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STRUCTURE OF MATTER AND QUANTUM CHEMISTRY

Attenuated Total Reflectance–Fourier Transform Infrared Microspectroscopy of Copper(II) Complexes with Reduced Dextran Derivatives*

G. S. Nikolić[a], M. Cakić[a], Ž. Mitić[b], B. Ilić[c], and P. Premović[c]

[a] *Faculty of Technology, Leskovac, Serbia,*

[b] *Faculty of Medicine, Department of Pharmacy, Niš, Serbia*

[c] *Laboratory for Geochemistry, Cosmochemistry and Astrochemistry, University of Niš, Niš, Serbia*

e-mail: goranchem_yu@yahoo.com, zak_chem2001@yahoo.com

Abstract—Dextran is a water-soluble, extracellular neutral polysaccharide with a linear flexible chain of α-(1 ⟶ 6)–linked α-D-glucopyranose units, in a single compounds. In alkali solutions Cu(II) ion forms complexes with reduced low-molar dextran (RLMD). The metal content and the solution composition depended on pH. The complexing process begins in weak alkali solution (pH > 7), and involves OH groups in C2 and C3 dextran monomer units. Synthesized copper(II) complexes with RLMD, of average molar mass M_w = 5000 g/mol were investigated by attenuated total reflectance–Fourier transform infrared (ATR–FTIR) spectroscopy and FTIR imaging microscopy. ATR–FTIR microspectroscopic data of synthesized complexes are rare in literature. The changes in intensity and width of the IR bands in region 1500–1000 cm^{-1} were related to changes in conformation and short-range interactions of the ligand dextran. FTIR microscopy images shows more and less ordered structures of the Cu(II)–RLMD complexes. ATR–FTIR microspectroscopic data shows homogeneity of the Cu(II)–RLMD samples and green color of the samples confirm existence of Cu(II) ions.

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INTRODUCTION

Dextran is a polysaccharide consisting of α-D-glucopyranose units coupled into long branched chains, mainly through the α-(1 ⟶ 6) and partly through the α-(1 ⟶ 3)-glycosidic linkages (Fig. 1). This unique linkage pattern gives dextran with distinctive physical properties. Due to these properties, dextran has been extensively used as a drug carrier system, including for antidiabetics, antibiotics, anticancer drugs, peptides and enzymes [1, 2]. Dextran is derivatized easily to control its solubility or provide reactive groups. Consequently, dextran and its derivatives have numerous potential food, pharmaceutical, and industrial applications [3, 4].

The aim of this work is to use attenuated total reflectance—Fourier transform infrared (ATR–FTIR) spectroscopy, and FTIR imaging microscopy as the main tools to verify the conformation and structure of this type of ligand around the copper(II) ions.

Many types of polysaccharides such as chitin [5], chitosan [6], heparin [7], alginate [8], inulin [9], dextran [10], and pullulan [11, 12] have been derivatized for biomedical applications. The numerous investigations have indicated that the polysaccharide dextran and its derivatives have the extraordinary power to forming the water–soluble complexes with various biometals [13, 14]. It has been established that the degree of Cu(II) ion binding within the complex depends primarily on the pH of the solution, as well as on the participation both of the OH groups and the H_2O molecules in the first coordination sphere of Cu(II) ion. Reduced low–molar dextran (RLMD), was chosen as a material for complexing, and the subsequent interactions with Cu(II) ions were investigated in this study. Copper(II) complexes were prepared from sodium salts, and investigated in the solid state. ATR–FTIR microspectroscopic data of synthesized complexes are rare in literature. The emergence of modern structural chemical methods such as ATR–FTIR spectroscopy and FTIR microscopy made it possible to assign the binding OH or other groups, and also to characterize the metal ion coordination of polysaccharides, monitoring the ligand conformation or/and configuration changes forced by the complexation processes [15–18]. The major goal of this work is to use of ATR–FTIR microspectroscopy and FTIR imaging as the main tools to verify the conformation and structure of this type of ligand around the copper(II) ions.

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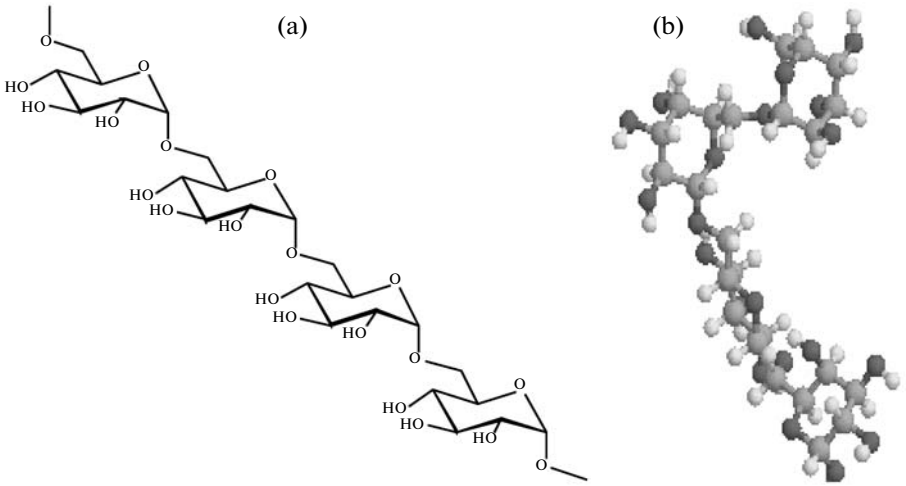

Fig. 1. Molecular structure of a dextran: (a) 2D model, (b) 3D model stick and ball.

EXPERIMENTAL

Copper(II) ion complex synthesis with RLMD have been described in detail by Mitić et al. [16]. FTIR microspectroscopy system, ATR–FTIR spectrometer Bruker Tensor-27 in conjunction with a FTIR Bruker Hyperion-1000/2000 microscopy attachment equipped with a 15× objective and a 250 μm liquid nitrogen cooled, narrow-band mercury–cadmium–telluride (MCT) detector (ATR objective GMBH, Germany) with the range of the IR spectrum from 4000 to 400 cm^{-1} was used in this work. The spectra were measured with 4 cm^{-1} resolution and 320 scans co-addition. The measurements were conducted in the reflection mode. In the region from 4000–400 cm^{-1} all spectra were Interactive polynomials baseline-corrected and area-normalized. A Kubelka–Munk arithmetic method was applied to enhance the resolution in this spectral region. Deconvoluted spectra were smoothed by the 40-point Fourier filter method.

Thus, various tests can be performed by the Bruker Hyperion microscope, such as transmission, reflection, polarized, and ATR–FTIR measurements, the linear scan and mapping techniques in terms of software, and optic video technology for true video analysis. In addition, spatial-resolution FTIR spectra and functional group imaging can also be acquired and analyzed. For measuring IR spectra by FTIR microscopy accurately, several primary parameters in the operation need to be selected and set first, which include aperture sizes, number of scans, resolution, velocity of motional mirror, and sampling background.

RESULTS AND DISCUSSION

Recently, FTIR spectroscopy was coupled with a microscope and a computer system, capable of microanalysis of minute samples by using a dedicated MCT detector. The resultant FTIR vibrational microspectroscopy can provide molecular information of samples with a high spatial resolution at microscopic level. Samples with microscopic size can be nondestructively analyzed by both vibrational microspectroscopies, particularly in the application of biomedical sciences [19–22]. Thus, the use of vibrational microspectroscopy has extensively become a great potential over other spectroscopic techniques for noninvasive investigation of chemical components of ultrastructual samples (carbohydrates, lipids, proteins, nucleotides) [23, 24]. More recently, FTIR and/or Raman microspectroscopic imaging systems have also been developed for applying to biosciences [25, 26]. ATR–FTIR spectra may be simultaneously collected at a time in a stepwise manner from different areas of a sample. We had to restrict ourselves to a few examples of wide potentialities of the method of FTIR spectroscopy in investigating the relationships between the structure and the properties of extracellular polysaccharide dextran and its complexes with

Characteristic bands in ATR–FTIR spectra of polysaccharide dextran and synthesized Cu(II)–RLMD complexes

$\bar{\nu}$, cm^{-1}	Assignation	Comment
3600–3400	ν(O–H)	CH–OH glucopyranose units, H–O–H
~2950	ν(C–H)	C–H
~1640	δ(HOH)	H–O–H
1450–1345	δ(C–H)	C–H
~1420	δ(O–H)	O–H
1150–1010	ν_{as}(C–O), ν_{as}(C–C)	glucopyranose units
1000–700	γ(C–H)	configuration

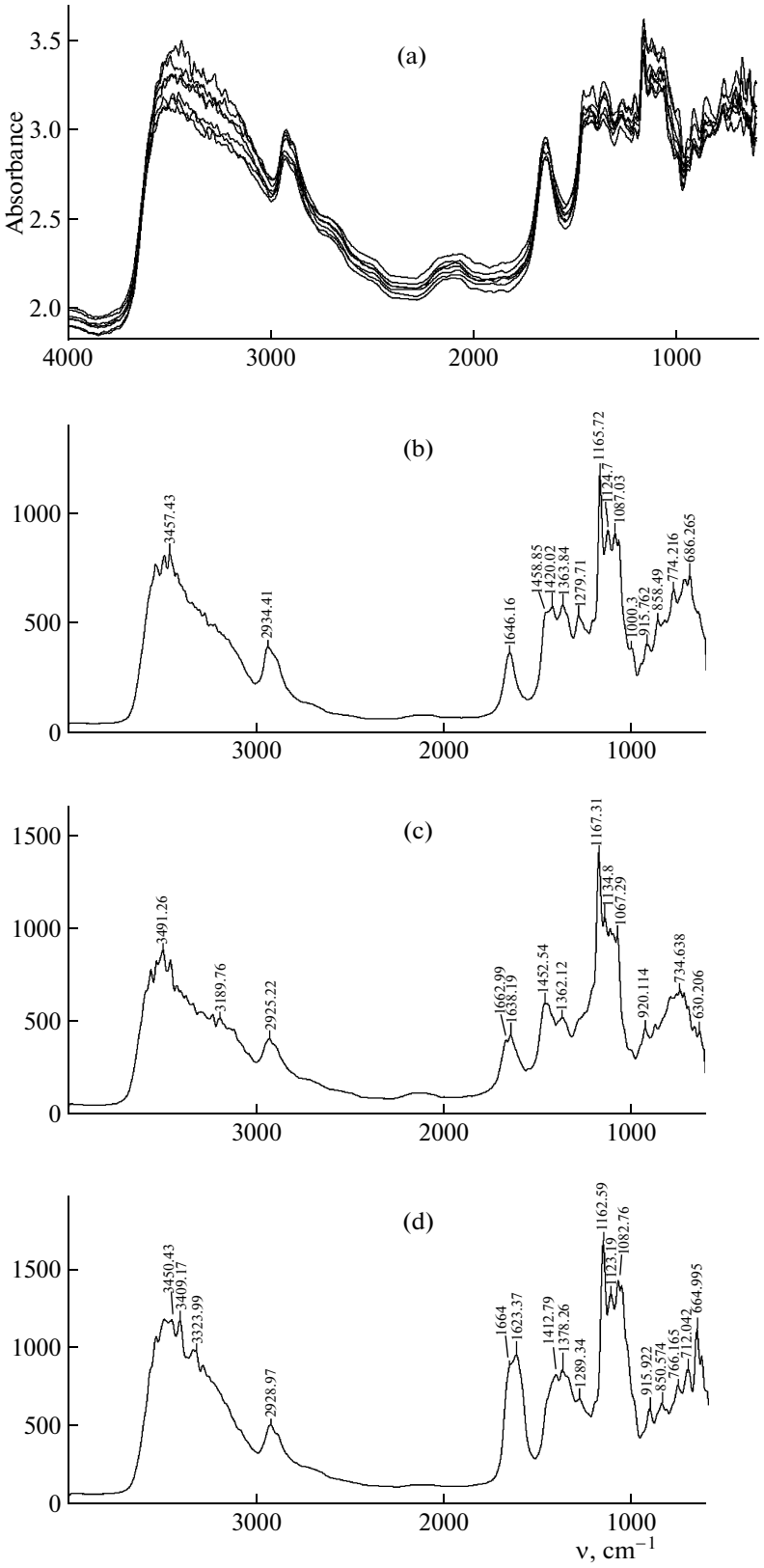

Fig. 2. Typical ATR–FTIR spectra of Cu(II)–RLMD complexes synthesized at boiling point and pH (a) 7.5, (b) 8.0, (c) 10.0, and (d) 12.0.

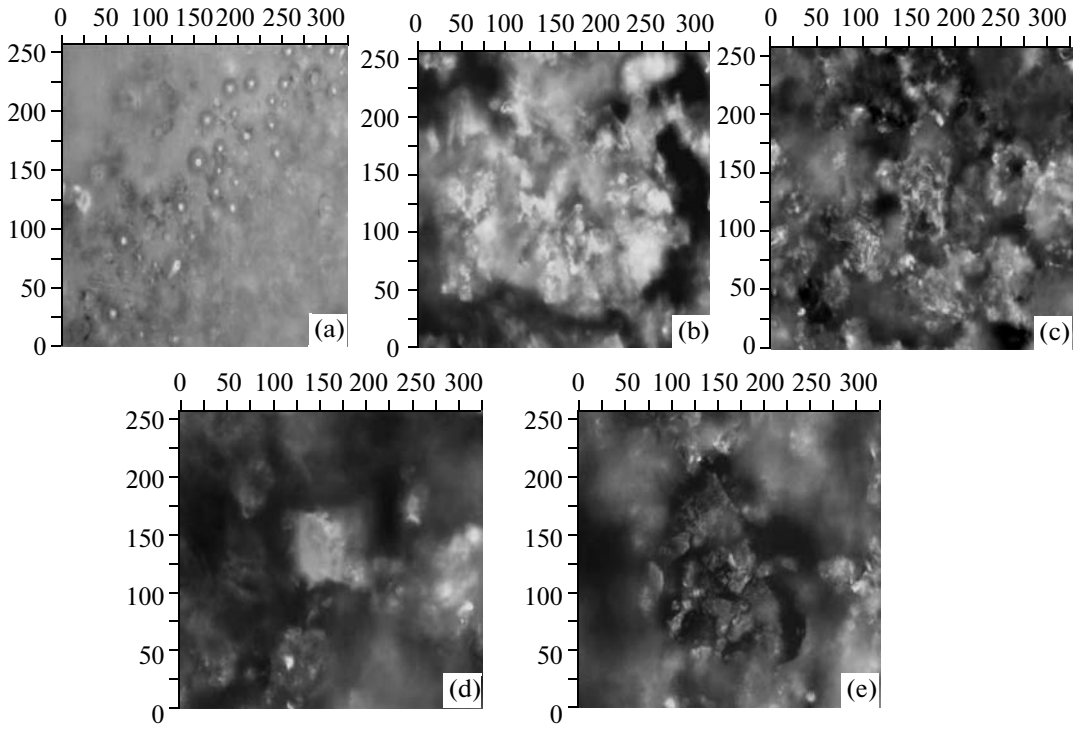

Fig. 3. FTIR microscopy images (250 μm × 300 μm) of (a) RLMD, $M_w = 5000$ g mol^{-1} and Cu(II)–RLMD complexes synthesized at boiling point and pH (b) 7.5, (c) 8.0, (d) 10.0, and (e) 12.0.

Cu(II) ion. The FTIR spectra of the RLMD and synthesized Cu(II) ion complexes contains following characteristic bands are given in table. The absorbance ATR–FTIR spectra of Cu(II)–RLMD complexes which were synthesized at pH 7.5, 8.0, 10.0, and 12.0 are shown in Fig. 2.

Spectroscopic ATR–FTIR study in a particular region of O–H (3400 and 1420 cm^{-1}) and C–H (2900, 1460, and 1350 cm^{-1}) vibrations indicates different binding between the central metal ion and ligand, depending on pH and copper(II) ion contents [15, 16].

The difference of the number, frequencies, intensity and shape of the bands (3600–3100 cm^{-1}) implies that in the complexes there is displacement of H_2O molecules by the hydroxyl groups. Cu(II)–RLMD complexes are formed by the displacement of H_2O molecules from the first coordination sphere of copper(II) ion by the OH groups. Copper(II) ion with RLMD unit (Glc) forms three different types of complex (pH 7–8: $Cu(II)(Glc)_2(H_2O)_2$, pH 8–10: $Cu(II)(Glc)_2(H_2O)(OH)$, pH 10–12: $Cu(II)(Glc)_2(OH)_2$) [15–17].

These results agree with a structural studies of the investigated complexes have been based on other spectroscopic techniques [27–32]. The changes in number, frequencies, intensity, and width of the IR bands in the particular region of ν(O–H) vibrations (3400 cm^{-1}), δ(C–H) vibrations (1500–1300 cm^{-1}) and ν(C–O) vibrations (1200–1000 cm^{-1}) were related to changes in the conformation and short-range interactions of the polysaccharide dextran. Very important changes can be observed in the range 1500–1300 cm^{-1} by detailed empirical analysis. Otherwise, the IR range is specific of bending vibrations of CH–OH groups. Namely, exchange position and intensity of complex bands can be registered in this range, where C–H and O–H bending vibrations from the CH–OH groups take part. An approximate effect exists in the stretching of the IR range of C–H vibrations (3000–2800 cm^{-1}). The appearance of bands at about 1460 cm^{-1} and 1370 cm^{-1} from δ(C–H) vibrations and the band at about 1420 cm^{-1} from δ(O–H) vibrations are characteristic for one of more possible positions of the CH–OH group, rotating around the C2–C3 and C3–C4 bond of the glucopyranose unit. The Cu(II) ions in solution have a possible influence on the rotation of CH–OH groups in the complexes.

RLMD and its complexes with the Cu(II) ion have one crystallographic type of the water molecule (1640 cm^{-1}) [15–17]. The band at 1079 cm^{-1} in the spectra of RLMD is attributed to the antisymmetric stretching vibration of C6–O–C1 glycosidic bridge with participation of the deformational vibrations of the C4–C5 bond. The band at 1079 cm^{-1} in the spectra of Cu(II) complex with RLMD is more pronounced than in the spectra of RLMD.

It is known that [33–35] the glucopyranose units exist in six different conformations (*1C*, *C1*, *1B*, *B1*, *3B*, and *B3*). The similarities of the γ(C–H) range

indicate that there is no difference in the conformation of the glucopyranose unit in the RLMD and Cu(II)–RLMD complex molecules and they probably exhibit *C1* chair conformation (916 and 850 cm^{-1}).

The absorbance of a band corresponding to a specific chemical component may be plotted as a map. ATR–FTIR spectra were presented in Fig. 2 from different areas of Cu(II)–RLMD complex (Fig. 3) and show high homogeneity of the sample. A new imaging capability has been established not only to image heterogeneous regions of the samples and simultaneously provide spectroscopic and spatial information, but also to show visually the concentrations of components and to highlight their effect from the three dimensional plot. The application of microscopic FTIR imaging system to the ligand RLMD and Cu(II)–RLMD complexes, were synthesized at pH 7.5–12, is shown in Fig. 3.

FTIR microscopy images of ligand RLMD, as well as images of the synthesized Cu(II)–RLMD complexes differ which also indicates that the complexation process and the creation of coordination compounds took place. FTIR microscopy images confirmed that the changes in the intensity of the analyzed bands are strongly associated with the alterations in the macromolecular order. These bands in the spectra of the complexes can be responsible for more and less ordered structures, respectively (Fig. 2). The changes in color contour may show the content and distribution of copper, and polysaccharides in Cu(II)–RLMD samples (Fig. 3). ATR–FTIR microspectroscopic data shows homogeneity and green color of the Cu(II)–RLMD samples confirm existence of Cu(II) ions (results from other spectroscopic techniques [13–17]).

CONCLUSIONS

Spectroscopic IR study in a particular region of O–H (3400 and 1420 cm^{-1}) and C–H (2900, 1460, and 1350 cm^{-1}) vibrations indicates different binding between the central metal ion and ligand, depending on pH and metal contents. The changes of the intensity on some bands were registered in RLMD complexes (in the ranges of a stretching vibration at about 2930 cm^{-1} and a bending vibration at about 1400 cm^{-1}). The IR band δ(HOH) at the frequency of 1640 cm^{-1} indicated the existence of water molecules in a complex structure. A part of IR spectra, in the range on 1000–700 cm^{-1} of Cu(II) ion complexes with RLMD, indicates no influence of complexing process on the conformation change of *C1* glucopyranose units. ATR–FTIR microspectroscopic data shows homogeneity and green color of the Cu(II)–RLMD samples confirm existence of Cu(II) ions.

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