

The investigations of bioactive copper(II) complexes with reduced low-molar dextran

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Abstract. The optimization of the reaction conditions (pH, T , t) to obtain stable copper(II) ion complexes with dextran derivatives were investigated in this paper. A complete synthesis of stable aqueous complexes can be realized with reduced low-molar polysaccharides, at an average molar mass 5000 g mol^{-1} and pH 7.5–8. Fourier-transform IR spectra of polysaccharide dextran and its compounds with copper(II) ion, recorded at room temperature, were analyzed in order to obtain the information about the structure and the conformation of these polymer compounds. The ESR parameters of the spectra indicate the square-planar coordination of Cu(II) ion with four O atoms. Copper(II) complex formation with dextran and its derivatives were analyzed by physicochemical methods. Synthesized complexes of Cu(II) ion with reduced low-molar dextran in comparison with commercial preparations showed the considerably lower acute toxicity (LD_{50} 1705).

Keywords: Complexes, copper(II) ion, dextran derivatives, physicochemical characterization, pharmacology

1. Introduction

Copper is a biometal, essential for living organisms. It is a hematopoetical active element [1] of some metalloenzymes regulating the iron absorption in intestines, maintaining, at the same time, the iron in a reduced state and influencing the iron incorporation into hemoglobin. The copper amount necessary is usually supplemented by a normal diet in both humans and animals. Copper deficiency causes a number of pathological states [2]. Complex compounds of Cu(II) ion are important for prevention and treatment of some anemia caused by iron deficiency. The carbohydrate type compounds as ligands have been of a considerable interest. Simple sugars and their derivatives with reduced and oxidized groups form metal ion complexes of a various composition and stability. In both human and veterinary medicine commercial copper preparations based on polysaccharide dextran and its derivatives are used for such purpose [3]. According to literature data, dextran has the ability of complex formation with various biometals (Co, Zn, Ca and Mg) [4–7]. Iron complexes with different polysaccharides have special importance, and they have been described in detail [8–12]. The interaction of Cu(II), Ni(II) and Fe(III) ions with dextran may be used for their speciation by ultrafiltration [13]. Synthesis procedures for the complex formation of Cu(II) ion with polysaccharides, including dextran, are described in scientific literature [14–16].

In the present work, we analyzed the IR and ESR spectra of Cu(II) ions complexes with reduced low molar dextran (RLMD). The stability of the synthesized complexes in HCl solution and the conductivity of water solutions were investigated. The acute toxicity expressed by LD_{50} values was determined.

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2. Experimental

2.1. Complex synthesis

Copper(II) ion complex synthesis with RLMD have been described in detail by Mitić et al. [16].

2.2. Spectroscopic and physicochemical characterization of complexes

For IR sample preparation KBr pastille method was used. The IR spectra as an average of 40 scans were recorded at room (298 K) temperature on FTIR spectrometer BOMEM MB-100 (Hartman & Braun) equipped with a standard DTGS/KBr detector, in the range of 4000–400 cm^{-1} with the resolution of 2 cm^{-1} , by Win-Bomem Easy software. For ESR measurements an X-band Bruker ESP 300 spectrometer was employed. ESR spectra of powder samples in quartz tubes (3 mm i.d.) were recorded at room temperature. The copper content of the complexes was measured by atomic absorption spectrometry (AAS) on the Philips Pye Unicam SP-9 spectrometer (Cu-lamp $\lambda = 324.8$ nm; the breadth of slot 0.5 nm; the flame: acetylene/air), under the standardized procedure [17]. The conductivity meter HANNA HI 8020 (HANNA Instruments) was employed for conductivity measurements. The conductivity meter was calibrated with HI 7031 standard solution at 25°C. The conductivity of water solutions (200 mg complex in 20 cm^3 of redistilled water) was monitored for 50 days and measured three times a day, at room temperature, with constant stirring on a magnetic stirrer. The fact that the conc. HCl entails the decomposition of the RLMD complex with Cu(II) in the investigation of its stability, the diluted 3 mol dm^{-3} HCl has been used. In order to study the Cu(II)–RLMD complex, hydrolytic stability against the strong acid was added, 2 cm^3 of 3 mol dm^{-3} HCl into 2 cm^3 of aqueous solution containing 300 mg of the complex. The time necessary for a complete change of color, from dark green of the complex solution to light blue was measured.

2.3. Pharmacological testing

The test of systematic acute toxicity of the Cu(II)–RLMD complex containing 19.85% of Cu(II) was executed by the standard pharmacological test used in the Biomedical Laboratory of “Zdravlje Actavis Co.”, Leskovac. White mice of both sexes, weighting 18–22 g, were tested by oral administration of 5%, 10%, 15% and 20% aqueous complex solution. Four groups of 10 animals were monitored for 10 days in order to determine LD_{50} value [18].

3. Results and discussion

The plan for the experimental synthesis of the Cu(II) complex with reduced dextran has required a detailed analysis of the synthesis procedure; both from the aspect of the reaction conditions of the synthesis and from the aspect of obtaining the stable and commercially applicable preparation of the complex. The analysis of the synthesis of similar complexes has pointed to the necessity of defining the physicochemical properties of commercial preparations. By their correlation, the undesired effects can be eliminated and thus a considerably improved pharmacological effect of the complex. For this reason, the choice and optimization of the low molar dextran in the capacity of the ligand have been made. Considering the importance of physicochemical parameters on the process and the synthesis results, the examination and optimization of ligands in relation to molar mass (M_w), as well as the reaction

Table 1
Reaction conditions related to synthesis of Cu(II) complexes with RLMD ligand

Procedure	Ligand	pH synth.	<i>T</i> synth.	<i>t</i> (min) synth.	Results of synth.
1	RLMD M_w 5000	7.5	Room	60	~60%
2	RLMD M_w 5000	7.5	Boiling	7	positive
3	RLMD M_w 5000	8.0	Boiling	7	positive
4	RLMD M_w 5000	10.0	Boiling	7	~65%
5	RLMD M_w 5000	12.0	Boiling	7	~60%
6	RLMD M_w 5000	7.5	Autoclave 130°C	60	~50%

Table 2
Characteristic physicochemical properties of synthesized Cu(II) complexes with RLMD

Procedure	pH synth.	Complex color	Cu (%) content	Water solubility	Stability (HCl effect) (min)	Stability (ageing, 6 months)
1	7.5	Light green	7.23	Soluble	3	Stable, clear solution
2	7.5	Green	19.85	Very soluble	12	Stable, clear solution
3	8.0	Green-blue	8.12	Soluble	6	Stable, clear solution
4	10.0	Blue-green	8.20	Sparingly soluble	4	Precipitate
5	12.0	Dark blue	6.97	Slightly soluble	2	Solution layering
6	7.5	Dark green	4.08	Slightly soluble	1	Turbid solution

conditions of the synthesis (pH, *T* and *t*) were investigated in this paper. The results of testing are shown in Table 1.

The basic characteristics of synthesized Cu(II) complexes with RLMD are given in Table 2.

Comparing the obtained complexes of Cu(II) with RLMD, either in solid state or in solution, it is obvious that, depending on pH values, various complex colors are obtained (Table 2). The change of the solutions color during the synthesis may be an indicator whether the syntheses of complexes were successful. The results obtained have shown that, in the range of pH 7.5–12, the color can vary from light green to dark blue. This is confirmed by the green solution color of the most stable complex of Cu(II) with RLMD (procedure 2, Table 2), in comparison with an indigo-blue alkali solution of decomposed Cu(II) at pH 13, where $[\text{Cu}(\text{OH})_4]^{2-}$ ions dominate. Water solubility of synthesized complexes of Cu(II) with RLMD is different. The most water soluble complex is obtained at pH 7.5 (Table 2). The solution is permanent and stable after a longer period of time (6 months). The complexes that are synthesized at higher pH are less soluble. The solution of the complexes obtained, following the procedure 5 (Table 1), after resting for long period of time, start layering, precipitate and become opalescent.

Medium pH is changed after adding Cu–salts and Cu(II) content in a complex is much influenced by it. Syntheses are performed at the same temperatures and within the same reaction period, but at different pH values (Table 1). The highest Cu(II) content was got at pH 7.5 (Table 2). The possibility of obtaining Cu(II)–RLMD complexes with a higher Cu(II) content has been tested with the increased concentration of Cu–salts. The expected results have not been obtained.

Solution pH probably has the influence on the way of binding of Cu(II) into a complex, i.e. on the type of a bond because, due to the change of pH value, the stability [19], the color and the solubility of the complex obtained are also changed (Table 2).

With Cu(II) complex with RLMD that is synthesized in the neutral and weak alkaline solution (pH 7.5–8) a very low conductivity values are noticed (105 and 215 $\mu\text{S cm}^{-1}$, respectively, Fig. 1), comparing with the conductivity of the Cu(II) ions water solution of the same concentration (2580 $\mu\text{S cm}^{-1}$).

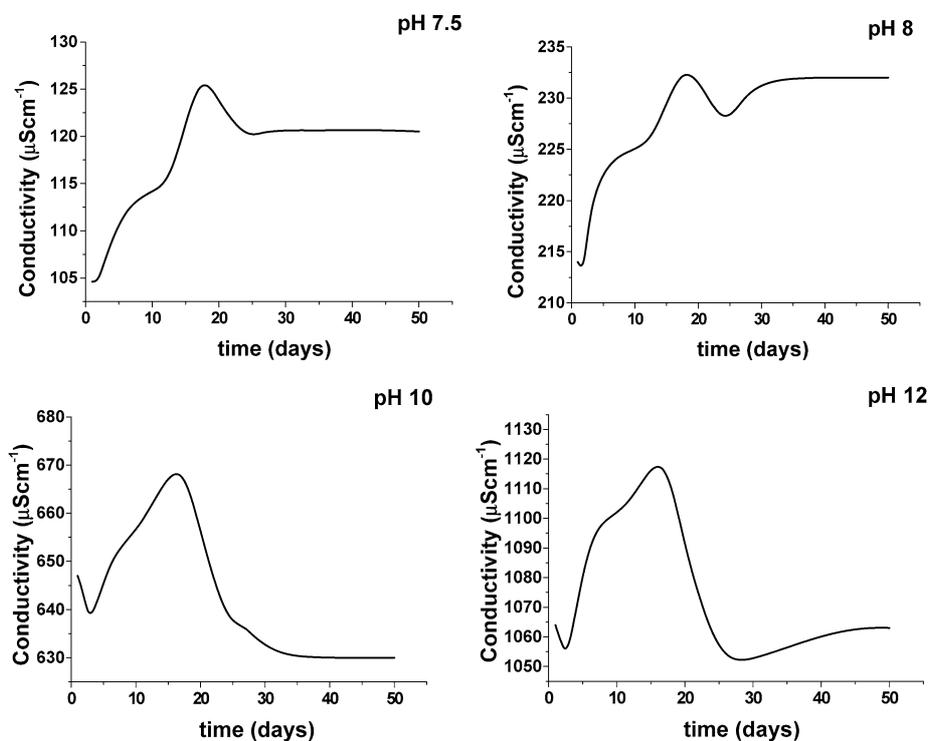


Fig. 1. Water solution conductivity of Cu(II) complexes with RLMD in function of time, at different pH synthesis values.

With the Cu(II) complex with RLMD that is synthesized in the alkaline solution (pH 10) the conductivity values are considerably higher ($640 \mu\text{S cm}^{-1}$), which indicates a more unstable complex. The conductivity growth during the first 20 days is a little bit increased than in the previous case ($30 \mu\text{S cm}^{-1}$), and then the conductivity value of the solution rapidly decreases below the initial value (Fig. 1). The fact indicates a precipitation of the insoluble $\text{Cu}(\text{OH})_2$. And, afterwards, it keeps the constant value, similar to the previous case. With Cu(II) complex with RLMD that is synthesized at pH 12, the conductivity value is 10 times higher comparing to the conductivity of the complex obtained at pH 7.5. The conductivity rapidly increases in the same period of time of 20 days, with the considerably higher growth of $60 \mu\text{S cm}^{-1}$, and then rapidly decreases to its initial value (Fig. 1).

Synthesized complexes differ in their stability to a hydrolytic effect of HCl. The complex of Cu(II) with RLMD obtained by the procedure 2 (Table 1), besides having the highest Cu(II) content (19.85%), also has the highest stability on 3 mol dm^{-3} HCl hydrolytic effect (12 min). Tolmachev et al. [20], in their paper of the influence of medium pH on binding Cu(II) with dextran, point out the possibility of a gradual complexing, i.e. a gradual forming of coordination bonds, with their reforming starting at pH 8. Thus, Cu(II) ions form three different types of complexes with deprotonized dextran monomer units [21]. In addition, depending on pH values, the complexes of Cu(II) with RLMD also behave differently, considering wavelength at which they show the absorption maximum. This range of wavelengths in the VIS spectra is 650–700 nm [14,16].

The results of FTIR spectroscopic investigations show that spectra of Cu(II)–RLMD complexes and ligand are basically similar (Fig. 2).

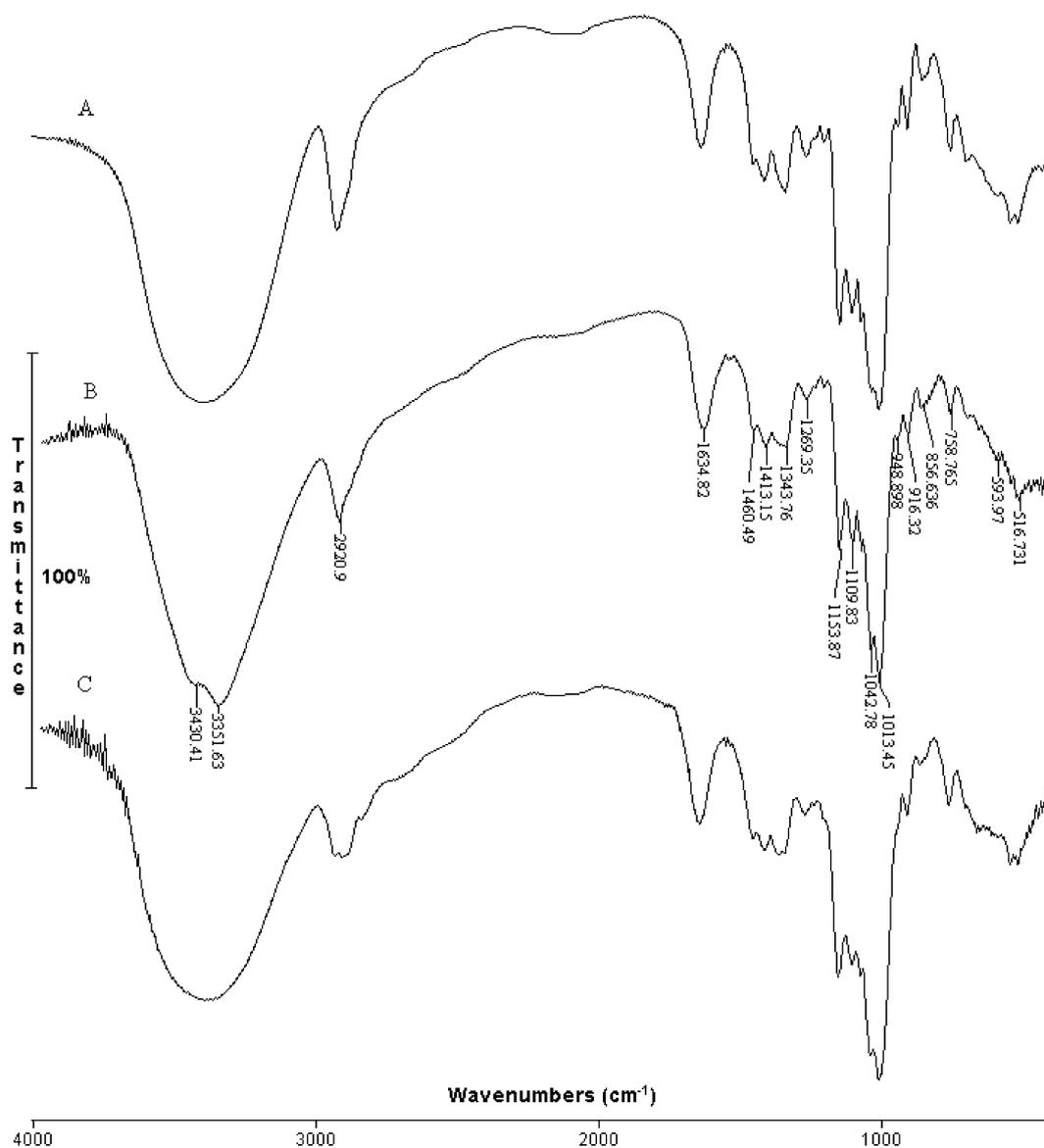


Fig. 2. FTIR spectra of: LM dextran (A); stable Cu(II)-dextran complex, with high metal content ($\sim 18\%$), obtained at pH 7–8 (B); unstable Cu(II)-dextran complex, with low metal content ($\sim 5\%$), obtained at pH 10–12 (C).

They are different in the range of stretching (O–H) and (C–H) vibrations. FTIR spectra of Cu(II)-RLMD complexes synthesized at different pH (pH 7–8 Fig. 2B, and pH 10–12, Fig. 2C) recorded at 298 K, show that the correlation between the O–H stretch frequency and the hydrogen bond strength. The difference in frequencies, intensity, and shape of these bands in the region $3600\text{--}3100\text{ cm}^{-1}$, implies that in complexes which were synthesized at pH 10–12 there is the displacement of H_2O molecules by O–H groups in the first coordination sphere of the copper(II) ion (Fig. 3).

Dextran and complexes with Cu(II) ion have one crystallographic type of water molecule [22,23]. The similarities of the bending (C–H) range indicate that there is no difference in the conformation of

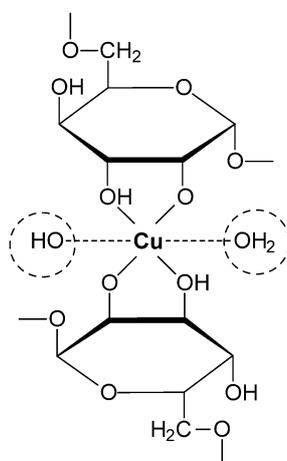


Fig. 3. Model of Cu(II) ion complex with RLMD at pH 8–10.

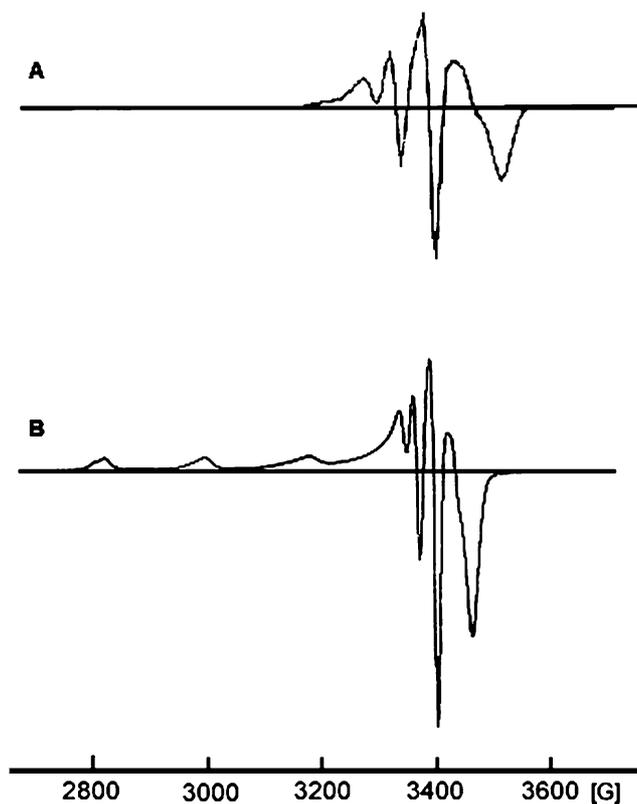


Fig. 4. ESR spectra of copper(II) complex with RLMD synthesized at pH 10 (A) and pH 12 (B).

the glucopyranose unit in the dextran and the complex molecule, and they probably exhibit C_1 chair conformation [24]. ESR parameters of spectra in Fig. 4 ($A_{\parallel} = 187 \times 10^{-4} \text{ cm}^{-1}$, $g_{\parallel} = 2.23$ and $g_{\perp} = 2.03$), for the complexes synthesized at higher pH values, were close to the values for the frozen

Table 3
Characteristic parameters of investigated acute toxicity Cu(II) complexes with RLMD

Tested species (18–22 g)	Concentration of complexes (%)	LD ₅₀	Equivalent Cu dose (mg Cu/kg of b.w.)
Mouse	5	1419	281.7
Mouse	10	1661	329.8
Mouse	15	1557	309.1
Mouse	20	1660	329.6

Pharmacological investigations were performed with the sample of the most stable complex obtained following the procedure 2 (Table 1).

Cu(II)–ethylene glycol complex, thus indicating the square-planar coordination of Cu(II) ion with four oxygen atoms. This conclusion corresponds with the results obtained by FTIR investigations [23], as well as with the results obtained by UV-VIS investigations [16].

Although the Cu(II) ion content of complexes synthesized at lower pH values was much higher (up to 18.95% 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 [25].

After physicochemical standardization of the most stable complex obtained according to the procedure 2 (Table 1), the preparation for the pharmacological test was provided. 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 (Table 3).

None of the applied complex doses at the concentration of 82–169 mg equivalent of Cu(II) per kg of a mouse's body weight was lethal in the tested mice. Therefore, in this case, a median lethal dose could not be determined. The application of higher doses (Table 3) has caused the mortality of one part of experimental animals. Thus, in this range, the preparation toxicity LD₅₀ of 1419–1661 was determined, which corresponds to the equivalent of Cu(II) dose of 281–329 mg per kg of the body weight, in the concentration 5–20% of the complex solution. Toxicity investigations of various commercial copper salts show a wide range of values for LD₅₀. The level of the acute toxicity is higher for more soluble than for less soluble Cu(II) salts [26]. The results of our pharmacological investigations point to the lower toxicity of Cu(II) complex with RLMD, what is much better than in the case of commercially applicable inorganic copper salts [2].

4. Conclusion

Synthesized Cu(II) complexes with RLMD as a ligand differ in color, stability, solubility in water and conductivity. The complex of Cu(II) with RLMD which is synthesized at pH 7.5 at boiling temperature is most stable on hydrolytic HCl action. According to the results obtained by the conductivity investigation it can be concluded that the complex synthesized in the weak alkaline solution (pH 7–8) shows the greatest stability. The differences in the region 3600–3100 cm⁻¹, imply that complexes originate from the displacement of H₂O molecules by O–H groups in the first coordination sphere of the Cu(II) ion. ESR spectra parameters indicate the axial symmetry of synthesized Cu(II) complexes and are typical of Cu(II) ion in 3d¹ electronic configuration. The results of preliminary pharmacological investigations showed the lower toxicity of complexes of Cu(II) with RLMD, which is much better than toxicity of commercially applicable inorganic salts.

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References

- [1] A.G. Lewis, *The Biological Importance of Copper*, Literature Review ICA Project, No. 223, British Columbia, 1995, pp. 7–17.
- [2] Expert group on vitamins and minerals, Draft review of copper, EVM/99/19, Secretariat, UK Government, London, May 2002.
- [3] Lj.A. Ilić, Ž.J. Mitić, G.S. Nikolić and M.D. Cakić, Synthesis and pharmacological activity of copper(II) and cobalt(II) complexes with dextran oligomers, in: *XVII Ulusal Kimya Kongresi*, Istanbul, TR, 2003, p. 190.
- [4] Ž.J. Mitić, M.D. Cakić, G.S. Nikolić, R.S. Nikolić and Lj.A. Ilić, The investigation of Co(II) dextran complexes, *Chem. Industr.* **61**(5) (2007), 257–262.
- [5] M. Cakić, G. Nikolić, Ž. Mitić and Lj. Ilić, Synthesis and spectroscopic characterization of zinc(II) complexes with dextran derivatives, in: *5th International Conference of the Chemical Societies of the South-East European Countries*, Ohrid, MK, 2006, p. 544.
- [6] Z.A. Lugovaya, V.N. Tolmachev, V.I. Naiden, A.I. Valakhanovich and R.V. Zaretskaya, Reaction products of low-molecular-weight dextran with copper, *Pharm. Chem. J.* **10**(11) (1976), 1536–1538.
- [7] B. Gyuresik and L. Nagy, Carbohydrates as ligands: Coordination equilibria and structure of the metal complexes, *Coord. Chem. Rev.* **203** (2000), 81–149.
- [8] Lj. Ilić, S. Ristić, M. Cakić, G. Nikolić and S. Stanković, Polynuclear complex Fe(III) with pullulan oligomers, process of its obtaining, and pharmaceutical preparations on the basis of the complex, PCT, Int. No. WO 02/46241 A2, Int. Cl. C08B 37/00, Int. Pub. Date 13 June, 2002.
- [9] G.S. Nikolić and M.D. Cakić, Physical investigation of the colloidal iron-inulin complex, *Coll. J.* **69**(4) (2007), 464–473.
- [10] B. Pekić and D. Cvetković, Int. Cl. C08B37/02, Appl. No. 564; YU Pat. 42 384 B, 1988.
- [11] M. Cakić and G. Nikolić, Iron complexes of pharmaceutical interest – Antianemics, *Chem. Industr.* **57**(10) (2003), 437.
- [12] G. Nikolić, M. Cakić, Lj. Ilić, S. Ristić and Ž. Cakić, Synthesis of some new antianemics I. Iron pullulan complexes of pharmaceutical interest, *Die Pharmazie* **57**(3) (2002), 155–158.
- [13] D. Solpan and M. Sahan, Concentration and separation of metallic ions from aqueous solutions by using EC-PEG 4000 alloy membranes, *J. Appl. Polym. Sci.* **48**(12) (1993), 2091–2093.
- [14] G. Nikolić, M. Cakić, Ž. Mitić, R. Nikolić and Lj. Ilić, Correlation between UV–VIS spectra and structure of Cu(II) complexes with hydrogenated dextran in alkaline solutions, *Chem. Industr.* **59**(1/2) (2005), 9–14.
- [15] Ž. Mitić, M. Cakić, R. Nikolić, Lj. Ilić and G. Nikolić, Polysaccharide complexes of pharmaceutical interest 1. Water solutions stability of Cu(II) complexes with reduced low-molecular dextran, *World Polym.* **7**(5) (2004), 225–229.
- [16] Ž. Mitić, G.S. Nikolić, M. Cakić, R. Nikolić and Lj. Ilić, Synthesis and spectroscopic characterization of copper(II) dextran complexes, *Russ. J. Phys. Chem.* **81**(9) (2007), 1433–1437.
- [17] Ž. Mitić, G. Nikolić, M. Cakić and Lj. Ilić, Physicochemical characterization of copper(II) and cobalt(II) complexes with dextran oligomers, in: *XVII Ulusal Kimya Kongresi*, Istanbul, TR, 2003, p. 206.
- [18] D.L. Eaton and C.D. Klaassen, Principle of toxicology, *Casarett and Doull's Toxicology – The Basic Science of Poisons*, 6th edn, McGraw-Hill, New York, 2000, pp. 17–24 (Ch. 2).
- [19] G. Nikolić, Ž. Mitić, M. Cakić and Lj. Ilić, Study of copper(II)–dextran complexes stability by conductometry method, in: *8th International Conference on Fundamental and Applied Aspects of Physical Chemistry*, Belgrade, Serbia, 2006, pp. 719–721.
- [20] L.I. Shevchenko, V.N. Tolmachev and A.Z. Lugovaya, Study of the complexing properties of the dextran carboxymethyl ether with transition metal ions in solutions, *Polym. Sci. USSR* **27**(9) (1975), 2242–2247.
- [21] E. Norkus, J. Vaičiuniene, T. Vuorinen and M. Heikkilä, Interaction of Cu(II) with dextran in alkaline solutions, *Carbohydr. Polym.* **50** (2002), 159–164.
- [22] G. Nikolić, M. Cakić, Ž. Mitić and Lj. Ilić, Copper(II) complexes of reduced low-molar dextran: a deconvoluted LNT-FTIR study, in: *8th International Conference on Fundamental and Applied Aspects of Physical Chemistry*, Belgrade, Serbia, 2006, 722–724.
- [23] G.S. Nikolić, M. Cakić, Ž. Mitić and Lj. Ilić, Deconvoluted Fourier-transform LNT-IR study of coordination copper(II) ion compounds with dextran derivatives, *Russ. J. Coord. Chem.* **34**(4) (2008), 1–7.
- [24] Ž. Mitić, G. Nikolić, M. Cakić, R. Nikolić and Lj. Ilić, Fourier-transform IR spectroscopy of Cu(II) ion complexes with different polyglucans, in: *XII European Conference on the Spectroscopy of Biological Molecules (ECSBM'2007)*, Bobigny, Paris, 2007, p. 319.

- [25] G.M. Nikolić, Lj. Ilić, M. Cakić, G.S. Nikolić, R. Nikolić and Ž. Mitić, Copper(II) complexes of reduced low-molecular dextran: an ESR study, in: *7th International Conference on Fundamental and Applied Aspects of Physical Chemistry*, Belgrade, Serbia, 2004, pp. 775–777.
- [26] J. Živanov-Čurlis, J. Tomin, P. Vasiljević, M. Vukelić, Lj. Đorđević and Ž. Mitić, The influence of long-term intake of copper and chromium compounds on reproductive ability and preadult development of *Drosophila melanogaster*, *Biotechnol. Biotec. Eq.* **20**(2) (2006), 62–66.



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