Int. J. Electrochem. Sci., 9 **(2014)** 7904 - 7915

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Estimation of the Antioxidative Properties of Amino Acids – an Electrochemical Approach

Anna Masek[1,*], *Ewa Chrzescijanska*[2], *Marian Zaborski*[1]

[1]Technical University of Lodz, Institute of Polymer and Dye Technology, Faculty of Chemistry, 90-924 Lodz, ul. Stefanowskiego 12/16, Poland
[2]Technical University of Lodz, Institute of General and Ecological Chemistry, Faculty of Chemistry, 90-924 Lodz, ul. Zeromskiego 116, Poland
[*]E-mail: anna.masek@p.lodz.pl

Received: 22 August 2014 / *Accepted:* 11 September 2014 / *Published:* 28 October 2014

The study antioxidant properties of amino acids is very important, because they are the building blocks of peptides and proteins, and they take part in many fundamental biochemical processes. Electrochemical methods provide big opportunities for investigation and assessment of antioxidant capacity of these compounds. The electrooxidation of cysteine, cystine, phenylalanine and alanine was investigated in a non-aqueous solution at a platinum electrode. The process of oxidation and its kinetics was investigated using cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The electrochemical oxidation of amino acids is an irreversible process that proceeds in two steps. Thus, this study demonstrated that cyclic voltammetry in aprotic solvents is a very useful technique for studying the reactivity of such compounds and their antioxidant activities due to the high sensitivity of the technique. Quantum chemical calculations were also performed for the amino acids. The molecular geometries of the amino acids in the gas phase were studied using ab initio quantum chemical calculations with the restricted Hartree–Fock (RHF). Structural investigations of the amino acids were conducted using FTIR. Mechanisms for the oxidation of cysteine and phenylalanine were then proposed.

Keywords: amino acids; electrochemical oxidation; voltammetry; FTIR spectroscopy; antioxidant capacity

1. INTRODUCTION

Electrochemical methods are an important class of diagnostic techniques that have been widely used for the investigation of biological properties of electroactive species [1-2]. One of the most important groups of electroactive species are amino acids [3-4]. Amino acids play an important role in protein metabolism, food, and pharmaceutical products [5-6]. Because of their great importance in all

basic biological processes in the cell, amino acids and proteins are among the most studied biological compounds [7].

Cystine (2-amino-3-[(2-amino-2-carboxyethyl)disulfanyl]propanoic acid) is a dimeric amino acid formed by the oxidation of two cysteine residues that covalently link to make a disulfide bond [8]. Cystine can be hydrolysed to cysteine in water. Cysteine, one of the 20 amino acids commonly found in natural proteins, is widely used in the food industry as an antioxidant and in the pharmaceutical industry as a biomarker or in drug formulation. Additionally, cysteine plays an important role in several biological processes [9-12]. Cysteine and its oxidised form, cystine, play an important role in the regulation of metabolic processes in the human body. In addition, cysteine is a powerful antioxidant and protects the human body against radiation and toxicants [13]. Phenylalanine (2-amino-3-phenylpropanoic acid), an essential amino acid, is a constituent of many central nervous system neuropeptides. It is widely used as a food or feed additive, as a dietary supplement or nutraceutical, in the chemical synthesis of clinically and pharmaceutically active compounds, and as an ingredient in cosmetics [14-15]. Another important amino acid is alanine (2-aminopropanoic acid), a nonpolar aliphatic amino acid [16].

Many techniques have been developed for the analysis of the chirality of amino acids, such as surface plasma resonance (SPR) [17], circular dichroism (CD) [18], chromatography [19-20], fluorescence [21], chemiluminescence [22], and enzyme-based electrodes [23]. However, these research methods require large equipment or relatively complex analytical tools and can therefore be inconvenient analytical tools.

Investigations of the redox behaviours of biologically occurring compounds by means of electrochemical techniques have the potential to provide valuable insights into the biological redox reactions of these molecules. Due to their high sensitivity and selectivity, voltammetric methods have been successfully used to study the redox behaviours of various biological compounds. Electrochemical techniques are excellent methods for the sensitive determinations of organic molecules [24-25], including amino acids [7, 26-29]. Cyclic voltammetry is frequently used for the characterization of electroactive systems [30-31]. The half-wave potential ($E_{1/2}$) is a useful parameter that provides information about the antioxidant activity of the compound under study [32-33]. The "low oxidation potential" corresponds to the "high antioxidant power". The electrochemical properties of amino acids have been described in previous research [34-37].

The aim of this study was to determine the electrochemical behaviours of amino acids during electrooxidation at a platinum electrode in a non-aqueous media and assessment of antioxidant capacity of these compounds. The structural investigations of the compounds were conducted using FTIR spectroscopy. Four biologically important amino acids, cystine, cysteine**,** phenylalanine and alanine, were investigated.

2. EXPERIMENTAL

2.1. Reagents

Pure amino acids: cysteine (2-amino-3-sulfhydrylpropanoic acid, $C_3H_7NO_2S$), cystine **(**2-amino-3-[(2-amino-2-carboxyethyl)disulfanyl]propanoic acid, $C_6H_{12}N_2O_4S_2$**),** phenylalanine (2-amino-

3-phenylpropanoic acid, $C_9H_{11}NO_2$), and alanine (2-aminopropanoic acid, $C_3H_7NO_2$) were obtained from Sigma-Aldrich (Germany) and used as received. Acetonitrile (CH_3CN, pure p.a.) was used to prepare the amino acid solutions and was from Sigma-Aldrich. Tetrabutylammonium perchlorate ($(C_4H_9)_4NClO_4$) was from Fluka (Germany) and was used as a supporting electrolyte. The substrates solutions were prepared by dissolving in 0.1 mol L^{-1} ($(C_4H_9)_4NClO_4$ in acetonitrile. Concentrations of amino acids it was 5.0×10^{-3} mol L^{-1}. All reagents used were of analytical grade.

2.2. Measurement methods

To assess the electrochemical oxidation mechanisms and the kinetics of the compounds under investigation, cyclic voltammetry (CV) and differential pulse voltammetry (DPV) were used with an Autolab electoanalytical unit (EcoChemie, Utrecht, The Netherlands) equipped with PGSTAT-12 and GPES softwares (Eco-Chemie). The experimental conditions for cyclic voltammetry (CV) was $\nu = 0.1$ V s^{-1}, and for differential pulse (DP) voltammetry were: pulse amplitude 25 mV, pulse width 70 ms, and scan rate 0.01 V s^{-1}. A three-electrode system was used for the measurements. Platinum was used as the anode and auxiliary electrode. The electrode potential was measured against a ferricinium/ferrocene reference electrode (Fc^+/Fc). All the solutions were degassed with argon prior to the measurements. During the measurements, an argon blanket was maintained over the solution. CV and DPV recorded in the potential range from 0 to 2 V vs. Fc^+/Fc. The effect of the scan rate on the electrooxidation of the amino acids in an non-aqueous medium was assessed. All of the experiments were performed at room temperature.

FTIR analysis. IR spectra were recorded from 3000-700 cm^{-1} using an FTIR Nicolet 6700 FTIR (Thermo Scientific). The measurement parameters were as follows: 128 scans, resolution 8 cm^{-1}, DTGS/KBr detector. The FT-Raman spectra of the compounds were also recorded over the range of 3000–1000 cm^{-1}.

The quantum chemical calculations were performed using the AM1 method with HyperChem program packages. The molecular structures of amino acids in gas phase were fully optimized by using ab-initio quantum chemical calculations at the restricted Hartree–Fock (RHF) level of theory.

3. RESULTS AND DISCUSSION

3.1. Voltammetric behaviours of amino acids

Cyclic voltammetry (CV) [38-40] and differential pulse voltammetry (DPV) [41-44] have become widely used tools to screen the antioxidant properties of organic compounds. The electrochemical oxidation behaviours of amino acids were studied here to evaluate their electron-transfer potentials. Oxidation potentials can be used to study the electron-donating capacity of a molecule as a general indicator of its radical scavenging ability. Electrochemical cyclic voltammetry allows for the determination of the redox properties of antioxidant molecules in solution. The cyclic and differential pulse voltammograms of the amino acids in acetonitrile solution are shown in Figs. 1-

4. The half-wave potential of the electrode reaction, as determined by cyclic voltammetry, corresponded to the peak potential from differential pulse voltammetry. The supporting electrolyte, 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$, showed no characteristic peaks within the potential range in which the compound oxidation peaks appeared (Fig. 1-4, curves 3).

The voltammograms presented in Figs.1-4 (curves 1 and 2) show that the amino acids are most likely irreversibly oxidised in two electrode steps at potentials lower than those of electrolyte decomposition.

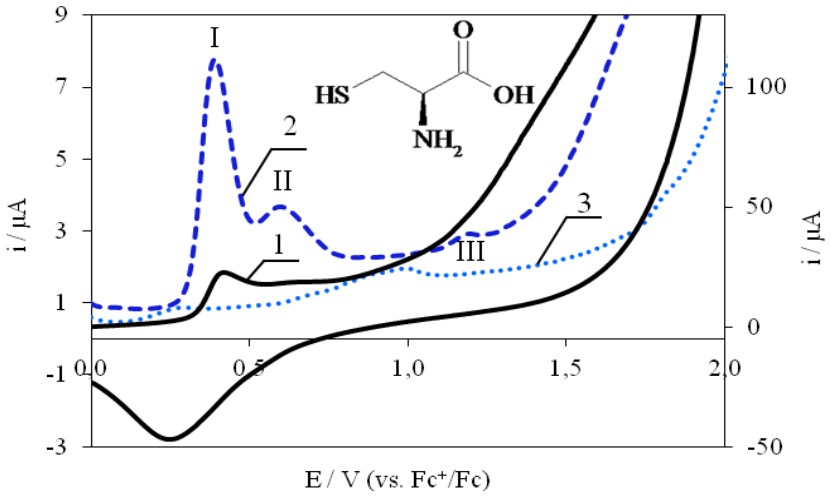


Figure 1. Voltammograms of cysteine oxidation at a Pt electrode; c = 5.0×10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}. 1: cyclic voltammogram, 2: differential pulse voltammogram, 3: differential pulse voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile.

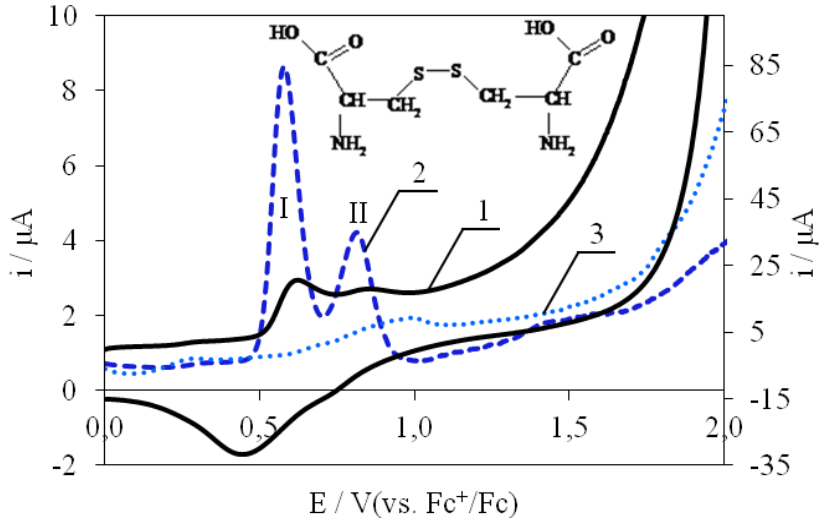


Figure 2. Voltammograms of cystine oxidation at a Pt electrode; c = 5.0×10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}. 1: cyclic voltammogram, 2: differential pulse voltammogram, 3: differential pulse voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile).

Cysteine was shown to oxidise easiest among the investigated amino acids (Fig. 1). As determined by cyclic voltammetry, the half-wave potential ($E_{1/2}$) of the first step in the cysteine oxidation was 0.38 V, which corresponded to the peak potential (E_p) from the differential pulse voltammetry measurements. The half-wave potential ($E_{1/2}$) of the second step was 0.58 V.

However, the oxidation of cystine required increased positive potential (Fig. 2). The half-wave potential ($E_{1/2}$) of the first step in the cystine oxidation was 0.57 V, which corresponded to the peak potential (E_p) from the differential pulse voltammetry measurements. The half-wave potential ($E_{1/2}$) of the second step was 0.83 V. The electroactive centres of both molecules were identical, but cystine contains a disulfide bridge between the two cysteine moieties, which explains the higher oxidation potential of cystine relative to cysteine [7].

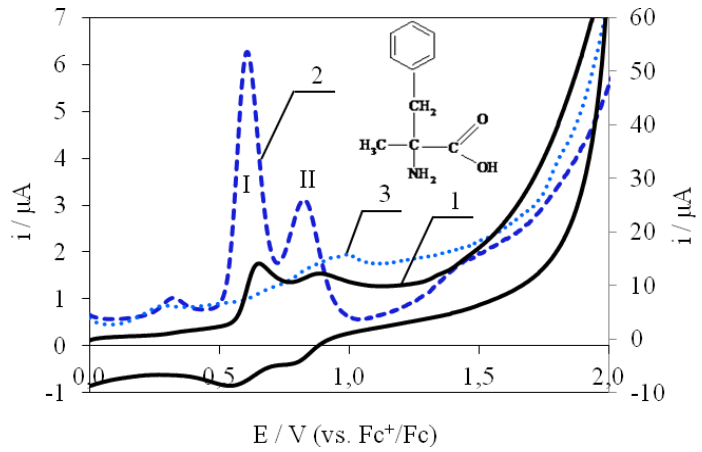


Figure 3. Voltammograms of phenylalanine oxidation at a Pt electrode; c = 5.0×10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}. 1: cyclic voltammogram, 2: differential pulse voltammogram, 3: differential pulse voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile).

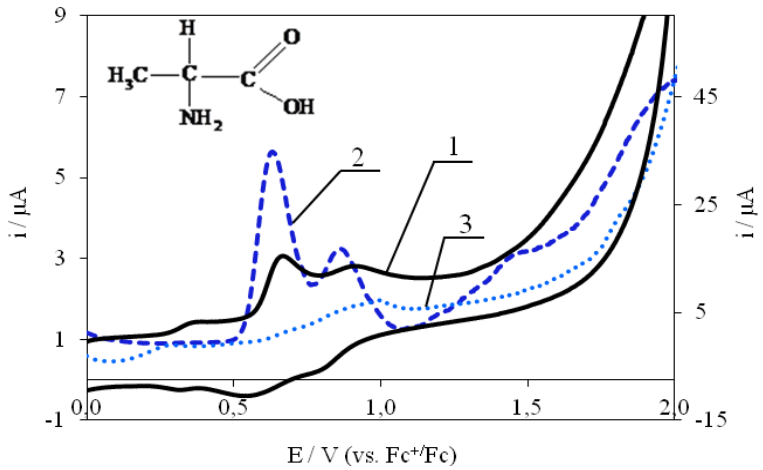


Figure 4. Voltammograms of alanine oxidation at a Pt electrode; c = 5.0×10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile; v = 0.1 V s^{-1}. 1: cyclic voltammogram, 2: differential pulse voltammogram, 3: differential pulse voltammogram of the supporting electrolyte (0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile).

Another amino acid that possesses very good antioxidant properties is phenylalanine. It was more difficult to oxidise phenylalanine than it was for cystine (Fig. 3). The half-wave potential ($E_{1/2}$) of the first step of electrooxidation for phenylalanine was 0.61 V, and the second step was 0.84 V. However, alanine was the most challenging amino acid to oxidise (Fig. 4). The half-wave potential ($E_{1/2}$) of the first step of electrooxidation for alanine was 0.61 V, while the second step was 0.86 V.

3.2. Kinetic parameters of amino acids electrooxidation

The recorded voltammograms, under linear diffusion during the first electrooxidation step, were used to determine the peak potential (E_{pa}), half-peak potential ($E_{pa/2}$) and half-wave potential ($E_{1/2}$). In addition, the voltammograms were used to calculate an anodic transition coefficient (βn_β) and a heterogeneous rate constant (k_{bh}) for the electrode process at the half-wave potential (see Table 1) [45].

Table 1. Values of peak potential (E_p) determined by differential pulse voltammetry, half-wave potential ($E_{1/2}$) determined by cyclic voltammetry, and anodic transition coefficient (βn_β) and heterogeneous rate constant (k_{bh}) determined for the half-wave potential of the first electrode step in the electrooxidation of amino acids at a platinum electrode; c = 5 x 10^{-3} mol L^{-1} in 0.1 mol L^{-1} $(C_4H_9)_4NClO_4$ in acetonitrile, v = 0.01 V s^{-1}.

Compounds	$E_{p\ Istep}$ (V)	$E_{1/2\ Istep}$ (V)	$E_{p\ IIstep}$ (V)	$E_{1/2\ IIstep}$ (V)	βn_β	$D \times 10^6$ ($cm^2\ s^{-1}$)	$k_{bh\ E1/2} \times 10^4$ (cm s^{-1})
cysteine	0.38	0.38	0.57	0.58	0.82	8.998	3.83
cystine	0.58	0.57	0.83	0.83	0.63	6.088	3.04
phenylalanine	0.60	0.61	0.84	0.84	0.64	6.926	3.24
alanine	0.62	0.63	0.86	0.86	0.81	11.304	4.27

The parameters were calculated from the following equations:

$$\beta n_\beta = \frac{1.857RT}{F\left(E_{pa} - E_{pa/2}\right)} \quad (1)$$

$$E_{pa} = -1.14\frac{RT}{\beta n_\beta F} - \frac{RT}{\beta n_\beta F}\ln\frac{k_{bh}^0}{D_{red}^{1/2}} + \frac{RT}{2\beta n_\beta F}\ln\beta n_\beta v \quad (2)$$

$$k_{bh} = k_{bh}^0 \exp\left(\frac{-\beta n_\beta FE}{RT}\right) \quad (3)$$

where: D_{red} – diffusion coefficient of reduced form ($cm^2\ s^{-1}$),

v – scan rate (V s^{-1}),

F – Faraday constant (96,487 C mol^{-1}),

R – universal gas constant (8.314 J K^{-1} mol^{-1}),

T – temperature (K),

k_{bh}^0 – heterogeneous rate constant at a peak potential *vs.* Fc^+/Fc (cm s^{-1}).

The heterogeneous rate constant (k_{bh}) determined for a specified potential E characterises the transfer rate of an electron through the electrode-solution interface. The electron transition coefficient characterises the symmetry of the activated barrier of an electrode reaction.

The diffusion coefficient (D_{red}) was estimated according to Hayduk and Laudie's equation [46]:

$$D = \frac{13.26 \times 10^{-5}}{\mu^{1.4} v_o^{0.589}} \quad (4)$$

where μ is the viscosity of a solvent (centipoises), and v_o is the molar volume ($cm^3\ g^{-1}\ mole^{-1}$).

Based on the results provided in Table 1, cysteine, with $E_{1/2}$=0.38 V, was most easily oxidised, while alanine, with $E_{1/2}$=0.62 V, was least easily oxidised. The calculated anodic transition coefficient (βn_β) for alanine, which was oxidised at the highest rate (i.e., 4.27×10^{-4} cm s^{-1}), was 0.81. Phenylalanine, cysteine and cystine were oxidised more slowly, with heterogeneous rate constants (k_{bh}) equal to 3.24×10^{-4}, 3.83×10^{-4} and 3.04×10^{-4} cm s^{-1}, respectively. Their anodic transition coefficients ranged from 0.63 to 0.82.

3.3. FTIR spectra of amino acids

FTIR spectroscopy (Fig. 5) was used for the evaluation and analysis of these compounds [47-48].

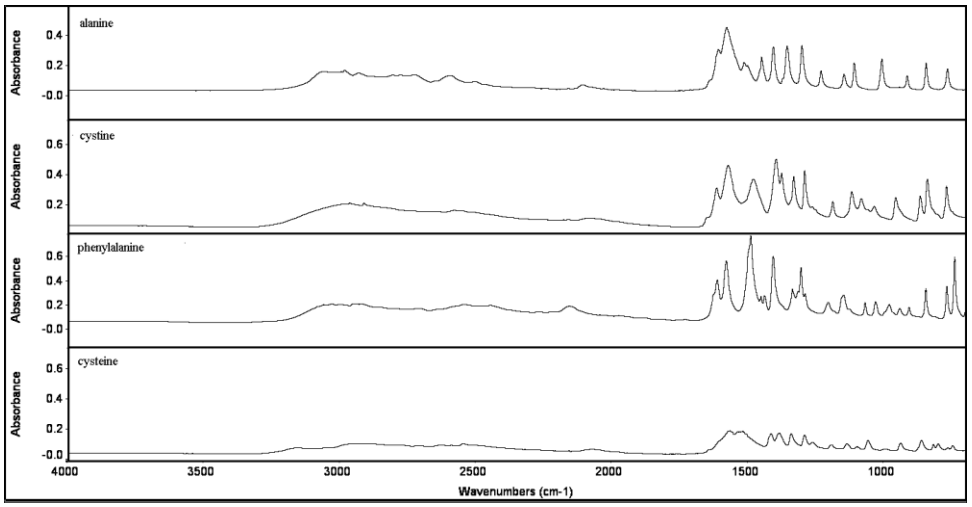


Figure 5. The FTIR spectra of the amino acids.

The FTIR spectra of the samples exhibited absorption from groups that are characteristic of amino acids. The absorption bands of amide groups were located at 3434 cm^{-1} (NH stretching), 1638 cm^{-1} (amide I, CO and CN stretching), 1545.51 cm^{-1} (amide II) and 1243 cm^{-1} (amide III). The FTIR absorption band of the C=O bond of the amide (HN–C=O) was located from 1630–1680 cm^{-1}. The spectra indicated that, although the geometrical parameters were identical, the frequencies were slightly different from each other. For example, CO ranged from 1738.0 cm^{-1} to 1884.0 cm^{-1}, and N–H ranged from 3487.2 cm^{-1} to 3607.3 cm^{-1}. The SH group of cysteine absorbed from 2550–2600 cm^{-1}.

A band for the ring mode of phenylalanine was located at approximately 1517 cm^{-1} and was easily detected in the amino acid absorbance spectra. Because an intense S-S band for the disulphide bond in cysteine requires a powerful corresponding C-S stretching vibration at approximately 704 cm^{-1}, the absorption corresponding to the S-S stretching was expected to appear at approximately 509 cm^{-1} at lower intensity. An asymmetric band at 3600–2900 cm^{-1} was associated with the hydrogen-bonded O–H stretching vibrations. The bands at 2923 cm^{-1} and 2853 cm^{-1} were due to the CH_2 asymmetric and symmetric stretching vibrations, respectively. The CH_2 bending and wagging vibrations caused two bands at 1452 cm^{-1} and 1337 cm^{-1}. Finally, the CH_3 amide group appeared at 1031 cm^{-1}, while the COC band appeared at 537 cm^{-1}.

3.4. Oxidation processes of amino acids

The as-determined parameters were confirmed using quantum chemical calculations. At present, the theoretical predictions of thermochemical quantities have achieved the level of reliability that approximate the accuracy of experimental measurements [49-51]. Amino acids are important to study because they are the building blocks of peptides and proteins and participate in many fundamental biochemical processes. The thermochemical data for amino acids and peptides are important to the understanding of different chemical and biochemical processes. The distribution of the electron charges in the investigated molecules was non-uniform and determined the reactivity of the particular positions (Fig. 6).

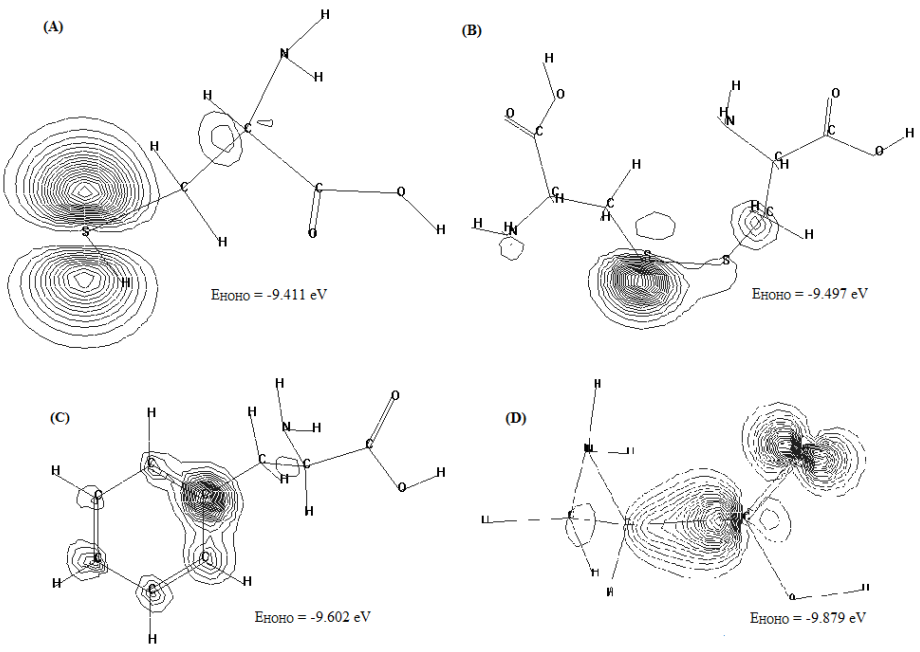


Figure 6. Electron density and probable sites in amino acids molecules susceptible electrooxidation.

The energy of the highest filled orbital (E_{HOMO}, or ionisation potential) determines the ease with which electrons are given up and indicates the site most susceptible to oxidation. The E_{HOMO}

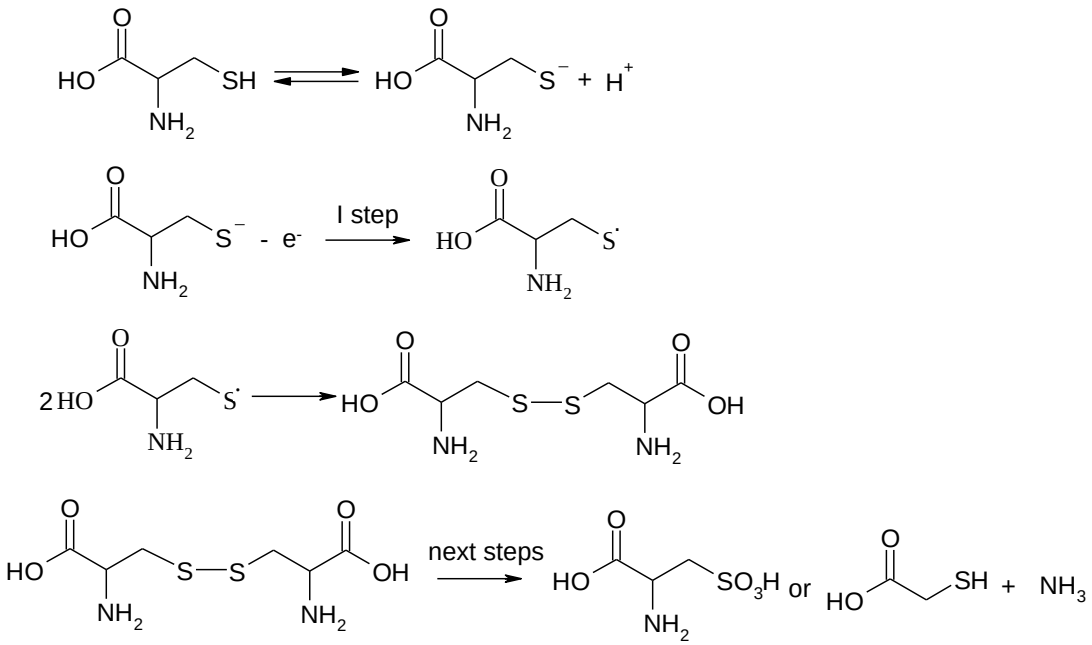
molecular orbital energies were calculated using the AM1 method, as implemented using the HyperChem software. The observed half-wave potential ($E_{1/2}$) should be linearly dependent on the energy of the HOMO (E_{HOMO}) [52]. The E_{HOMO} values for all the studied amino acids were determined via calculation and correlation to the $E_{1/2}$ of the first electrooxidation step (Table 2).

Based on the designated $E_{1/2}$ and calculated E_{HOMO} values for the amino acids, cysteine and cystine were oxidised most easily, followed by phenylalanine. Alanine was the most difficult to oxidise. On the basis of the heat of formation, we confirmed that cystine was the most durable molecule, while phenylalanine was the least durable.

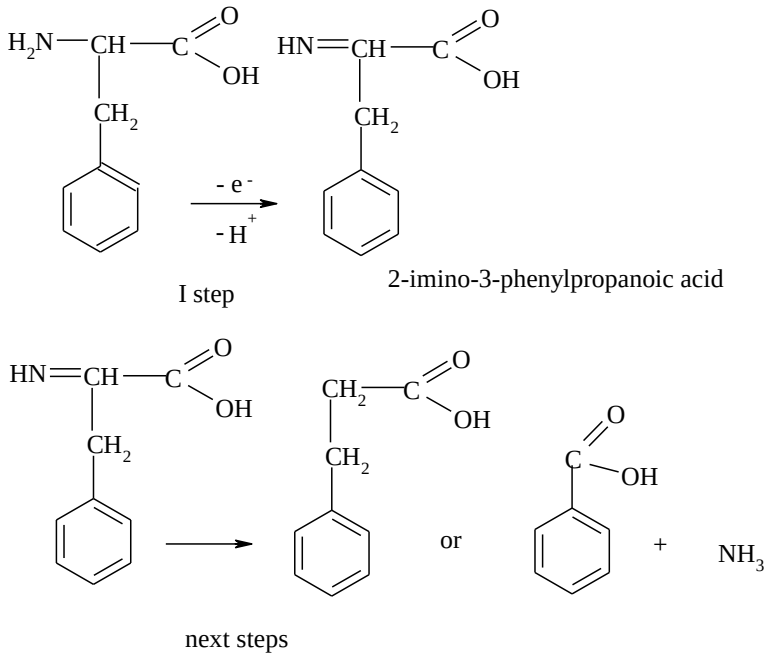
Table 2. Values of the half-wave potential ($E_{1/2}$) of electrooxidation, molecular orbital energies (E_{HOMO}) and heat of formation (ΔH_f) for amino acids.

Compounds	$E_{1/2\ Istep}$ (V)	E_{HOHO} (eV)	ΔH_f (kcal mol^{-1})
cysteine	0.38	-9.431	-89.026
cystine	0.57	-9.497	-172.551
phenylalanine	0.61	-9.602	-72.331
alanine	0.63	-9.879	-101.263

Based on the electroanalytical investigations, quantum chemical calculations and the literature data [38, 53-55], the mechanisms shown in Schemes 1 and 2 were proposed here for the oxidation of cysteine and phenylalanine.



Scheme 1. Proposed mechanism for cysteine electrooxidation.



Scheme 2. Proposed mechanism for phenylalanine electrooxidation.

4. CONCLUSIONS

The amino acids: cystine, cysteine, phenylalanine and alanine, can undergo electrochemical oxidation at Pt electrodes. Cysteine was shown to oxidise most easily, followed by cystine and phenylalanine, while alanine was the most difficult to oxidise. The low oxidation potentials of the amino acids indicated that they are excellent scavengers of free radicals (high antioxidant power). Of the compounds studied, cysteine exhibited the best antioxidant properties (i.e., the lowest half-wave potential $E_{1/2}$). Electrochemical research and quantum chemistry calculations (i.e., the energy of the highest occupied molecular orbital) allowed for the proposal of a mechanism for the electrochemical oxidation of cysteine and phenylalanine. The data obtained were consistent with the available knowledge and suggested that voltammetry may provide a convenient method to elucidate the electrochemical oxidation mechanisms of these types of compounds in non-aqueous media.

ACKNOWLEDGEMENT
This study was supported by Ministry of Science of Higher Education IP 2012 037072.

References

1. T. A. Enache and A. M. Oliveira-Brett, *Bioelectrochemistry*, 89 (2013)11.
2. M. Somji, V. Dounin, S. B. Muench, H. Schulze, T. T. Bachmann and K. Kerman, *Bioelectrochemistry*, 88 (2012) 110.
3. C. Vilaseca, M. C. Quintana, J. Vicente, P. Hernández and L. Hernández, *Anal. Biochem.*, 379 (2008) 91.
4. J. Xu, Y. Wang, Y. Xian, L. Jin and K. Tanaka, *Talanta*, 60 (2003)1123.
5. Y. Iwasaki, K. Ikeda and M. Kinoshita, *J. Neurol. Sci.*, 107 (1992) 219.
6. S. Akamatsu and T. Mitsuhashi, *J. Food Compos. Anal.*, 30 (2013) 47.

7. T. A. Enache and A. M. Oliveira-Brett, *Bioelectrochemistry*, 81 (2011) 46.
8. T. Andres, L. Eckmann and D. K. Smith, *Electrochim. Acta*, 92 (2013) 257.
9. Y. Dong, L. Pei, X. Chu, W. Zhang and Q. Zhang, *Electrochim. Acta*, 55 (2010) 5135.
10. P. C. White, N. S. Lawrence, J. Davis and R. G. Compton, *Anal. Chim. Acta*, 447 (2001)1.
11. Ch. C. Udenigwe and R. E. Aluko, *J. Food Sci.*, 71 (2012) R11.
12. L. Nie, H. Ma, M. Sun, X. Li, M. Su and S. Liang, *Talanta*, 59 (2003) 959.
13. L. G. Shaidarova, S. A. Ziganshina and G. K. Budnikov, *J. Anal. Chem.*, 58 (2003) 577.
14. J. Bongaerts, M. Kramer, U. Muller, L. Raeven and M. Wubbolts, *Metab. Eng.*, 3 (2001) 289.
15. H. Zhou, X. Liao, T. Wang, G. Du and J. Chen, *Bioresour. Technol.*, 101 (2010) 4151.
16. V. De Salles Painelli, B. Saunders, C. Craig Sale, R. C. Harris, M. Y. Solis, H. Roschel, B. Gualano, G. G. Artioli and A. H. Lancha Jr., *Amino Acids* 46 (2014) 1207.
17. H. L. Guo, M. K. Charles and B. K. Richard, *Synth. Met.*, 101 (1999) 44.
18. S. Allenmark, *Chirality*, 15 (2003) 409.
19. P. G. Rigas, *Anal. Bioanal. Chem.*, 405 (2013) 7957.
20. A. Leggio, E. L. Belsito, R. de Marco, M. L. Di Gioia, A. Liguori, C. Siciliano and M. Spinella, *J. Food Sci*., 77 (2012) S170.
21. M. Jaworska, M. Stańczyk, M. Wilk, G. Kłaczkow, E. Anuszewska, J. Barzał and P. Rzepecki, *Amino Acids*, 43 (2012) 1653.
22. N. A. Alarfaj, M. A. E. Abdalla and A. M. Al-Hamza, *Int. J. Electrochem. Sci.*, 7 (2012) 7888.
23. Z. M. Zain, S. A. Ghani and R. D. O'Neill, *Amino Acids*, 43 (2012)1887**.**
24. E. Wudarska, E. Chrzescijanska, E. Kusmierek and J. Rynkowski, *Electrochim. Acta*, 93 (2013) 189.
25. A. Masek, E. Chrzescijanska and M. Zaborski, *Electrochim. Acta*, 107 (2013) 441.
26. L. C. Umuhumuza and X. Sun, *Eur. Food Res. Technol.*, 232 (2011) 425.
27. K. S. Prasad, G. Muthutaman and J. M. Zen, *Electroanalysis*, 20 (2008)1167.
28. Z. Feng, M. Li, Y. Yan, T. Jihai, L. Xiao and Q. Wei, *Tetrahedron: Asymmetry*, 23 (2012) 411.
29. A. Abbaspour and A. Ghaffarinejad, *Electrochim. Acta*, 53 (2008) 6643.
30. A. Masek, E. Chrzescijanska and M. Zaborski, *Food Chem.*, 148 (2014) 18.
31. Y. Yardim, *J. Food Sci.*, 77 (2012) C408.
32. J. B. He, S. J. Yuan, J. Q. Du, X. R. Hu and Y. Wang, *Bioelectrochemistry*, 75 (2009)110.
33. A. Masek, M. Zaborski and E. Chrzescijanska, *Food Chem.*, 127 (2011) 699.
34. A. Nezamzadeh-Ejhieha and H. S. Hashemi, *Talanta*, 88 (2012) 201.
35. S. Kakhki and E. Shams, *J. Electroanal. Chem.*, 704 (2013) 249.
36. M. F. Barroso, T. Santos, M. G. F. Sales, C. Delerue-Matos and M. C. V. F. Vaz, *Port. Electrochim. Acta*, 25 (2007) 119.
37. P. Liu, M. O. Torrens-Spence, H. Ding, B. M. Christensen and J. Li, *Amino Acids*, 44 (2013) 391.
38. A. Safavi, R. Ahmadi and F. A. Mahyari, *Amino Acids*, 46 (2014) 1079.
39. E. Chrzescijanska, E. Wudarska, E. Kusmierek and J. Rynkowski, *J. Electroanal. Chem.*, 713 (2014) 17.
40. C. V. Krishnan and M. Garnett, *Int. J. Electrochem. Sci.*, 6 (2011) 3607.
41. Š. Komorsky-Lovrić and I. Novak, *J. Food Sci.*, 76 (2011) C916.
42. R. Luo, W. Zhang, W. Cheng, D. Zhao, Y. Li, X. Lin, F. Dong and S. Ding, *Int. J. Electrochem. Sci.*, 8 (2013) 3186.
43. D. Telsnig, A. Terzic,T. Krenn, V. Kassarnig, K. Kalcher and A. Ortner, *Int. J. Electrochem. Sci*., 7 (2012) 6893.
44. S. Skrzypek, V. Mirčeski, W. Ciesielski, A. Sokołowski and R. Zakrzewski, *J. Pharm. Biomed. Anal.*, 45 (2007) 275.
45. Z. Galus, Fundamentals of Electrochemical Analysis, Ellis Horwood/Polish Scientific Publishers PWN, New York/Warsaw (1994).
46. J. A. Schranke, S. F. Murphy, W. J. Doucette and W. D. Hintze, *Chemosphere*, 38 (1999) 2381.

47. K. Kargosha, S. H. Ahmadi, M. Zeeb and S. R. Moeinossadat, *Talanta*, 74 (2008) 753.
48. Y. Wang, H. Huang, J. Zang, F. Meng, L. Dong and J. Su, *Int. J. Electrochem. Sci.*, 7 (2012) 6807.
49. B. B. Koleva, T. M. Kolev and M. Spiteller, *Spectrochim. Acta, Part A*, 68 (2007) 1187.
50. O. V. Dorofeeva and O. N. Ryzhova, *J. Chem. Thermodyn.*, 41 (2009) 433.
51. M. M. Kabanda, I. B. Obot and E. E. Ebenso, *Int. J. Electrochem. Sci*., 8 (2013) 10839.
52. R. E. Sioda and B. Frankowska, *J. Electroanal. Chem.*, 568 (2004) 365.
53. K. F. Babu, R. Sivasubramaniana, M. Noelb and M. A. Kulandainathan, *Electrochim. Acta*, 56 (2011) 9797.
54. M. Hasanzadeh, N. Shadjou, S. T. Chen and P. Sheikhzadeh, *Catal. Commun.*, 19 (2012) 21.
55. O. Chailapakul, E. Popa, H. Tai, B. V. Sarada, D. A. Tryk and A. Fujishima, *Electrochem. Commun.*, 2 (2000) 422.