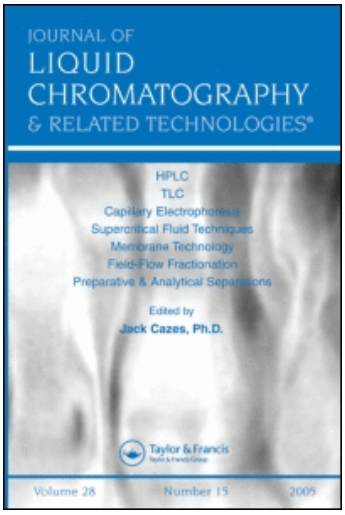

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ELECTROCHEMICAL DETECTION OF UNDERIVATIZED AMINO ACIDS WITH A Ni-Cr ALLOY ELECTRODE

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ABSTRACT

A nickel-chromium (80:20) alloy is employed as electrode material in the amperometric detection of underivatized amino acids in flow injection and high performance liquid chromatographic experiments. Cyclic voltammetric results show that amino acids are oxidized by a surface catalyzed process, proposed to involve Ni(III) oxyhydroxides, which are formed on the electrode surface at approximately 0.43 V (Ag/AgCl reference electrode) in 0.10 N NaOH. The hydrodynamic voltammograms of different amino acids show current plateaus at potentials above ca. 0.48 V. Preliminary HPLC experiments show that the nickel-chromium alloy is useful for the amperometric detection of underivatized amino acids following anion exchange separations.

INTRODUCTION

Determination of amino acids has a great importance in food analysis, clinical chemistry, biotechnology and fermentation control. Several strategies for amino acids determination have been developed, including direct detection by ultraviolet absorbance, refractive index and fluorescence.

However, derivatization of these molecules is necessary to attain high sensitivities in ultraviolet absorbance and fluorescence detection because most of the amino acids lack a chromophore group.

Electrochemical detection in Flow Injection Analysis (FIA) and High Performance Liquid Chromatography (HPLC) has become widely accepted nowadays. There are various modes for the electrochemical detection of amino acids.[1] Direct oxidation of amino acids can be performed with varying degree of success using constant potential amperometric detection[2] or pulsed amperometric detection.[3,4] Chemically modified electrodes can also be used for the electrochemical detection of amino acids.[5] Still, derivatization has become important in improving their electrochemical response at conventional electrode materials (e.g. glassy carbon) because a very few amino acids are electrochemically active.

Derivatization approaches involving the use of nitroaryl reagents,[6,7] o-phthaldehyde,[8,9] phenylisothiocyanate,[10] and ferrocene derivatives[11] have been described in the literature. However, pre- or post-column derivatization methods add complexity to the detection scheme.

Nickel electrodes have been previously used for the amperometric detection of organic molecules in alkaline solutions.[2,12,13,14] However, the sensitivity and the long term response reproducibility of the electrode was poor when compared with copper electrodes.[2] One advantage of the nickel electrode is its relatively low background current at the usual working potentials (approximately 4-6 $\mu A/cm^2$ at 0.5 V vs Ag/AgCl).

Nickel alloys have demonstrated good catalytic activity and proved useful in the amperometric detection of carbohydrates after high performance anion exchange separations.[15] Moreover, high sensitivity and good response reproducibility were achieved using the nickel-chromium (80:20) (Ni-Cr) alloy.[16] An increase in sensitivity by a factor of approximately ten was observed at the Ni-Cr alloy electrode as compared with pure nickel electrodes. Easy implementation, very low background current, and short settling time are another advantages of the Ni-Cr alloy electrode.

In this paper, we report the use of Ni-Cr alloy electrodes for the electrochemical detection of underivatized amino acids following anion exchange separations. Cyclic voltammetric (CV) experiments show that oxidation of amino acids is associated with the Ni(II)/Ni(III) redox couple. The set potential for the electrochemical detection of amino acids is optimized on a Signal-to-Noise ratio (S/N) analysis at different potential values. Good electrode performance was obtained in preliminary HPLC experiments with an anion exchange column for amino acids separation.

MATERIALS AND METHODS

Reagents

Standard amino acid (Sigma, USA) and sodium hydroxide (Merck, Argentina) solutions were daily prepared with triply distilled water. All reagents were analytical degree.

Equipment

Nickel-chromium (80:20) (Goodfellow, England) working electrodes for CV experiments were prepared with 0.1 cm diameter wires embedded in Teflon shrinkable tubes. The working electrodes for the liquid chromatographic experiments were made of the same Ni-Cr wires embedded in Kel-F blocks of 0.5x1.0x1.0 inches.

The electrodes were polished successively with emery paper of 400, 600 and 0000 grit, and finished to a mirror surface with 1, 0.3 and 0.05 μm alumina particles suspended in water on a microcloth pad, sonicated, and then thoroughly washed with triply distilled water.

A Ag/AgCl (3 M KCl) reference electrode was used in the CV experiments.

Cyclic voltammetric experiments were performed with an EG&G PARC Model 273 computerized potentiostat. An EG&G PARC model 175 Universal Programmer was used as the waveform generator.

The current-voltage (i-E) output was recorded with either the PC's printer or with a Houston 2000 XY recorder. A conventional three electrode glass cell was used for the CV experiments.

The chromatographic system consisted of a model 307 pump (Gilson, France), a model 7125 inyector (Rheodyne, USA) with a 20 μL injection loop, and a Wescan Anion-R, 250x4.6 mm (Hamilton Co., USA). A home made potentiostat was used as an amperometric detector.

The electrochemical signal was fed to a Personal Computer (PC) equipped with Peak Simple (SRI, USA) data processing software. The electrochemical cell for the flow experiments consisted of a home made working electrode, a stainless steel auxiliary electrode and a Ag/AgCl (3 M KCl) reference electrode. The dead volume of the flow cell was approximately 5 μL.

RESULTS AND DISCUSSION

Previous studies carried out in our laboratory showed that the voltammetric behavior of Ni-Cr electrodes in alkaline solutions is complex.[17] A featureless i-E trace is obtained in the potential range from 0.00 V to -1.10 V after the first few 3-4 potential cycles at 0.050 V/s. A reversible redox system is observed in the potential range from 0.00 V to 0.60 V.

The complexity of both oxidative and reductive waves in this potential range increases as the number of CV cycles increases. An increment of the peak current, peak potential, and peak charge is observed on the oxidative wave during approximately 50 CV cycles. After this number of cycles, only the peak current and peak charge of the same wave increase. The peak remains at approximately 0.43 V

The peak potential of the reductive wave on the reverse scan is approximately 0.35 V. A decrease of the peak current and electrochemical charge of this wave is observed as the number of CV cycles increases. Simultaneously, a shoulder develops on this reductive wave at approximately 0.29 V. After approximately 500 CV cycles at 0.050 V/s the shoulder has changed into a totally defined peak, and the original reductive peak is almost not detected.

Finally, a steady i-E response is obtained after approximately 600 CV cycles, using the same potentiodynamic conditions. A square reaction diagram, with different Ni oxy-hydroxide species, was proposed[17] to explain the peak multiplicity observed at Ni-Cr electrodes in the potential range from 0.00 V to 0.60 V.

The steady i-E trace obtained with Ni-Cr electrodes after nearly 600 CV cycles in the background solution, is shown in Figure 1 (solid line). The oxidative wave observed at approximately 0.43 V was attributed to the oxidation of Ni(II) to Ni(III).

A reductive wave, which may be attributed to Ni(III)/Ni(II) reduction, is obtained at approximately 0.29 V on the reverse scan. No other features are observed in the cyclic voltammogram of Ni-Cr electrodes in this potential range.

Cyclic voltammograms, of different glycine concentrations at the Ni-Cr electrode, are also shown in Figure 1 (dotted lines). An increment in the charge and peak current under the anodic wave on the positive scan, and a decrease of these parameters during the negative scan are the main effects due to the addition of glycine.

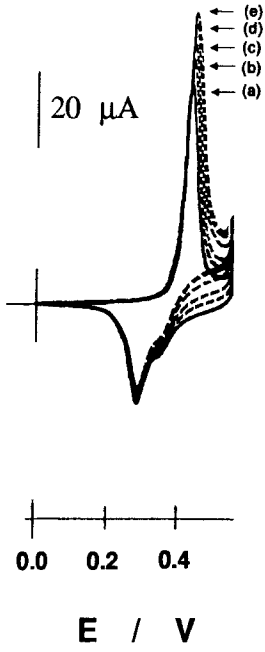

Figure 1. Cyclic voltammogram of a Ni-Cr electrode in 0.10 N NaOH (a), and with different glycine concentrations: (b) = 0.5 mM; (c) = 1.0 mM; (d) = 1.5 mM; (e) = 2.0 mM. v = 0.050 V/s, T = 298 K.

This behaviour is typical of electrocatalytic mechanisms,[18] and was previously observed in the oxidation of carbohydrates at Ni-Cr electrodes in alkaline solutions.[16] The proposed mechanism is shown below in the general pathway indicated in reactions (1)-(2):

$$Ni(OH)_2 + OH^- \underset{}{\overset{k(E)}{\rightleftharpoons}} NiOOH + H_2O + e \quad (1)$$

$$NiOOH + \text{organic molecule} \xrightarrow{k} Ni(OH)_2 + \text{products} \quad (2)$$

The active species, Ni(III), is generated from Ni(II) species on the electrode surface at approximately 0.43 V via electron transfer (reaction (1)). Electrogenerated Ni(III) species then oxidize the analyte via redox reactions (reaction (2)). The rate constant for the first reaction is potential dependent. The analytically important experimental observable is that for an electron

transfer which is kinetically slow (k(E) small), the application of a greater potential difference will increase the reaction rate. On the other side, reaction (2) has been proposed[19] as the rate limiting step of the reaction pathway.

The same basic mechanism may be proposed for the oxidation of glycine (and other amino acids) at the Ni-Cr alloy electrode since only the Ni(II)/Ni(III) redox couple seems to be involved in the reaction. However, mixed oxides and hydroxides of both Ni(II) and Ni(III) may be involved, as well as their different crystalline forms (α, β and γ). Thus, not one species, such as NiO, $Ni(OH)_2$, or NiO(OH) suffices to describe the surface chemical entities involved in the reaction.

Both peak current and oxidative charge have a linear relationship with the concentration of glycine. Calibration plots of the anodic peak current as a function of glycine concentration give straight lines. The linearity of the plot is extended up to approximately $3x10^{-3}$ M. These results indicate that the Ni-Cr alloy may be used as an amperometric detector for amino acids in flowing systems (e.g. HPLC and FIA).

Sensitivity is a detector parameter of major analytical importance, and it is defined as the ratio of current generated to concentration present. Sensitivity depends mainly on two sorts of processes: mass transfer and charge transfer.[20] Mass transfer refers to how easily a solute can reach the electrode surface. Charge transfer is related to the ease of the analyte to undergo electron transfer once it has reached the electrode surface.

The oxidation of amino acids at Ni-Cr electrodes in alkaline solutions was previously proposed as an electrocatalytic process. The whole process of electron transfer from the analyte to the electrode is composed of two steps, reactions (1) and (2) in the reaction scheme. The rate constant for reaction (1) is potential dependent. Thus, the rate for this process may be controlled by selecting the appropriate working potential.

On the other side, the rate at which reaction (2) proceeds, may not compromise the detector's sensitivity, assuming that the analytes remain enough time into the flow cell chamber. This assumption is valid for most of the usual chromatographic working conditions. For example, a 25-cm column with 15,000 theoretical plates will have a peak width of about 10 s for k'=1.[21]

Hydrodynamic voltammetric studies provide information about the working potential range in which the detector's sensitivity is only dependent on the mass transfer rate. Thus, we performed hydrodynamic voltammograms of various amino acids by FIA experiments using the Ni-Cr electrode in alkaline solutions (Figure 2). The initial working potential was 0.40 V. After obtaining

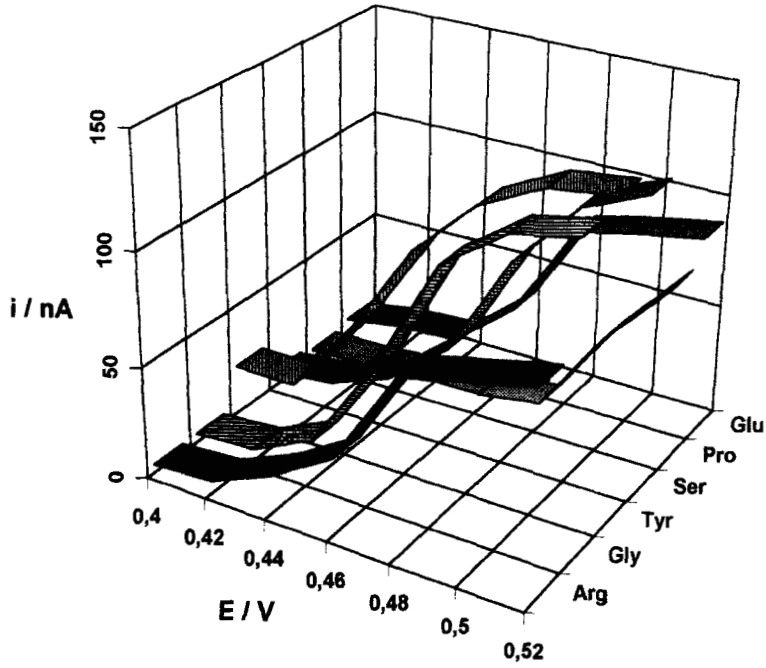

Figure 2. Hydrodynamic voltammogram of various amino acids at Ni-Cr electrodes in 0.10 N NaOH. Glutamic acid (Glu), Proline (Pro), Serine (Ser), Tyrosine (Tyr), Glycine (Gly), and Arginine (Arg). Flow rate = 0.50 mL/min; concentration of the analytes: 1.0×10^{-4} M each; injection amount: 2 nmol each.

a stable, drift-free baseline at the set potential, ten separate injections of each amino acid were analyzed. The working potential was then increased by increments of 0.02 V, and the FIA experiments repeated. In the region between 0.40 V to approximately 0.44 V, there is essentially no evidence for amino acid oxidation (except for tyrosine, which gives a small oxidative current at this low potential). At potentials greater than 0.44 V the current increases and, then, plateaus at values above ca. 0.48 V. The current for Proline oxidation increases only after 0.48 V, and this trend seems to be an exception for the behavior of the amino acids in this study.

At potentials above 0.52 V, oxygen evolution starts as characterized by an increase in the background noise level. Thus, the optimum set potential for amino acid detection with the Ni-Cr electrode in alkaline solution, should lay in the potential range between 0.44 V and 0.50 V.

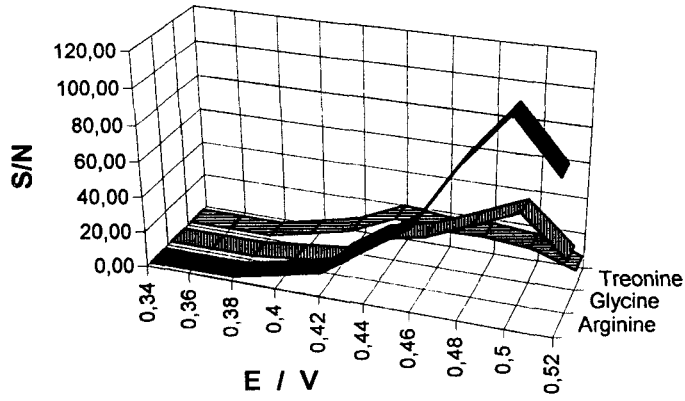

Figure 3. Signal to Noise ratio analysis of different amino acids at Ni-Cr electrodes in 0.10 N NaOH. Flow rate = 0.50 mL/min. Injection amount = 2 nmol each amino acid. T = 298 K.

In order to be able to use any theoretical expression for signal intensity in the calculation of optimum conditions, some knowledge of the noise in the system is needed. Poppe et al. studied the noise in a flow-through channel electrode.[22] The sources which they gave for noise showed a linear relationship between noise intensity and electrode area. Taking these findings into account, the optimum set potential for electrochemical detection should be that, where the highest Signal-to-Noise ratio is observed.

The S/N analysis for different amino acids was performed by HPLC experiments using the Ni-Cr electrode in alkaline solution. Figure 3 shows the results of this analysis at various working potentials. The initial working potential was 0.34 V. After obtaining a stable, drift free baseline, a record of this background current was obtained at high sensitivity, in order to analyze the noise. Then, various separate injections of the amino acid mixture were analyzed, and the S/N ratio for each amino acid computed. The working potential was then increased by 0.02 V and the study repeated.

The potential for the highest Signal-to-Noise ratio in the analysis of amino acids with Ni-Cr electrodes in 0.10 N NaOH is approximately 0.48-0.50 V (Figure 3). This working potential range, can be taken as the best[23] for HPLC analysis of amino acids. Although, the current plateaus at potentials over 0.48V in the hydrodynamic voltammograms, it has to be noticed that, the S/N ratio for each analyte has a maximum value in the above mentioned

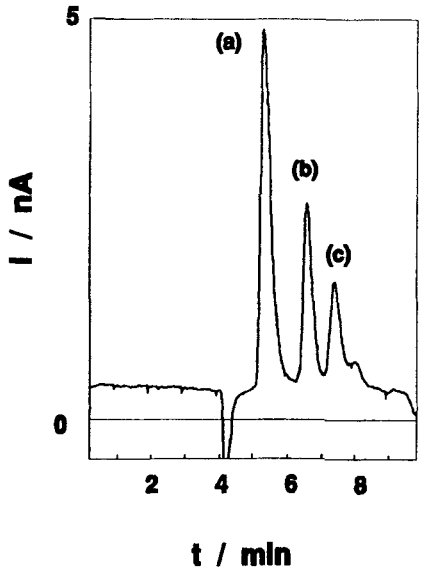

Figure 4. High Performance Anion Exchange chromatogram of three amino acids separated with an anion exchange column. Injection amount = 2 nmol each amino acid. Flow rate - 0.50 mL min. T = 298K. (a) Arginine, (b) Glycine, (c) Treonine.

potential range. This phenomena may be attributed to the increased background noise observed at potentials over 0.48 V. Thus, the S/N ratio is lowered over 0.50 V.

An anion exchange high performance chromatogram of glycine, treonine and arginine at a working potential of 0.48 V is shown in Figure 4. These compounds can be easily separated with the anion exchange column, and detected with the Ni-Cr alloy electrode. The effectiveness of the chromatographic separation is indicated by the excellent resolution at a flow rate of 0.5 mL/min.

CONCLUSIONS

Amino acids separated by anion exchange columns, are easily detected with a Ni-Cr alloy electrode in 0.10 N NaOH. It is believed, that the target molecules are oxidatively detected by a surface catalyzed process, proposed to involve Ni(III) species. The hydrodynamic voltammogram of various amino acids show that the mass-transfer control of the current is obtained at potentials above 0.48 V.

The signal-to-noise ratio analysis at different working potentials, shows that the range from 0.48 V to 0.50 V can be taken as the best for the analysis of amino acids with Ni-Cr electrodes.

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