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Voltammetric Characterization and Determination of Histidine Dipeptides - Carnosine and Anserine

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This study reports the characterization of two histidine dipeptides, carnosine (*β* -alanyl-L-histidine) and anserine (*β*–alanyl-3-methyl-L-histidine), by differential pulse voltammetry. Both molecules showed one oxidation peak, which decreased with successive scans. This effect was more pronounced in carnosine, indicating the adsorption of the oxidation products and blocking the electrode surface. The proposed oxidation mechanism includes the transfer of one electron and one proton and is pH-dependent.

Keywords: carnosine, anserine, voltammetry, oxidation

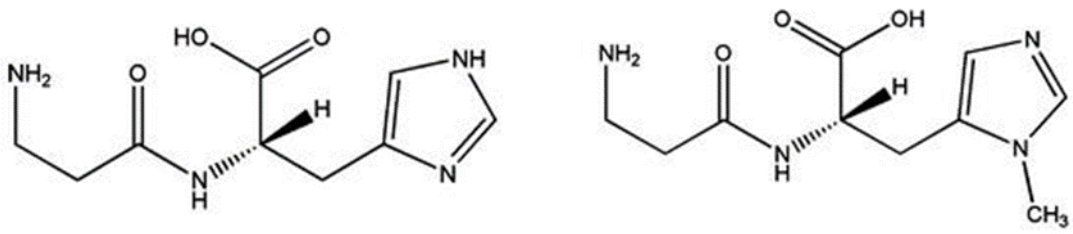
1. INTRODUCTION

Carnosine and anserine are histidine dipeptides that are found in high concentrations in the skeletal muscles and central nervous system of vertebrates [1,2]. These substances have a variety of physiological roles. They improve learning ability and reduce muscle fatigue because of their antioxidative effects [3]. They also have buffering capacities due to the presence of an imidazole group.

L-Carnosine (*ß*-alanyl-L-histidine) is a dipeptide composed of ß-alanine and L-histidine, which performs multiple biological functions including anti-oxidation, anti-glycation, anti-aging effects, pH buffering and chelation of divalent metal cations [4-6], and it acts as a scavenger of reactive oxygen species [7].

Anserine (*ß*-alanyl-N-methyl histidine) is an N-methylated analog of carnosine with similar properties, but it is found predominantly in birds, fish, and other non-mammalian species.

The chemical structure of carnosine and anserine are shown in Fig. 1.



Carnosine (*β*-alanyl-L-histidine) Anserine (*β*-alanyl-3-methyl-L-histidine)

Figure 1. Chemical structure of carnosine and anserine.

Interest in histidine dipeptide quantification is constantly rising. The available colorimetric methods include the diazo procedure [8], which produce colorful compounds with carnosine. Additionally, fluorometric methods can be used for determination in biological tissues [9].

Chromatographic methods are used for the determination of histidine dipeptides present in meat products, biological materials, etc. Such techniques include a) high performance liquid chromatography (HPLC) with UV [10] or fluorescent detection [11], b) a new HPLC procedure based on hydrophilic interaction chromatography (HILIC) [12], c) new gradient reversed-phase HPLC methods [13], d) reversed phase HPLC using an electrospray ionization mass spectrometry (ESI-MS) detector [14], e) high-performance anion-exchange chromatography (HPAEC) with integrated amperometric pulse detection [15], and f) micellar liquid chromatography (MLC) with UV detection [16]. HPLC is the most commonly applied method for the analysis of histidine dipeptides due to its good separation efficiency. However, HPLC suffers from several disadvantages including long analysis time and limited column lifetime.

The presence of carnosine and anserine can also be determined in meat products, urine samples, and biological materials via capillary electrophoresis with UV detection [17-19], laser-induced fluorescence (LIF) [20], electrospray mass spectrometric detection [21], and microchip electrophoresis with chemiluminescence detection [22].

Electrochemical methods are rarely used for the determination of histidine dipeptides. However, the use of cyclic voltammetry [23-26] and potentiometry [27-29] have been reported.

The objective of this investigation was to study the electrochemical properties of the histidine dipeptides carnosine and anserine in order to elucidate their mechanism of oxidation which can be applied in the studies of real samples.

2. EXPERIMENTAL

2.1 Chemicals and apparatus

All chemicals used in this research were of reagent grade and used without further purification. L-carnosine was purchased from Acros Organics (Geel, Belgium) and L-anserine nitrate was

purchased from Bachem (Bubendorf, Switzerland). All other chemicals were obtained from Kemika (Zagreb, Croatia). Carnosine and anserine stock solutions ($c = 1\times10^{-2}$ mol dm^{-3}) were prepared in high purity water. The solutions were kept in the refrigerator and were stable for at least 3 weeks. Prior to use, the stock solutions were diluted to the desired concentration with a buffer supporting electrolyte (I_c = 0.1 mol dm^{-3}). The buffer supporting electrolyte solutions were also 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. Buffer solutions were used as follows: a phosphate buffer solution with a pH = 5.5 to 8.0 and an acetate buffer solution with a pH = 3.5 to 5.0.

Electrochemical experiments were performed on a PalmSens potentiostat/galvanostat (PalmSens BV, Utrecht, The Netherlands). The instrument was driven by PSTrace 4.2 software. A conventional three-electrode cell was used. Glassy carbon was used as a working electrode, Ag/AgCl as a reference electrode, and a platinum wire as a counter electrode. The glassy carbon working electrode was polished with coarse diamond polish (1 μm, ALS, Japan) and after that with polishing α-Al_2O_3 (0.05 μm, ALS, Japan) before each measurement. The used differential pulse voltammetry conditions were: scan increment 5 mV, pulse amplitude 25 mV, pulse width 70 ms, and scan rate 5 mV s^{-1}.

3. RESULTS AND DISCUSSION

3.1. Differential pulse voltammetry studies of carnosine and anserine

Differential pulse voltammograms of carnosine and anserine shown in Fig. 2 revealed one oxidation peak for carnosine at 1.15 V, and one oxidation peak for anserine at 1.18 V and a pH = 6.1.

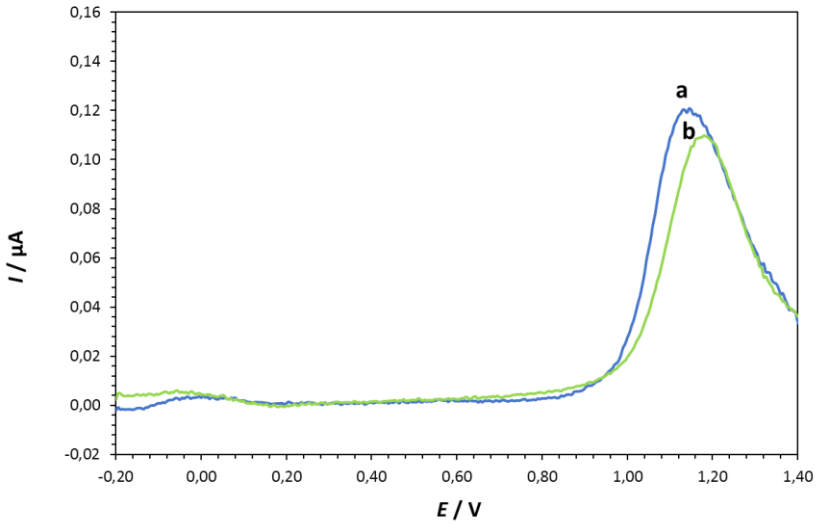


Figure 2. Differential pulse voltammogram of a) carnosine ($c = 3\cdot10^{-4}$ mol dm^{-3}) and b) anserine ($c = 3\cdot10^{-4}$ mol dm^{-3}) at the glassy carbon electrode (I_c = 0.1 mol dm^{-3}, pH =6.1).

The oxidation peak of carnosine decreased significantly with successive scans (Fig. 3). This may indicate the adsorption or bonding of the carnosine oxidation product onto the glassy carbon electrode surface [30]. The anserine oxidation peak also decreased with successive scans. However, the height change was somewhat less dramatic compared with carnosine.

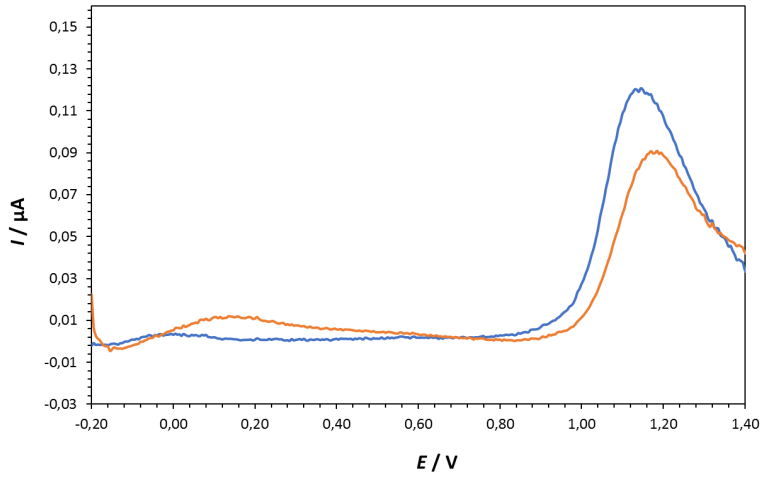
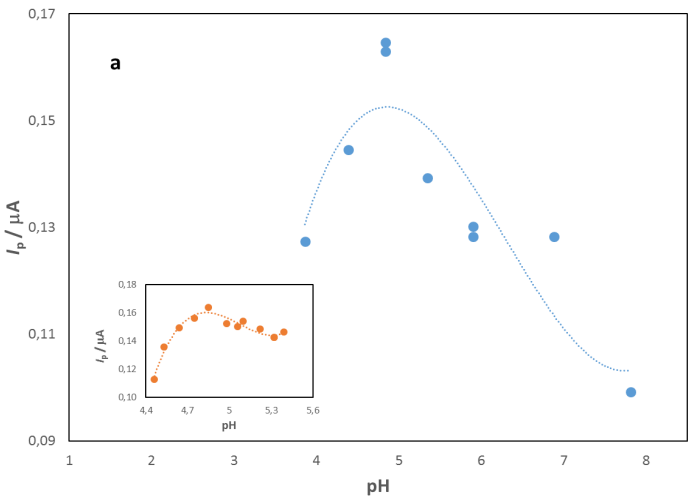


Figure 3. Differential pulse voltammogram of carnosine (c = 3·10^{-4} mol dm^{-3}) at the glassy carbon electrode (I_c = 0.1 mol dm^{-3}, pH =6.1) v = 5 mV/s. First scan (—), second scan (—).

The influence of pH on carnosine oxidation peak current (I_p) in the pH interval from pH = 4 to 8 was investigated. As shown in Fig. 4a and 4c, the highest peak current occurred at a pH of approximately 4.8, and it was lower under more acidic and alkaline conditions. The graph of the peak potential (E_p) *vs.* pH was linear within the pH range from 4 to 8 (Fig. 4b), with a slope of 58.4 mV. This corresponds to an oxidation mechanism where an equal number of electrons and protons are exchanged and is pH-dependent in acidic and neutral media [31].



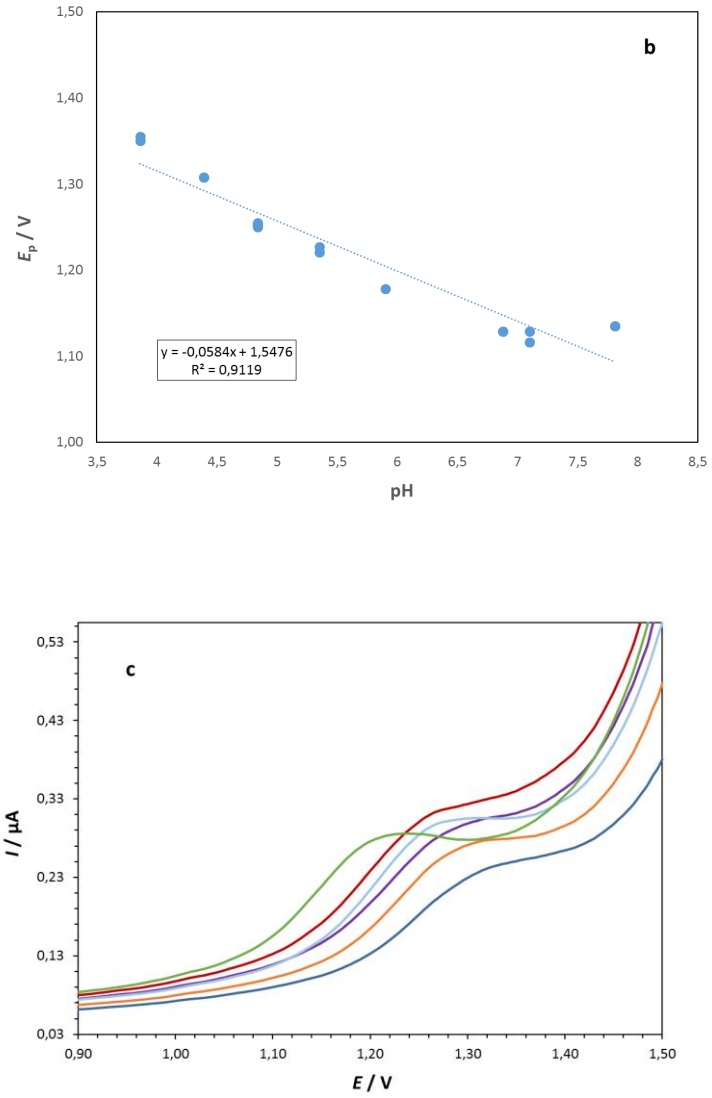


Figure 4. Carnosine (c = $3 \cdot 10^{-4}$ mol dm^{-3}) peak current (I_p) (4a), and peak potential (E_p) (4b) as a function of pH. Differential pulse voltammograms of carnosine as the function of pH (4c): pH= 4.4 (—), 4.5 (—), 4.6 (—), 4.7 (—), 4.8 (—) and 5.3 (—).

The anserine peak current (I_p) as a function of pH (from a pH of 4.5 to 8) was also investigated. As shown in Fig. 5a and 5c the highest peak current occurred at a pH of approximately 6.1, and it was lower under more acidic and alkaline conditions. The graph of the peak potential (E_p) *vs.* pH showed linearity within the pH interval from 4.5 to 8 (Fig. 5b), with a slope of 66.8 mV. This corresponds to an oxidation mechanism where an equal number of electrons and protons are exchanged and is pH-dependent in acidic and neutral media [31]. The dissociation diagram of carnosine and anserine (Fig. 6) shows that spontaneous deprotonation occurs at a pH = 1. This means that the deprotonated forms (H_2Car^- and H_2Ans^-) of both molecules take part in electrochemical oxidation reactions, and undergo adsorption up to a pH = 5. At higher pH values (pH = 5 to 8), spontaneous deprotonation continues, and other dissociated species of carnosine and anserine are formed.

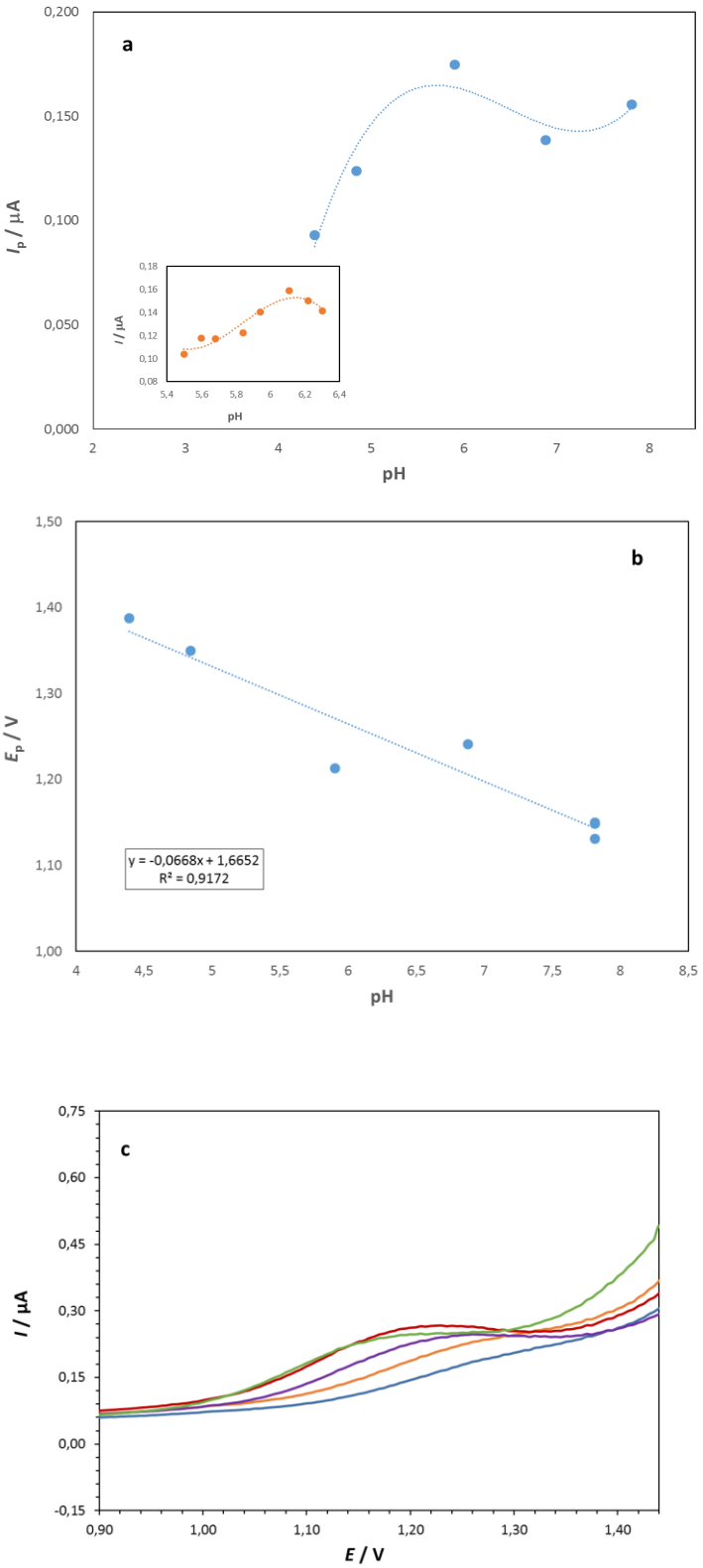


Figure 5. Anserine (c = $3 \cdot 10^{-4}$ mol dm^{-3}) peak current (I_p) (5a), and peak potential (E_p) (5b), as the function of pH. Differential pulse voltammograms of anserine as the function of pH (5c): pH= 5.5 (—), 5.6 (—), 5.9 (—), 6.1 (—) and 7.8 (—).

This increases the likelihood of occurring of complex electrochemical and adsorption processes. Dissociation constants of carnosine (pKa_1 = 2.64; pKa_2 = 6.83; pKa_3= 9.51) and anserine (pKa_1 = 2.64; pKa_2 = 7.04; pKa_3= 9.49) are taken from the literature data [32,33].

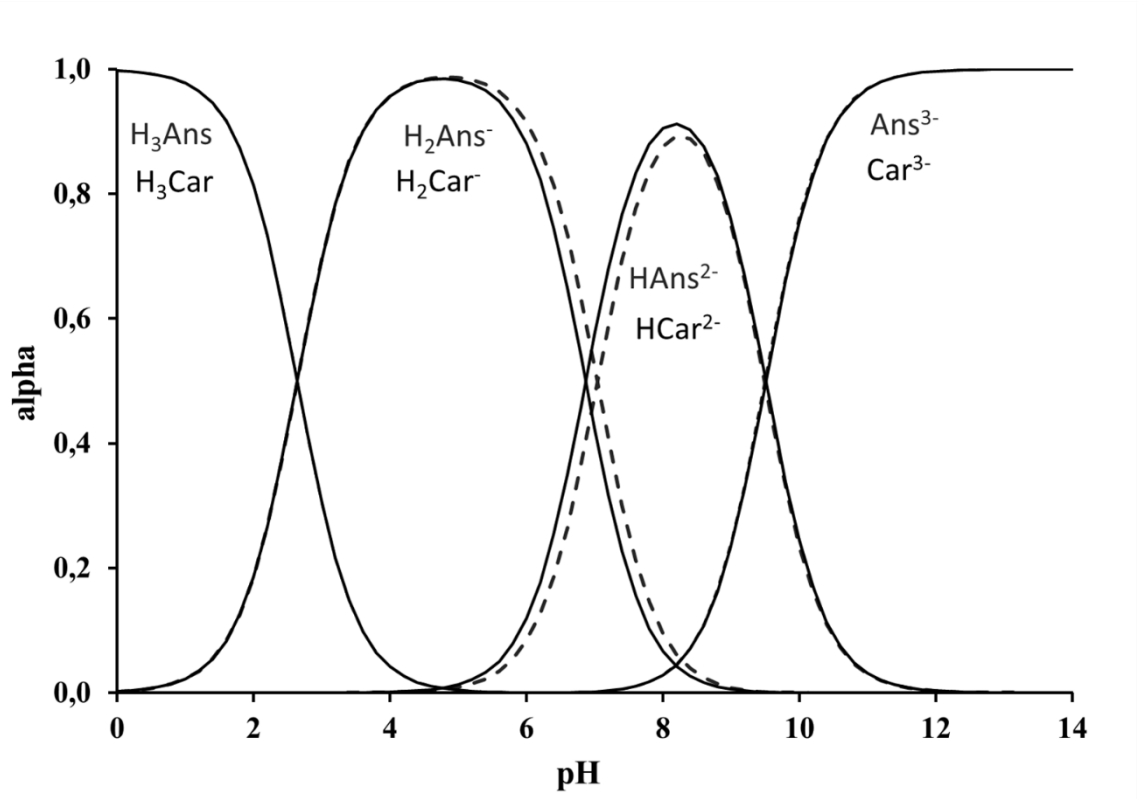
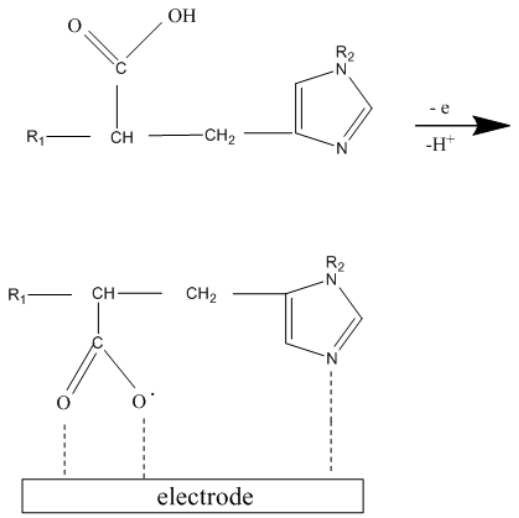


Figure 6. Dissociation diagram of carnosine (—) and anserine (---): undissociated forms (H_3Car and H_3Ans), deprotonated forms (H_2Car^-, H_2Ans^-, $HCar^{2-}$, $HAns^{2-}$, Car^{3-} and Ans^{3-}).



Scheme I. Proposed mechanism for the electrochemical oxidation of carnosine and anserine. R_1 = H_2NCH_2COHN; R_2 = H (in carnosine), CH_3 (in anserine).

The proposed oxidation mechanism is shown in Scheme I. The oxidation of both molecules includes the loss of one electron and one proton from a histidine carboxyl group, which leads to the formation of a carnosine/anserine radical. The radical form of the molecule is adsorbed onto the electrode surface through the carboxyl radical and amine group from the imidazole ring, which blocks the electrode surface and causes the observed decrease in the oxidation peak current [1,30].

Carnosine and anserine peak current and peak potential of increase with increase of their concentration. This can be explained by kinetic limitations in the reaction between the redox sites of the glassy carbon electrode and the investigated histidine dipeptides. The peak current and the carnosine and anserine concentration show linear dependence in the concentration range of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ mol dm^{-3} (Fig. 7 and 8). A linear regression equation for carnosine, $I_p = 0.061c + 0.0159$, was obtained, with a correlation coefficient of $R^2 = 0.9763$ (I_p is the oxidation peak current, and c is the carnosine concentration (10^{-4} mol dm^{-3}) (Fig. 7)).

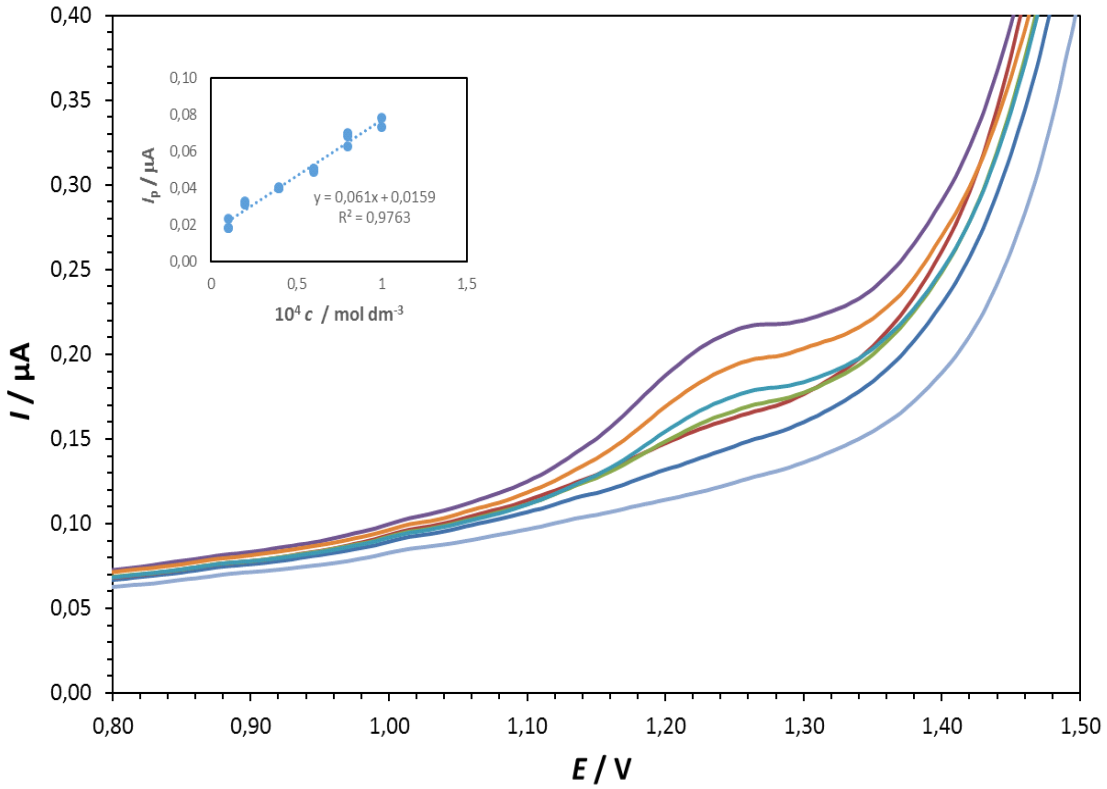


Figure 7. Differential pulse voltammograms in solutions of carnosine with concentrations of 0, $1 \cdot 10^{-5}$, $2 \cdot 10^{-5}$, $4 \cdot 10^{-5}$, $6 \cdot 10^{-5}$, $8 \cdot 10^{-5}$, and $1 \cdot 10^{-4}$ mol dm^{-3}, at a glassy carbon electrode (I_c = 0.1 mol dm^{-3}; pH = 4.8). v = 5 mV/s.

A linear regression equation, $I_p = 0.0629c + 0.0084$, with a correlation coefficient of $R^2 = 0.9908$ was obtained for anserine, where I_p is the oxidation peak current, and c is the anserine concentration (10^{-4} mol dm^{-3}) (Fig. 8).

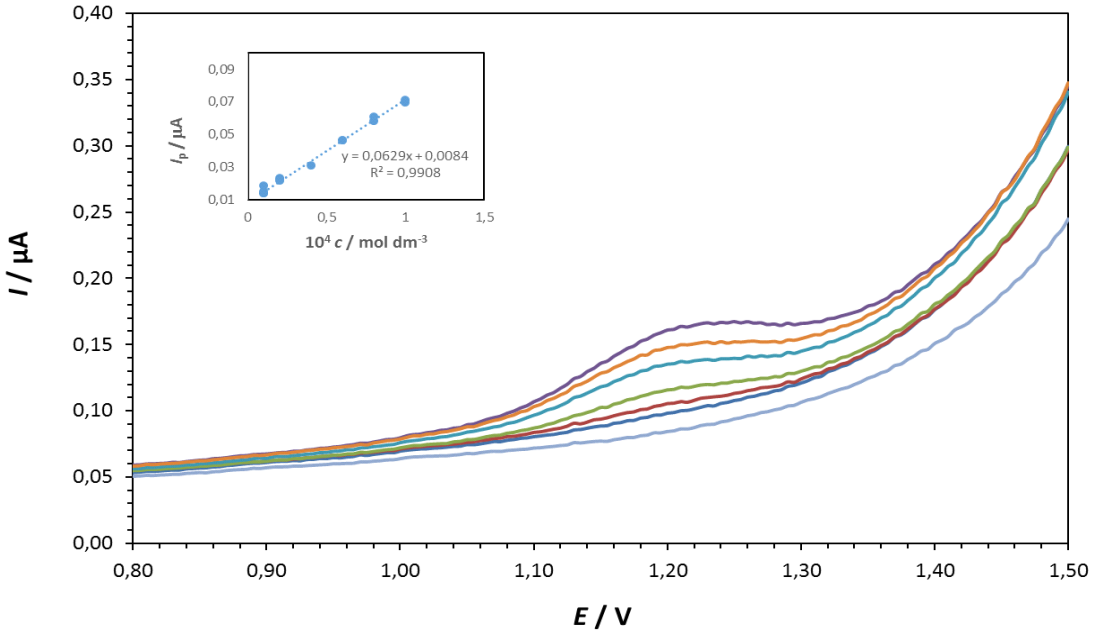


Figure 8. Differential pulse voltammograms in solutions of anserine with concentrations of 0, $1 \cdot 10^{-5}$, $2 \cdot 10^{-5}$, $4 \cdot 10^{-5}$, $6 \cdot 10^{-5}$, $8 \cdot 10^{-5}$, and $1 \cdot 10^{-4}$ mol dm^{-3} at a glassy carbon electrode (I_c = 0.1 mol dm^{-3}; pH = 6.1). v = 5 mV/s.

4. CONCLUSION

In this paper, two histidine dipeptides, carnosine and anserine, were characterized by differential pulse voltammetry. Both dipeptides are oxidized, and their oxidation mechanism involves the transfer of one electron and one proton. The optimal pH for the oxidation of carnosine and anserine is approximately 4.8 and 6.1, respectively. The oxidation product of both investigated compounds adsorb on the glassy carbon electrode surface. A linear relationship between the peak current and the carnosine and anserine concentration in the range of $1 \cdot 10^{-5}$ to $1 \cdot 10^{-4}$ mol dm^{-3} was established.

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