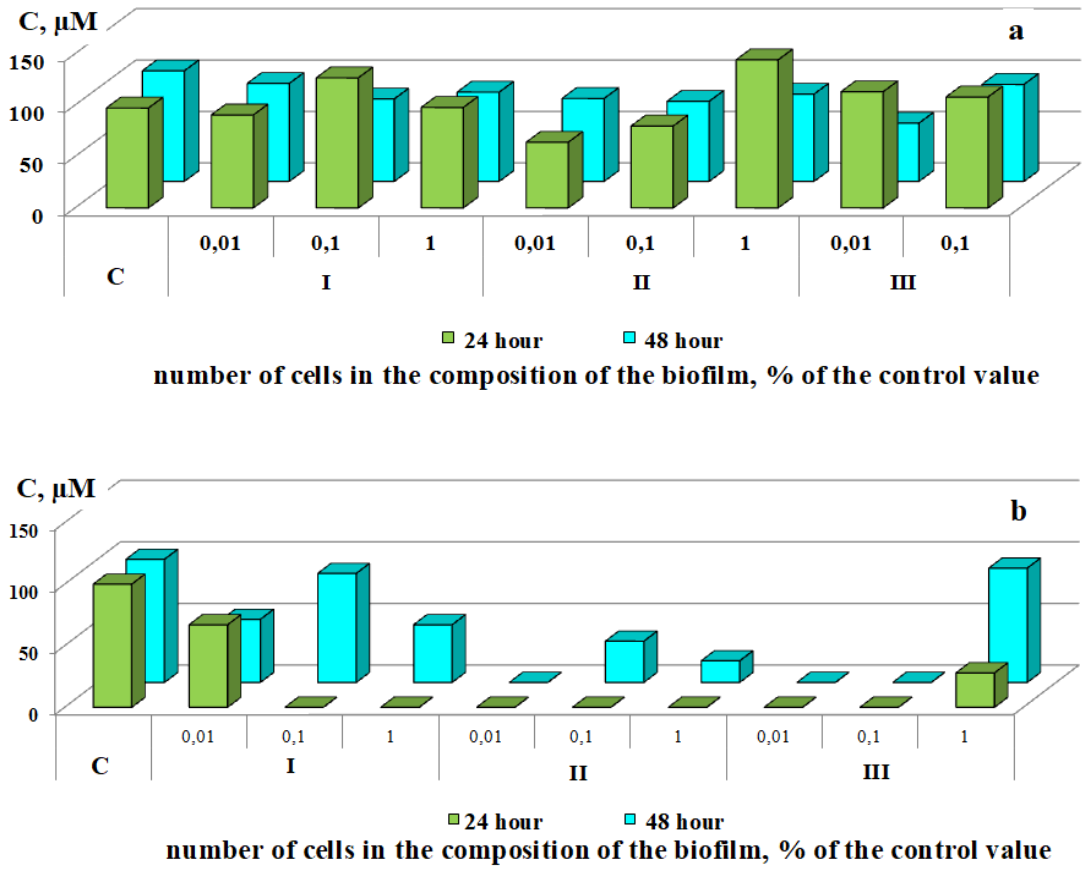


Fig. 6 – Activity of Nd^{III} complexes with H_4EDDS against Candida albicans cells, which are part of the biofilm, during cultivation in Saburo (a) and Spider (b) media.

CONCLUSIONS.

1. f-d-Heteronuclear ethylenediaminedisuccinate complexes of Nd^{III} with Co^{II} and Zn^{II} were obtained by the «block synthesis» method. The complexes belong to the «closed» type complexes, in which the ligand-complexon realizes the maximum dentateness to Nd^{III}, and the octahedral coordination sphere of the 3-d cation is formed by the carboxyl groups of EDDS and intraspherical water molecules. The coordination polyhedron of Nd^{III} corresponds to a square antiprism (C_{4v}) with a Ln^{III} coordination number of 8. In solutions and in the solid state, the complexants have a similar structure.

2. Experiments were conducted to detect the antifungal activity of heterometallic complexes of neodymium and 3-d metals against phytopathogenic strains of myxomycetes *Candida albicans*. It was shown that, regardless of the environment (Spider or Saburo), the complexes show a significantly greater inhibitory effect than ethylenediaminediansuccinic acid, which served as a control. It was established that in Saburo nutrient medium, all investigated complexes are more effective against *C. albicans* cells at all stages of cultivation than in Spider medium.

3. The yeast-like form of the *C. albicans* cell was characterized by sensitivity to complexes, the level of which was 4–6 times higher than the sensitivity to pure complexone. Hyphal elements turned out to be more sensitive to the action of the studied compounds than yeast-like cells of *C. albicans*. Regarding yeast-like elements, the antifungal activity of the studied compounds was observed at the beginning of biofilm formation, in contrast to hyphal forms, which were more sensitive in the composition of associations.

4. The most active was the heterometallic complex NdEDDSCo with a concentration of 1 μM, which inhibited the growth of *C. albicans* cells in suspension culture by 4 times compared to the control value. Lower concentrations of this complex had a less pronounced antimycotic effect. Other studied complexes (NdEDDS and NdEDDSZn) were characterized by an antifungal effect that was about 50–55% compared to the control (EDDS). The variant of the environment and, accordingly, the predominant form of development of the microorganism do not have a significant effect on the effectiveness of the studied compounds.

5. The complexes were characterized by a significantly greater effect on the cells of the biofilm formed in the Spider nutrient medium, that is, on the hyphal form of microcete development. Therefore, hyphal elements were more sensitive to the action of complexes than yeast-like elements of *C. albicans*. When using almost all complexes, both in the early stages of association cultivation and later stages, inhibition of the biofilm formation process was observed. As for yeast-like elements, more pronounced antifungal activity of the studied compounds was observed at later stages of biofilm formation.

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КОМПЛЕКСИ Nd(III) І 3d-МЕТАЛІВ НА ОСНОВІ ЕТИЛЕНДИАМІНДИБУРШТИНОВОЇ КИСЛОТИ ЯК ПОТЕНЦІЙНІ АНТИФУНГАЛЬНІ ЗАСОБИ

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Методом «блокового синтезу» отримано f-d-гетероядерні етилендиаміндисуксинатні комплекси Nd^{III} з Co^{II} та Zn^{II}. Комплекси належать до комплексів «закритого» типу, в яких ліганд-комплексон реалізує максимальну дентатність до Nd^{III}, а октаедрична координаційна сфера 3d-катіону утворена карбоксильними групами EDDS та внутріш-

ньосферними молекулами води. Координаційний поліедр Nd^{III} відповідає квадратній антипризмі (C_{4v}) із координаційним числом Ln^{III} 8. У розчинах і твердому стані комплексанти мають близьку будову. Проведено дослідження чутливості різних морфологічних форм *Candida albicans* до комплексів неодиму з етилендиаміндисукцинатом: NdEDDS(**I**), NdEDDSZn(**II**) NdEDDSCo(**III**) у середовищах Сабуро та Спайдер. Штам *Candida albicans* ATCC 18804 отримано з музею культур мікроорганізмів кафедри мікробіології, вірусології та біотехнології ОНУ ім. І. І. Мечникова. Показано, що незалежно від середовища комплекси виявляють значно більший пригнічуючий ефект, ніж етилендиаміндибурштинова кислота, яка слугувала як контроль. Встановлено, що у поживному середовищі Сабуро всі досліджені комплекси є більш дієвими щодо клітин *C. albicans* на всіх стадіях культивування, ніж у середовищі Спайдер. Дріжджоподібна форма клітини *C. albicans* виявляла чутливість до комплексів, рівень якої у 4–6 разів був вищим, ніж чутливість до чистого комплексону. Гіфальні елементи виявилися більш чутливими до дії досліджуваних сполук, ніж дріжджоподібні клітини *C. albicans.* Щодо дріжджоподібних елементів, то антифунгальну активність досліджуваних сполук спостерігали на початку формування біоплівки на відміну від гіфальних форм, які виявилися більш чутливими у складі асоціацій. Найбільш активним виявився гетерометалічний комплекс NdEDDSCo з концентрацією 1 мкМ, який пригнічував у 4 рази ріст клітин *C. albicans* у суспензійній культурі порівняно з контрольним значенням. Менші концентрації цього комплексу мали менш виражену антимікотичну дію. Іншим досліджуваним комплексам (NdEDDS та NdEDDSZn) притаманний антифунгальний ефект, який становив близько 50–55 % порівняно з контролем (EDDS). Варіант середовища та відповідно переважна форма розвитку мікроорганізму не мають суттєвого впливу на ефективність досліджуваних сполук. Комплекси здійснюють суттєво більший вплив на клітини біоплівки, яка утворювалася у поживному середовищі Спайдер, тобто на гіфальну форму розвитку мікроцетів. Отже, гіфальні елементи виявилися більш чутливими до дії комплексів, ніж дріжджоподібні елементи *C. albicans.* При використанні практично всіх комплексів, як на ранніх стадіях культивування асоціацій, так й на більш пізніх, спостерігали гальмування процесу утворення біоплівки. Щодо дріжджоподібних елементів, то більш виражену антифунгальну активність досліджуваних сполук спостерігали на більш пізніх стадіях формування біоплівки.

Ключові слова: етилендиаміндисукцинатні комплекси, неодим, кобальт(II), цинк (II), антифунгальні властивості.

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