

SYNTHESIS AND STABILITY STUDIES IN PHYSIOLOGICAL-LIKE CONDITIONS OF TWO UREA COMPLEXES OF COPPER (II) AND MANGANESE (II)

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In order to obtain antioxidants with low molecular weight, suitable for pharmacological applications, two new water-soluble urea complexes of metallic centres with biological relevance, $\text{Cu}(\text{OC}(\text{NH}_2))_2\text{Cl}_2$ and $\text{Mn}(\text{OC}(\text{NH}_2))_4\text{Cl}_2$ were synthesised and characterised. Their bonding and stereochemistry were characterised by spectroscopic methods and conductivity measurements. Stability studies in physiological-like conditions were carried out by absorption electronic spectroscopy. The results justify the further investigations of their antioxidant activity *in vitro* and *ex vivo* and allow their use as intermediates in the synthesis of other potential antioxidants complexes, with mixed ligands.

Introduction

Cardiac dysfunction has been largely attributed to the detrimental effects of reactive oxygen species (ROS). Inhibitors, antioxidants, ROS scavengers, have been shown to reduce the severity of the heart injury during the post-ischæmia reperfusion. However, the cytotoxicity, the pro-oxidant activity and the high molecular weight limited the application of these compounds as cardio-protective agents [1÷3].

It was reported that some copper proteins also exerted cardio-protective effects [4,5]. However, as proteins, some difficulties of their pharmacological formulation and administration may occur. Many recent studies dealt with the design and construction of Cu (II) and Mn (II) centres in antioxidant compounds [6÷12].

A recent study [13] revealed novel cardiac protective effects of urea. Urea is the most common nitrogen-containing end product of protein catabolism and its synthesis is involved in the regulation of the chronic acid-base disturbances [14]. Urea also stimulates gene transcription and expression [15] and it has an antioxidant effect, protecting brain liver and heart from peroxidation of lipids.

The present work represents a part of a larger study concerning new potential antioxidants with the following pre-established characteristics: reproducibility of the active sites of some metal-proteins with proved antioxidant properties, significantly lower molecular weight than the reported metal-proteins, compatibility with and stability in physiological mediums. The urea complexes reported here may represent potential antioxidants by themselves or are intermediates in the aqueous synthesis of other compounds, with mixed ligands. The already reported related Cu(II) and Mn(II) compounds [16÷19] displayed some properties unsuitable with the pharmacological applications.

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Experimental

Syntheses and analytical procedures

All reagents were of commercial analytical quality and were used without further purification. Chemical analysis of metals and chlorine was performed using volumetric methods and by combustion for carbon and nitrogen.

The *Cu-urea* complex was prepared by adding drop wise, on ice, a cold isopropyl alcohol solution of copper (II) chloride to an aqueous urea solution (in minimum of water) (in 4:1 molar ratio urea : CuCl_2 and 50 : 1 volumes ratio isopropyl alcohol : H_2O). The mixture was allowed to stir on ice during 30 min. The water-soluble bluish-green solid compound was filtered, washed with isopropyl alcohol and dried. The elemental analysis indicates that its composition is $\text{Cu}(\text{urea})_2\text{Cl}_2$, Cu: 24,9 %; C: 9,4%, N: 22,0%; Cl: 27,8%. Requires for $\text{CuC}_2\text{O}_2\text{N}_4\text{H}_8$: Cu: 25,1 %; C: 9,2 %, N: 22,4%, Cl: 28,0 %. Yield 76%, m.p. 437,5K.

The *Mn-urea* complex was prepared similarly, from ethylic solutions of manganese chloride and urea in the molar ratio 1 : 6. The pale pink compound is water-soluble. The elemental analysis results for $\text{Mn}(\text{urea})_4\text{Cl}_2$: Mn: 15,1%, C: 13,1%, N: 30,6%, Cl: 19,4%; requires for $\text{MnC}_4\text{O}_4\text{N}_8\text{H}_{16}$: Mn: 15,1%, C: 12,9 %, N: 30,1 %, Cl: 19,7%. Yield 62%, m.p. 435K.

Apparatus and methods

The *melting points* were measured using a SMP-3 apparatus. *IR spectra* were recorded with a BIO RAD FTIR 135 spectrometer, using the potassium bromide technique, in the range $4000\div 400\text{cm}^{-1}$. *Electronic spectra* by diffuse reflectance technique, with MnO standard, were recorded in the range $380\div 1100\text{ nm}$, on a VSU2-P-Zeiss Jena spectrometer; absorption spectra were recorded in the range $200\div 1100\text{ nm}$, on a Jasco V 530 spectrometer, in aqueous solutions, in non-modified and modified pH conditions, using aqueous NaCl solutions 0,9% and HCl 0,1n; the pH values were measured with a Mettler 335 apparatus. The molar conductivities were measured on a CONSORT C533 apparatus.

Results and Discussions

The structure of urea involves resonance between three structures, the observed values of the inter-atomic distance indicating 30% double bond character for the carbon-to-nitrogen bonds, leaving 40% for the carbon-to oxygen bond; as a result, the urea molecule may coordinate with metal ions through one nitrogen as well as the oxygen atom.

The IR spectra of the urea-complexes were interpreted having as reference the assignments of the observed frequencies of urea; on these bases, an earlier IR study [20] divided the metal-urea complexes into two groups, one with nitrogen, the other with oxygen as donor atom, particularly by the presence or absence of a carbonyl band near 1700 cm^{-1} and by the presence or absence of shifted NH_2 peaks.

For the free urea (symmetry C_{2v}) there are two types of NH_2 in-plane deformation vibrations: NH_2 *bending motions*, in the region about 1600 cm^{-1} , symmetric (A_1), mixed

with the carbonyl vibrations), and anti-symmetric (B_1), respectively symmetric and anti-symmetric *in-plane rocking*, at about 1155 cm^{-1} .

The IR spectra recorded for the to urea complexes (shown in Fig. 1, comparatively with those of free urea) display significant differences, discussed below.

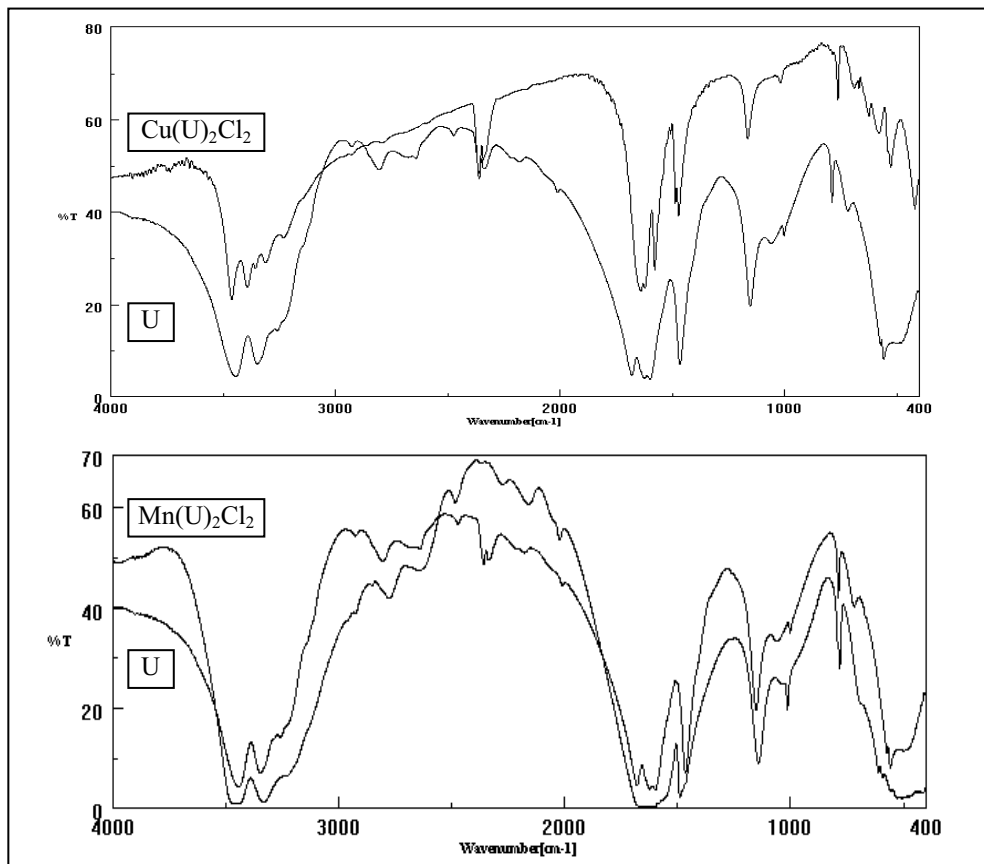


Fig. 1: IR spectra of Cu(II) and Mn(II) urea complexes versus urea

The presence of a carbonyl vibration in the IR spectrum of the metal-urea complexes near 1700 indicates that a coordinate bond is formed between nitrogen and the metal atom and that oxygen is not a donor atom. The shift in this region has two reasons: the formation of the N-M bond increases the electronic demand by the donor nitrogen atom and blocks the resonance between this nitrogen atom and the carbonyl group; both of these factors bring about increased C=O double bond character. In addition to the two bands attributed to the N-H stretching vibrations of free urea, found in the region 3000 cm^{-1} , the bands at lower frequencies in the IR spectra of metal urea-complexes are assigned to the N-H stretching vibrations of the coordinated NH_2 groups. As a result of the formation of the N-M bond, the corresponding N-C bond acquires more single bond character, which shifts the CN absorption peaks at lower frequencies.

Infrared N-H stretching vibrations bands at almost the same frequencies (of about $3400\div 3300\text{ cm}^{-1}$) in the spectra of both urea and urea-complexes indicate that urea does not coordinate through nitrogen atoms. The formation of the O-M bonds in these complexes tends to reduce the coupling the CO stretching and the NH_2 bending vibrations, making them more separable: the group CO is no longer terminal, coordination increases its single bond character and, therefore, the CO and CN vibrations and corresponding bands become less separable. Supplementary absorptions in the region $400\div 550\text{ cm}^{-1}$ are assigned to the M-N and M-O frequencies.

The IR data (Table 1) suggest that coordination occurs through oxygen atoms in the Mn(II) compound and both through nitrogen and oxygen atoms in the Cu(II) compound.

Table 1. IR data

Assignments	Absorption maxima (cm^{-1})		
	Urea	$\text{Cu(urea)}_2\text{Cl}_2$	$\text{Mn(urea)}_4\text{Cl}_2$
N-H stretching	3423,3	3461	3451
	3346,8	3391	330,5
	3259	3355 3310 3231	3235
C=N	2359	2360	
	2340	2341	
$\nu_{\text{CO}} + \text{NH}_2$ bending (symm.)	1680,6 (A_1)	1638	1667,1
NH_2 bending	1624,7 (B_1)	1622	1635,4
NH_2 bending, ν_{CO}	1598,7 (A_1)	1577	1582
ν_{CN} , antisymm.	1465,6 (B_1)	1486,8 1471,4	1489,7
NH_2 rocking (B_1)	1152,2	1163	1142
ν_{CN} (symm.)	1059,7 (A_1)	1017	1035,5
δNH_2 rocking	788,7	772,7	781,9
φCN	718,3	689,4 669	687,5
δNCO	573,3	624	611,3
δNCN	558,3	579	593
ν_{MN}		527	507
ν_{MO}		424	

The electronic spectra by diffuse reflectance (Fig. 2), displaying maximal values at about 795 nm (CuU) and 900 nm (MnU), seem to indicate an octahedral environment of metal ions in both urea-complexes.

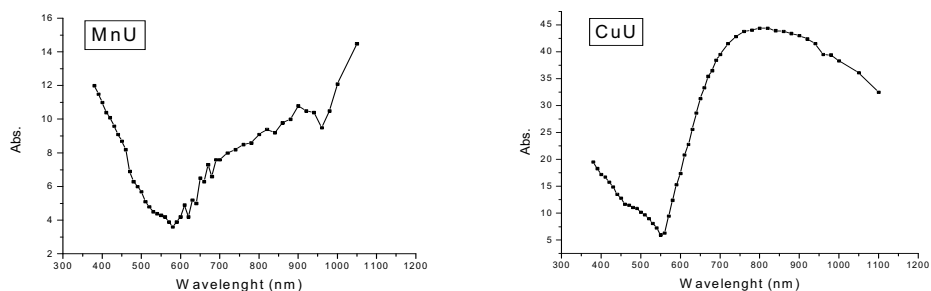


Fig. 2: Electronic spectra by diffuse reflectance of Cu(II) and Mn(II) urea complexes

The conductivity measurements were performed in aqueous solutions (10^{-3} M). The conductivity values $(\text{Cu(urea)}_2\text{Cl}_2)$: $1,74\Omega^{-1}\text{cm}^2\text{mol}^{-1}$; $(\text{Mn(urea)}_4\text{Cl}_2)$: $1,95\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ indicate a non-electrolytic nature of the two complexes.

The stability studies were performed by absorption spectroscopy in aqueous solutions, in physiological-like conditions. The stability at pH 7,2 was studied using aqueous NaCl solutions 0,9%, absorption spectra of the same sample being recorded immediately after dissolution ($t = 0$), after 1,5 and 3 hours. The pH conditions were modified using appropriate amounts of HCl 0,1n. Figs. 3 and 4 illustrate significant areas of the absorption spectra of the two urea-complexes.

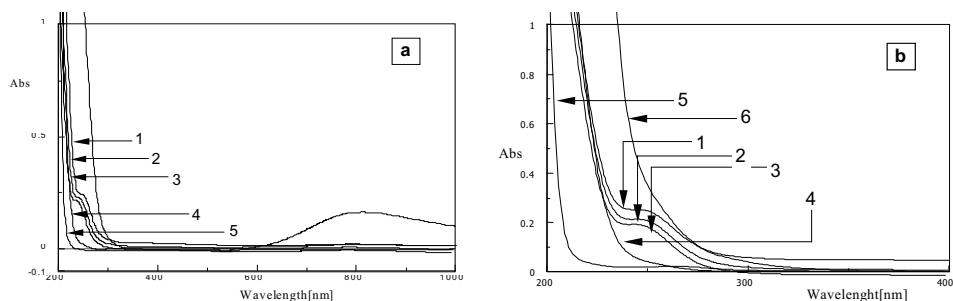


Fig. 3: Absorption spectra of aqueous solutions of $\text{Cu(urea)}_2\text{Cl}_2$: **a**: at pH=7,2, in time (1: at $t = 0$; 2: 1,5 hours; 3: 3 hours; 4: CuCl_2 ; 5: urea) ; **b**: in different pH conditions (1: 7,2; 2: 6,5; 3: 5,5; 4: 4,5; 5: urea; 6: CuCl_2)

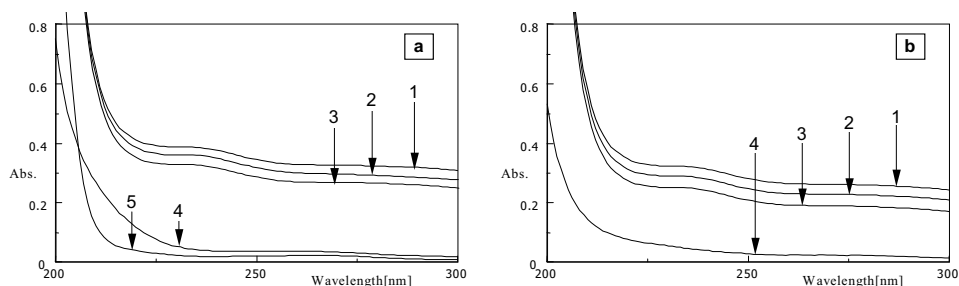


Fig. 4: Absorption spectra of aqueous solutions of $\text{Mn(urea)}_4\text{Cl}_2$: **a**: at pH=7,2, in time (1: at $t = 0$; 2: 1,5 hours; 3: 3 hours; 4: MnCl_2 ; 5: urea) ; **b**: in different pH conditions (1: 7,2; 2: 6,5; 3: 5,5; 4: 4,5)

The stability studies reveal that both synthesized urea-complexes display reasonable stability in time in aqueous solutions at pH 7,2 and in moderate acidic solutions; at pH values more acidic than 5,5 the complexes are not entirely destroyed to hydrated metal ions, but their nature changes significantly.

Conclusions

The urea complexes reported here may represent potential antioxidants by themselves or are intermediates in the aqueous synthesis of other compounds, with mixed ligands. Both potential uses inquire moderate acidic mediums (above pH 5).

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