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Potentiometric, electrochemical and UV/VIS investigation of a copper (II) complex with β-alanyl-L-histidine

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The main goal of this study was to investigate complexation of L-carnosine and its constituent amino acids β-alanine and L-histidine with copper (II) in a buffer at pH = 10. For this study the following methods were used: potentiometry, cyclic and differential pulse voltammetry and UV/VIS spectroscopy. The results have shown that L-histidine and L-carnosine formed a complex with copper in a 1:1 ratio, while no complexation with β-alanine was observed.

Keywords: potentiometry, voltammetry, copper, carnosine, metal complex

1. INTRODUCTION

L-carnosine (β-alanyl-L-histidine) is a dipeptide composed of two amino acids: β-alanine and L-histidine (Fig. 1). It can be found in high concentrations in the skeletal muscles, brain, and gastrointestinal tissue of vertebrates, where it shows neurotransmitter, buffer and antioxidant activity. The antioxidant activity of carnosine is related to its ability to chelate metal ions and scavenge reactive oxygen species (ROS) and other free radicals. Carnosine is a substrate of enzyme carnosinase, which can be activated or stabilized by different metal cations. Since carnosine is a major source of β-alanine in a human organism, an investigation of its hydrolysis and metal complexes of carnosine are important for central metabolic processes [1]. Copper is a trace element essential for optimal functioning of all living organisms and can be found in humans in amounts between 70 mg to 80 mg. It is concentrated in organs with high metabolic activity, such as the liver, heart, and brain, and can be toxic in higher concentrations [2].

Carnosine is a polydentate ligand with six potential binding sites: the two imidazole nitrogen atoms, one carboxylate and one amino group, and the peptide linkage. It has three groups that undergo

acid-base reactions in the pH range from pH = 1 to pH =10: the carboxylic acid group (pK_1 = 2.6), the protonated N imidazole group (pK_2 = 6.7), and an ammonium group (pK_3 = 9.2) [3]. Carnosine can chelate with many metal cations such as Ru^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Mg^{2+}, Ca^{2+}, and Fe^{2+}, and form complexes [4,5]. Because of its chelating activity, it has been proposed as a cure for Alzheimer's disease and Wilson's disease [6,7]. Some studies have shown that by chelation of copper ions, carnosine may act as antioxidant in vivo and can be involved in the regulation of anaerobic glycolysis of skeletal muscle [8].

So far, carnosine was studied through different methods because of its important roles in living organisms. These methods include high-performance liquid chromatography (HPLC) with different detectors (UV, fluorescent and amperometric) [9], liquid chromatography with mass spectrometry (LC-MS) [10], capillary electrophoresis (CE) [10], HPLC using an electrospray ionization mass spectrometry (ESI-MS) [10], microchip electrophoresis with chemiluminescence detection [11], capillary electrophoresis with laser induced fluorescence detection [12], potentiometry [13,14] and voltammetric methods [15-18]. Complexes of carnosine and metal cations were also investigated by cyclic voltammetry [19], spectroscopy (IR [19], ESR [19], NMR [19], UV/VIS [20], Raman [21]) and potentiometry [22].

In this study, we have investigated the complexation of L-carnosine and its amino acid components β-alanine and L-histidine with copper in a buffer at pH = 10, by potentiometry, voltammetry (cyclic and differential pulse) and UV/VIS spectroscopy. The main goal was to get more information regarding structure of carnosine complex with copper ion in a solution. Since carnosine prevents toxic effects of copper in brain signalling [23], the investigation of carnosine-copper complexation can be applied to biological systems.

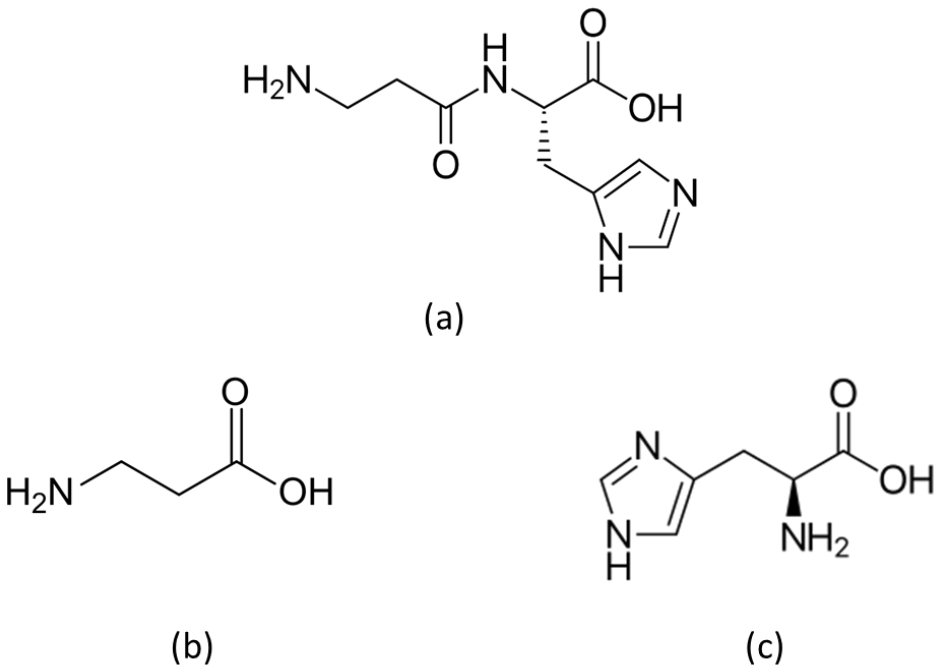

Figure 1. Chemical structures of L-carnosine (a), β-alanine (b) and L-histidine (c).

2. EXPERIMENTAL

2.1 Chemicals and solutions

All commercially available chemicals were of reagent grade and used as purchased. Copper (II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) was purchased from Acros Organics, Belgium. L-histidine ((S)-2-amino-3-(4-imidazolyl) propionic acid), β-alanine (3-aminopropanoic acid) and L-carnosine (β-alanyl-L-histidine) were purchased from Sigma-Aldrich, USA. Sodium hydroxide (NaOH) and sodium hydrogen carbonate ($NaHCO_3$) were obtained from T.T.T., Croatia. Standard solutions of copper (II) nitrate, β-alanine, L-histidine and L-carnosine ($c = 1 \cdot 10^{-2}$ mol dm^{-3}) were prepared in high purity water from a TKA, GenPure Ultra Pure Water System (TKA, Niederelbert, Germany), resistivity greater than or equal to 18 MΩ cm. Standard solutions were diluted with buffer pH = 10 (prepared with 0.1 mol dm^{-3} NaOH and $5.0 \cdot 10^{-2}$ mol dm^{-3} $NaHCO_3$ in high purity water) to the desired concentration before each measurement.

2.2. Apparatus and Procedure

A Dosimat 765 (Metrohm, Switzerland) with homemade software and combined with a Metrohm 780 pH meter (Metrohm, Switzerland) was used to dose the chelating reagent coper (II) nitrate in a buffer solution pH = 10 with β-alanine, L-histidine or L-carnosine. The solutions were magnetically stirred during titrations using a 728 Titration Stand (Metrohm, Switzerland). A platinum redox electrode (Metrohm, Switzerland) was used as potentiometric sensor and Ag/AgCl electrode (inner electrolyte solution 3 M KCl) (Metrohm, Switzerland) was used as a reference electrode in order to study the Cu^{2+} ion complexation with β-alanine, L-histidine and L-carnosine. The Dosimat was programmed to work in DET (Dynamic Equivalence point Titration) Mode with a signal drift of 5 mV/min and an equilibrium time of 30 s. The waiting time before each increment addition was 120 s. All the measurements and titrations were performed at room temperature. In potentiometric titrations the solution of $Cu(NO_3)_2$ ($c = 1 \cdot 10^{-2}$ mol dm^{-3}), was used as the titrant, while β-alanine, L-histidine or L-carnosine were used as analyte during the potentiometric titrations. The volume of the solution used for all of the titrations was 22 cm^3, while the concentration of ligand in the solution was $1 \cdot 10^{-3}$ mol dm^{-3}.

Electrochemical experiments were performed on PalmSens potentiostat/galvanostat (PalmSens BV, Utrecht, The Netherlands) driven by PSTrace 4.2 software. A three-electrode electrochemical cell was used with a glassy carbon (geometrical area 0.018 cm^2) as a working electrode, Ag/AgCl (inner electrolyte solution 3 M NaCl) as a reference electrode and a platinum wire as a counter electrode. The glassy carbon working electrode was electrochemically cleaned in 1 mol dm^{-3} H_2SO_4 (10 cycles, scan rate 50 mV/s from -1.0 V to 1.0 V vs Ag/AgCl electrode). Cyclic voltammetry scan rate varied from 50 mV/s to 300 mV/s. The differential pulse voltammetry conditions were: scan increment 5 mV, pulse amplitude 25 mV, pulse width 70 ms and scan rate 5 mV/s.

UV/VIS spectra were recorded on a UV-1700 Pharma Spec spectrophotometer (Shimadzu, Japan). Standard solutions were diluted with a buffer pH = 10 to $c = 4 \cdot 10^{-3}$ mol dm^{-3} and recorded from 200 nm to 1000 nm using standard 1.00 cm quartz cells.

3. RESULTS AND DISCUSSION

3.1. Potentiometry

Potentiometric titration curves and their first derivatives of the different analytes, L-carnosine (Fig. 2A), L-histidine (Fig. 2B) and β-alanine (Fig. 2C) in a buffer pH = 10 are shown in Figure 2. In the titration, 2 mL of the analyte ($c = 1 \cdot 10^{-2}$ mol dm^{-3}) in a buffer pH = 10 was titrated with $1 \cdot 10^{-2}$ mol dm^{-3} $Cu(NO_3)_2$. Well-defined inflexions in Figures 2A and 2B were observed, suggesting complexation of L-carnosine and L-histidine with Cu^{2+}. The complexation was confirmed visually by colour change of the solution from colourless (initial solution of the analyte) to blue (final solution colour after addition of Cu $(NO_3)_2$). The volume of the titrant at the end-point of both titrations was at V = 2 mL indicating formation of a 1:1 complex. This result agrees with previous study where a monomeric 1:1 carnosine complex with copper at room temperature was detected [24]. Formation of a dimeric complex of carnosine and copper at neutral pH [22] and formation of a 1:1 complex of L-histidine and copper at pH = 6 was also reported [25]. The titration curve of β-alanine (Fig. 2C) showed no inflexion, and the colour of the solution was not changed during titration, so no complexation of β-alanine with Cu^{2+} was observed.

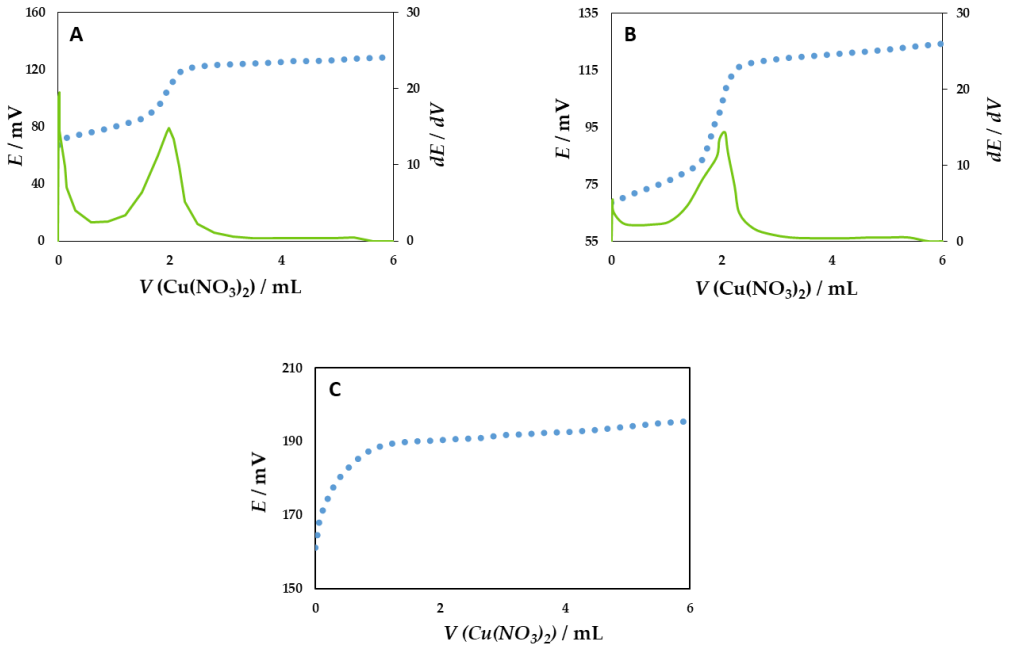

Figure 2. Potentiometric titration curves (••••) and their first derivatives (▬) of: A) L-carnosine, B) L-histidine and C) β-alanine ($c = 1 \cdot 10^{-2}$ mol dm^{-3}) in a buffer pH = 10, using $Cu(NO_3)_2$ ($c = 1 \cdot 10^{-2}$ mol dm^{-3}) as a titrant.

3.2. Cyclic voltammetry

Electrochemical properties of L-carnosine (Fig. 3A), L-histidine (Fig. 3B) and β-alanine (Fig. 3C), $c = 1 \cdot 10^{-4}$ mol dm^{-3} were studied in a buffer pH = 10 in a potential range from -0.800 V to 1.000 V *vs*. Ag/AgCl reference electrode. At this pH value all investigated ligands were completely deprotonated [3,26] and no oxidation/reduction peaks were observed. Cyclic voltammograms of copper (II) nitrate (Fig. 3) showed two anodic peaks at potentials, E = 0.040 V (A1), which corresponded to the oxidation of copper from Cu^0 to Cu^+, and E = 0.110 V (A2), which corresponded to the oxidation of copper from Cu^+ to Cu^{2+}. Two reduction peaks were observed at potentials E = -0.310 V (C2), which corresponded to the reduction of Cu^{2+} to Cu^+, and E = -0.690 V (C1), which corresponded to the reduction of Cu^+ to Cu^0. This result agrees with results of a previous study [19].

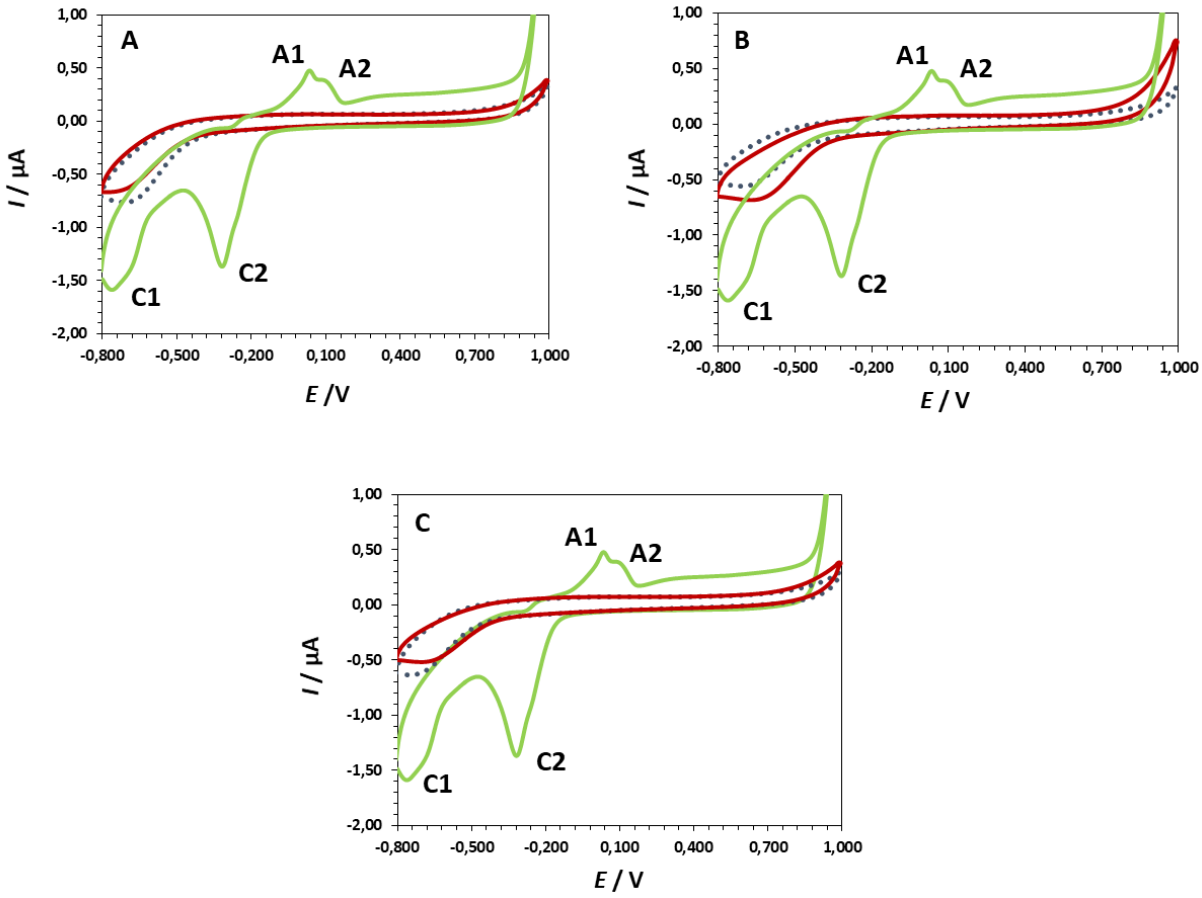

Figure 3. Cyclic voltammograms of blank solution (••••), $Cu(NO_3)_2$ (▬) and A) L-carnosine (▬), B) L-histidine (▬) and C) β-alanine(▬) ($c = 1 \cdot 10^{-4}$ mol dm^{-3}) in a buffer pH = 10. Scan rate, 100 mV/s, number of cycles, n = 3.

Redox properties of the Cu^{2+} complex with L-carnosine (Fig. 4A) and L-histidine (Fig. 4B) in a 1:1 ratio were also investigated. The cyclic voltammogram of the L-carnosine complex with copper showed two anodic peaks, (A1) at a potential E = -0.015 V, which corresponded to the oxidation of copper in a complex, and A2 at potential E = 0.860 V, which corresponded to the oxidation of the

imidazole ring. The cyclic voltammogram of the L-histidine complex with copper showed one anodic peak (A1) at the potential E = 0.030 V, which also corresponded to the oxidation of the metal centre in a complex. Complexation of β-alanine with Cu^{2+} was not detected (Fig. 4C) since oxido-reduction reactions were only observed for free copper (Fig. 3) were observed. In a previous research formation of Cu^{+} complex with carnosine was detected at lower metal : ligand ratio, while with the increase of carnosine concentration more stable Cu^{2+} complex with carnosine was formed [19].

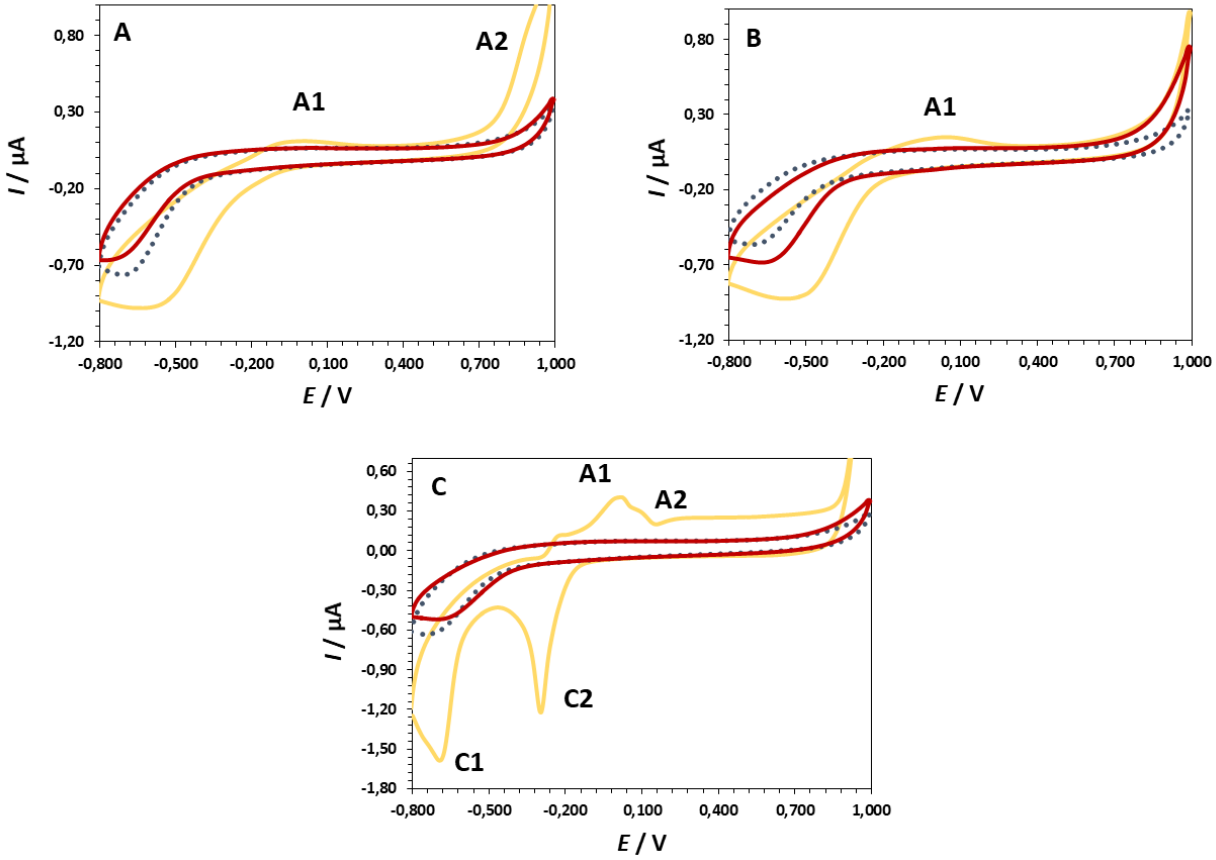

Figure 4. Cyclic voltammograms of blank solution (••••), A) L-carnosine (▬), B) L-histidine (▬) and C) β-alanine(▬) (c = 1·10^{-4} mol dm^{-3}) and their complex with Cu^{2+} in a 1:1 ratio (▬) in a buffer pH = 10. Scan rate, 100 mV/s, number of cycles, n = 3.

3.3. Differential pulse voltammetry

Additional information regarding the electrochemical behaviour of the studied ligands and their complexes with copper were obtained by differential pulse voltammetry. Differential pulse voltammograms of L-carnosine (Fig. 5A) and L-histidine (Fig. 5B) revealed one oxidation peak (A3) at the potential E = 0.860 V, which corresponded to the oxidation of the imidazole ring of both ligands, while β-alanine (Fig. 5C) showed no oxidation. Two anodic peaks of copper nitrate at potentials E = 0.025 V (A1) and E = 0.075 V (A2), which corresponded to copper oxidation from Cu^{0} to Cu^{+} and Cu^{+} to Cu^{2+} respectively, were also detected.

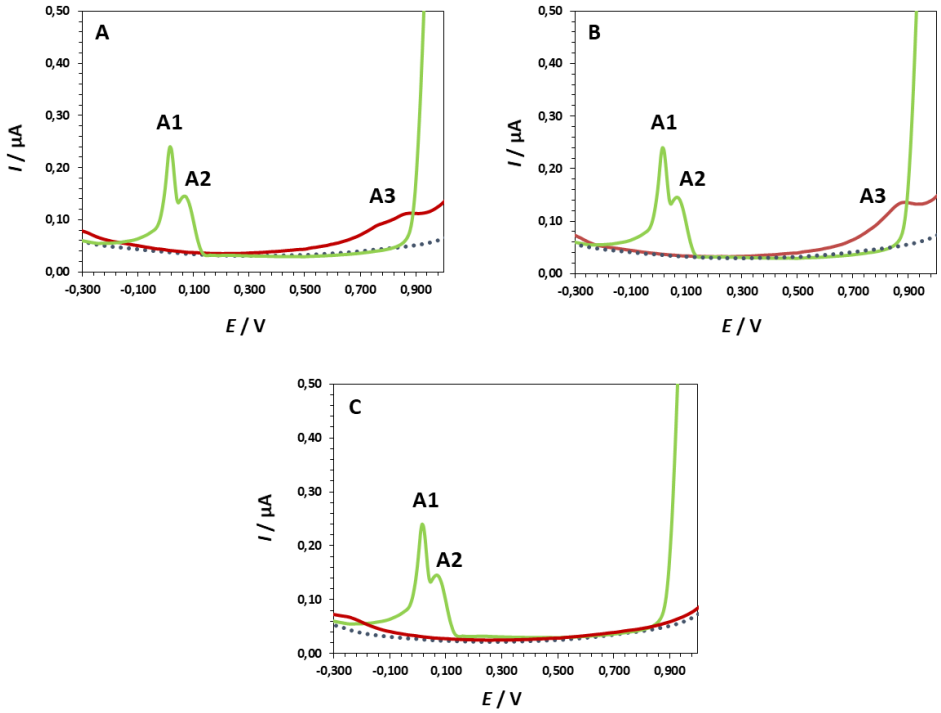

Figure 5. Differential pulse voltammograms of blank solution (••••),$Cu(NO_3)_2$ (▬) A) L-carnosine (▬), B) L-histidine(▬) and C) β-alanine (▬) (c = 1·10^{-4} mol dm^{-3}) in a buffer pH = 10. Scan rate, 5 mV/s.

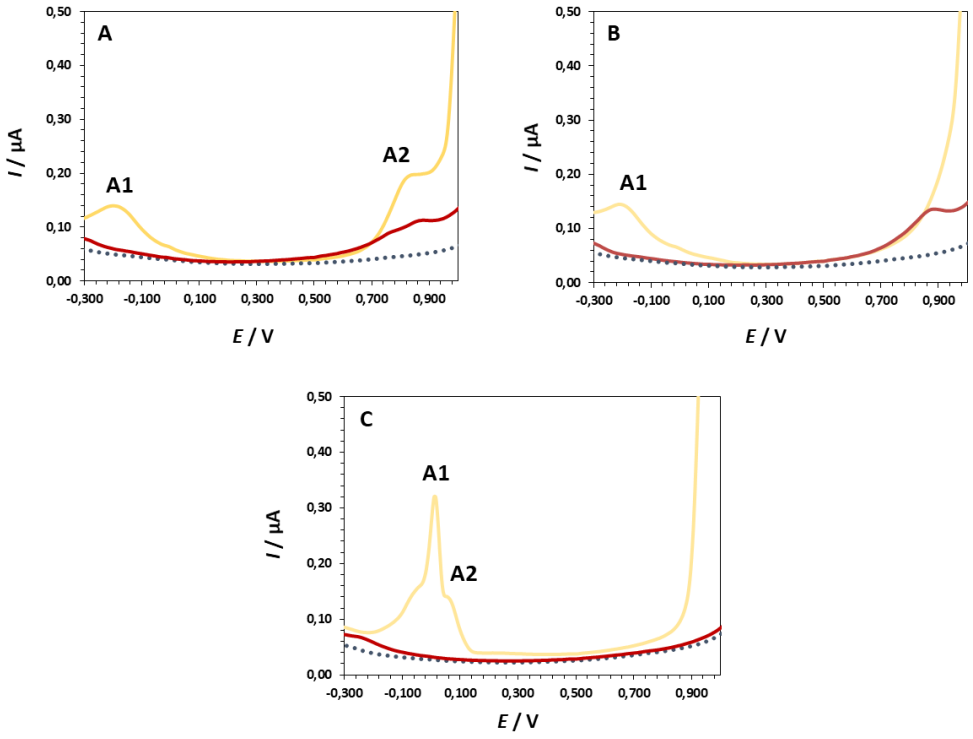

Figure 6. Differential pulse voltammograms of blank solution (••••), A) L-carnosine (▬), B) L-histidine (▬) and C) β-alanine (▬) (c = 1·10^{-4} mol dm^{-3}) and and their complex with Cu^{2+} in a 1:1 ratio (▬) in a buffer pH = 10. Scan rate, 5 mV/s.

Differential pulse voltammogram of L-carnosine complex with copper in a 1:1 ratio (Fig. 6A) showed two anodic peaks, (A1) at the potential E = -0.180 V, which corresponded to the oxidation of complexed copper, and (A2) at the potential E = 0.820 V, which corresponded to the oxidation of the imidazole ring. The L-histidine complex with copper (Fig. 6B) showed one anodic peak (A1), at a potential of E = -0.200 V, which also corresponded to oxidation of a complexed metal centre, while the oxidation peak of the imidazole ring was not observed. Complexation of β-alanine with Cu^{2+} ion was not detected (Fig. 6C) since two oxidation peaks for free copper (Fig. 5) were observed.

3.4. UV/VIS spectroscopy

In Figure 7A, the UV/VIS spectra of 4 mmol dm^{-3} L-carnosine (a), $Cu(NO_3)_2$ (b), and the Cu^{2+} complex with L-carnosine in a 1:1 ratio (c) are shown. It can be seen that L-carnosine showed an absorbance band in the UV region at 277 nm, which can be assigned to the n→π* transition, while copper (II) nitrate showed a broad band approximately 723 nm, which can be assigned to the d-d transition. The spectra of Cu^{2+} ion with L-carnosine complex showed an absorbance band at 603 nm which can be assigned to the metal to ligand charge transfer, while the carnosine band at 277 nm was no longer visible. Earlier research showed that at low pH <4.8 carnosine solution with copper showed absorption maximum at 780 nm and a shoulder near 730 nm. Above pH = 6.5 the first absorption maximum at 780 nm decreased while the second at 730 nm increased and shifted to 610 nm, which was followed by colour change and the increase in molar absorption. These changes indicated formation of carnosine copper complex in which two imidazole nitrogen atoms and one deprotonated nitrogen from peptide bond of carnosine were bonded to the Cu^{2+} ion [24].

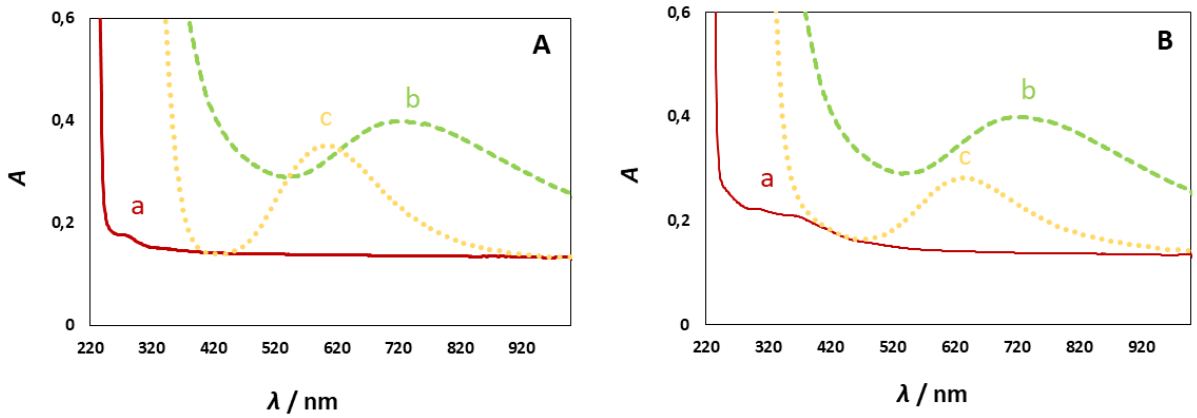

Figure 7. UV-Vis absorption spectra of $Cu(NO_3)_2$ (- - -), A) L-carnosine (▬) and B) L-histidine(▬) and their complex with Cu^{2+} in a 1:1 ratio (••••), $c = 4 \cdot 10^{-3}$ mol dm^{-3} in a buffer pH = 10.

Recent study has shown that the UV/VIS spectra of the Cu^{2+} complex with carnosine and its derivatives (1-[2-(β-alanyl-L-histidine)ethoxy)]-β-D-glucopyranoside and 1-[2-(β-alanyl -L-histidine) ethoxy]-(β-D-galactopyran osyl) -(1→4) -β-D-glucopyranoside) showed no significant difference [27].

In Figure 7B, the UV/VIS spectra of 4 mmol dm^{-3} L-histidine (a), $Cu(NO_3)_2$ (b), and the Cu^{2+} complex with L-histidine in a 1:1 ratio (c) are shown. L-histidine showed an absorbance band in the UV region at 309 nm, which can be assigned to the $n \rightarrow \pi^*$ transition, while the spectra of Cu^{2+} ion with L-histidine complex showed an absorbance band at 632 nm, which can also be assigned to the metal to ligand charge transfer. The histidine absorbance band was not visible in a spectrum of the histidine complex with Cu^{2+}.The colour of two complexes was different, the L-carnosine complex with Cu^{2+} was dark blue, while the L-histidine complex with copper was light blue.

4. CONCLUSION

In this study complexation of β-alanine, L-histidine and L-carnosine with copper was studied by potentiometry, cyclic and differential pulse voltammetry and UV/VIS spectroscopy in a buffer at pH = 10. At that pH value, all ligands (L-carnosine, L-histidine and β-alanine) were completely deprotonated. The concentrations of Cu^{2+} and ligands were adjusted for every method in order to obtain optimal results and they varied from $c = 1 \cdot 10^{-4}$ mol dm^{-3} in the voltammetric measurements, and $c = 1 \cdot 10^{-3}$ mol dm^{-3} in the potentiometry to $c = 4 \cdot 10^{-3}$ mol dm^{-3} with UV/VIS spectroscopy. The results have shown that Cu^{2+} complexation with L-histidine and L-carnosine occurred in a 1:1 ratio, while complexation with β-alanine was not observed by any of the methods used in this study. It could be concluded that the histidine part of carnosine molecule (imidazole ring and carboxylate group) was involved in complexation with Cu^{2+}.

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