Cathodic Behaviour of Cysteine at a Platinum Electrode

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Abstract

The electroreduction behaviour of cysteine was investigated using cyclic, square wave and differencial pulse voltammetric techniques at a platinum working electrode.

The reduction of cysteine occurs at a potential of -0.36 V independent of pH. It is a reversible process, controlled mainly by diffusion and in the mechanism of reduction 1 electron per molecule is involved. Using the voltammetric techniques: Cyclic Voltammetry, Square Wave Voltammetry and Differencial Pulse Voltammetry, different parameters (pH, frequency, step potential, pulse amplitude, scan rate) were optimized in order to develop an electrochemical procedure for determination of cysteine in pharmaceutical products. The repeatability, reproducibility, precision and accuracy of the methods were studied. No electroactive interferences from the excipient were found in the pharmaceutical compounds.

Keywords: cysteine, voltammetry, platinum electrode, pharmaceuticals, reduction.

Introduction

Cysteine (Cys) (2-amino-3-mercaptopropanoic acid) is a thio-containing compound (Fig. 1) biologically important in living systems [1], as an active site in the catalytic activity of enzyme and it's found in the structure of vasopressin, an antidiuretic hormone [2]. This substance is involved in a variety of important cellular functions, such as protein synthesis, detoxification and metabolism. Disorders of Cys metabolism include cystinosis, an antosomal recessive disease produced by a defect in lysosomal transport and cystinuria, a common heritable disorder of amino acids transport [3,4]. Furthermore, plasma thiol groups have been shown to be susceptible to oxidative damage and, therefore, can serve as diagnostic markers for cardiovascular diseases [5]. Cys has several pharmaceutical applications; it is used in some antibiotics and for treatment of skin damages [6] and as radioprotective agent [7].

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Cys is added to wheat flour by the baking and pasta industry to improve dought flexibility, which improves the baked product and reduces baking time. It is the major component of the recipe for the meaty flavouring used in savoury snacks and pet foods and it is effective as an antioxidant in natural fruit juices and other foodstuffs [8] under the E-number 910 or 920.
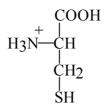
$$\underset{H_3N}{\overset{+}{}}\text{—CH} \begin{matrix} \text{COOH} \\ | \\ \text{CH} \\ | \\ \text{CH}_2 \\ | \\ \text{SH} \end{matrix}$$

Figure 1. Structural formula of Cys.

Different analytical methods have been developed to quantify Cys and there metabolites in biological matrices (urine, human salivary, blood), pharmaceutical preparation and foods. The most commonly employed techniques are the chromatographic such as liquid chromatography (LC) [9-15], and gas chromatography (GC) [16]. Many of these methods require several manipulation steps which are time-consuming and use significant amounts of solvent, and they require sophisticated instrumentation and training. Considering that voltammetric techniques are relatively simple to apply, quite rapid and reasonably cheap, several procedures for quantification of Cys based on electrochemical methods have been developed.

The electrochemical oxidation of Cys has been the subject of many investigations. This amino acid can be easily oxidized to cystine by suitable oxidizing agents [17] or using platinum, gold, vitreous carbon [8,18-19] but the oxidation of Cys occurs at high potentials. Mercury electrodes are also used [17, 20], but the formation of mercury compounds is today an environmental concern.

The aim of this work is the study of the electrochemical reduction behaviour of Cys at a platinum electrode using voltammetric techniques, and the development of an analytical procedure to quantify Cys in pharmaceutical compounds.

Experimental

Equipment

All voltammetric measurements were performed using a computer controlled pontentiostat/galvanostat Autolab PSTAT (EcoChemie, Netherlands), and a Metrohm VA Stand containing a three-electrode cell (all Metrohm): a platinum electrode or a glassy carbon electrode as a working electrode, a Ag/AgCl/KCl 3.00 mol L^{-1} reference electrode and a glassy carbon rod counter electrode. The system was controlled by means of a General-Purpose Electrochemical System (GPES) software package, version 3.0, from Ecochemie.

pH was measured with a Metrohm E-520 pH meter with combined electrode.

Solid working electrodes were mechanically cleaned before each experiment by polishing its surface using a polishing kit (Metrohm 6.2802.010), first with α-Al$_2$O$_3$ (0.3 μm) until a shining surface was obtained, and after with only water.

Reagents and solutions

Cys (Fluka) was purchased from Riedel-deHaen and used without further purification. All other chemical were Merck pro-analysis grade and all solutions were prepared using purified water (conductivity < 0.1 μS cm^{-1}) obtained from a Barnstread E-pure 4 system.

The supporting electrolyte in voltammetric determination was KNO$_3$ solution (0.5 mol L^{-1}). The pH of the solutions was adjusted with 0.1 mol L^{-1} HCl and 0.1 mol L^{-1} NaHO.

Standard and sample preparations

Stock solutions of Cys (1.0×10^{-2} mol L^{-1}) were prepared with an exact weight of the pure amino acid dissolved in water. The standard solutions used for the optimization studies and plotting calibration curves were prepared by dilution of these stock solutions with water. These solutions are stable for at least 1 week if kept in the dark at + 4 ºC.

Five tablets of Phakan (each tablet contains 0.290 mg of Cys) were accurately weighed and finely powdered and mixed. An adequate amount of this powder was weighed, and transferred into a 50 mL calibrated flask; the volume was completed with water. The content of the flask was sonicated for 5 min to achieve complete dissolution. Appropriate solutions were prepared by taking suitable aliquots and diluting with KNO$_3$ solution.

Procedures

The electrochemical behaviour of 5.0×10^{-4} mol L^{-1} Cys was studied in KNO$_3$ solutions (0.5 mol L^{-1} ionic strength) over a wide pH range (3.4 – 10.9) at platinum electrode, using three voltammetric techniques: Cyclic Voltammetry (CV), Square Wave Voltammetry (SWV) and Differential Pulse Voltammetry (DPV). A known volume of Cys solutions, together with 10.0 mL of KNO$_3$ solution, was purged with purified nitrogen for 2 min. The electrode surface was polished between two consecutive scans. The scan was conducted from 0.0 V to -1.0 V.

Interferences

Various possible interfering substances, citric acid, ascorbic acid, sucrose and glutamic acid, all of which commonly present in the pharmaceutical products, were tested under the same experimental conditions. The effect of interference with different concentrations on the peak current of a solution containing 5.0×10^{-4} mol L^{-1} of Cys was studied. It was generally considered to interfere with the analysis when its presence produced a variation in the peak current of the sample larger than 5%.

Results and discussion
Preliminary study

In order to obtain some information of the electrochemical reduction behaviour of Cys in this preliminary study, a glassy carbon electrode was used over a wide pH range (3.4 – 10.9). The square wave voltammograms of 5.0×10^{-4} mol L^{-1} Cys in KNO$_3$ solution showed that this amino acid presents a reduction peak at a potential of -1.38 V vs. AgCl/Ag (Fig. 2). The drawback of this solid electrode is that the reduction of this amino acid occurs at a high negative potential. Using the platinum electrode, Cys presents electrochemical reduction activity. At the same conditions, the scans showed in the cathodic direction a very well-defined peak at a potential of -0.36 V vs. AgCl/Ag independent of pH (Fig. 2). So it was selected, as working electrode, a platinum electrode.
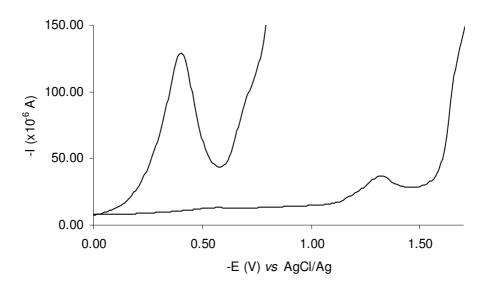

Figure 2. Square wave voltammograms of 5.0×10^{-4} mol L^{-1} Cys in KNO$_3$ (0.5 mol L^{-1}) solution at: A) glassy carbon electrode, and B) platinum electrode.

Cyclic Voltammetry

The reversibility of Cys reduction was studied using CV. The voltammograms carried out showed one single well-defined peak in the cathodic and anodic scan, indicating that the reaction is a reversible process. The peak current of the cathodic (i_{pc}) and anodic scan (i_{pa}) of Cys were measured and it was verified that $|i_{pc}/i_{pa} =1|$, this way probing the reversibility of the electroreduction of Cys [21].

The influence of the scan rate on the cathodic peak current (i_{pc}) was studied within the range 0.010 – 0.50 V s^{-1} (Fig. 3) for 5.0×10^{-4} mol L^{-1} Cys in KNO$_3$ solution at pH 4.4. The cycles carried out within the increased values of scan rates produced a linear relationship with the square root of the scan rate. This indicates that the process at the surface of the electrode was mainly controlled by diffusion following eq. 1:

$$-i_{pc} (\times 10^{-5}) = 2.03 \times 10^{-1} v^{1/2} + 5.04 \times 10^{-6} \tag{1}$$

where i_{pc} is the peak current of cys (Ampere); ν is the scan rate (mV s^{-1}), with a correlation coefficient (r) of 0.997.

Using CV at a platinum working electrode, the number of electrons was determined by the application of Randles and Sevcik mathematical equation [21] (eq. 2):

$$\frac{i_{pc(cys)}}{i_{pc(Fe)}} = \frac{n_{(cys)}^{3/2} D_{(cys)}^{1/2} C_{(cys)}}{n_{(Fe)}^{3/2} D_{(Fe)}^{1/2} C_{(Fe)}} \tag{2}$$

where i_{pc}(Cys), i_{pc} (Fe) are the peak current (A); $n_{(cys)}$, $n_{(Fe)}$ are the number of electrons; $D_{(cys)}$, $D_{(Fe)}$ are the diffusion coefficients (cm^2 s^{-1}); and $C_{(cys)}$, $C_{(Fe)}$ are the same concentration (mol L^{-1}) in the same solvent, of Cys and hexacyanoferrate (III), respectively. This equation was applied assuming that $D_{(Cys)}$ is equal to $D_{(Fe)}$. Knowing that potassium hexacyanoferrate (III) involved one electron per molecule [21], and applying the Randles and Sevcik equation, the number of electrons involved in the reduction of Cys was 1.29 ± 0.05 electron per molecule.
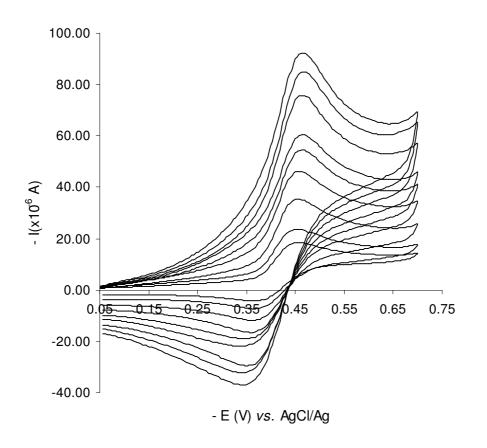

Figure 3. Cyclic voltammograms of 5.0×10^{-4} mol L^{-1} Cys solution in KNO$_3$ solution with a platinum electrode: A↑ (sense of increase scan rate) 0.010 V s^{-1} to 0.50 V s^{-1}.

Using a scan rate of 0.40 V s^{-1} and pH 4.4, a calibration curve was plotted that had a linear response from 2×10^{-4} to 1.0×10^{-3} mol L^{-1} Cys in KNO$_3$ solution with r = 0.998, (eq. 3):

$$-i_{pc}(\times10^{-6}) = 5.84 \pm 0.08 \ (\times10^{-4}) \ C - 9.44 \pm 0.08 \ (\times10^{-6}) \tag{3}$$

where i$_{pc}$ is the peak current of cys (Ampere); C is the Cys concentration (mol L^{-1}).

Square Wave Voltammetry

After knowing the electroreduction behaviour of Cys, a SWV technique was used in order to evaluate the influence of pH, frequency (*f*), step potential and pulse amplitude on the peak shape and peak height.
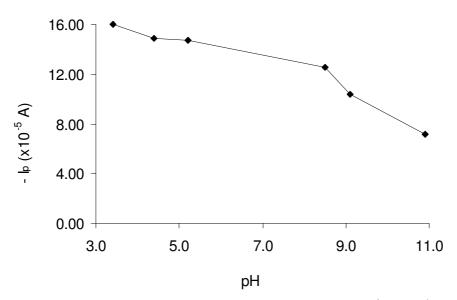

Figure 4. Influence of pH on the intensity of peak current of 5×10^{-4} mol L^{-1} Cys.

The influence of pH on the reduction of 5.0×10^{-4} mol L^{-1} Cys was investigated using KNO$_3$ solutions over a wide pH range (3.4 – 10.9) and a frequency of 50 Hz. It was verified that Cys presents electrochemical activity in every range of pH studied, having a maximum peak current at pH 3.4 (Fig. 4). However, for the next studies it was chosen the pH 4.4; with this pH the peak is more well-defined and repeatability and reproducibility are higher.

The frequency was varied from 10 to 250 Hz. i$_p$ increased with the frequency until 200 Hz; above this value the peak became distorted. Hence, this frequency was chosen for all subsequent studies.

The influence of step potential was studied in the range of 0.001 to 0.150 V, presenting an i$_p$ maximum at 0.001 V; above this value i$_p$ decreased. The pulse amplitude parameter was evaluated from 0.001 V to 0.100 V presenting a maximum activity at 0.100 V. For the next analysis were used step potential of 0.001 V and pulse amplitude 0.100 V.

With the optimised experimental conditions (pH =4.4; f =200 Hz; step potential = 0.001 V; pulse amplitude = 0.100 V) linearity studies were performed on peak current and Cys concentration (Fig. 5) within the range of 8.0×10^{-5} to 1.0×10^{-3} mol L^{-1} below the eq. 4:

$$-i_{pc} (\times 10^{-6}) = 5.6 \pm 0.2 \ (\times 10^{-4}) \ C \ -11.5 \pm 0.9 \ (\times 10^{-6}) \tag{4}$$

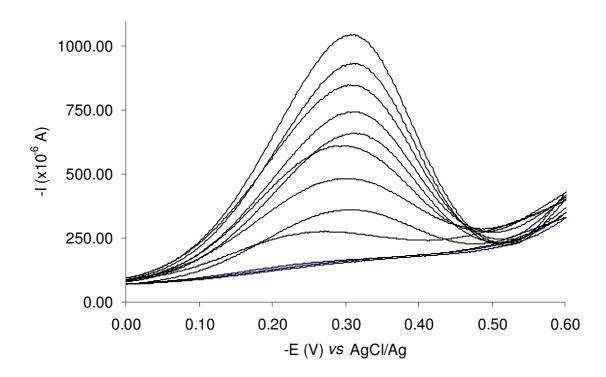where i_{pc} is the peak current of cys (Ampere); C is the Cys concentration (mol L^{-1}), with r = 0.997.

Figure 5. Square wave voltammograms of standard solutions of Cys at platinum electrode (pH 4.4; f= 200 Hz) A↑(sense of increase of Cys concentration): (8×10^{-5}; 9×10^{-5}; 1.0×10^{-4}; 2.0×10^{-4}; 4.0×10^{-4}; 5.0×10^{-4}; 6.0×10^{-4}; 7.0×10^{-4}; 8.0×10^{-4} and 1.0×10^{-3} mol L^{-1}).

Differential Pulse Voltammetry (DPV)
Using DPV (Fig. 6), the parameters step potential and pulse amplitude were optimized. The step potential was studied in the range of 0.002 V to 0.010 V obtaining a maximum peak current with the value 0.007 V for the step. The pulse amplitude was changed within 0.001 V and 0.100 V with a maximum at 0.100 V. With this technique a good linearity was obtained within the i_{pc} and concentration of Cys (2.0×10^{-5} to 1.0×10^{-3} mol L^{-1}) following eq. 5:

$$-i_{pc} (\times 10^{-6}) = 11.30 \pm 0.02 \ (\times 10^{-4}) \ C \ - 5.6 \pm 0.6 \times 10^{-6} \tag{5}$$

where i_{pc} is the peak current of cys (Ampere); C is the Cys concentration (mol L^{-1}), with r = 0.996.

Table 1 presents the calibration data obtained using the optimised condition for the three voltammetric techniques used. Each point of calibration curve corresponds to the mean value obtained from 10 independent measurements. The limit of detection (LOD) and limit of quantification (LOQ) values were calculated according to Mocak [22].
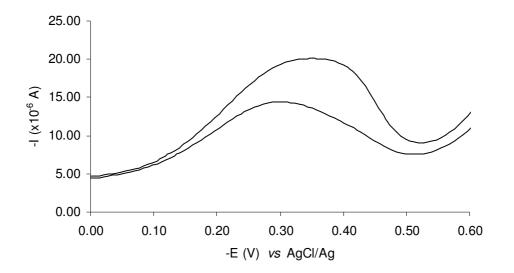

Figure 6. Differential pulse voltammograms of standard solutions of Cys at platinum electrode. A – 1.0×10^{-4} mol L^{-1}, B – 2.0×10^{-4} mol L^{-1}.

Table 1. Mean values (± standard deviation) of 10 independent calibrations of Cys using CV, SWV and DPV.

	CV	*SWV*	*DPV*
Linear concentration range ($\times 10^{-4}$ mol L^{-1})	2.0 - 10.0	0.80 - 10.0	0.20 - 10.0
Slope ($\times 10^{-4}$ A mol^{-1} L)	5.84 (± 0.08)	5.6 (± 0.2)	11.30 (± 0.02)
Intercept ($\times 10^{-6}$ A)	9.44 (± 0.08)	11.5 (± 0.9)	5.6 ± (0.6)
Correlation coefficient (n = 10)	0.998	0.997	0.996
LOD ($\times 10^{-6}$ mol L^{-1})	4.0	8.0	4.5
LOQ ($\times 10^{-5}$ mol L^{-1})	1.2	2.4	1.4
RSD % [a]	2.3	1.5	2.0

[a] Concentration of Cys equal to 5.0×10^{-4} mol L^{-1}. LOD: Limit of detection; LOQ: Limit of quantification.

Repeatability, reproducibility and accuracy
Repeatability, reproducibility and accuracy of Cys measurements under optimised conditions using the three voltammetric techniques were confirmed after three successive determinations of two different concentrations of Cys over

one day (intraday assay) and over a week (interday assay). Results obtained are reported in Table 2 and point out the good precision and accuracy of Cys electrochemical measurements.

Table 2. Analytical precision and accuracy of Cys assays by the proposed voltammetric procedure.

Concentration ($\times 10^{-4}$ mol L^{-1})	*Intraday[a]*				*Interday[b]*			
	Found ($\times 10^{-4}$ mol L^{-1})	R (%)	Bias (%)	RSD (%)	Found ($\times 10^{-4}$ mol L^{-1})	R (%)	Bias (%)	RSD (%)
CV method								
5.5	5.3	96.4	-3.6	1.2	5.2	94.5	- 5.5	2.9
8.6	8.9	103.5	+3.5	3.7	8.5	98.8	- 1.2	3.0
SWV method								
5.3	5.0	94.3	-5.7	2.5	5.4	101.9	+1.9	2.3
8.2	8.0	97.6	-2.4	1.8	8.1	98.7	-1.2	1.5
DPV method								
6.2	6.1	98.4	-1.6	2.3	6.3	101.6	+1.6	1.3
8.5	8.6	101.2	+1.2	1.4	8.4	98.8	-1.2	1.8

[a] Average of three measurements over a day; [b] average of three measurements over a week; R: recovery; RSD: relative standard deviation.

Interferences

To test the efficiency and selectivity of the three proposed analytical method to pharmaceutical formulations, a systemic study was made. Solutions containing a fixed amount of Cys (5.0×10^{-4} mol L^{-1}) were spiked with excess amount of each excipient of pharmaceutical preparation under the optimum experimental conditions.

Experiments showed that no serious interference occurred from the addition of the studied compound. However, citric acid and ascorbic acid interfere in the signal of Cys when the concentration of these interferences is 3×10^{-4} mol L^{-1} for citric acid and 8×10^{-4} mol L^{-1} for ascorbic acid. Nevertheless, this is not a problem because these compounds appear in pharmaceuticals in smaller concentration.

Analysis of Cys in pharmaceuticals (Phakan)

The optimized voltammetric methods were applied to the determination of Cys in pharmaceuticals (Phakan). This analysis was carried out using the three techniques evaluated: (CV, SWV and DPV) and as supporting solutions KNO_3 solution. An aliquot of each diluted sample solution was added to the

voltammetric cell. The quantification of Cys was assessed by analysing samples in three independent measurements and calculated using the calibration curve. Mean values of Cys concentration, standard deviations, and recovery results are presented in Table 3.

Table 3. Voltammetric quantification of Cys in pharmaceutical product (Phakan)

Concentration ($\times 10^{-4}$ mol L^{-1})	Found ($\times 10^{-4}$ mol L^{-1})	Bias (%)	Recovery %)
CV methods			
0.90	0.81 ± 0.01	- 10.0	97.0
1.00	0.93 ± 0.07	- 7.5	97.0
SWV method			
0.90	0.89 ± 0.02	- 1.1	97.3
1.66	1.90 ± 0.03	+ 7.8	99.8
DPV method			
0.70	0.68 ± 0.03	- 2.9	83.5
0.80	0.83 ± 0.04	+ 3.75	83.5

Conclusions
On the basis of the electroreduction of Cys at a platinum electrode, three voltammetric procedures were optimized in order to quantify this amino acid in pharmaceuticals. These voltammetric techniques are a good alternative because they are simpler, faster and less expensive, than the classical procedures and were applied directly to the analysis of pharmaceutical products without complex sample preparation such as time-consuming extraction steps.

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