NANO-BIOCOMPLEXES BASED ON OLIGOSACCHARIDES AND THEIR DERIVATES*

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Nanotechnology refers to the branch of science and engineering dedicated to materials, having dimensions in the order of 100th nm or less. Many ions of transition or noble metals can form complex compounds of nanoparticle size. These compounds are of scientific and practical interest, because of their proven or potential application in different areas. Nano-biocomplexes of iron, copper, cobalt and silver with oligosaccharides and their derivatives are important in medicine, cosmetics and veterinary medicine. The development of new nano-biocomplexes based on oligosaccharides and their derivatives and non-toxic or "green" silver nanoparticles syntheses, the influence of the ligand constitution on the synthesis outcome, stability, structure and pharmaco-biological properties are the subject of this paper.

Keywords: nano-biocomplexes, iron, copper, cobalt, silver, oligosaccharides, pharmaco-biological properties

Introduction

Materials of nano dimension, from 1 to 100 nm, have specific characteristics that differ from those of the same chemical composition with macroscopic dimensions. Metal nanoparticles, (MNPs), have unique catalytic, electrical, magnetic, optical and mechanical characteristics, as well as biological activity [1-3], thanks to the large specific surface area in terms of volume and large surface energy. Many ions of some transition metals such as copper, iron, cobalt can form complex nano size particles under appropriate reaction conditions. Ions of precious metals like silver, gold, palladium, platinum, titanium in the presence of a reducing agent, having a role of the stabilizing agent at the same time, can also form the nanoparticles. Complex compounds are not only of scientific but also of practical interest since many of them have found application not only in different industrial areas but also in medicine, cosmetics, veterinary and nanotechnologies [4-9]. A lot of investigations of synthesis and characterization of different metal complexes with biological ligands or synthetic ligands, serving as a model of complex biomolecular structures, has been conducted. Bioligands, or synthetic ligands are mainly natural chemical compounds of macromolecular type, such as oligosaccharides, proteins, and nucleic acids. In this group of products, chemical compounds of polysaccharide type or their derivatives containing cations of different transition biometals (Cu(II), Co(II), Zn(II) and Fe(III)) with proven or potential pharmacological activity, are very important [10-12]. The preparations based on these compounds are used in human and

veterinary medicine since they contain polysaccharide as an energy source and a bio-element as a significant factor in the metabolic processes. Commercial parenteral preparations based on the water-soluble complex of Fe(III) with dextran oligosaccharides are used in the treatment of sideropenic anemia. Such complexes can also be obtained with other polysaccharide-type ligands, such as inulin (polyfructofuranoside) and pullulan (polymaltotriosis). Except for iron, the presence of other hematopoietically active biometals, such as copper, cobalt and zinc, is necessary for the successful medical treatment. In human and veterinary medicine, the products with polymicroelements are of special interest, since they provide a complex treatment of different diseases simultaneously.

Nano particles of precious metals have practical applications in various fields. Silver, gold, palladium, and platinum nanoparticles are particularly important in the fields of electronics, medicine, pharmacy and cosmetics. For this reason, the processes of synthesis, characterization and application of such particles have been studied intensively over the past decades. Among the various metal nanoparticles, silver nanoparticles (AgNPs) are practically used particularly for their potential antibacterial, antifungal and antiproliferative, antioxidant and anticancer activities in different areas [12-21]. Silver nanoparticles can be obtained by different methods [22]. Increasing application of silver nanoparticles based on environmental principles implies, among other things, an increasing need for non-toxic or "green" syntheses. One of the ways to develop

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the non-toxic syntheses is the usage of a biopolymer as a matrix. Polysaccharides are the most widely used polymers from the following biopolymers: dextran, pullulan, alginate, agar, starch, chitosan, and glycogen, as well as extracts obtained from natural products [23].

The development of new nano-biocomplexes based on oligosaccharides and their derivatives, as well as the influence of the ligand constitution on the synthesis outcome, stability, structure and pharmaco-biological properties, are the subjects of this paper.

Iron(III) complexes with Dextran, Pullulan and Inulin oligomers

Synthesis of complexes

Many preparations based on the organic and inorganic bivalent and trivalent iron compounds are used for the prevention and treatment of sideropenic anemia in human and veterinary medicine. A wide spectrum of preparations was pharmacologically and clinically examined into details earlier, while the solutions of trivalent iron (polynuclear ironoxyhydroxide, with β_2 -FeOOH macromolecule) with carbohydrates and their derivatives have been used as parenteral antianemics more recently [24]. Water soluble Fe(III) polynuclear complexes can be produced from low molecular weight dextran (LMD), its hydrated derivative (RLMD), and other oligosaccharide type ligands such as hydrated low molecular pullulan (polymalthotriose, RLMP) and inulin (polyfructofuranoside, IN).

Figure 1. Structure of polysaccharide dextran with α -(1 $\rightarrow$ 6) glycosidic linkages in main chain; branching with α -(1 $\rightarrow$ 3) glycosidic linkage, a) polysaccharide pullulan with α -(1 $\rightarrow$ 6) repeating dominant maltotriose units, b) and inuline c)

Molecular forms of these oligosaccharides are presented in Fig 1. A new, so called single-step synthesis process, which differs from the two-step process, was used in the preparation of the component β_2 -FeOOH for the synthesis of complexes with RLMP, RLMD and IN [25-28]. The optimal properties of the ligand and complexes as potential parenteral antianemics were defined by studying the influence of various physico-chemical factors on the synthesis course and outcomes for the synthesis of the complex with inulin, dextran and pullulan with the optimal M_w in the range of 2.000-4.000, 3.000-6.000, and 5.000-9.000 gmol^{-1} , respectively, and reducing sugar content to less than 0.5%. The optimum iron : ligand ratio was 1:5, pH 10, synthesis at boiling temperature for 30 – 120 min [27].

Gel chromatography of the complex

Gel chromatographic (GPC) investigations on Sephadex G-75 columns showed the presence of 2 fractions in the complexes: (1) the fraction of true complex with bound ligand (colored fraction of about 14 cm^3 with a molar mass higher than 100.000 gmol^{-1} and a fraction of 60-90%) and (2) a free ligand fraction (a colorless fraction of about 18 cm^3 with a molar mass less than 2.000 gmol^{-1} with a contribution of 10-40 %).

Spectra-structural correlations

The structure of polynuclear Fe(III) complexes with oligosaccharides was not explained in details. Namely, β_2 -FeOOH and oligosaccharides can be bound by covalent or hydrogen bond [24,29]. The type of bonding in the complex cannot be verified with certainty by spectroscopic method because of the covering band effect from free oligosaccharides. Iron(III) complexes with dextran, pullulan and inulin oligomers had been studied earlier by UV-Vis methods [26]. These complexes had shown an absorption maximum at $\lambda_{\text{max}} = 505 \text{ nm}$ with the crystal split energy of 2.56 eV, and the crystalline field splitting energy of $E_m = 237.02 \text{ kJmol}^{-1}$ (Vis), corresponding to $p \rightarrow d$ transition of Fe (III) with octahedral coordination [26]. FTIR spectroscopy was used for further investigation of the structure. The presence of β_2 -FeOOH in the complex structure was verified by the band at $\sim 700 \text{ cm}^{-1}$ of $\gamma(\text{OH})$ vibrations [30]. The complexes, isotopic exchanged and refined by gel-chromatography, were confirmed as hydrates by FTIR study. Water protons take part in the formation of weak hydrogen bonds; $\text{O}_w \dots \text{O}$ distances were estimated as 280 and 283 pm for inulin and their complex, respectively. In the case of dextran and pullulan the distances were 284 and 290 pm, respectively [31]. The FTIR spectra of RLMD, RLMP and their Fe(III) complexes showed two bands at 930 and 850 cm^{-1} in the $\gamma(\text{CCH})$ region (1000-700 cm^{-1}), characteristic for $^4\text{C}_1$ conformation of glucopyranosyl units. Significant shifts was noticed in 1500-1300 cm^{-1} region, characteristic for methylene groups bending vibration. The decrease of bands intensity from methylene groups, $\delta(\text{CH})$ at $\sim 1450 \text{ cm}^{-1}$ and $\nu(\text{CH}) \sim 2900 \text{ cm}^{-1}$, was controlled by

C5-C6 rotation in the glucopyranose ring of isomers due to dipole moment changes characteristic for this conformation [31]. In the FTIR spectra of gel-refined compounds, bands from $\nu(\text{OH})$ vibration was shifted to lower vibrations for 90 cm^{-1} , 60 cm^{-1} and 40 cm^{-1} for dextran, pullulan and inuline complexes, respectively, compared to the non-refined complexes and FeOOH oligosaccharides. These results showed a stronger hydrogen bond between FeOOH and oligosaccharides compared to initial compounds. FTIR results, ESR spectroscopy and electron microscopy showed that particles sizes were 3-6 nm (FeOOH), 20-30 nm (inuline complex), 9-21 nm (dextran complex) and 12-26 nm (pullulan complex), and 12 nm (ferritin); the shape factor was 0.83, (spherocolloid particles). The GPC suggests that complexes particles have a solid FeOOH core with a convolute oligosaccharides sheath. Based on the physico-chemical and morphological investigations, a two-layer spherical model of the complex is proposed (Fig. 2) according to Muller and Cox models. In this model the polymer particle $\beta_2\text{-FeOOH}$ is wrapped with the oligosaccharide of adequate M_w forming a stronger hydrogen bond while the second layer is made of oligosaccharides with lower molar masses, bound together with the oligosaccharides from the first layer by weaker hydrogen bonds.

Figure 2. Structure models of polynuclear iron(III) complexes with oligosaccharides: Muller (a), Cox and Kawaguchi (b) and spherical, multilayer hypothetical (c)

Pharmacological study

In the global context, the investigated synthetic Fe(III) complexes are similar to the biological protein complex with iron-ferrite, as well as the results of the pharmacological tests. Similarity of Fe(III) complexes to human blood-grain-erythrocyte allows their undisturbed moving through body fluids in vivo [26]. By testing acute toxicity, in the iron-inulin complex (Fe content 50 mgcm^{-3} and inulin oligomers 150 mgcm^{-3}), an average lethal dose of $\text{LD}_{50} = 525\text{ mgFekg}^{-1}\text{ m.w.}$ for mice was determined. For the iron-pullulan complex (Fe content 50 mgcm^{-3} and pullulan oligomers 145 mgcm^{-3}), LD_{50} was $1429\text{ mgkg}^{-1}\text{ m.w.}$, while for the iron-dextran (Fe content 50 mg/cm^3 and dextran oligomers 190 mgcm^{-3}) LD_{50} was higher than $2500\text{ mkg}^{-1}\text{ m.w.}$, which meets the requirements of most pharmacopoeias. Resorption studies of these complexes on rabbits have shown satisfactory results. The maximum iron concentration was achieved 36 hours after iron-inulin and iron-dextran complexes applications, as well as 24 hours after iron-pullulan complex application.

Nano-Biocomplexes of Dextran and Pullulan with Copper and Cobalt

Synthesis

Schematic presentation of synthesis and characterization of synthesized M(II)-RLMP and M(II)-RLMD complexes ($\text{M} = \text{Cu}, \text{Co}$) is shown in Fig. 3., and described in details [10,32-34].

Figure 3. Schematic presentation of synthetic procedure and characterization of synthesized $\text{M(II)-RLMP/ M(II)-RLMD}$ complexes.

The pH of the reaction medium was changed after Cu(II)-salt addition, reflecting the dependence of pH values of the Cu(II) ion content in the Cu(II)-RLMP complex. In fact, when synthesis were performed at the same temperature and within the same reaction period, the highest Cu(II) ion content was obtained at pH 7.5. Additionally, if the solution pH values increased from 7.5 to 12, the percentage of the bound Cu(II) in the complex decreased from 13 to ~8% (AAS). The increase of solution pH from 7.5 to 13.5, the percentage of the bound Co(II) ion in the Co(II)-RLMP complex increased from ~2 to ~8% (AAS). The solubility of the synthesized Co(II)-RLMP complexes varies in water. For comparison, the copper content in Cu(II)-RLMP complexes varied from ~8 to ~13% (AAS). The most water-soluble Co(II)-RLMP and Co(II)-RLMD complexes were obtained at pH 13.5, since increased pH value favored formation of ionized forms of synthesized complexes leading to their better aqueous solubility. Optimal conditions for copper complexes, with respect to the metal content, are pH 7-8, $T = 100\text{ }^{\circ}\text{C}$, and $t = 7\text{ min}$ for the complex; Cu contents were 19.8, and 13% for Cu(II)-RLMD and Cu(II)-RLMP, respectively. Optimal conditions for cobalt complexes, with respect to the metal content are: pH 12-13.5; $T = 100\text{ }^{\circ}\text{C}$, and $t = 7\text{-}15\text{ min}$; Co contents were 8.35 and 12.9% for Co(II)-RLMD and Co(II)-RLMP, respectively.

UV-Vis study

The synthesis and spectroscopic characterization of the Cu(II) ion complexes with RLMP and RLMD is described in detail [33,34,37]. The formation of Cu(II)-hydroxyl complexes with deprotonated dextran monomer unit was observed in the range of pH 8-12. The results of spectrophotometric analysis indicate the possibility of the formation of coordination bonds which rearrangement begins at pH > 8. Synthesized Cu(II)-complexes are decomposed at pH > 12. Complexes of Cu(II)-RLMD and Cu(II)-RLMP show different maxima of UV-Vis absorption as a function of pH. There are typical hypsochromic (blue) shifts due to the interaction of orbitals of Cu(II) ion and ligands. From the Vis spectra of Cu(II) complexes, the wavelength interval of the absorption maximums was found to be within $\lambda = 630 - 750\text{ nm}$ for dextran complexes and $\lambda = 650 - 700\text{ nm}$ for pullulan complexes. According to literature, these energy values (1.8 to 2 eV) are in the area characteristic for the octahedral ligand field (Δ_o).

The synthesis and spectroscopic characterization of the Co(II) ion complexes with RLMD and RLMP is described in detail [35,36,38,39]. With the increase in pH the absorbance maxima of complex solutions showed red shift compared to $[\text{Co}(\text{H}_2\text{O})_6]^{2+}$ ion (bathochromic effect). From the Vis spectra of Co(II) complexes, synthesized under different reaction conditions, it was possible to see that the absorbance maxima were in the range of 583 – 624 nm for dextran complexes, and 525 – 675 nm for pullulan complexes. The corresponding ligand crystal field splitting energies (Δ_o), according to the literature

data, are characteristic for the O_h ligand field.

The spectrophotometric parameters of the investigated complexes are characteristic for Co(II) ion in octahedral or tetragonal distorted octahedral coordination with O ligand atoms.

FTIR study

FTIR spectroscopy method is usually the technique of choice for structural analysis of polysaccharides [40-43]. The FTIR spectra of complexes with Cu(II)-RLMD and Cu(II)-RLMP contain the following characteristic bands: $\nu(\text{O-H}) \sim 3400\text{ cm}^{-1}$, $\nu(\text{C-H}) \sim 2930\text{ cm}^{-1}$, $\delta(\text{HOH}) \sim 1645\text{ cm}^{-1}$, $\delta(\text{C-H}) \sim 1450$ and $\sim 1345\text{ cm}^{-1}$, $\delta(\text{O-H}) \sim 1420\text{ cm}^{-1}$, a complex band $\nu(\text{C-O}) 1200 - 1000\text{ cm}^{-1}$, $\gamma(\text{C-H}) 1000 - 700\text{ cm}^{-1}$. The spectra appearance in this region is very similar for Co(II) complexes. It is already known that complexation of copper(II) ion with RLMP [41] and RLMD [42] gives different types of complexes, depending on the pH value. With the assumption that Co(II) ions formed similar complexes, bands in this region should be attributed to the stretching vibrations of ligand OH groups and coordinated H_2O molecules for the complex synthesized in a weak alkaline solution. It should also be associated with ligand OH groups and the OH^- ions in the first coordination sphere of the Co(II) ion for the complexes, synthesized in alkaline and strong alkaline solutions. No effect after complexation on the conformation change of $\alpha\text{-D-glucopyranose}$ units with ${}^4\text{C}_1$ chair conformations was observed for the M(II)-RLMD and M(II)-RLMP complexes as evidenced by the appearance of FTIR spectra in the range of $1000\text{--}700\text{ cm}^{-1}$ [38,41,42].

FTIR spectra of RLMD and Co(II)-RLMD complexes were compared with the deconvoluted spectra of their analogues recrystallized from D_2O [43-45]. FTIR spectra were measured in order to find the specific spectral peculiarities, i.e. collecting the information about structure and conformation of these macromolecules. In the range of $\nu(\text{O-D})$ vibrations one new band appears at about 2495 cm^{-1} in the FTIR spectrum of deuterated RLMD and this is obviously the consequence of isotopic exchange in some OH groups whose $\nu(\text{OH})$ vibrations are manifested by the presence of a broad intensive band at about 3350 cm^{-1} which is an evidence of water molecules presence in RLMD structure. As for the Co(II)-RLMD complex, a new band appears in the FTIR spectrum of deuterated complex synthesized at pH 13.5 at about 2483 cm^{-1} in the range of $\nu(\text{O-D})$ vibrations. Partners of these vibrations are expected to be $\nu(\text{O-H})$ vibrations manifested by the broad intensive band at about 3400 cm^{-1} from water molecules. Sharp band at 3636 cm^{-1} in the deconvoluted FTIR spectrum of Co(II)-RLMD complex may be attributed to $\nu(\text{O-H})$ vibrations of the free OH groups which are not included in the formation of hydrogen bonds. Complete deuteration of these OH groups results in the disappearance of this band and appearance of a new sharp band at 2580 cm^{-1} , originating from $\nu(\text{O-D})$ vibrations in the FTIR spectrum of deuterated Co(II)-RLMD complex.

By using Falk criteria [46], it can be concluded that both RLMD and Co(II)-RLMD complex have one crystallographic type of H_2O molecule. According to the correlation of Berglund et al. [47], estimated Ow...O distances are 283.1 pm for RLMD and 281.8 pm for Co(II)-RLMD complex, and H_2O protons take part in the formation of relatively weak hydrogen bonds. Co(II)-RLMD complexes are probably formed by the displacement of H_2O molecules from the first coordination sphere of Co(II) ion by the OH groups of ligand.

An effect of conformation change was observed in recrystallized Cu(II)-dextran complex, especially when glucopyranose units with ${}^4\text{C}_1$ chair conformations are present, the IR spectra exhibit one band in the region between 925 to 885 cm^{-1} and another one around 860 to 820 cm^{-1} , which are assigned to mixed CCH deformation vibrations. In the particular region, of 1000 to 700 cm^{-1} a certain band at 880 cm^{-1} was considered as characteristic for the conformation change ${}^4\text{C}_1$ -chair to 3B-boat of glucopyranose units (Fig. 4) [43].

Figure 4. Change of ${}^4\text{C}_1$ chair conformation to B1 or 3B boat conformation of the glucopyranose dextran units.

ATR-FTIR study

There are differences between FTIR microscopy images of RLMP and RLMD ligands and synthesized M(II)-RLMP and M(II)-RLMD complexes, confirming that the complexation process has taken place. The changes in color contour may show the content and distribution of metal and ligand in the samples. The diameter and shape of the particles of synthesized complexes are different and depend on pH. ATR-FTIR spectra and FTIR microscopic images from different areas of complexes synthesized at pH 7.5-13.5 also show high homogeneity of the samples [48].

ESR

The ESR spectra of Cu(II)-RLMD complexes indicate the axially elongated tetragonal distorted octahedral symmetry of synthesized Cu(II) ion complexes and are typical for the Cu(II) ion with one unpaired electron in 3d subshell [33]. Specific values of $A_{||}$ and $g_{||}$ for $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ ion are $A_{||} = 137 \times 10^{-4} \text{ cm}^{-1}$, $g_{||} = 2.41$. ESR parameters of the spectra for the complexes synthesized at higher pH values were close to the values for the frozen Cu(II)-ethylene glycol complex indicating the square-planar or tetragonal distorted octahedral coordination of Cu(II) ion, with four oxygen atoms in the same plane. The appear-

ance and ESR parameters for the spectra of complexes obtained at pH 8 and 12 were very similar to the ESR spectra of some Cu(II) ion complexes with carboxymethyl-dextran and cellulose obtained at high pH values [34,37]. Although the Cu(II) ion content of the complexes synthesized at lower pH values was much higher (up to 13.04% for the complex synthesized at pH 7.5), the ESR signal of these complexes was lacking due to strong spin-spin interactions of neighboring Cu(II) ions, and probably due to the formation of polynuclear Cu(II) complexes. All ESR parameters observed from pH 7.5-12 differ, especially in $A_{||}$ and $g_{||}$ from the parameters of $[\text{Cu}(\text{H}_2\text{O})_6]^{2+}$ ion and $[\text{Cu}(\text{OH})_4]^{2-}$ ion ($A_{||} = 194 \times 10^{-4} \text{ cm}^{-1}$, $g_{||} = 2.271$). This indicates that O- donors from deprotonated OH groups may participate in Cu(II) ion coordination. A decrease of $g_{||}$ values with the increase of pH complex solutions also confirms the presence of different types of complexes. This conclusion is in accordance with the results obtained by UV-Vis investigations.

XRD

The crystallinity/amorphicity changes caused by the complexation of RLMP with Co(II) ions was observed by using XRD analysis. The crystallinity of the final product gradually rose with increased concentration of introduced Co(II) ions. Furthermore, in the XRD pattern of Co(II)-RLMP complex obtained at pH 13.5, only several diffraction peaks at 2θ values of 19° , 32.5° , and 38° were present, which suggests that Co(II)-RLMP complexes still had low degree of crystallinity after the complexation [32].

MALDI-TOF MS

Shape of peak intensities distribution in the 1000-10000 Da range with apparent maximum around 3500 Da indicates that additional depolymerization of pullulan occurred during complex synthesis. Adjacent peaks in this m/z range differ by approximately 162 Da which corresponds to the anhydro-glucopyranose moiety. The MALDI-TOF/TOF MS in the 450-2000 Da range also contains peaks differing by 162 Da, but some adjacent peaks differ by approximately 59 Da corresponding to the cobalt loss from these low molar mass fragments. Since peaks with m/z difference of 59 Da do not appear in the 1000-10000 Da range it can be concluded that Co(II)-HNMP complex synthesized at pH 13.5 is very stable, while Co(II) ion binding in low molar mass fragments is much weaker. The MALDI-TOF/TOF MS of different Co(II)-HNMP complexes also show the repeating unit of mass 162 Da which corresponds to the anhydro-glucopyranose moiety. In addition to the series of peaks with a 162 Da gap, there is a minor series with a 486 Da increment corresponding to the mass of anhydro-maltotriose moiety [32].

Structure

Based on the correlation of data obtained by different spectroscopic techniques for the characterization

of synthesized M(II)-RLMP and M(II)-RLMD complexes, the structure with tetragonal distorted Oh coordination for the synthesized complexes was proposed. M(II) ions form three different types of complexes with glucopyranose units (Glc_p): a) M(II)(Glc_p)₂(H₂O)₂ at pH 7-8, b) M(II)(Glc_p)₂(H₂O)(OH) at pH 8.5-10, and c) M(II)(Glc_p)₂(OH)₂ at pH 11-13.5. The proposed structures of the complexes synthesized at different pH values are given in Fig. 5.

Figure 5. Proposed structures of M(II)-RLMP and M(II)-RLMD complexes synthesized at different pH values: a) at pH 7-8: Co(II)(Glc_p)₂(H₂O)₂, b) at pH 8.5-10: Co(II)(Glc_p)₂(H₂O)(OH), c) at pH 11-13.5: Co(II)(Glc_p)₂(OH)₂.

Pharmacology

The preparation was tested pharmacologically with the aim of determining systemic acute toxicity expressed as a median lethal dose (LD₅₀) and as an equivalent of Cu(II) dose per kg of a mouse body weight. The application of higher doses caused the mortality of a number of experimental animals. The preparation toxicity LD₅₀ of 1419–1661 was determined in this range which corresponds to the equivalent of Cu(II) dose of 281–329 mg per kg of the body weight (concentration of the complex solution 5–20%). The results of our pharmacological investigations suggest to the lower toxicity of Cu(II) complex with RLMD, what is much better than in the case of commercially applicable inorganic copper salts [49]. This result is very important for the development of new pharmaceutical preparations and it can be applicable in medicine and cosmetology.

Nanoparticle (complexes) of biometals Cu and Co with CMD and DS

Physico-chemical characteristics of the Cu and Co complexes

Copper and cobalt complexes with carboxymethyl dextran (CMD, *M_w* = 80000 g·mol⁻¹) and sodium salts of dextran sulfate (DS, *M_w* = 500000 g·mol⁻¹, see Fig. 6) were synthesized from aqueous solutions of Cu(CH₃COO)₂ and CoCl₂·6H₂O in the molar ratio of 2:1 at boiling temperature. The synthesis of cobalt and copper complexes was monitored by color change from colorless to pink and green, respectively, and new absorption maxima appearance in the visible part of the spectra. The higher metal content and yield were achieved after 60 minutes

of reaction. The molar ratio of both metals with CMD and DS was 2:1 and 1:1, respectively [22, 50].

Figure 6. Structure of carboxymethyl dextran, CMD (a) and sodium salt of dextran sulphate, DS (b)

Spectra-structural correlations

Copper-CMD and -DS complexes showed bends maxima at ~750 and ~510 nm, respectively, in the UV-Vis spectra, while cobalt-CMD and -DS complexes showed bends maxima at ~710 and ~500 nm, respectively. The absorption maxima for DS complex were at lower wavelengths (~10 or ~40 nm) compared to maxima of CMD complex, indicating stronger metal-O interactions in DS complexes. The absorption maxima of cobalt complex exhibit a hypochromic effect in relation to the corresponding copper complexes, indicating stronger Co-O interactions compared to Cu-O. Calculations of molar energy content (161 kJ·mol⁻¹) and crystalline field splitting energy (1.74 eV), indicate the formation of tetragonal deformed complexes of octahedral configuration in Cu-CMD, while for Cu-DS the corresponding values are 171 kJ·mol⁻¹ and 1.85 eV [22, 50].

The efficiency of synthesis process was confirmed by spectra-structural correlations based on FTIR studies, changes in the vibrations of all types of OH functional groups, and the absence of ν(C-OH) vibration. This vibration is absent in the spectra of CMD and DS, but not in the spectra of complexes. In the same time, the ν(Co-O) and ν(Cu-O) vibrations were found at ~580 cm⁻¹ in the spectra of complexes, but not in CMD and DS spectra. The similarities of the γ(C-H) ranges, in a low frequencies part of the spectra, indicate that there is no difference in the conformation of the ⁴C 1 glucopyranose unit between CMD and DS and synthesized Cu(II) or Co(II) complexes. Analysis of FTIR spectra in the region of valent (C=O) vibration showed that -COOH group acts as bidentate ligand, while the compounds of Cu(II) or Co(II) with DS metal ions are in the region of four oxygen atoms of two adjacent sulfo groups. The presence of crystalline water was determined by isotopic substitution of H₂O with D₂O molecules. Comparison of spectra measured at room temperature (RT) and liquid nitrogen temperature (LNT) allowed detection of water molecules libration indicating that they are coordinated complementing coordination sphere of Cu(II) ions to six. The bands of valent (S=O) vibrations in Cu and Co complexes were at lower frequencies for ~12 cm⁻¹ compared to DS. The similarity of FTIR spectra, originating from DS sodium salt or Cu and Co complexes, indicates the predominantly ionic character of these compounds. A band of ν_{as}(O-S-O) at ~810 cm⁻¹

(vibrations of DS sulfo group in the equatorial position) was shifted to $\sim 850\text{ cm}^{-1}$ in the spectra of Cu and Co complexes, indicating a change in the position of this group in the axial plane [22,50]. The complexes were of $M(II)\cdot(CMD)_2\cdot(H_2O)_2$ or $M(II)\cdot DS\cdot(H_2O)_2$ type (Fig.7).

Figure 7. Proposed structures of $M(II)$ -CMD and $M(II)$ -DS complexes

Silver Nanoparticle with CMD and DS and Water extracts of *Fumaria officinalis* L.

Synthesis procedure

The synthesis was performed until the defined Ag:L (DS or CMD) ratio (from 1:1 to 1:2) at 100°C was achieved with continuous stirring in the duration of 120 min. A successful synthesis of the AgNPs-CMD or AgNPs-DS complex was identified through the color changes of the reaction mixtures, from white to yellow [51,52]. Water extracts (E) of *Fumaria officinalis* L. (earth smoke), according to literature data [53], contain alkaloids, flavonoids, carbohydrates, glycosides, tannins, saponins, proteins, steroids and organic amino acids, the phytochemicals with different functional groups which can reduce Ag^+ to Ag^0 additionally acting as capping agents. Green synthesis of AgNPs with the extract of *Fumaria officinalis* L. was performed in alkaline solution at room and boiling temperatures [54].

UV-Vis study

Strong absorption band in UV-Vis spectra, called SPR (surface plasmon resonance) band at 420 nm, is characteristic for AgNPs formation. Its exact position mostly depends on AgNPs size, while intensity depends on AgNPs concentration [55]. Changes in this band position were used as a criterion of the AgNP aggregation stability monitoring. The position of this band in AgNPs-DS was changed during synthesis. After 2 h of synthesis it remained constant at 425 nm indicating particle size of $\sim 40\text{ nm}$. SPR band in the AgNPs-CMD spectrum, synthesized in different molar ratio, was located at 420 nm indicating particle size of 20-40 nm; the intensity of this band is proportional to AgNPs-CMD concentration. Unchanged position of SPR band is an evidence of high aggregation stability of the reaction mixture for 3 months at room temperature. The region of UV spectra below 300 nm was monitored for first time according to available literature [51,52,54]. An intensive band at 215 nm ($\pi\rightarrow\pi^*$ transition of the carboxyl group) in the UV spectra of formed complex [56] indicates red shift effect compared

to CMD. This phenomenon is an additional indicator of Ag ions and CMD interaction i.e. AgNP-CMD complex formation.

According to UV-Vis study it can be concluded that synthesis of AgNPs-E at boiling temperature was much faster (30 min) compared to synthesis at room temperature (90 min). The unchanged position of the SPR band indicates good aggregates stability of particles with the size of approximately 18 nm. The intensity of $\pi\rightarrow\pi^*$ and $n\rightarrow\pi^*$ bands (200-350 nm) for AgNPs-E were significantly reduced compared to extract itself due to reduction of silver ions by functional groups which possess free electron pairs (CO or amide).

XRD study

Crystal structure of AgNPs-DS, AgNPs-CMD and AgNPs-E nanoparticles was confirmed by X-ray diffraction (XRD) technique. For surface-centered cubic grid of silver the characteristic peaks are at 2θ , 38.2° , 44.4° , 64.7° and 77.4° corresponding to the (111), (200), (220) and (311) reflection planes, respectively. All samples exhibited the characteristic peaks at this 2θ positions. The average size of the AgNPs was estimated from the reflection width in the X-ray diffraction pattern according to the Scherrer equation, using the Bragg reflection at $2\theta = 38.2^\circ$ and 44.4° . The average crystallite size of the AgNPs-E synthesized at room and boiling temperature was 20 ± 1 and $18 \pm 1\text{ nm}$, respectively; in the case of AgNPs-DS the average crystallite size was $40 \pm 4\text{ nm}$ [51,52,54,57].

SEM and EDX analysis

The morphology of the AgNPs-CMD, AgNPs-DS and AgNPs-E was studied by SEM analysis. In the AgNPs-CMD stable and spherical particles of 10-60 nm are dominant. The size of AgNPs-DS was similar, but the appearance of aggregates with an irregular structure without a well-defined morphology was noticed [52,54]. SEM micrographs of AgNPs-E nanoparticles showed spherical particles with an average size of 25 nm and the presence of small aggregates.

EDX spectroscopy was used for qualitative and quantitative analysis of silver involved in formation of AgNPs-DS and AgNPs-E. All samples showed strong signals corresponding to elemental silver at 3 keV. Lower signals from S or C and O confirm the formation of AgNPs as a part of AgNPs-DS or AgNPs-E. After calibration, the content of Ag was approximately determined by INCA software as 35% and 70% in the AgNPs-E formed at room and boiling temperatures, respectively [52,54].

FTIR study

The FTIR spectra of AgNPs-CMD and AgNPs-DS were basically very similar to the spectra of copper and cobalt complexes with these ligands. The $^4\text{C}_1$ conformation of the glucopyranose unit i.e. the high content of α -1,6 linkage remained unchanged after complexation. Here, the carboxyl group of CMD again played the role

of a bidentate ligand. There were strong interactions between Ag and two O atoms from the -COO groups in the O_h environment, bonding the CMD with AgNPs. There was a difference in the position of $\nu_{as}(S=O)$ band, in the FTIR spectrum of AgNPs-DS compared to DS, for about 22 cm^{-1} . A similar situation was in the complexes with copper and cobalt, indicating predominantly ionic interactions between sulfo groups from DS with AgNPs.

The detection of water molecules libration was enabled using isotope substitution method (deuteration) and the spectra measured by RT and LNT comparing. The presence of coordinated complementing coordination sphere of Cu(II) or Co(II) ions to six was indicated. The absence of a band at $\sim 1380\text{ cm}^{-1}$ in the spectra of dialyzed analogues, that originates from $\nu_{as}(O=N=O)$ of residual nitrate ions, practically confirms the real origin of this band.

The comparison of FTIR spectra measured for AgNPs-E and E, in amide region (Amid I – $\nu(C=O)$ and Amid II – $\delta(N-H)$ at 1620 and 1530 cm^{-1} , respectively), and/or $\nu(C=O)$, $\nu(C-O)$, $\nu(C-O-C)$ and $\delta(OH)$ vibration, leads

to the conclusion that bonds were formed between secondary amide group $(NH)C=O$, keto or hydroxyl groups from the AgNPs-E and Ag ions. The compounds participating in reduction and stabilization of AgNPs could be polyphenols, flavonoids, triterpenoids, proteins or acids and polysaccharides [54]. Bearing in mind everything mentioned above, the proposed structure of all AgNPs is presented in Fig.8.

Figure 8. Proposed structures of AgNPs with CMD, DS and E

Table 1. Radial diameters of inhibition zones for bacterial and fungal strains treated by AgNPs in different concentrations

Temperature		Room T		boiling T		boiling T			
		AgNPs-E		AgNPs-E		AgNPs-CMD	AgNPs-DS		
Concentration (mg ml ⁻¹)		1	0.5	1	0.5	0.5	1	0.5	1
Fungal strain	<i>B. cereus</i>	25	17	25	21	18	19	12	14
	<i>B. luteus</i>	25	23	20	22	21	24	13	20
	<i>B. subtilis</i>	x	x	x	x	17	19	x	x
	<i>L. monocitogenes</i>	22	20	23	20	17	18	x	x
	<i>P. aeruginosa</i>	26	22	24	23	24	26	-	12
	<i>K. Pneumoniae</i>	24	22	21	22	18	19	14	15
	<i>E. coli</i>	25	23	23	22	18	21	x	x
	<i>P. vulgaris</i>	28	25	25	22	14	15	x	x
	<i>C. albicans</i>	18	13	17	15	-	16	-	11

Comparative analysis of AgNPs antimicrobial activity

It is already known that silver nanoparticles exhibit the antimicrobial activity [12-21,58]. Radial diameters of inhibition zones for gram positive (G+) and gram negative (G-) bacterial as well as fungal strains, treated by different concentrations of synthesized AgNPs with CMD, DS and E, are presented in Table 1.

The size of radial inhibition zones decreases in the order: AgNPs-E > AgNPs-DS > AgNPs-CMD. The inhibition zones are higher in almost all cases where the concentration of AgNPs is higher. The synthesized AgNPs-E showed better antimicrobial properties compared to starting water extracts of *Fumaria officinalis* L. which is explained by synergistic effect of silver and extract compounds [54]. These results indicate the potential use of synthesized AgNPs-E as a raw material in various pharmaceutical and cosmetic preparations design

References

- [1] J. J., Ramsten (2014) What is Nanotechnology? Applied Nanotechnology, Second Edition. <http://dx.doi.org/10.1016/B978-1-4557-3189-3.00001-4>
- [2] V. Trajković, Z., Marković, (2010) Nanomedicina: Stanje i perspektiva. In book: Biomaterijali. Rakovic D., Uskoković D. (eds), Institut tehničkih nauka Srpeke akademije nauke i umetnosti, Beograd, 762
- [3] S. Horikosi, N. Sorpone, Microwaves in Nanoparticle Synthesis: Fundamentals and Applications 1. Introduction to Nanoparticles Published Online: 26 APR 2013 DOI: 10.1002/9783527648122.ch1
- [4] V. Gibson, E. Marshall, Comp. coord. Chem. II, 9, (2003), 411
- [5] P.O. Brien, N. Pickett, Comp. coord. Chem. II, 9 (2003) 1005
- [6] B. Graczykowski, A. Dobek, J. Coll. Interf. Sci., 363 (2011) 551
- [7] I. Iakovidis, I. Delimaris, S.M Piperakis, Mol. Biology

- Internat. Article ID 594529, 13 pages, (2011) doi: 10.4061/2011/594529
- [8] S. Tsiliou, S. Kefala, L.A. Perdih, F. Turel, I. Kessissoglou, D.P. Psomas G, *Europ. J. of Med. Chem.* 48 (2012) 132
- [9] M. Gielen, ER., Tiekink, *Metallotherapeutic drugs and metal-based diagnostic agents: the use of metals in medicine.* John Wiley & Sons 2005
- [10] Ž. Mitić, M. Cakić, *Acta Med. Medianae* 53(4) (2014) 54-63.
- [11] G. Nikolić and M. Cakić, *Fourier Transforms-New Analytical Approaches and FTIR Strategies.* Goran Nikolić, (Eds) InTech, Rijeka 2011.
- [12] Y. Mohan, K. Lee, T. Premkumar, K. Geckeler, *Polym.* 48(1) (2007) 158
- [13] V. Thomas, M. Yallapu, B. Sree dhar, S. Bajpai *J. Coll. Interf. Sci.* 31 (2007) 389
- [14] A. Panáček, M. Kolář, R. Večeřová, R. Prucek, J. Soukupová, V. Kryštof, P. Hamal, R. Zbořil, L. Kvítek, *Biomat.* 30: (2009). 6333
- [15] Q. H. Tran, V. Q. Nguyen and A.T. Le, *Nanosci. Nanotechnol.* 4 03300 (2013) 20
- [16] J. Devis, B. Bhimba (2012) 1: 242. doi:10.4172/scientificreports.242, Page 1-5.
- [17] S. Bekkeri, *IOSR J. Of Pharm.*, 4 (7) 2014 38
- [18] Y. Mohanta, Sujogya K. Panda, K. Bastia, T. Mohanta, *Frontiers in Microbiol.* 8 (2017) 1
- [19] D. Van Phu, Duy, Q. Hien, *Rad. Phys. Chem.* 88: (2013) 90-4.
- [20] J. Kim., B. V. der Bruggen, *Environmen. Poll.* 158(7) (2010) 2335
- [21] S. Arora, J. Rajwade, P. Omay S. Khandelwal K. Paknikar, *Mol. Pharmacol.* 6(5) (2009) 1388
- [22] S. Glišić, *Doktorska disertacija, Tehnološki fakultet Leskovac*, 2017
- [23] S.K., Srikar, D.D. Giri, D.B. Pal, P.K. Mishra, S.N. Upadhyay, *Green Sustainb. Chem*, 6 (2016) 34
- [24] A. Muller, *Arzneim. Forsch. (Drug Res.)*, 24, 6. (1974) 880
- [25] Lj. Ilić, S. Ristić, M. Cakić, G. Nikolić, S. Stanković, *PCT WO 02/46241 A2* June 2002, IP Cl. C08B 37/00.
- [26] G. Nikolić, *Sinteza, Fizičko-hemijska svojstva i struktura gvožđa(III) kompleksa sa oligosaharidima*, Doktorska disertacija, Tehnološki Fakultet Leskovac, 2001.
- [27] M. Cakić, G. Nikolić, D. Cvetković, Lj. Ilić, *Kompleksi Fe(III) sa oligosaharidima – Antinemići*, Monografija, Tehnološki fakultet, Leskovac 2007
- [28] G. Nikolić, M. Cakić, *Colloid. J.* 69(4) (2007) 464
- [29] J. Cox, G. Kenedy, P. Marshall, D. Rutherford, *J. Pharm. Pharmacol.* 24 (1972) 513
- [30] G. Nikolić, M. Cakić, D. Cvetković, Lj. Ilić, *Spectrosc. Lett.* 29, (1996) 7
- [31] M. Cakić, G. Nikolić, Lj. Ilić, *Bull. Chem. Technol. Macedonia*, 21 (2). (2002) 135
- [32] Ž. Mitić, G.M. Nikolić, M. Cakić, G.S. Nikolić, S. Živanović, S. Mitić, S. Najman, *Carbohydr. Pol.* 200 (2018), 25
- [33] Ž. Mitić, M. Cakić, G.M. Nikolić, R. Nikolić, G.S. Nikolić, R. Pavlović, E. Santaniello, *Carbohydr. Res.* 346(3) (2011), 434.
- [34] Ž. Mitić, G.S. Nikolić, M. Cakić, R. Nikolić, Lj. Ilić, *Russ. J. Phys. Chem. A*, 81(9) (2007), 1433
- [35] G. Nikolić, M. Cakić, Ž. Mitić, 2th European Chemistry Congress, Torino, Italy, 2008, CD-ROM
- [36] G. Nikolić, M.D. Cakić, Ž.J. Mitić, G.S. Nikolić, R.S. Nikolić, Lj.A. Ilić, *Hem. Ind.* 61(5) (2007), 257
- [37] G. Nikolić, M. Cakić, Ž. Mitić, R. Nikolić, Lj. Ilić, *Hem. Ind.* 59(1-2) (2005), 9
- [38] Ž. Mitić, G. M. Nikolić, M. Cakić, S. Mitić, G. S. Nikolić, S. Najman, *Acta F. Med. Naiss.*, 35(1) (2018), 37
- [39] G. Nikolić, M. Cakić, S. Glišić, D. Cvetković, Ž. Mitić, D. Marković, in *Fourier Transforms - High-tech Application and Current Trends*, G. Nikolic, M. Cakic, D. Cvetkovic (Eds.), InTech, Rijeka 2017, p. 149.
- [40] Ž. Mitić, A. Stolić, S. Stojanović, S. Najman, N. Ignjatović, G. Nikolić, M. Trajanović, *Mat. Sci. Eng. C*, 79 (2017), 930-949.
- [41] M. Cakić, Ž. Mitić, G.S. Nikolić, Lj. Ilić, G.M. Nikolić, *Spectrosc. Int. J.*, 22(2-3) (2008), 177
- [42] Ž. Mitić, G.S. Nikolić, M. Cakić, P. Premović, Lj. Ilić, *J. Mol. Struct.*, 924-926 (2009), 264
- [43] G. Nikolić, M. Cakić, Ž. Mitić, Lj. Ilić, *Russ. J. Coord. Chem.*, 34(5) (2008), 322
- [44] Ž. Mitić, M. Cakić, G. Nikolić, *Spectrosc. Int. J.*, 24(3,4) (2010), 269
- [45] Ž. Mitić, M. Cakić, G.S. Nikolić, Lj. Ilić, M. Stanković, *Hem. Ind.*, 64 (1) (2010), 9
- [46] G. Brink, M. Falk, *Spectrochim. Acta A* 27(9) (1971), 1811
- [47] B. Berglund, J. Lindgren, J. Tegenfeldt, *J. Mol. Struct.* 43(2) (1978), 179
- [48] G.S. Nikolić, M. Cakić, Ž. Mitić, B. Ilić, P. Premović, A. Russ. *J. Phys. Chem. A*, 83(9) (2009), 1520
- [49] M. Cakić, Ž. Mitić, G. Nikolić, I. Savić, I. Savić, *Expert Opin Drug. Disc.*, 8(10) (2013), 1253
- [50] S. Glišić, G. M. Nikolić, M. D. Cakić, N. V. Trutić, *Russ. J. Phys. Chem. A*, 89(7), (2015), 1254
- [51] S. Glišić, M. Cakić, G. Nikolić, B. Danilović, *J. Mol. Struct.*, 1084 (2015), 345-351.
- [52] M. Cakić, S. Glišić, G. Nikolić, G. M. Nikolić, K. Cakić and M. Cvetinov, *J. Mol. Struct.*, 1110 (2016), 156-
- [53] Lj. Stanojević, J. Zvezdanović, B. Danilović, D. Cvetković, J. Stanojević, D. Ilić, M. Cakić, *Adv. Technol*, 7(2) (2018) 31.
- [54] M. D. Cakic, S. Glisic, D. Cvetkovic, M. Cvetinov, Lj. Stanojevic, B. Danilovic, K. Cakic, *Coll. J.* 80 (6) (2018) 803
- [55] N. Bednar, PhD Thesis, University of Novi Sad, Serbia, (2014).
- [56] S.A. Hapse, P.T. Kadaskar, A.S. Shirsath, *Der Pharm. Lett.* 3 (6) (2011) 18
- [57] A. Clearfield, J. Reibenspies, and N. Bhuvanesh, *Principles and Applications of Powder Diffraction*, New York: Wiley, 2008.
- [58] Bar, H., Bhui, D.K., Sahoo, G.P., Sarkar, P., De, S.P., and Misra, A., *Colloids Surf. A*, 339, (2009), 134.

Izvod**NANO-BIOKOMPLEKSI NA BAZI OLIGOSAHARIDA I NJIHOVIH DERIVATA**

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Predmet ovog rada je razvoj novih nano-biokompleksa gvožđa, kobalta i bakra na bazi oligosaharida (dekstran, pullulan, inulin) kao i kompleksa bakra i kobalata sa dekstran sulfatom i karboksi metil dekstranom i nanočestica srebra sa derivatima ovih oligosaharida i vodenim ekstraktom *Fumaria officinalis* L. Ispitivan je uticaj konstitucije liganda na ishod sinteze, prikazani su i rezultati fizičko-hemijskih i spektroskopskih metoda ispitivanja (HPLC, GFC, FTIR, ESR, XRD, EDX, SEM, TEM, UV-Vis, AAS) na osnovu kojih su predloženi modeli strukture ispitivanih nano-biokompleksa. Rezultati ispitivanja stabilnosti i farmako-biološke aktivnosti ukazuju na moguću primenu za izradu različitih farmaceutskih, kozmetičkih i drugih preparata za primenu u humanoj medicini, veterini, kozmetici i drugim oblastima.

Ključne reči: nano-biokompleksi, gvožđe, bakar, kobalt, srebro, oligosaharidi, farmako-biološke karakteristike.