



# Copper dispersed into polyaniline films as an amperometric sensor in alkaline solutions of amino acids and polyhydric compounds

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Received 12 March 1996; revised 1 July 1996; accepted 19 July 1996

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## Abstract

A chemically modified electrode composed of copper microparticles dispersed into a polyaniline (PANI) film was investigated as an amperometric sensor of scarcely electroactive compounds possessing –OH and –NH<sub>2</sub> groups. Glassy carbon was used as an electrode material and modified firstly by a PANI film, then allowed to stand in contact with a solution of copper ions, and finally, the electroreduction was done at –0.3 V. The electrochemical behaviour of the resulting modified electrode in alkaline medium was examined by cyclic voltammetry and flow-injection amperometry. Using some representative compounds, the effect of copper loading and pH on the electrode response was investigated. Constant-potential amperometric detection was applied in conjunction with anion-exchange chromatographic (AEC) separations of amino acids and carbohydrates. At an applied potential of 0.55 V vs. Ag/AgCl, the detection limits ( $S/N=3$ ) for all analytes investigated ranged 5–15 pmol, and the linear dynamic range was three–four orders of magnitude above the detection limits. The resulting modified electrode was found to retain 95% of its initial response in flowing streams for 3 h of operating time.

*Keywords:* Liquid chromatography; Copper electrocatalyst; Polyaniline; Amino acids; Carbohydrates

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## 1. Introduction

Electrochemical detection (ED) in liquid chromatography (LC) is very attractive owing to its high sensitivity and wide dynamic range in regard to several classes of compounds. However, the analytical performances strongly depend on the applied potential, electrode material, its morphological state

and the analytes involved. Chemically modified electrodes (CMEs) containing surface-confined chemical functionalities have shown some advantages over conventional electrode materials.

Polymer films have been employed in CMEs and are used in biosensors to protect the electrode surface from fouling, immobilize a biocomponent, extend the linear range of the sensor and to trap or incorporate a catalytic species [1–3]. Indeed, transition metals deposited into viologen-based polymer [4], poly(vinylacetic acid) [5], poly(4-vinylpyridine) [6,7],

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nafion, polyaniline, cellulose acetate, polypyrrole, etc. [8–17] have been used as amperometric sensors in electroanalytical applications. Metals electrodeposited in thin organic films appear as microparticles dispersed in a three-dimensional array confined to the polymer matrix [5–17]. The possibility of dispersing metallic particles into organic polymers provides a good physical separation of the catalytic centres leading to a highly active electrode surface suitable for efficient electrocatalysis. Moreover, the advantages that can be accrued from this type of electrode are mechanical stability and improved reproducibility, which can be achieved by the controlled electrodeposition of metal into the polymer.

Very often, polymeric membranes are also used as size and/or charge exclusion barriers in order to improve selectivity and stability of CMEs [18–21].

Polyaniline (PANI), in particular, is a conducting polymer which is easy to deposit from aqueous solutions, adheres strongly to the support and chemically stable [10–12,19–30]. A fibril-type PANI film, with a fibril diameter of approximately 100 nm, was obtained by electrochemical oxidation of aniline in acidic aqueous medium, and the subsequent electroreduction of metal catalysts gave rise to a uniform dispersion of microparticles in the fibrils [10,22]. Electrochemical polymerized PANI films or metal microparticles dispersed into the polymer on glassy carbon (GC) electrodes have been previously described as amperometric sensors in acidic or neutral media [10–12,19,20,23]. For example, PANI films modified with platinum particles seem to substantially enhance the electro-oxidation current of methanol offering an effective protecting matrix for the metals microparticles against contamination from the bulk solutions, especially catalyst leaching [10–12,23].

Although PANI films have been characterized and extensively used in acidic and neutral media as electroactive polymeric membranes, no study has been reported in alkaline media, where the PANI film is an insulator. Considering that, in alkaline media, many transition metals show the maximum catalytic activity towards the electro-oxidation of several organic molecules, the characterization of new amperometric sensors based on the nonconducting PANI films appears interesting.

Here, we describe a chemically modified electrode prepared by copper microparticles entrapped into a PANI film electrochemically deposited on GC. Upon deposition of the PANI film, the uptake of copper was realized by electroreduction of copper ions incorporated into the conducting film. This modification procedure yielded reproducible electrode surfaces as long as the experimental parameters were well controlled. The described Cu–PANI modified GC electrode (Cu–PANI) was characterized by cyclic voltammetry and flow-injection analysis (FIA) in alkaline solutions as an amperometric sensor for the detection of some important organic molecules as amino acids and polyhydric compounds. Chromatographic separations, using an anion-exchange column, are reported.

## 2. Experimental

### 2.1. Reagents

All solutions were prepared from analytical-reagent grade chemicals without further purification using doubly distilled, degassed and deionized water. Aniline (99.5%), carbohydrates (99%), amino acids (99%),  $\text{CuCl}_2$  (99.9%) and NaOH (97%) were purchased from Aldrich (Germany), and  $\text{H}_2\text{SO}_4$  (96%) suprapur grade was purchased from Merck (Germany). Individual 100 mM standard solutions of carbohydrates and amino acids (Aldrich) were prepared daily in distilled water. Appropriate dilutions were performed with 0.1 M NaOH. Unless otherwise specified, experiments were performed using 0.1 M NaOH as a background electrolyte. Alkaline solutions were protected from oxygen in cyclic voltammetry experiments by purging with high-purity nitrogen.

### 2.2. Apparatus

A Model 273 Princeton Applied Research (EG&G) potentiostat/galvanostat controlled by the 270 Electrochemical Analysis Software was used for electrochemical measurements. Cyclic voltammetry (CV) was done in a three-electrode cell using a Cu–PANI working electrode, a saturated calomel reference electrode (SCE) (4 M KCl) and a platinum foil

counter electrode. The GC electrode used in CV, having a geometric area of  $0.125 \text{ cm}^2$ , was purchased from EG&G. All current densities reported in this paper are quoted in terms of apparent Cu–PANI surface area.

Constant-potential amperometric measurements in flowing streams were performed using a Model 400 Electrochemical Detector (EG&G) with a flow-through thin-layer electrochemical cell consisting of a Cu–PANI as working electrode, an Ag/AgCl (4M KCl) reference electrode and a stainless steel counter electrode. The output signal was recorded by a Model 868 Amel recorder. Flow-injection experiments were carried out with a Varian 2510 pump equipped with a Model 7125 Rheodyne injector using a  $50 \mu\text{l}$  sample loop. The mobile phase was purged from oxygen using an on-line degasser system (Hewlett-Packard Series 1050).

Chromatographic separations were performed with a CarboPac PA1 ( $250 \times 4 \text{ mm}$  i.d., Dionex) anion-exchange column, using 0.10 M and 0.15 M NaOH as the mobile phase for the analysis of amino acids and carbohydrates, respectively. All experiments were carried out at ambient temperature.

### 2.3. Electrode preparation

Prior to its modification, the GC electrode was polished with  $0.05 \mu\text{m}$   $\alpha$ -alumina suspension on a microcloth polishing pad and washed with doubly distilled water for few minutes. The PANI films were prepared by cycling the potential continuously between  $-0.1$  and  $1.1 \text{ V}$  for five cycles at  $50 \text{ mV s}^{-1}$  in  $85 \text{ mM}$  aniline and  $0.1 \text{ M}$  sulphuric acid solution. Usually, a charge of  $4.5\text{--}6.0 \text{ mC}$  was consumed during the PANI film deposition on GC with  $0.125 \text{ cm}^2$  as geometric area. It has been reported that  $80 \text{ mC cm}^{-2}$  is required to deposit a  $1 \mu\text{m}$  thick film [10,24], therefore, the films prepared in this study were approximately  $0.5 \mu\text{m}$  thick. The polymer electrode was allowed to remain in contact with a  $50 \text{ mM}$   $\text{CuCl}_2$  and  $0.1 \text{ M}$   $\text{H}_2\text{SO}_4$  solution for 5 min prior to the deposition. Copper was electrodeposited onto the PANI films at a constant potential of  $-0.3 \text{ V}$  vs. SCE for 3 min. The amount of copper deposited, calculated from the total charge passed during the deposition process, was estimated by assuming that the reduction  $\text{Cu}^{2+}$  to Cu is 100% efficient.

## 3. Results and discussion

### 3.1. Electrode characterization

The formation of PANI films by electropolymerization of aniline in acidic medium has been reported by several authors [10–12,22,24–30]. The voltammogram (not shown) of PANI in acidic solutions ( $0.1 \text{ M}$   $\text{H}_2\text{SO}_4$ ) obtained between  $-0.1$  and  $1.1 \text{ V}$  vs. SCE is characterized by four peaks with anodic potentials at  $+0.25$ ,  $+0.48$ ,  $+0.62$  and  $+0.85 \text{ V}$  vs. SCE. Continued potential cycling between  $-0.1$  and  $1.1 \text{ V}$  decreases the peak currents relevant to both processes at  $+0.25$  and  $+0.85 \text{ V}$ , while other peak currents increase. It has been previously reported that oxidation of the polymer to the imine form at potentials greater than  $+0.85 \text{ V}$  results in the hydrolysis of imine nitrogen-carbon bonds to a quinone form [10–12,24,29,30]. Thus, in order to prevent polymer degradation, potentials greater than  $+0.6 \text{ V}$  should be avoided [10]. Fig. 1a shows a typical CV of a PANI film on GC after repeated potential cycling (80 cycles) between  $-0.1$  and  $+0.6 \text{ V}$ . To ascertain the stability of the PANI film in alkaline media, the modified electrode was cycled in  $0.1 \text{ M}$  NaOH between  $-0.1$  and  $+0.6 \text{ V}$  for 30 min and polarized at  $+0.55 \text{ V}$  for 4 h, then it was washed with water and further cycled in  $0.1 \text{ M}$   $\text{H}_2\text{SO}_4$ . The corresponding voltammetric pattern is shown in Fig. 1b, and agrees very well with that reported in Fig. 1a, suggesting a good chemical

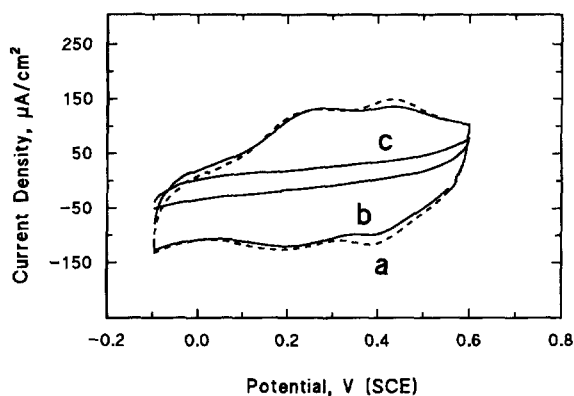


Fig. 1. (a) Cyclic voltammogram in  $0.1 \text{ M}$   $\text{H}_2\text{SO}_4$  of a polyaniline film growth on GC; (b) as (a) after cycling for 30 min between  $-0.1$  and  $+0.6 \text{ V}$ , and subsequent polarization at  $+0.55 \text{ V}$  for 4 h in  $0.1 \text{ M}$  NaOH; (c) voltammogram obtained in  $0.1 \text{ M}$  NaOH. Sweep rate,  $50 \text{ mV s}^{-1}$ .

stability of the film in alkaline solutions as well. The CV of a GC/PANI electrode acquired in 0.1 M NaOH is also reported (curve c) in Fig. 1. As can be seen, the electrochemical behaviour is fully suppressed because the polymer in the neutralized free-base form is electrochemically inactive [30].

Upon electrochemical deposition of the PANI film on a GC electrode, it was immersed in 50 mM  $\text{CuCl}_2 + 0.1 \text{ M H}_2\text{SO}_4$  solution. The chronoamperometric curves for the electroreduction of  $\text{Cu}^{2+}$  following a negative step in applied potential, that is  $-0.3 \text{ V vs. SCE}$ , are shown in Fig. 2. The elapsed times after the immersion of the PANI film electrode are 5 min in curve (a) and 0 min in curve (b). It was observed that upon an elapsed time of 5 min, the polymeric film was found fully saturated with copper species. When elapsed time longer than 5 min was considered, the amount of copper deposited onto the PANI film electrode remained practically constant (not shown). The total charge relevant to the electroreduction of copper ions, passed during 180 s of applied potential ( $-0.3 \text{ V}$ ), measured after an immersion time of 5 min (or higher) is about 32 mC, while the charge estimated when the GC/PANI film was immediately immersed before the electroreduction is about 28 mC. These results indicate that a certain amount of  $\text{Cu}^{2+}$  is able to penetrate the polymeric matrix, corresponding to about 12% of the total amount of copper deposited, when the film is allowed to remain in contact with  $\text{Cu}^{2+}$  for at least

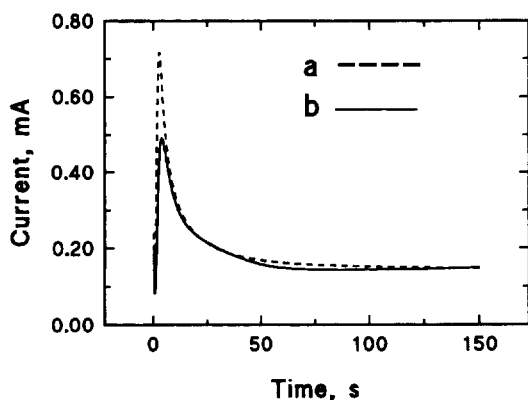


Fig. 2. Current–time curves observed when a PANI film was polarized at  $-0.3 \text{ V vs. SCE}$  in  $0.1 \text{ M H}_2\text{SO}_4 + 50 \text{ mM CuCl}_2$  solution: (a) electrode left in contact for 5 min prior to electroreduction; (b) immediate application of potential upon immersion.

5 min. Therefore, using an immersion time of 5 min and a deposition time of 3 min, reproducible amounts of copper particles were deposited.

### 3.2. Electrocatalytic behaviour of Cu–PANI film electrodes

#### 3.2.1. Voltammetric response

Representative CVs obtained at a Cu–PANI film electrode in 0.1 M NaOH containing 2.0 mM proline (a) and 2.1 mM glucose (b) are depicted in Fig. 3. The copper loading was about 32 mC. Large anodic waves were observed in the positive potential scan between ca.  $+0.3$  and  $+0.8 \text{ V vs. SCE}$  with current magnitude proportional to analyte concentration up to about 50 mM. On the reverse scan, the oxidation processes were only observed in the region from  $+0.8$  to  $+0.4 \text{ V}$ . A similar behaviour was observed for all examined compounds including mannitol, lactose, glutamine, glycine, serine, lysine and alanine. The anodic peak current vs. the square root of the scan rate was linear up to  $200 \text{ mV s}^{-1}$ , while the peak potential shifted towards more positive potentials. The peak potential also shifted in the anodic direction on increasing the substrate concentration. These results suggest that the electro-oxidation mechanism at the modified electrode is under kinetic control.

#### 3.2.2. Effect of copper loading

The influence of the copper loading on the catalytic activity was examined in voltammetric experiments.

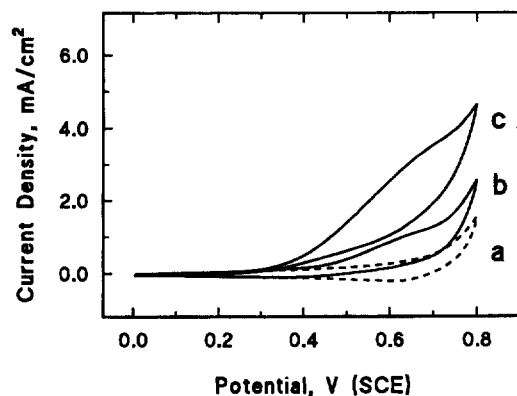


Fig. 3. Cyclic voltammograms of a Cu–PANI film electrode in 0.1 M NaOH (a, dashed line) in the presence of 2.0 mM proline (b) and 2.1 mM glucose (c); sweep rate,  $50 \text{ mV s}^{-1}$ .

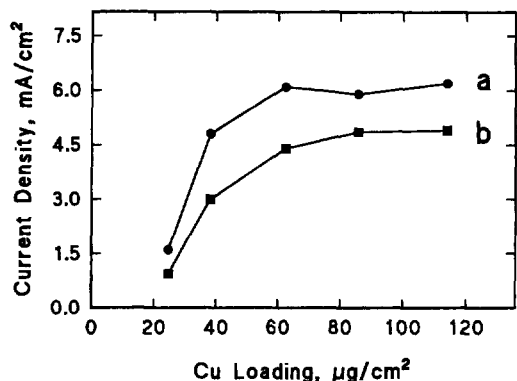


Fig. 4. Current density of 4 mM mannitol (a) and 6 mM proline (b) as a function of copper loading at a Cu-PANI film electrode. The current densities were determined by cyclic voltammetry at +0.55 V, in 0.1 M NaOH.

Fig. 4 illustrates the changes of current density measured at +0.55 V in the presence of mannitol (curve a) and proline (curve b), as a function of the amount of copper deposited. As can be seen, the current density increases proportionally with the copper loading up to a maximum value of 80–90  $\mu\text{g cm}^{-2}$ , corresponding to about 3 min of deposition time at  $-0.3$  V, then values become nearly constant. Therefore, all experiments were carried out with PANI films loaded with the highest amount of copper.

### 3.2.3. Effect of pH

It is known that all copper-based modified electrodes exhibit their catalytic activity in the presence of relatively high concentration of hydroxide ions, thus the effect of pH was investigated in the range 11–14. As test compounds, 5 mM proline and 5 mM mannitol were used in solutions maintained at constant 1.0 M ionic strength with  $\text{NaNO}_3$ . A remarkable increase of the oxidation current was observed up to pH 13.3 with both compounds (not shown). At higher pH, the current densities of mannitol leveled off, whereas proline exhibited a decrement. A similar behaviour was observed for other amino acids investigated. Such a different behaviour of amino acids and polyhydric compounds may be explained if one takes into account the involvement of hydroxide ions in the catalytic electro-oxidation mechanism. Hydroxyl radicals are probably formed in the  $\text{OH}^-$  oxidation at Cu(III) catalytic centres; these react with the

organic molecules through the abstraction of a hydrogen atom from the carbon in  $\alpha$ -position with respect to  $-\text{OH}$  or  $-\text{NH}_2$  group [31–35]. Since amino acids and carbohydrates seem to react with a similar electro-oxidation mechanism [31], the difference noted at high pH most likely reflects a very favourable competitive oxidation of hydroxide ions compared to amino acids.

### 3.3. Flow-injection measurements

Hydrodynamic voltammograms (HDVs) of cysteine (a), proline (b), glucose (c) and mannitol (d), obtained under flow-injection conditions using a thin-layer electrochemical cell, are shown in Fig. 5. In accordance with the CV behaviour, the maximum response was found in the potential range 0.50–0.60 V vs. Ag/AgCl. In contrast to a simple diffusion-limited process, the HDVs at potential values higher than +0.6 V exhibit a decreased electrode response which is similar for all investigated analytes. Therefore, at potentials higher than +0.6 V, the hydroxide oxidation process takes place with subsequent evolution of oxygen gas, leading to a partial suppression of the adsorption/oxidation processes of the organic molecules. A constant potential of +0.55 V was usually chosen both to maintain a low background current (generally lower than 0.5  $\mu\text{A}$ ) and to increase the long-term stability of the modified electrode.

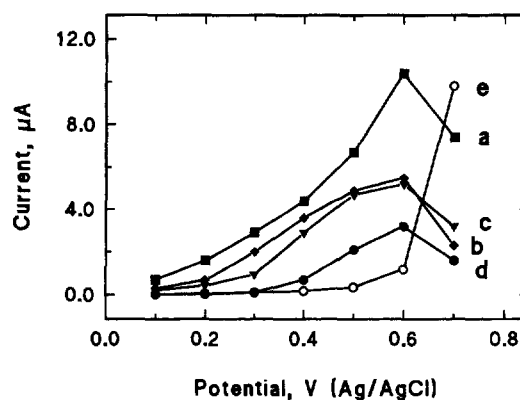


Fig. 5. Hydrodynamic voltammograms in FIA at a Cu-PANI film electrode for (a) 0.40 mM cysteine, (b) 0.12 mM mannitol, (c) 0.15 mM glucose and (d) 0.44 mM proline. Curve (e) represents the background current. Injection volume, 50  $\mu\text{l}$ ; electrolyte/carrier, 0.1 M NaOH; flow rate: 1.0  $\text{ml min}^{-1}$ .

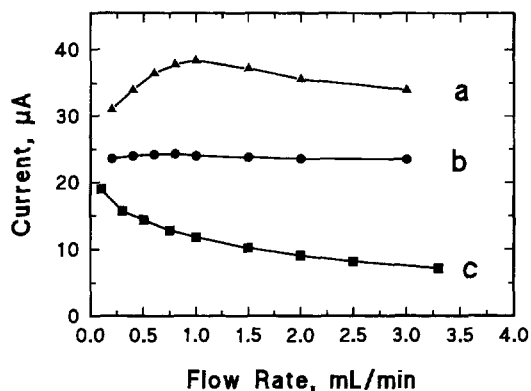


Fig. 6. Dependence of the peak current upon the carrier flow rate: (a) glucose, (b) cysteine, and (c) glycine. Concentrations: 1.0 mM of each compound; applied potential: +0.55 V vs. Ag/AgCl. Other experimental conditions as in Fig. 5.

Note that, while the amperometric response for carbohydrates and cysteine is acceptable at relatively low applied potentials, i.e. 0.2–0.4 V, other amino acids do not show a significant response in the same potential window. Earlier works involving amperometric detection at copper electrodes suggested that enhancements in the anodic current at low applied potentials could be attributed to the complexation process of analytes by Cu(II) species [35].

The effect of flow rate on the response of Cu-PANI film electrodes for glucose (a), cysteine (b), and glycine (c) using 1 mM concentrations, is illustrated in Fig. 6. Whereas cysteine presented minimal flow rate effect in the range 0.2–3.0 ml min<sup>-1</sup>, the peak current of glycine and glucose was strongly dependent upon the flow rate. In particular, glycine exhibited a sharp decrease in current on increasing the flow rate, while the current response of glucose was first found to increase up to about 1.0 ml min<sup>-1</sup>, thereafter it showed a decrease at higher flow rates. This general behaviour can be interpreted in terms of a surface-confined rate-limiting step. The difference of behaviour between glucose and glycine may reflect different reaction rate on the electrode surface.

In order to provide a clear overview of the electrode capability towards the catalytic oxidation of -NH<sub>2</sub> and -OH containing compounds, the molar response factors in flow-injection experiments were evaluated. The results of some representative analytes are reported in Table 1. In the first approximation, the amperometric response is related to the number of

Table 1

Flow-injection response of some carbohydrates and amino acids at a Cu-PANI film electrode

Compounds	Sensitivity (nA (cm <sup>2</sup> µM) <sup>-1</sup> )
Glucose	304
Fructose	288
Sorbitol	384
Mannitol	336
Lactose	170
Sucrose	152
Glycerol	216
Glycine	94
Proline	48
Alanine	26
Glutamine	29
Lysine	52
Serine	110
Methionine	46
Cysteine	190
Glucosamine	203

Applied potential +0.55 V vs. Ag/AgCl, flow rate 1.0 ml min<sup>-1</sup>, carrier/electrolyte 0.1 M NaOH.

hydrogen atoms in  $\alpha$ -position, the functional group -OH or -NH<sub>2</sub>, the steric hindrance at the  $\alpha$ -hydrogen and the molecular size of analytes. The molar response ratios of some representative carbohydrates and amino acids obtained at the Cu-PANI film electrode with those reported by Xie and Huber [31] using a cuprous oxide/carbon paste electrode, Cu<sub>2</sub>O-CPE as presented in Table 2. Adopting glucose as a reference compound with response equal to 100, the molar response ratios of carbohydrates and amino acids show a different trend. Indeed, whereas the data of carbohydrates are fully comparable, amino acids exhibit a molar response ratio significantly greater

Table 2

Comparative response ratios of some carbohydrates and amino acids between the Cu-PANI film electrode and the Cu<sub>2</sub>O-CPE [31]

Compound	Cu-CME	Cu <sub>2</sub> O-CPE
Glucose	100	100
Lactose	56	58
Sucrose	50	51
Glycerol	71	45
Glycine	31	13
Proline	16	4.2
Alanine	8.5	2.6
Methionine	15	4.2

Experimental conditions as in Table 1. The data are reported with respect to glucose taken as 100%.

than that reported at the  $\text{Cu}_2\text{O}$ -CPE. The inductive electronic effects of PANI films and/or the particular three-dimensional dispersion of the catalyst are probably the main factors which positively affect the electro-oxidation mechanism of amino acids at the Cu-PANI electrode.

### 3.3.1. Chromatographic separations, calibration, repeatability and detection limits

Anion-exchange chromatography under alkaline conditions represents a valid approach for the separation of carbohydrates and amino acids. Fig. 7 shows a chromatogram obtained with a standard mixture of some representative amino acids using a Carbowac PA1 column, 0.1 M NaOH solution as the mobile phase, and a flow rate of  $0.5 \text{ ml min}^{-1}$ . The analytical results are reported in Table 3.

Fig. 8 shows a typical separation of some carbohydrates and alditols, using the same anion-

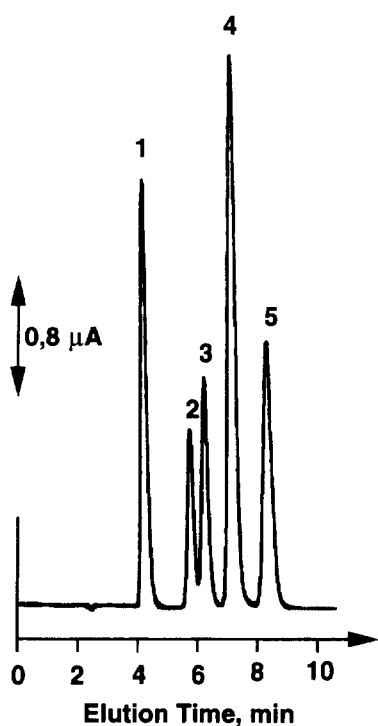


Fig. 7. Chromatogram of a mixture containing (1)  $64 \mu\text{M}$  lysine, (2)  $63 \mu\text{M}$  glutamine, (3)  $68 \mu\text{M}$  alanine, (4)  $35 \mu\text{M}$  glycine, and (5)  $69 \mu\text{M}$  methionine at a Cu-PANI film. Applied potential:  $+0.55 \text{ V}$  vs. Ag/AgCl. Column: Dionex Carbowac PA1 ( $250 \times 4 \text{ mm}$  i.d.); isocratic elution with  $0.1 \text{ M}$  NaOH; flow rate:  $0.5 \text{ ml min}^{-1}$ ; sample loop:  $50 \mu\text{l}$ .

Table 3  
Quantitative parameters of amino acids in LC-EC detection at a Cu-PANI film electrode

Compounds	LOD <sup>a</sup> (pmol)	Linear range (M)	$r^2$	RSD <sup>b</sup> (%)
Alanine	13	$7.2 \times 10^{-4}$ – $2.7 \times 10^{-7}$	0.997	2.9
Glycine	5	$5.6 \times 10^{-4}$ – $8.7 \times 10^{-8}$	0.999	2.6
Lysine	10	$5.1 \times 10^{-4}$ – $2.1 \times 10^{-7}$	0.999	2.6
Methionine	9	$5.6 \times 10^{-4}$ – $1.9 \times 10^{-7}$	0.999	3.5
Glutamine	15	$5.2 \times 10^{-4}$ – $3.1 \times 10^{-7}$	0.999	4.2

Amperometric detection at  $+0.55 \text{ V}$  vs. Ag/AgCl; isocratic elution with  $0.1 \text{ M}$  NaOH, flow rate:  $0.5 \text{ ml min}^{-1}$ ; column: Dionex Carbowac PA1 ( $250 \text{ mm}$ ,  $4 \text{ mm}$  i.d.).

<sup>a</sup>Limit of detection.

<sup>b</sup>Relative standard deviation obtained from five repetitive injections of about  $0.02 \text{ mM}$  sample solutions.

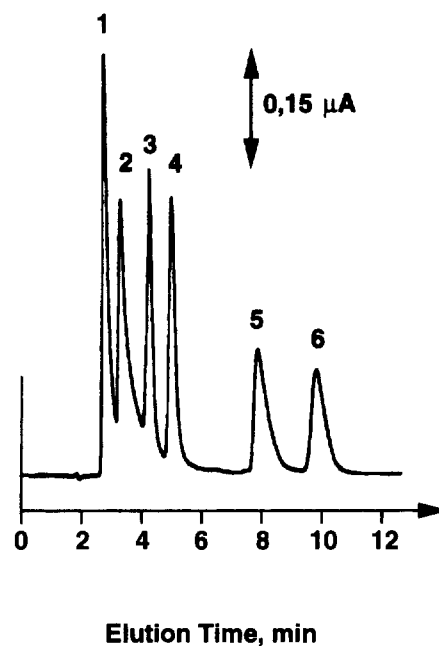


Fig. 8. Chromatogram of a mixture containing (1)  $6.6 \mu\text{M}$  xylitol, (2)  $6.1 \mu\text{M}$  sorbitol, (3)  $6.4 \mu\text{M}$  glucosamine, (4)  $6.4 \mu\text{M}$  glucose, (5)  $8.0 \mu\text{M}$  lactose, (6)  $8.1 \mu\text{M}$  sucrose. Isocratic elution with  $0.15 \text{ M}$  NaOH; flow rate:  $0.6 \text{ ml min}^{-1}$ . Other experimental conditions as in Fig. 7.

exchange column, with a flow rate of  $0.6 \text{ ml min}^{-1}$ . In this case, a better separation was obtained with  $0.15 \text{ M}$  NaOH as the mobile phase. Analytical figures of merit, including limit of detection (LOD), linear range and relative standard deviation, are summarized in Table 4. The linear dynamic ranges for all

Table 4  
Quantitative parameters of carbohydrates in LC-EC detection at the Cu-PANI film electrode

Compounds	LOD (pmol)	Linear range (M)	$r^2$	RSD (%)
Xylitol	2	$7.4 \times 10^{-4}$ – $4.4 \times 10^{-8}$	0.999	3.1
Sorbitol	6	$7.6 \times 10^{-4}$ – $1.2 \times 10^{-7}$	0.997	5.2
Glucosamine	2	$5.8 \times 10^{-4}$ – $4.1 \times 10^{-8}$	0.999	3.3
Glucose	2	$5.4 \times 10^{-4}$ – $4.3 \times 10^{-8}$	0.999	4.1
Lactose	3	$8.8 \times 10^{-4}$ – $6.4 \times 10^{-8}$	0.998	4.6
Sucrose	4	$1.0 \times 10^{-4}$ – $8.1 \times 10^{-8}$	0.999	5.1

Isocratic elution with 0.15 M NaOH, flow rate:  $0.6 \text{ ml min}^{-1}$ . Other experimental conditions as in Table 3.

amino acids and carbohydrates investigated were about three–four orders of magnitude above the detection limits. The LOD, determined at a signal to noise ratio of 3 from the lowest injected concentration, comprised between 2 and 15 pmol. These values are comparable with those obtained with other amperometric detectors based on metallic surfaces [36–39], but are lower than those obtained with other CMEs: poly(ester) sulphonic acid/Ni [16], nafion/Cu [17], carbon pastes/Ru [40,41] or cobalt phthalocyanine [42]. Moreover, although the detection limits of carbohydrates are comparable with those obtained using a cuprous oxide/carbon paste electrode [31], these values are significantly lower for amino acids.

Precision, estimated in terms of relative standard deviation (% RSD) by five repetitive chromatographic analyses (over about 1 h) of a solution containing 0.021 mM lysine, 0.020 mM glutamine, 0.033 mM alanine, 0.012 mM glycine and 0.034 mM methionine ranged between 2.6% for lysine and 4.2% for glutamine. The precision of polyhydroxyl and related compounds, estimated by five successive injections of a solution containing 0.016 mM xylitol, 0.011 mM sorbitol, 0.013 mM glucosamine, 0.015 mM glucose, 0.019 mM lactose and 0.020 mM sucrose ranged between 3.1% xylitol and 5.2% sorbitol.

Detector stability was tested by measuring the amperometric peak current during 12 consecutive chromatographic separations of 0.55 mM lysine, 0.47 mM alanine and 0.62 mM methionine. After an initial stabilization of the electrode response, which took about 1 h, the subsequent 3 h of operation showed an average decrease of the signal of about 5%. However, after about 8 h of operation in flowing

streams the amperometric response exhibited an averaged lowering of ca. 23%. The progressive dissolution of the catalyst from the PANI film may be the main cause of such a behaviour. Note that for analytical applications, the use of internal standards for calibration purposes will correct for any change in the electrode sensitivity.

Repetitive preparations of the Cu-PANI film electrodes result in highly reproducible loading with subsequent homogeneity of the film and distribution of the catalyst. In a series of three deposition trials, the modified electrode tested in flow-injection yielded  $48 \text{ nA (mM cm}^2)^{-1}$  and  $336 \text{ nA (mM cm}^2)^{-1}$  as the mean current values for the oxidation of 1.0 mM proline and 0.98 mM sorbitol, respectively, with a per cent RSD of about 8%.

#### 4. Conclusions

The novelty of this study lies in the use of PANI films in alkaline media as an inert and stable organic matrix of copper-catalytic species. Such microparticles entrapped within the polymeric skeleton exhibit powerful catalytic activity toward the electro-oxidation of scarcely electroactive compounds as carbohydrates and amino acids. The suitability of the Cu-PANI as an amperometric sensor of carbohydrates and amino acids in flowing streams, including LC, has been investigated. Under constant applied potential, the Cu-PANI film electrode offers good temporal stability, low LODs and wide linear range. The good chemical stability of the PANI films in alkaline media, the ease of preparation of the sensing electrode and the possibility of exploring several metals' combinations dispersed in the nonconducting PANI matrix confirm the interest of this design for the EC detection of organic molecules in LC.

Further studies regarding the potential permselective effects of the inert PANI membranes to improve selectivity performances and practical applications are in progress.

#### Acknowledgements

The financial support of the Italian National Research Council (CNR, Rome) and Ministero



dell'Università e della Ricerca Scientifica (MURST, Rome) are gratefully acknowledged.

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