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Lactic acid and M(II) d-metals (Cu, Co, Mn, Cd) milli- and micro- quantities interaction: FTIR and ESI-MS analysis

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This study investigated the interaction of M(II) d-biometal ions (Cu, Co, Mn) and toxic Cd(II) ion with lactic acid, which is present in human body as a result of intake or as a product of biological processes, at milli- and micro-molar level, under approximately physiological conditions by FTIR and ESI-MS spectroscopy. Spectroscopic investigations showed that the lactic acid in the tested system behaves as a monodentate ligand, it comes to interact at all concentration levels, and the most pronounced interaction is in the model system with Co(II) ion.

Keywords: M(II) d-metals, Milli- and Micro-interactions, Spectroscopy

INTRODUCTION

In biological systems d-biometal cations easily interact with molecules of water and various parts of different organic and inorganic biomolecules, as natural constituents or substances introduced into the human body. These complex associates, built *via* O-, N- and S- donor atoms, have an important role in biological processes [1-3].

Copper is a biogenic element found in numerous enzymes' systems. The Cu(II) ion (d^9 configuration) can give coordination compounds of various coordination and geometry through O-, N-, and S-donor atoms of different biomolecules and small molecules [1, 4].

Cobalt as a micro-element has a role in the metabolism of proteins and amino acids as the constituent parts of metaloenzymes. The Co(II) ion (d^7 configuration) easily interacts with parts of other molecules and builds complex particles with a coordination number 4, as well as a coordination number 6 *via* the O-, but also *via* the N-donor atom [1].

Manganese is an element weakly present in biological systems, but it also has an irreplaceable role in detoxification from oxygen free radicals as a cofactor of numerous enzymes. The Mn(II) ion (d^5 configuration) easily builds complexes with a coordination number 6 which are only slightly stable and easily interact with other molecules, which leads to changes in the ligands and the building of new products [1, 5].

Cadmium is a toxic metal, and it is not a biometal. In the human body this metal can enter through the food chain. Toxicity of cadmium is partly a consequence of its ability to bind instead of biometals with similar physicochemical characteristics over -SH of the active sites of some enzymes and block their activity, because of higher affinity for thiol groups. This metal induces oxidative damage to red blood cells in different tissues [6,7].

The carboxyl group as a part of many compounds (pharmaceuticals, supplements) is a "universal" O-donor ligand which can form complexes of different coordination, and can act as a monodentate or a bidentate ligand [8, 9].

α-Hydroxycarboxylic acids are constitutive components of many biochemical processes in the living world. They are often used in pharmaceutical preparations [10,11]. Lactic acid, 2-hydroxypropanoic acid, (LA) is formed by fermentation of sugars and other carbohydrates under the action of anaerobic bacteria and it can be ingested with food into the body. In medicine it is used as a component of the Ringer's and Hartman's solutions [12]. Lactic acid is a suitable molecule for model systems for the study of interaction of biometals with O-donor ligands in biosystems. With M(II) metal ions from solutions of molar concentrations, depending on the conditions of complexation, properties and synergistic effect of the metal ions, LA can form different types of complex associates [10, 11, 13, 14] With M(II) d-metal ions LA can form complexes of the type $[M(LA)_2R]$, where it behaves as a bidentate ligand, and R is molecule of solvent [15]. Cu(II) ion can form mononuclear and binuclear complexes with aliphatic α-hydroxycarboxylic acids at different pH values [10, 11, 16].

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Our previous studies have shown that there is a mobility of metal ions from biological hard mineral tissue of teeth that had been exposed to the impact of various media (lactic acid, acetic acid, etc.) as a result of the interaction of metal ions with the test media [13, 17]. In the literature there are no enough data about interactions of d-metals and LA under approximately physiological conditions, and at the level of micro-molar concentration at which they are present in human body.

Products of interaction of M(II) d-biometal ions (Cu, Co, Mn) and toxic Cd(II) ion with LA were investigated at milli-molar and micro-molar level, under approximately physiological conditions by FTIR and ESI-MS spectroscopy.

EXPERIMENTAL

Experimental procedure for FTIR characterization of M(II)–LA interactions.

The solid products of the M(II)-LA interaction were obtained by mixing a solution of M(II) ions, and a ligand in a molar ratio of 1:2 in favor of the ligand, at room temperature, under conditions in which the hydroxide is not precipitated (pH~5.5 for Cu, and approximately physiological pH for others M(II) ions), and separated by centrifugation after 7 days. FTIR spectra were recorded on a FTIR instrument Bomem MB-100 (Hartmann & Braun, Canada), with a DTGS/KBr (deuterated triglycine sulfate/KBr) detector in the wave number range of 4000-400 cm^{-1}, and were processed with ACD/Labs 10.08 software.

Experimental procedure for ESI-MS characterization of M(II)–LA interactions.

Binary solutions M(II)–LA were prepared by mixing stock solutions of M(II) ion (100 µmol/L) and ligand (100 µmol/L) in various proportions in order to obtain different metal/ligand ratios. ESI-MS analysis was performed 30 min after mixing without pH adjustment.

ESI-MS analysis of the model solutions was performed in negative ionization mode by using LCQ DECA Ion Trap mass spectrometer (Thermo Finnigan, USA). ESI-MS spectra and LOOP chromatograms (total ion current chromatograms of loop injected samples) were processed with Xcalibur LCQ Advantage 1.4 software. M(II) ion-ligand interaction strength was estimated by comparing the areas of ESI-MS ion current chromatograms for pure ligand solution (P$_1$) and M(II)–ligand solution (P$_2$), their relative difference being expressed as:

$$\Delta P = \frac{P_1 - P_2}{P_1} \times 100\% \qquad (1)$$

The value of ΔP is actually the fraction of the ligand involved in ion-ligand interaction so that the larger ΔP value indicates stronger interaction and increased binding of M(II) ions to ligand on micromolar level [18, 19].

RESULTS AND DISCUSSION

FTIR

FTIR spectrum of LA is characterized by the presence of absorption bands of carbonyl group (Figure 1). The band at 2633 cm^{-1} originates from the intra-molecular hydrogen bonds. The band appearing at 2500-3300 cm^{-1} can be attributed to vibrations of –O–H from iz –COOH group, while the band of free –O–H group appears at 3230-3550 cm^{-1}. Together, these bands cover the region 2500-3550 cm^{-1}, and thus cover bands that originate from the C-H vibration. FTIR spectrum of LA is characterized by a strong band at 1730 cm^{-1} ν(C=O) and bands at 1430 cm^{-1} ν(C–O–H in plane), 1240 cm^{-1} ν(C–O), i 930 cm^{-1} ν(C–O–H out of plane) [20, 21].

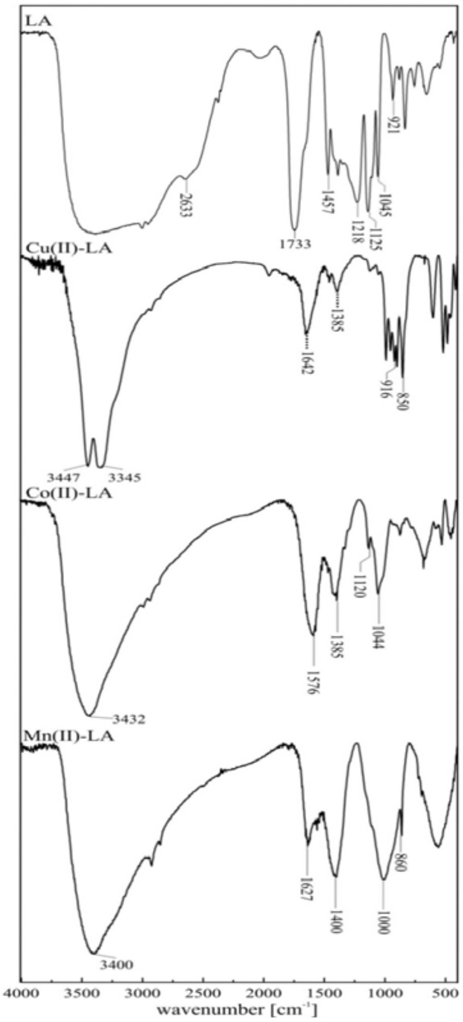

Fig. 1. FTIR spectra of LA, and solid products of interactions of M(II) d-biometal ions with LA.

The FTIR spectra of the obtained solid products of the interaction of the investigated M(II) d-biometal ions with lactic acid are shown in Fig. 1. The disappearance of the intra-molecular hydrogen bond band indicates potential interaction between M(II) ions and O-donor atoms of the ligand. The shifts of ν(C=O) band (ν_{asym}(C=O)) and ν(C–O–H) band (ν_{sym}(C–O)) are very indicative since frequencies of ν_{asym}(C=O) and ν_{sym}(C–O) are the main features of transition metal complexes with carboxylate ligands. The difference $\Delta\nu = \nu_{asym}(COO) - \nu_{sym}(COO)$ may be used to determine the coordination of M(II) ion with O-donor atoms of LA carboxylic group [1, 8, 22-24]. In the investigated model systems *Cu(II)-LA*, *Co(II)-LA* and *Mn(II)-LA* the shifts of ν_{asym}(C=O) bands were to 1642 cm^{-1}, 1576 cm^{-1} and 1627 cm^{-1} respectively; and the shifts of ν_{sym}(C–O) bands were to 1385 cm^{-1}, 1385 cm^{-1} and 1400 cm^{-1}, respectively. The $\Delta\nu$ values of 257 cm^{-1} (Cu(II)-LA), 191 cm^{-1} (Co(II)-LA) and 221 cm^{-1} (Mn(II)-LA) indicate that lactic acid behaves as a monodentate ligand [10, 11, 15, 20, 22].

The investigated system with the toxic Cd(II) ion behaved spectroscopically in a similar way to the system with Mn(II) ion, probably because these two ions are spectroscopically similar (full/semi-full d-level) [18, 23, 24].

The strength of metal-ligand interactions according to the position of R-C-O---M vibrations, based on values of $\Delta\nu$, decreases in the series Co(II) > Cu(II) ~ Mn(II)/Cd(II) probably due to the physico-chemical properties of the investigated M(II) ions [25, 26].

ESI-MS

Figure 2 shows the ESI-MS ion current chromatogram in the m/z range from 88 to 90 and the mass spectrum recorded in negative ionization mode for the loop injection of lactic acid at a flow rate of 100 µL/ min. One of the most prominent peaks in the mass spectrum of LA was that of lactate anion $[LA–H]^-$ at m/z = 89 with relative intensity of 100% and it served for adjusting optimal working conditions of the instrument. Ions $[LA–MeOH]^-$ at m/z = 121 and $[2LA–H]^-$ dimer at m/z = 178 could also be identified.

Figure 3 shows the ESI-MS ion current chromatogram in the m/z range from 88 to 90 and the mass spectrum recorded in negative ionization mode for the loop injection of LA with Cu(II) ion at a flow rate of 100 µL/min. In comparison to the mass spectrum in Figure 1, the peak intensities for $[LA–H]^-$ and $[2LA–H]^-$ decrease by about 20% and 10%, respectively. However, new ionic species appeared and we could identify $[LA+Cu]^-$ at m/z = 152. These spectral changes indicate that interactions between Cu(II) ion and LA occurred even at the micromolar level.

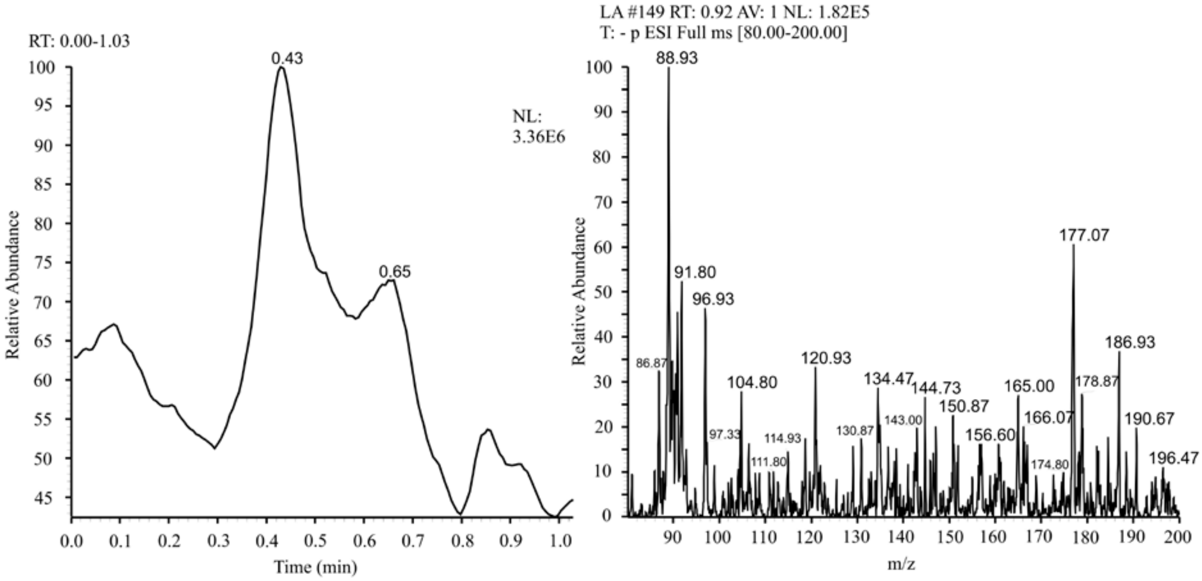

Fig. 2. ESI-MS ion current chromatogram in the m/z range from 88 to 90 and mass spectrum recorded in negative ionization mode for the loop injection of LA at a flow rate of 100 µL/min (c_{LA} = 5.00 µmol/L, solvent: MeOH/H_2O = 50/50).

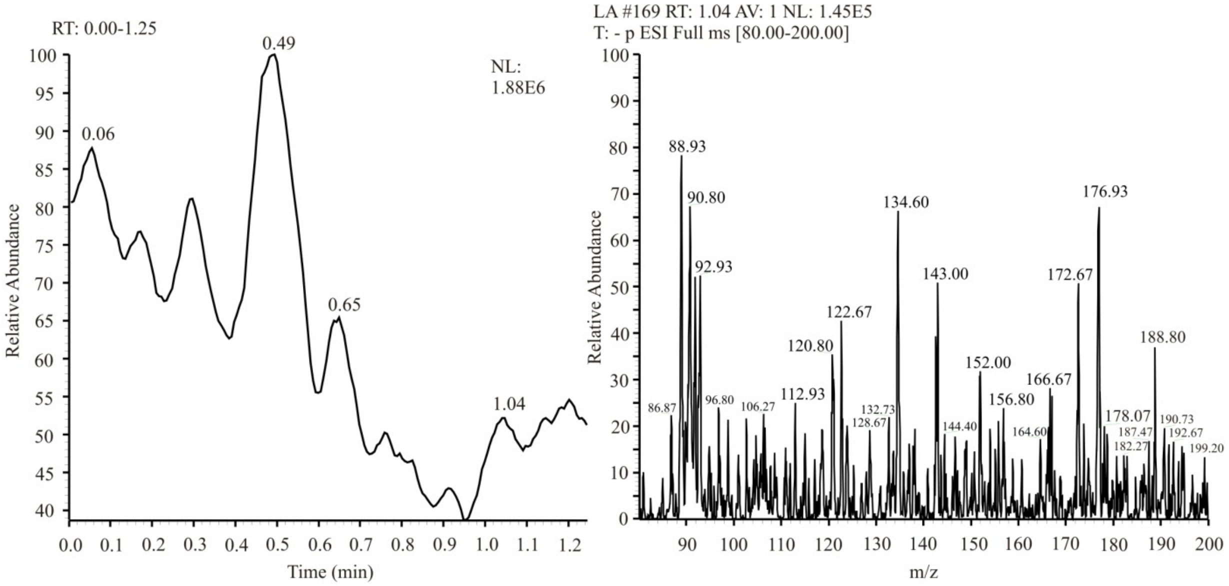

Fig. 3. ESI-MS ion current chromatogram in the m/z range from 88 to 90 and mass spectrum recorded in negative ionization mode for the loop injection of LA with Cu(II) ion at a flow rate of 100 µL/min (c_{LA} = 1.00 µmol/L, $c_{Cu(II)}$ = 9.00 µmol/L, solvent: MeOH/H_2O = 50/50).

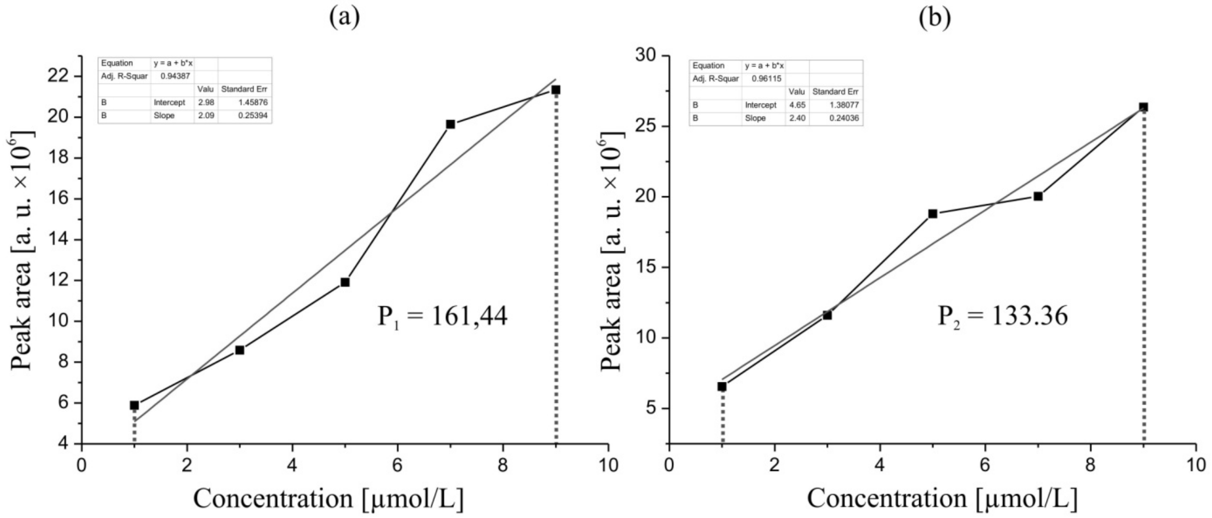

Fig. 4. Plot of the peak areas of the ESI-MS chromatograms of the loop injection *vs.* LA concentration for pure LA (a) and Cu(II)–LA system (b).

The quantitative analysis of the lactate anion, $[LA–H]^–$, was performed by injecting exact volumes of LA solution of known concentration by using LOOP apparatus. Areas below LOOP chromatograms (Fig. 4a and b) were determined with Xcalibur software. Calibration curve for LA was obtained by linear fitting of areas in LOOP chromatographic peaks for the m/z range 88-90 as a function of LA concentration. Integral area value for the LA system was P_1 = 164.44 (R^2 = 0.9439, Fig. 4a), and for the Cu(II)–LA system the value was P_2 = 133.36 (R^2 = 0.9612, Fig. 4b).

The obtained results for investigated model systems were analysed as mentioned before for the the Cu(II)-LA model system [18]. The values of the sub-integral areas of the investigated binary systems and their ΔP (Eq. 1) values are shown in Table 1.

Table 1. Values of sub-integral areas and their ΔP values

	LA	Cu-LA	Co-LA	Mn-LA	Cd-LA
P [a.u.]	161.44	133.36	107.55	146.17	147.84
R^2	0.9439	0.9612	0.9439	0.9468	0.9457
ΔP [%]	/	17.39	33,38	9.46	8.42

The area under the peaks in the LOOP chromatograms in the presence of the investigated metal ions is lower than the analog area of the lactic acid without metal ions, which is in accordance with results of other similar studies [18, 19]. These results indicate that there is considerable interaction of M(II)-LA at the micromolar level, which decreases in the order: Co(II)-LA > Cu(II)-LA > Mn(II)-LA ~ Cd(II)-LA.

According to the results of spectroscopic examinations it can be noticed that the interaction is most pronounced between Co(II) ion and lactic acid and less pronounced between the toxic Cd(II) ion and lactic acid, because Cd(II) ion has a lower affinity for O-donor atoms, considering hat it is a softer acid in regard to d-metals (HSAB theory) [6, 25, 26].

According results, substitution of metals with similar physico-chemical properties of the active centers of some biological systems (e.g. enzymes, hard mineral and different media) on micro-molar level could be expected [13, 17].

CONCLUSION

The spectroscopic investigations showed that lactic acid interacts with the investigated M(II) d-biometal ions and the toxic Cd(II) ion at both investigated levels of concentration. FTIR characterization of the isolated products of interaction of M(II)-LA showed that LA acts as a monodentate ligand. Lower values of surface of ESI-MS LOOP chromatograms of the examined system of M(II)-LA compared to the pure ligand (LA) indicated that the interaction between M(II) d-biometal ions or the toxic Cd(II) ion, and lactic acid occurs at the micro-molar level. According to the spectroscopic results Co(II)-LA interaction was the most pronounced compared to the other investigated metals..

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ИЗСЛЕДВАНЕ НА ВЗАИМОДЕЙСТВИЕТО МЕЖДУ МЛЕЧНА КИСЕЛИНА И M(II) d-МЕТАЛИ (Cu, Co, Mn, Cd) НА МИЛИГРАМОВО И МИКРОГРАМОВО НИВО ЧРЕЗ FTIR И ESI-MS АНАЛИЗ

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(Резюме)

Взаимодействието на M(II) d-биометални йони (Cu, Co, Mn) и токсичния Cd(II) йон с млечна киселина, която се намира в човешкото тяло в резултат на поглъщане или като продукт от биологични процеси на мили- или микромоларно ниво, е изследвано при приблизително физиологични условия с помощта на FTIR и ESI-MS спектроскопия. Установено е, че млечната киселина в изследваната моделна система се отнася като монодентатен лиганд; тя взаимодейства на всички концентрационни нива и най-силно изразеното взаимодействие е с Co(II) йон.