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Applications of a copper-modified gold electrode for amperometric detection of polar aliphatic compounds by anion-exchange chromatography

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Abstract

An Au/Cu bimetallic electrode is investigated as a sensor in amperometric flow-through detection in alkaline solutions. Experimental conditions such as detection mode, pH, copper loading and flow-rate on sensitivity and stability are evaluated. Constant-potential detection at 0.550 V or 0.350 V and pulsed detection modes are tested after anion-exchange chromatography of alditols, carbohydrates and amino acids. The detection limits (S/N=3) of carbohydrates at 0.550 V and 0.350 V ranged between 4.6–9.0 pmol and 1.0–4.0 pmol, respectively. Those of amino acids were 9.0–39 pmol. In pulsed amperometric detection mode, molar sensitivities and detection limits were higher than those obtained in DC mode. Linear dynamic ranges spanned over four (alditols and carbohydrates) and three (amino acids) orders of magnitude. The Au/Cu CME retained 94–96% of its response after several hours operations in flowing streams. As an application, some alditols, carbohydrates and amino acids were determined in soluble coffee and red wine.

Keywords: Amperometric detection; Detection, LC; Copper-modified gold electrodes; Alditols; Amino acids; Carbohydrates

1. Introduction

The good performances of column liquid chromatography (LC) for the separation of organic compounds gives rise to a substantial interest in developing sensitive and effective detectors. This is particularly true when the eluates do not have chromophore and/or fluorophore groups.

Electrochemical detection in liquid chromatography (LC–ED) is of considerable interest, since it offers good sensitivity, selectivity and wide linear dynamic ranges [1,2]. Electrode materials and chemical/morphological status are critical parameters in ED, since specific surface catalytic sites control electrode mechanisms and products. Noble metals are extensively used as electrode material for the LC–ED of many aliphatic compounds [2–7], but they require surface cleaning/regeneration steps by using pulsed amperometric detection (PAD) modes, to avoid fouling resulting from strong surface adsorption phenomena and to obtain stable amperometric responses. Transition metals such as copper, nickel and ruthenium are also used in constant-potential amperometric detection of carbohydrates

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and amino acids in alkaline media [1,8–12]. Some chemically modified electrodes (CMEs), characterised by either surface-confined or three-dimensional dispersions of transition metals catalytic sites, proved competitive over conventional bulk metal electrodes [13–19].

Noble metal-based binary electrodes, either obtained by underpotential deposition of foreign metal atoms (i.e. Cd, Re, Ru, Sb, etc.) or by metal alloying, often display an enhanced electrocatalytic activity [20–23], likely because submonolayers of electroactive species are less affected by electrode fooling. Recently, a bimetallic electrode made by dispersing copper microparticles onto the surface of a bulk gold electrode (Au/Cu CME) which leads at a sensing electrode with strong catalytic activity in a wide range of potentials. This electrode displayed a long-term stability when used as electrochemical sensors of glucose in alkaline media [24]. In this work, it is used as an amperometric detector in LC analysis of some carbohydrates and amino acids in real samples.

2. Experimental

2.1. Reagents

Solutions were prepared from analytical-reagent grade chemicals without further purification and from double distilled and deionised water. Individual 0.15 *M* solutions of alditols, carbohydrates, amino acids and organic acids (Aldrich) were prepared daily in distilled water and diluted, when necessary, with 0.2 *M* NaOH solutions and distilled water. Unless otherwise specified, the background electrolyte was 0.2 *M* NaOH.

During cyclic voltammetry experiments (CV), solutions were purged with high-purity nitrogen. All experiments were carried out at ambient temperature. Before injection, samples of red wine were diluted with distilled water 1:50 and filtered. Soluble coffee (300 mg) was dissolved in 70 ml of distilled water, sonicated at 60°C for 60 min, filtered and diluted with water 1:20.

2.2. Apparatus

A Model 273 Princeton applied research (PAR EG&G) potentiostat/galvanostat was used for electrochemical measurements. CV was done in a three-electrode cell using the Au/Cu CME working electrode, a SCE (4.0 *M* KCl) reference electrode and a platinum foil counter-electrode. The gold electrode used in CV (geometric area equal to 0.125 cm^2) was purchased from PAR. All current densities are quoted in terms of apparent geometric area (mA/cm^2) of the electrode.

Amperometric measurements in flowing streams were performed by using a PAR Model 400 electrochemical detector and a flow-through thin-layer electrochemical cell consisting of the Au/Cu CME as working electrode, an Ag/AgCl (4 *M* KCl) reference electrode and a stainless steel counter electrode. A Model Servogor 120 BBC recorder recorded the signal. Flow injection experiments were carried out by a Varian 2510 pump equipped with a Model 7125 Rheodyne injector using a 50-μl sample loop. The eluent was purged by an on-line degasser system (Hewlett-Packard Series 1050).

LC separations were performed by using a CarboPac PA1 (Dionex) anion-exchange column (250×4 mm I.D. with particle size of 10 μm) and a CarboPac MA1 (Dionex) anion-exchange column (250×4 mm I.D. with particle size of 8.5 μm). The last column was coupled with a CarboPac MA1 (50×4 mm I.D.) guard column.

2.3. Electrode preparation

After removal of any trace of copper from the gold surface (by soaking for few minutes in 18% w/w hydrochloric acid), the electrode was polished with 0.05-μm α-alumina powder on a polishing microcloth and washed with doubly distilled water. Copper was electrodeposited onto the gold surface at −0.3 V vs. SCE for 90 s in 50 m*M* $CuSO_4$. The copper loading (typically 25–30 $\mu g/cm^2$) was estimated from the total charge passed by assuming a 100% faradic efficiency.

3. Results and discussion

3.1. Electrochemical characterisation

Representative CVs obtained at the Au/Cu CME

in alkaline medium are reported in Fig. 1A. The two small cathodic peaks present at about 0.0 V (Ic) and 0.58 V (IIc) (vs. SCE) can be ascribed to the reduction of AuO formed during the anodic sweep [25–29] and, respectively, to the reduction of Cu(III) to Cu(II) [10,12,17,30–32]. The solid curve in Fig. 1A is characterised by a large anodic wave at −0.2–0.8 V both in cathodic and anodic scans. Two peaks can be observed at about 0.380 V (anodic scan) and at 0.0 V (cathodic scan). The gold substrate is the main electrocatalyst around 0.0 V. Other alditols and carbohydrates exhibited similar current–potential curves. Fig. 1B displays some CVs obtained in the presence of 5.0 m*M* glycine (curve a) and 4.5 m*M* cysteine (curve b). The electrochemical behaviour of cysteine appears more complex than that of glycine. Alanine, lysine, glutamine, proline and methionine, behaved similarly to glycine. Fig. 1C shows the CV behaviour of salicylic (curve a) and tartaric acid (curve b). Large oxidation currents are observed in the 0.45–0.65 V potential region.

These results suggest that the electrooxidation of amino acids and some organic acids should involve Cu(III) surface oxides, while other organic acids, such as ascorbic, oxalic, uric and gluconic, exhibited a CV behaviour similar to that of sorbitol (see Fig. 1A). Thus, the Au/Cu CME seems to allow the catalytic oxidation of several classes of organic compounds in the 0.0–0.6 V potential range (vs. SCE). Moreover, combining gold and copper catalytic activity makes an increase in selectivity possible, since alditols, monosaccarides and some organic acids are electrooxidised at low potential values, while amino acids, polysaccarides and several aliphatic acids need higher detection potential values, where Cu(III) species are the catalysts.

3.2. Flow-injection measurements

Fig. 2 shows the dependence of current density on copper loading obtained in FIA conditions. Reproducible and stable electrode performances were observed at 25–30 $\mu g/cm^2$ copper loading.

Since copper- and gold-based electrodes usually require basic eluents, the effect of pH at constant ionic strength (2.0 *M* $NaNO_3$) was also investigated in FIA. The amperometric response of 0.5 m*M* solutions of xylitol, glucose, saccharose and serine,

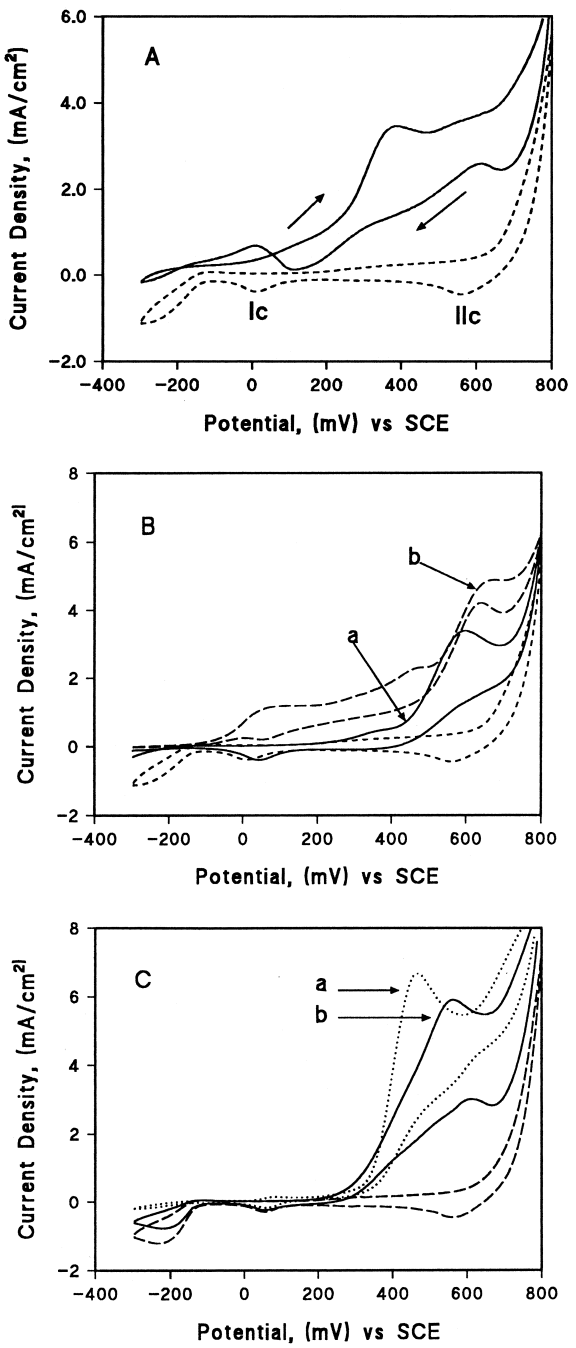

Fig. 1. Cyclic voltammograms at a Au/Cu CME (dashed curves) obtained in 0.2 *M* NaOH solutions; (A) (solid curve): 4.0 m*M* sorbitol; (B) (curve a): 5.0 m*M* glycine, B (curve b): 4.3 m*M* cysteine; (C) (curve a): 8.5 m*M* salicylic acid, C (curve b): 8.5 m*M* tartaric acid. Sweep rate: 50 mV/s.

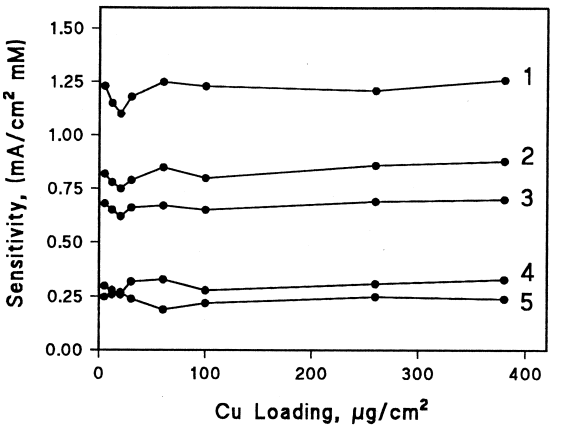

Fig. 2. Sensitivity vs. copper loading for xylitol (1), glucose (2), glucosamine (3), tartaric acid (4) and serine (5). Current densities were determined in FIA at 0.550 V; eluent: 0.2 *M* NaOH; flow-rate: 1.0 ml/min; sample loop: 50 μl.

measured at 0.550 V and at 1.0 ml/min flow-rate, reached a maximum value at pH 13 or higher. To improve buffer capacity and electrode stability an eluent containing 0.2 *M* or 0.4 *M* NaOH was used in all experiments.

Molar sensitivities of some alditols, carbohydrates, amino acids and organic acids at 0.350 V and 0.550 V are compared in Table 1 with those obtained by PAD. PAD is mandatory to preserve catalytic performances of bulk gold electrodes at potential values lower than 0.350 V, where gold species only are significantly active [2–5,7]. In this work, best PAD results were obtained by pulsing first at 0.200 V (detection potential) for 0.4 s, then at 0.650 V for 0.2 s and at last back at −0.200 V for 0.2 s.

Table 1 confirms the good molar sensitivities of the Au/Cu CME for the detection of alditols, monosaccarides and some organic acids, either when used in DC (i.e. 0.550 V or 0.350 V) or PAD mode. A good sensitivity in detecting most amino acids (with exclusion of cysteine and tryptophan) and some organic acids can only be obtained in DC mode at 0.550 V.

The above results confirm that the bimetallic Au/Cu CME profitably couples the activity of hydrous gold oxide and copper oxyhydroxide to detect several classes of organic compounds in PAD or DC amperometric modes and exhibits an improved selectivity and an uncompromised analytical sensitivity.

Table 1
Flow-injection responses of alditols, carbohydrates, amino acids and organic acids at a Au/Cu CME in DC and PAD detection mode

Compounds	Sensitivity (mA/mM cm^2)		
	(DC) 0.550 V	(DC) 0.350 V	PAD
Xylitol	1.10	0.45	0.27
Mannitol	1.00	0.40	0.24
Sorbitol	1.24	0.55	0.31
Glucose	0.92	0.24	0.24
Ribose	0.84	0.44	0.30
Galactose	0.88	0.28	0.19
Arabinose	0.92	0.32	0.22
Fructose	0.64	0.28	0.16
Lactose	0.52	0.06	0.13
Saccharose	0.36	0.02	0.10
Maltose	0.20	0.01	0.09
Raffinose	0.52	0.03	0.09
Glucosamine	0.72	0.08	0.33
Cysteine	0.59	0.59	0.22
Methionine	0.03	0.002	0.003
Serine	0.28	0.011	0.15
Glutamine	0.015	0.001	0.004
Proline	0.03	0.003	0.008
Alanine	0.02	0.001	0.004
Glycine	0.10	0.002	0.01
Tryptophan	0.28	0.14	0.10
Tartaric acid	0.37	0.072	0.09
Ascorbic acid	0.89	0.89	0.18
Uric acid	0.48	0.21	0.14
Gluconic acid	1.50	0.72	0.30

Au/Cu CME with 25 μg/cm^2 copper loading copper; flow-rate 1.0 ml/min; carrier electrolyte 0.2 *M* NaOH; sample loop 50 μl.

3.3. Chromatographic separations, calibrations, reproducibility and detection limits

Fig. 3a–c shows the chromatograms of the same standard mixture of alditols and carbohydrates obtained by operating the Au/Cu CME in DC mode at 0.550 V and at 0.350 V and, respectively, in PAD mode using a CarboPac PA1 column: alditols were best detected in DC mode at 0.550 V and 0.350 V, while carbohydrates (in particular polysaccharides) in PAD mode.

Fig. 4 shows a typical chromatogram of a mixture containing some alditols, carbohydrates and amino acids obtained in DC at 0.550 V using a CarboPac MA1 column.

Limits of detection (LOD), determined at the lowest injected concentration as a signal-to-noise

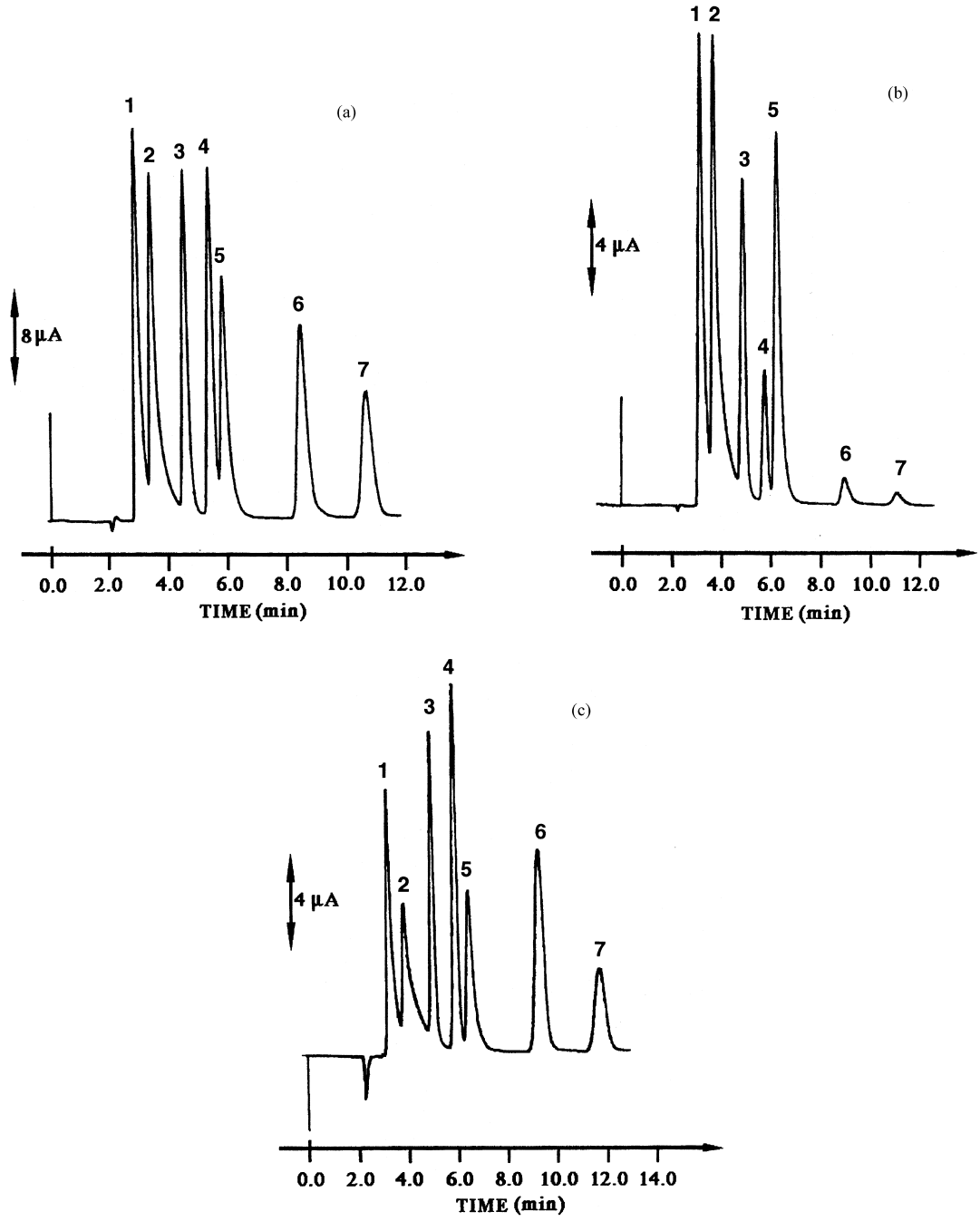

Fig. 3. (a) LC of a standard mixture containing 0.2 m*M* (1) xylitol, (2) sorbitol, (3) arabinose, (4) glucose, (5) fructose, (6) lactose, (7) saccharose. Applied potential: 0.550 V vs. Ag/AgCl. Column: Dionex Carbopac PA1 (250×4 mm I.D.); isocratic elution; eluent: 0.2 *M* NaOH; flow-rate: 0.6 ml/min; sample loop: 50 µl. (b) LC of the standard mixture of alditols and carbohydrates of (a) but at 0.350 V. LC conditions as in (a). (c) LC of a standard mixture of alditols and carbohydrates as in (a). Pulsed detection mode: E_{det}: 0.200 V; t_{det}: 0.4 s; E_{oxd}: 0.650 V; t_{oxd}: 0.2 s; E_{red}: −0.200 V; t_{red}: 0.2 s. LC conditions as in (a).

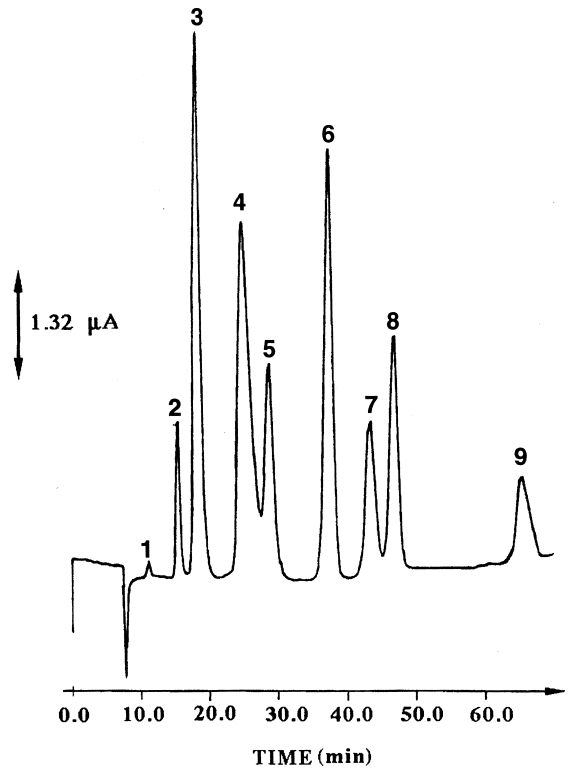

Fig. 4. LC of a standard mixture of (1) 1.0 m*M* methionine, (2) 1.0 m*M* lysine, (3) 0.2 m*M* xylitol, (4) 0.2 m*M* sorbitol, (5) 0.2 m*M* mannitol, (6) 0.2 m*M* glucose, (7) 0.2 m*M* fructose, (8) 1.0 m*M* glycine, (9) 1.0 m*M* proline. Applied potential: 0.550 V. Column: Dionex Carbopac MA1 (250×4 mm I.D.); eluent: 0.4 *M* NaOH; flow-rate: 0.3 ml/min; loop: 50 μl.

ratio equal to 3 are shown in Table 2. Whatever the detection mode, linear dynamic ranges of all investigated polyhydric compounds spanned over 3–4 orders of magnitude above the detection limit, and precision (relative standard deviation, R.S.D.%, of ten repetitive chromatographic analyses of a solution containing 40 μ*M* of each analyte over about 2 h) ranged from 1.2 to 4.8%. LOD, linear range and precision are almost comparable with those obtained with other amperometric sensors based on pure gold or copper metals [1,2,4,10,12,16,17,32]. Moreover, when operated in DC detection mode at low applied potential (i.e. 0.350 V) the Au/Cu CME exhibits a better analytical response than previously described electrodes.

The detector stability was tested by 20 repetitive injections of a standard mixture of carbohydrates and

Table 2
Quantitative parameters in LC–EC of some alditols, carbohydrates and amino acids at an Au/Cu CME

Compounds	LOD[a] (pmol)		
	DC, 0.550 V	DC, 0.350 V	PAD
Xylitol	4.6	1.1	39
Sorbitol	9.0	2.7	45
Arabinose	4.0	1.4	14
Glucose	4.0	4.0	4.5
Fructose	9.0	1.3	20
Lactose	4.0	3.2	18
Saccharose	9.0	1.6	30
Lysine	39	–	18
Serine	9.0	–	16

LC: Carbopac PA1 column; eluent, 0.2 *M* NaOH; flow-rate, 0.6 ml/min; sample loop, 50 μl.
[a] Limit of detection.

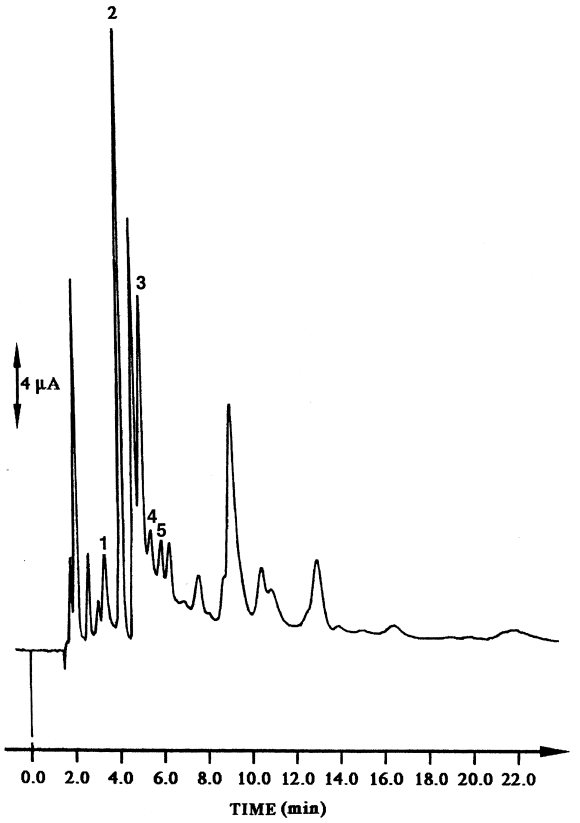

Fig. 5. LC of soluble coffee: 300 mg dissolved in 70 ml of distilled water, the solution was filtered and diluted 1:20. (1) mannitol, (2) arabinose, (3) glucose+galactose, (4) xylose, (5) methionine. Column: Carbopac PA1. Applied potential: 0.550 V vs. Ag/AgCl. The sample was treated as in Section 2.

amino acids (6 h operation by using a Carbopac PA1 column): after about 20 min to stabilise the background current, an average decrease of 3–5% of the signal was observed in DC mode at 0.550 V while an increase of 4–7% was observed in PAD mode. Noticeably, repetitive changes in detection modes, from DC (0.550 V) to PAD and vice versa, did not affect catalytic performances. Over a 2-month period under discontinuous operation, the electrode response in DC at 0.550 V decreased by about 20%.

3.4. Analytical applications

LC analyses of free carbohydrates in a commercial red wine were performed with a Carbopac MA1 column by using a 0.4 *M* NaOH as the eluent, while soluble coffee samples were analysed using a Carbopac PA1 column with a 0.2 *M* NaOH as the eluent. Analytes concentrations were determined by a standard addition method.

A typical chromatogram of a soluble coffee is shown in Fig. 5. Some results are listed in Table 3. Almost negligible percent differences were observed when testing the same carbohydrates by different detection modes. Only glucose in soluble coffee exhibited a larger difference (i.e. 17%) between the results obtained by DC mode at 0.550 V and by PAD mode. This can be likely ascribed to the overlapping [33] of glucose and galactose responses. Galactose is an important component of coffee samples [34,35]. As reported in Table 1, a 20% difference between sensitivity factors of glucose and galactose was observed, so that the multifunctional character of the Au/Cu bimetallic electrode represents a simple way of checking peak purity.

4. Conclusions

A Cu/Au CME was successfully applied to the determination of several organic compounds in flow-through conditions. The electrode favourably combines the catalytic activity of gold and copper. FIA experiments confirmed the good sensitivity and catalytic stability in different detection mode. The availability of different detection modes allows significant improvements of electrode selectivity. The Au/Cu CME allowed the LC determination of alditols, carbohydrates and amino acids in real matrices. Ease of preparation and good time stability confirmed the potential interest of this bimetallic electrode as amperometric sensor in analytical applications.

Acknowledgements

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Table 3
Determination of free alditols, carbohydrates and amino acids in a commercial red wine and soluble coffee

	Detection mode			
	DC (0.550 V)	DC (O.350 V)	PAD	Δ^c (%)
Red wine[a] (g/l)				
Mannitol	0.182	0.179		1.6
Sorbitol	0.118	0.121		2.5
Glucose	0.270		0.264	2.2
Fructose	0.182		0.188	3.2
Lysine	1.22			
Proline	3.20			
Soluble coffee[b] (mg/g)				
Mannitol	0.157		0.153	2.5
Glucose	4.78		3.94	17

[a] Carbopac MA1 column was used for LC; eluent 0.4 *M* NaOH; flow-rate 0.3 ml/min.
[b] Carbopac PA1 column was used for the LC; eluent 0.2 *M* NaOH; flow-rate 0.6 ml/min.
[c] Δ% represents the percent differences between the data obtained with the different detection modes.
The precision expressed as R.S.D.% ranged between 2 and 4% and was obtained from four repetitive LC analysis.

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