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Conductometric studies on the stability of copper complexes with different oligosaccharides

Research Article

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Abstract: Bioactive copper complexes with pullulan or dextran oligosaccharides are the subject of intensive research mainly because of their possible application in veterinary and human medicine. The thermal stability and stability under oxidative conditions of the Cu(II) complexes with reduced low-molar pullulan or dextran were investigated in this paper, using a conductometric method. The influence of ligand constitutions on the stability of the complexes was examined on the basis of ligand property. Forced degradation studies were performed on bulk sample of complexes by using heat (25, 40 and 60ºC) and an oxidation agent (0.1, 0.5, 1.0 and 10.0% v/v hydrogen peroxide). It can be concluded, according to the results obtained (by examining conductivity during the forced degradation studies), that Cu(II) complexes show a large pharmaceutical stability for both tests.

Keywords: *Copper(II) • Polysaccharide • Complex • Conductometry • Stability*

1. Introduction

Many types of carbohydrate derivatives (reduced or oxidized) were synthesized for biomedical applications. Pullulan and dextran are polysaccharides that are used in a drug delivery, because of theirs solubility and biocompatibility. Pullulan is a water-soluble, extracellular, neutral polysaccharide with a linear flexible chain of α-(1,6)-linked maltotriose units. The structure is intermediate between pullulan and amylose structures, because it has both α-(1,6) and α-(1,4)-glycosidic bonds [1]. Dextran is a branched glucane with chains of varying lengths, made from many glucose molecules [2]. The straight chains consist of α-1,6 glycosdic bonds between glucose molecules, while branches occurs with α-1,4 bonds (in some cases with α-1,2 and α-1,3 bonds). Inspite of their many ionic groups, pullulan and dextran are neutral molecules.

The copper(II) ion is a biologically active, essential ion, its clearing ability, and positive redox potential allows participation in biological transport reactions. Cu(II) complexes display a wide range of biological activity, and are among the most potent antiviral, antitumor and anti-inflammatory agents [3]. On the other hand, metal complexes with polysaccharides and their derivatives are important in medicine and pharmacy. For example, polypher is a well-known blood substitute [4]. New blood substitutes with hemostimulating and antianemic function, which are complexes of dextran or pullulan with Fe(II), Fe(III), Cu(II) and Co(II) ions, differ from the existing analogues, in good bio- and hemocompatibility, and more pronounced and prolonged action [5-8]. Magnetic complexes, based on polysaccharide derivatives with Fe, Ca, Zn, Co, Ni and Cu oxides, are used in roentgenologic studies. These complexes must be very stable during prolonged storage and non-toxic [9,10].

Reduced low-molar pullulan (RLMP) and low-molar dextran (RLMD) were chosen as new materials for complexing, and subsequent interactions with Cu(II)

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ions. In alkaline solutions Cu(II) ions form complexes with these ligands [11,12]. The complexing process begins in a mild alkaline solution (pH > 7), and involves OH groups on C(2), C(3) and C(4) in dextran, or C(2), C(3) and C(6) in pullulan monomer units (a-D-glucopyranose) (Fig. 1). Complexes of Cu(II) ions with reduced low-molar pullulan or dextran were synthesized in the water solutions, at the boiling temperature, and pH values of 7.5. The metal content and the solution composition depend on ligand constitutions and pH values [13]. In the solid state these complexes are very stable during prolonged storage at room temperature and are non-toxic [14]. Potential structures of the bioactive copper(II) complex with dextran or pullulan oligosaccharide (Fig. 1) were confirmed by physicochemical and spectroscopic characterization [15-17].

Bioactive copper(II) complexes with polysaccharides of dextran or pullulan, as an active pharmaceutical compound, can be use for making a new antihypocuprermical formulations. These complexes are not yet official in any pharmacopoeia. The patent literature has only data about the synthesis procedure, with physicochemical and spectroscopic characterization [11,18]. The results of correlations between a structure and stability study of copper(II) complexes with RLMD by conductometric methods was well described in the literature [14]. However, the pharmaceutical stability study of bioactive copper complexes with oligosaccharides has not been described. Forced degradation studies or stress testing are important for new active substances for drug development. The most relevant information about product-related degradations can be obtained by determining the stability studies. Stress testing should include the effects of temperature, humidity, hydrolysis, oxidation and photolysis on the drug substance and drug product.

A conductometric method is useful for determining the properties of complex in aqueous systems [19,20]. In this paper a conductometric method was used to assess the stability of the complex during the forced degradation studies. Electrical conductivity or specific conductance is a measure of the ability of materials to conduct electricity. The conductivity of an aqueous solution is dependent on the amount of dissolved salts and sometimes other chemical species, which tend to ionize in the solution. The conductance of a solution depends on the number and types of ions. Generally, small and highly charged ions conduct current better than larger and smaller charged ions. The size of ions is important, because it determines the speed at which they travel through the solution. Small ions can move more rapid than larger ones. The measured conductance is the total conductance of all ions in the solution. Since all ions contribute to the conductivity of solution, the method is not particularly useful for qualitative analysis, *i.e.*, the method is not selective. The major use for the conductometric method is the monitoring of the total conductance of solution.

Total Dissolved Solids (TDS) is the measure of total ions in the solution. Electrical conductivity (EC) is a measure of the ionic activity of the solution. In diluted solution, TDS and EC are reasonably comparable [21]. TDS of water soluble samples can be calculated by using the following equation:

$$TDS\,(mg\ dm^{-3}) = 0.5 \times EC\,(dS\ m^{-1}) = 0.5 \times 1000 \times EC\,(mS\ cm^{-1}) \quad (1)$$

If the solution becomes more concentrated (TDS > 1000 mg dm^{-3}, EC > 2000 mS cm^{-1}), the close proximity of the ions decreases their activity and ability to transmit current, although the amount of dissolved solids are greater. At higher TDS values, the ratio TDS/EC is increased, and the relationship tends TDS = 0.9×EC. In these cases, the previously mentioned relationship should not be used; each sample should be characterize separately.

The aim of this paper is monitoring of the thermal and oxidative stability of bioactive copper(II) complexes with pullulan or dextran, as potential active substances, of new antihypocuprermical formulations by using the conductometric method. Forced degradation studies were analyzed on bulk samples of complexes by using heat (25, 40 and 60ºC) and an oxidation agent (0.1, 0.5, 1.0 and 10.0% v/v hydrogen peroxide).

2. Experimental Procedure

2.1. Samples

The bioactive copper(II) complex with dextran was synthesized by original procedure [12]. The complex synthesis of copper(II) with pullulan is described in detail by Nikolic *et al.* [11]. Bioactive complex of copper(II) with RLMP contained 13.1% of copper, and copper(II) with RLMD contained 19.8% of copper. The neutral aqueous solutions of complexes were filtered at room temperature through membrane filter (Millipore 0.45 μm), in order to remove possible traces of impurities. The concentration of copper ions and ligand in the filtrate was determined by atomic absorption spectroscopy (AAS) and spectrophotometry [11,12], respectively.

2.2. Dialysis of complex solutions

The complex solutions were dialyzed by capillary dialyzer (Drake Willock dialysis system, Portland, USA) with membrane AQM-1681. The basic parameters of

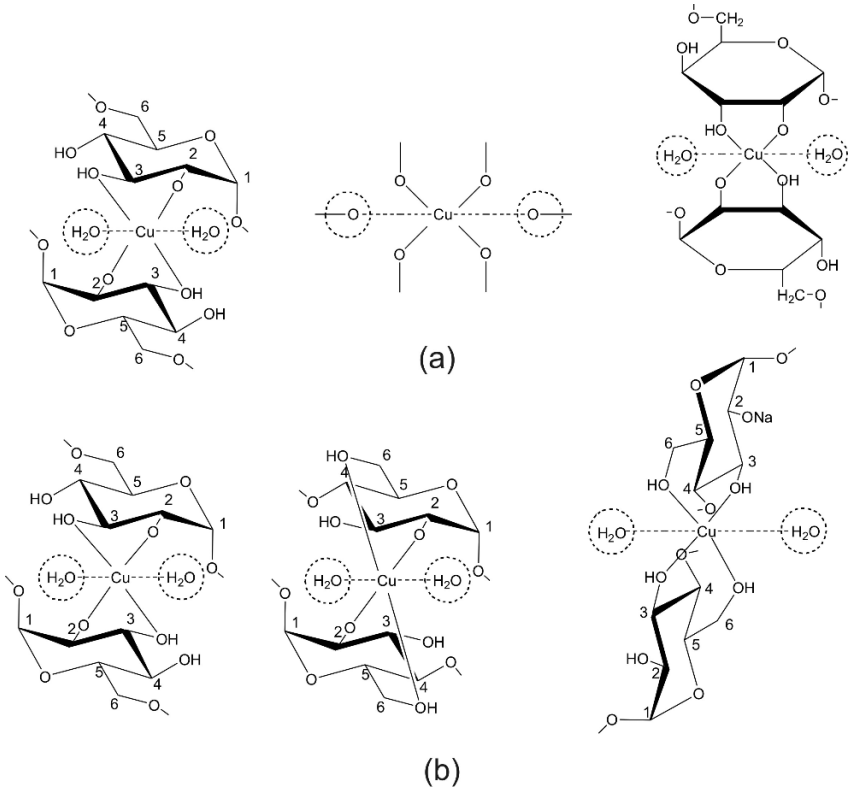

Figure 1. Structure models of Cu(II) complexes with dextran (a) and pullulan (b)

dialysis: input pressure of 150 mm Hg, output pressure of 130 mm Hg, pump flow of 400–500 cm^3 min^{-1}, dialysis time of 120 min.

2.3. Conductometric study

Conductometric measurements were carried out by Hanna HI 8020 conductometer. The conductometer was calibrated by using HI-7031 standard solution at temperature of 25°C, with temperature coefficient of β = 2% $°C^{-1}$.

2.4. Stability study

For monitoring the thermo stability, 50 mg of copper(II)-pullulan or copper(II)-dextran complex was dissolved in deionized water in a volumetric flask of 50 cm^3. The conductivity of the prepared solution was monitored for 60 min at temperatures of 25, 40 and 60°C with constant stirring (by magnetic stirrer). For monitoring the stability under oxidative conditions, 50 mg of copper complexes were dissolved separately in 0.1, 0.5, 1.0 and 10.0% v/v hydrogen peroxide solutions and filled up with the solvent solutions to the mark with the same solvent. The conductivity of these sample solutions was monitored for 60 min at temperature of 25°C with constant stirring (by magnetic stirrer).

3. Results and Discussion

The copper(II) complex was synthesized at pH values from 7 to 8 at the boiling temperature of the reactant solution with reduced low-molar pullulan (RLMP, M_w 6000 g mol^{-1}), or dextran (RLMD, M_w 5000 g mol^{-1}) and $CuCl_2$ [11,12]. The obtained complexes were green (Fig. 2), amorphous, almost odorless and freely soluble in water at 25°C [22]. Cu(II) complexes were investigated in the solid state, and in the aqueous solution. ATR-FTIR spectroscopic characterization was used for studying the composition of complex carbohydrate systems, molecular interactions, molecular orientation and conformational transitions of polysaccharides [23,24]. The application of a microscopy imaging system to the ligands (dextran and pullulan), as well as Cu(II) complexes synthesized at pH 7.5, is presented in Fig. 2. Optical microscopic images on Figs. 2a-2h from different areas of the Cu(II) complex shown high homogeneity of the samples. The appearance of the microscopic images of the ligand are different than images of the synthesized Cu(II) complexes, which indicating the formation of coordination compounds. Microscopic images confirm that the changes in the color of the analyzed compounds are strongly associated with the alterations in the macromolecular order. These changes of complexes

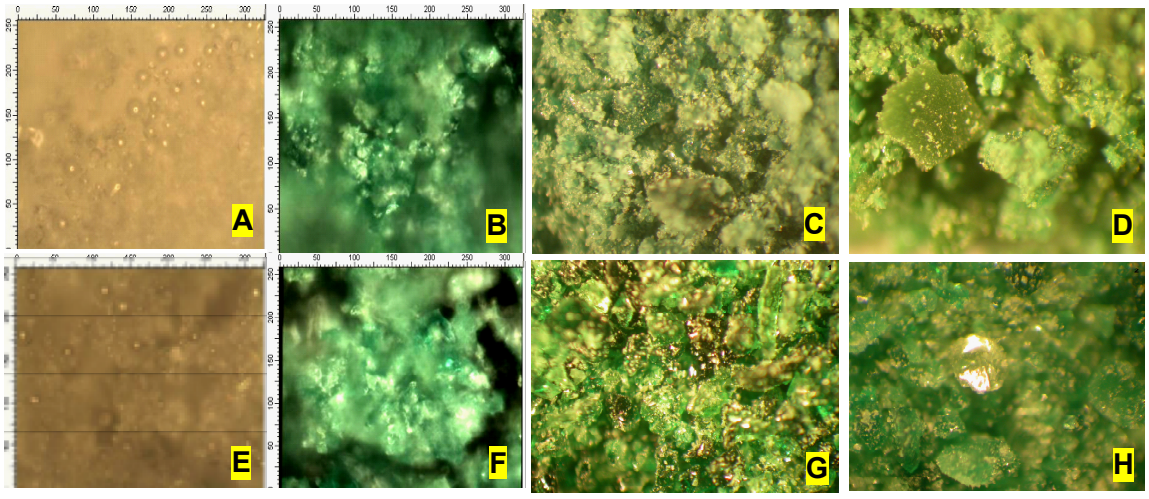

Figure 2. Optical microscopy images (300×250 μm) of: dextran (M_w=5.000 g mol^{-1}) (A), Cu(II)–dex complexes (B,C,D), pullulan (M_w=6.000 g mol^{-1}) (E), and Cu(II)–pull complexes (F,G,H)

Table 1. Temperature and conductivity requirements of pure water (values from USP24 – NF19, 5th Supplement, 645)

Temperature (°C)	Water conductivity (μS cm^{-1})	Temperature (°C)	Water conductivity (μS cm^{-1})
20	1.1	50	1.9
25	1.3	55	2.1
30	1.4	60	2.2
35	1.5	65	2.4
40	1.7	70	2.5
45	1.8	100	3.1

can be responsible more or less for the structure order. The changes in color in Fig. 2 show the content and distribution of copper, as well as polysaccharides in the Cu(II) complexes.

In the stability test, the complexes of Cu(II)-dextran and Cu(II)-pullulan were exposed to the forced degradation, by heating (25, 40 and 60°C). The thermal stability testing of the Cu(II) complexes with reduced low-molar pullulan or dextran were carried out by the conductometric method. Conductivity values of Cu(II)-RLMD were very low, compared to the conductivity of Cu(II)-RLMP under the same conditions. Conductivity values of Cu(II)-RLMP complex are much higher, which is indicated in the instability of the complex.

The untreated aqueous solution of Cu(II)-dextran complex (concentration 1 mg cm^{-3}) displayed a very low conductivity (12 μS cm^{-1}) at room temperature during 24 h. That value of conductivity was close to the conductivity of chemically pure water (1.3 μS cm^{-1}) at room temperature (Table 1). For example, water at pH value of 5.8, must have conductivity below 2.4 μS cm^{-1}. If the conductivity value is higher, the quality of this water is not sufficient, and it cannot be used for manufacturing of the pharmaceutical products.

For comparison, conductivity of the aqueous $CuCl_2$ solution was 2580 μS cm^{-1} in the case of the same copper concentration. During the termal treatment of Cu(II)-dextran complex solution (25-60°C), the conductivity in all tests increased slightly (Fig. 3a). After the treatment at different temperatures (25, 40 and 60°C), the slight increase was detected in the first 60 min. In this case, temperature was destabilized the complex and acted as a catalyst. The kinetic energy was greater after increasing the temperature, because of the increased mobility of dextran molecules. The digression of dextran molecules leads to the weaker intermolecular hydrogen bonds (between OH groups), as well the interaction between copper ions and hydroxyl groups, of glucopyranosyl dextran units. All of these lead to a negligibly small destabilization of the complex, and the increase of free copper ions in the solution. The conductivity of solution is a consequence of partly liberated Cu(II) ions from the complex. This experience indicated a high stability complex.

The conductivity of the Cu(II)-pullulan complex at 25°C was increased during 10-15 minutes. A difference in conductivity for the first 15 min was found between the thermal behavior of Cu(II)-dextran and Cu(II)-

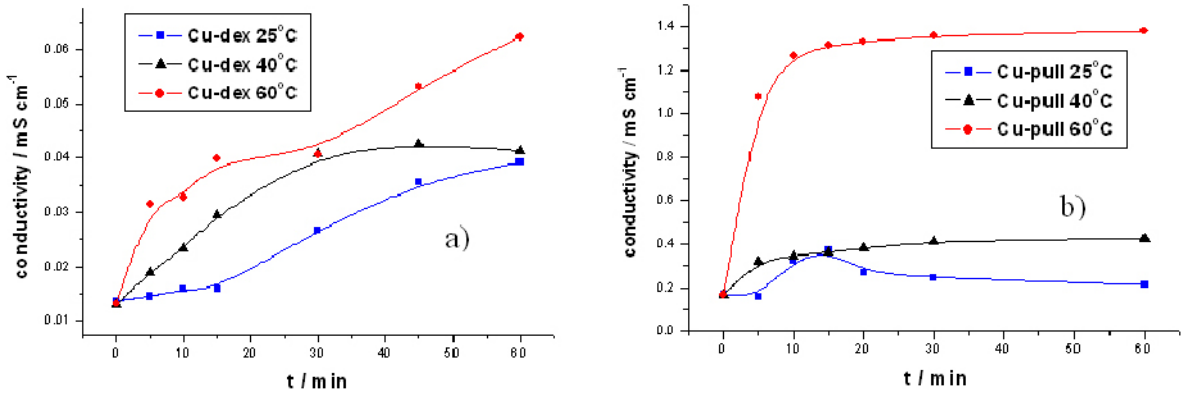

Figure 3. The functionality of conductivity and time for (a) Cu(II)-dextran, and (b) Cu(II)-pullulan complexes at different temperatures (25, 40 and 60°C)

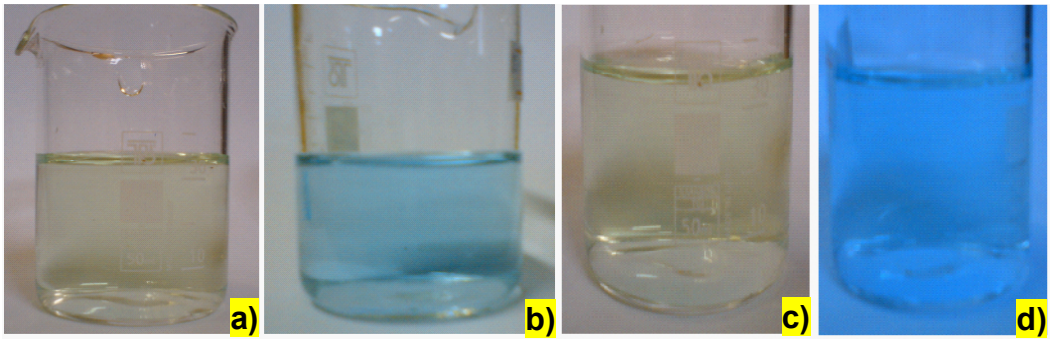

Figure 4. The color solution during the thermal treatment at 25°C (a,c) and 60°C (b,d) for Cu(II)-dextran and Cu(II)-pullulan complex, respectively

Table 2. Total Dissolved Solids (TDS) as the measure of total ions in the solution of copper(II)-pullulan and copper(II)-dextran complexes

Time	Cu(II)-pullulan complex						Cu(II)-dextran complex					
	Temperature (°C)			H_2O_2 (%)			Temperature (°C)			H_2O_2 (%)		
(min)	25	40	60	0.1	0.5	1.0	25	40	60	0.1	0.5	1.0
0	84	84	84	84	84	85	7	7	7	4	82	160
5	79	160	538	167	185	215	7	10	16	5	110	218
10	173	170	633	214	459	624	8	12	16	5	118	232
15	186	180	657	277	443	653	8	15	20	6	124	245
20	134	190	666	-	-	-	-	-	-	-	-	-
30	122	205	680	307	382	601	13	20	20	7	137	263
45	-	-	-	361	311	551	18	21	27	7	151	270
60	111	220	689	428	306	551	21	21	31	8	179	275

pullulan complexes. The conductivity of the Cu(II)-pullulan complex was increased rapidly in the first 10 min (Fig. 3b). It reached a maximum value of 370 μS cm^{-1}, after an rapid decrease of the conductivity to 200 μS cm^{-1}. This behavior, was probably due to partial hydrolysis and reorganization of the complex. The conductivity was increased by increasing the temperature. The highest conductivity (1320 μS cm^{-1}) was achieved after treatment

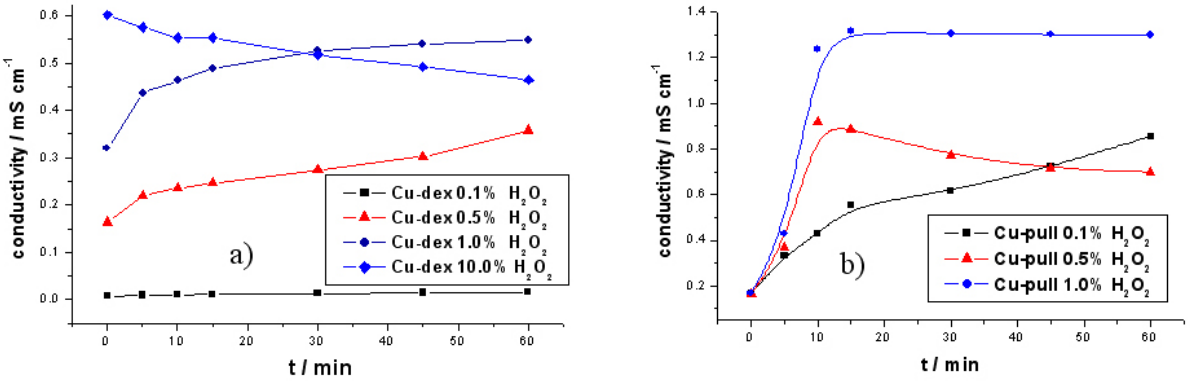

Figure 5. The functionality of conductivity and time in the case of different concentration of oxidant: a) Cu(II)-dextran and, b) Cu(II)-pullulan

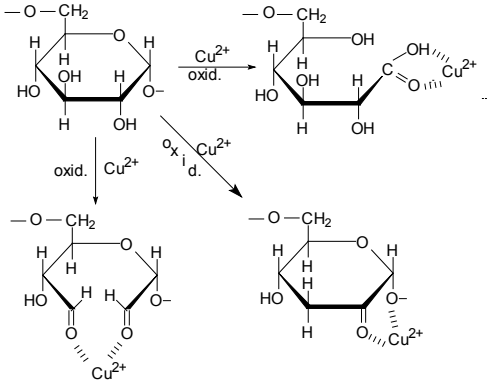

Figure 6. Pathway of destruction ligand in the presence of Cu^{2+} ions for the process of oxidation

at 60°C, in the previous case. The results showed a greater destabilization of the complex and fast release of copper ions. The explanation of the changes was the same as in previous case. But, the forty times higher conductivity is associated with a different constitution of ligands. Pullulan, a malthotriose polymer, is in a stericly unfavorable position during the thermal treatment, thus, it can sustain weak non-covalent bond with copper ions. After 15 min, at temperature of 25°C, the conductivity of Cu(II)-pullulan complex was slight lower. Probably, there was a global reorganization of complex form, with rebinding of copper ions on stericly favorable centers of glucopyranosyl units. The bigger particles have higher resistance during the movement, the concentration of free copper ions was therefore lower, and thus the conductivity was also lower. The light blue solution was unchanged after the treatment temperature, which is also a confirmation of the hypotheses (Fig. 4).

The lower conductivity value, of the Cu(II)-dextran complex during the thermal treatment, as well as after treatment with oxidation agent, and in according to the Total Dissolved Solids (Table 2), was showed a higher stability of the dextran complex compared to the pullulan complex.

The destabilization of the complex was higher, after oxidative treatment at room temperature, with the increase of H_2O_2 concentrations from 0.1 to 1.0%. There was no change in system after the treatment with 0.1% H_2O_2 (Fig. 5a). The conductivity of the complex solution was almost the same as the conductivity of water (Table 1). This fact suggests that the complex did not have any electric charge and thus was not involved in the conductance. The complexes are destabilized rapidly, as the concentration of the oxidation agents increases to 0.5%. It is known [2,25-27] that OH groups of glucopyranosyl units under this conditions were oxidized to carbonyl (C=O) groups making dialdehyde or acetone derivatives (Fig. 6). As a result, there is the releasing of copper ions from the complex and increasing of conductivity in the solution. On the other side, the hydroxyl groups at the end of glucopyranosyl units in dextran chain could be oxidized to carbonyl groups, and formed derivatives of dextran-carboxylic acid. These derivatives additionally increased the conductivity of tested solution, because of their charge. These changes were characteristic in the first 10 min of treatment (Fig. 5a). The hypotheses about the derivation was upheld by the color change from light-green, for the starting solution, to brown-yellow after 10 min (Fig. 7).

During further oxidation treatment, after 15 min, a black colored solid phase was formed in the system, while the conductivity stagnated. Beside the destruction of ligand, free copper(II) ions formed CuO as a solid phase. The treating of the Cu(II)-dextran complex with 10% oxidation agent, causes the rapid decrease of the conductivity (Fig. 5a).

In the case of oxidative treatment of Cu(II)-pullulan, with the concentration increase of hydrogen-peroxide, much more the complex is destabilized in the first 5-10 min (Fig. 5b). The largest conductivity (1325 μS cm^{-1}) was observed after the treatment with 1% H_2O_2. Similar to other cases, the significant changes were noted in the system during the treatment with 0.5% H_2O_2, after

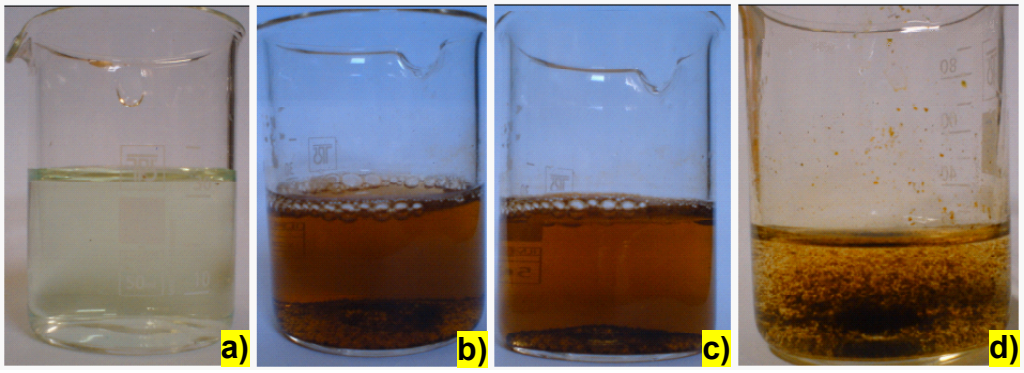

Figure 7. Photo of the changes in the aqua solution of Cu(II)-dextran in the presence of H_2O_2 for different time intervals: a) 0 min, b) 5 min, c) 10 min and d) 15 min

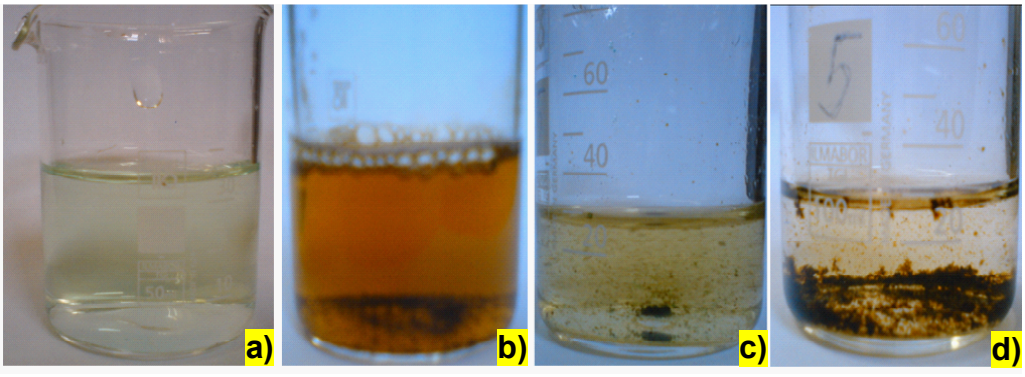

Figure 8. Photos of the changes in the aqueous solution of Cu(II)-pullulan in the presence of H_2O_2 for different time intervals: a) 0 min, b) 5 min, c) 10 min and d) 15 min

10 min (Fig. 8). The conductivity was decreased rapidly after 10 min. The interaction between free copper(II) ions and dialdehyde forms of pullulan or pullalan carboxylic acid was the explanation of pullulan derivation at the beginning of treatment. At the same time, one part of copper(II) ions were involved in forming of CuO, as a new phase in the system, which was confirmed by the color change of the solution, as well the forming of a brown-black sediment (Fig. 8).

The changed conductivity of aqueous solutions of the complex, with increasing temperature or concentration of oxidating agens, is indicated by the instability complex. It can be concluded that the stability of the complex is proportional to temperature and oxidant concentration. The electrical conductivity of the untreated complex was 12 μS cm^{-1}. This is probably a consequence of the poor binding of copper ions in the complex. The lower conductivity shows that the stronger Cu(II)-ligand coordination in the complex, leads to higher stability of complex. The conductivity of the treated complex was increased during 10-15 min. It was slight increased in all case of the thermal treatment, and in all case of oxidative degradation. The conductivity values of the complex, as well as the fact of the structure has two coordinated water molecules, can explain the conductivity increases, due to the free copper ions. After the conductivity achieved maximum value, it then decreased rapidly. This reduction in conductivity is a consequence of deposition CuO.

During the oxidation, the color changes of the tested solutions from light green through light yellow to brown with black sediment, indicate the change in the strenght of ligand field around Cu(II) ions. A green color is the characteristic of the most stable Cu(II) complexes with oligosaccharides. A yellow color is the characteristic of the destruction of the oligosaccharide ligand, and brown-black of the derivation of Cu(II) decomplexed ions in CuO compounds.

4. Conclusion

The thermal and oxidation stability of Cu(II) complexes with different oligosaccharides (dextran or pullulan) has been monitored by conductometric method in 1 mg cm^{-3} of

aqueous solution. The effect of temperature and oxidant concentrations on the stability of the complexes was studied and the corresponding TDS parameters were calculated and discussed. The increase in conductance is probably due to the release of the Cu(II) ions, from the complexes, during the decomposition complex. In the case of forced oxidation studies, the decrease of conductivity is due to: (a) the volume increase of degradation products formation, which is accompanied by decreasing value of diffusion coefficient of particle, (b) the reduction of charge of the newly formed ligand ion through covalent bond formation with copper ion, and (c) the charge reduction of Cu(II) ions through formation of CuO. The complex stability of Cu(II)-dextran was higher than Cu(II)-pullulan.

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