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Determination of electroactive organic acids by anion-exchange chromatography using a copper modified electrode

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Abstract

An ion-chromatographic method combined with electrochemical detection at a copper-based chemically modified glassy carbon electrode (Cu–GC) has been shown to provide a simple analytical approach for the determination of some common organic acids in alkaline medium. Under the optimized isocratic chromatographic conditions (i.e. 0.1 *M* NaOH plus 80 m*M* CH_3COONa), organic acids such as gallic, ascorbic, gluconic, lactobionic, galacturonic and glucuronic acid could be separated in less than 20 min. Under constant potential amperometric detection (i.e. 0.55 V vs. Ag–AgCl) the Cu–GC modified electrode allowed detection limits between 2 and 5 pmol for all investigated organic acids while the linear dynamic range spanned generally over three orders of magnitude. Examples of applications included the separation and quantitation of some common organic acids in vinegar, honey and tea samples, are given.

Keywords: Electrochemical detection; Food analysis; Honey; Tea; Vinegar; Organic acids

1. Introduction

Organic and sugar acids are commonly present in foods, beverages, drug, industrial plants, soil and a variety of samples of analytical interest. Acids of low molecular weight, occurring widely in soils, are intermediate in the metabolism of larger molecular mass compounds, such as carbohydrates, peptides and lipids. In addition, organic residues, derived from agriculture and food engineering, are under investigation for their applications as sources of chelating agents able to remove heavy metals from polluted materials. In fact, sugar acids are known to be effective chelators under alkaline conditions [1]. The simultaneous determination of acids, as well as the ratios of the specific components proved to be of particular importance regarding information on the quality, freshness and storability of foods products and in soil systems is of paramount importance since the acids can affect or mediate microbial metabolism, pedogenesis, plant–microorganism relationships and allelopathic activity. Traditional methods of organic acids analysis include gas- and liquid-chromatographic techniques as separation procedures. Liquid-chromatographic methods used for the separation of these classes of molecules are generally based on reversed-phase (RP) HPLC [2–4] and ion chromatography (IC) [4–9] in ion-exchange or ion-exclusion modes. In particular, ion-exchange chromatography has been recognized as a useful technique for the separation of organic weak acids, based

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on a ion-exchange resin depending on their first dissociation constant and the hydrophobicity character. Further influences on the partition behaviour of organic acids can be derived from size-exclusion effects and Van der Waals forces. However, over the past decade, capillary electroseparation techniques have emerged as powerful tools for the separations of aliphatic and aromatic acids and now provide a real option for replacing measurements by liquid chromatography [10–14].

The major problem in organic acids analysis in LC is that these analytes lack chromophores and fluorophores, thus limiting the use of traditional spectrophotometric methods to identify and quantify these compounds. In fact, acids are not readily detected by conventional spectrophotometric methods without tedious and time-consuming derivatization procedures. Thus, indirect absorbance methods have been extensively used for the analysis of non-absorbing acids in foods and biological samples [11,15,16]. However, problems derived from baseline instability can seriously affect the analytical sensitivity of the indirect UV methods. Electrochemical detection (ED), which is typically operated in amperometric mode, has gained some popularity for the detection of underivatized sugars, sugar acids, alditols and uronic acids [8,12,17–23]. A major shortcoming of amperometric detection at noble metal electrodes (i.e. Pt and Au) for a constant applied potential has been the loss of activity during anodic detections of organic substances. Pulsed amperometric detection based on a multistep waveform overcomes the problem of lost activity on noble metals by alternating cathodic and anodic polarizations to clean and reactivate the electrode surface [23–26]. However, slow dissolution of metal particles and higher background noise levels can occur during applications of the multistep waveforms. Thus, we are motivated to search for new electrode materials that can be operated at constant applied potential without loss of electrode activity. In this respect, investigations using Cu-based working electrodes have shown that various electroactive organic molecules can be determined with good sensitivity and reproducibility at constant applied potential in LC detection schemes [12,17–22]. In order to improve the amperometric performance and to decrease the undesired poisoning effects of working electrodes, the use of appropriately designed chemically modified electrodes (CMEs), in which the redox mediator with highly active electrode surface appears finely dispersed on the inert electrode substrate or in polymer matrices, is particularly encouraged in analytical applications [26–28]. Nevertheless, one of the problems in the use of CMEs is the gradual change in the mechanical integrity of the catalytic film for extended periods of time under applied potentials and flowing streams conditions. Thus, the modified electrode can be subject to a slow but continuous erosion of the redox mediator loading with a subsequent loss of the catalytic activity. In order to overcome this problem, innovative modification strategies of the CMEs in which both electrochemical activity of the redox mediator and its film deposition on the electrode surface can be performed in the same experimental conditions (i.e. pH, background electrolyte and applied potentials) are required to preserve the catalytic performance. Therefore, catalyst lost from the electrode surface during prolonged operation can be immediately replaced by a continuous deposition of new active redox particles. In our laboratory, several chemically modified electrodes were prepared by direct electrodeposition of various transition metals on glassy carbon or noble metals substrates which exhibited a promising electrochemical activity towards the electrooxidation of different organic compounds [22,27,29–31]. In particular, a copper oxide/hydroxide film was anodically electrodeposited onto a glassy carbon (GC) substrate from alkaline solutions containing cuprous cyanide ions [32]. The Cu–GC modified electrode showed a powerful catalytic activity towards the electrooxidation of alditols, carbohydrates, amines, amino acids, etc. According to these findings it can also be used as an amperometric sensor for organic acids detection.

Thus, the objective of the present work was to devise an anion-exchange chromatographic method using an amperometric detection with a constant applied potential for the determination of several electroactive organic and sugar acids in alkaline medium. The applicability of the proposed method has been demonstrated by the analysis of some organic acids typically contained in various foods and beverages.

2. Experimental

2.1. Reagents

Solutions were prepared from analytical-reagent grade chemicals without further purification and by using doubly distilled deionized water. Sodium hydroxide pellets (99%), cyanocuprate (I), organic acid compounds, alditols and carbohydrates (Aldrich) were prepared daily in distilled water. Unless otherwise specified, experiments were performed by using 0.1 or 0.15 *M* NaOH as background electrolyte. All experiments were carried out at ambient temperature.

2.2. Apparatus

A Model 273 Princeton Applied Research (PAR, EG&G) potentiostat–galvanostat was used for electrochemical measurements. Cyclic voltammetry (CV) was done in a three-electrode cell using the Cu–GC working electrode, a SCE (4 *M* KCl) reference electrode and a platinum foil counter-electrode. The glassy carbon electrode (geometric area: 0.125 cm^2) used in CV experiments was purchased from PAR. All current densities are quoted in terms of mA/cm^2 of apparent geometric area of the electrode).

Amperometric measurements in flowing streams were performed by using a Dionex Model ED 40 Amperometric detector and a flow-through thin-layer electrochemical cell (Dionex) consisting of the Cu–GC as working electrode (1.0 mm diameter), an Ag–AgCl (4 *M* KCl) reference electrode and a titanium cell body serving as the counter electrode. A personal computer equipped with a Kontron PC INTEGRATION PACK software allowed acquisition and processing of chromatograms. Flow injection and chromatographic experiments were carried out by a Labservice Analytica (mod. LabFlow 3000, Italy) isocratic pump equipped with a Model 7125 Rheodyne injector using a 50-μl sample loop.

Chromatographic separations were performed by using a CarboPac PA1 (Dionex) anion-exchange column (250×4 mm I.D.) coupled with a guard Carbopac PA1 column (50×4 mm I.D.).

2.2.1. Sample treatment

2.2.1.1. Tea powder

A sample of tea (1.5 g) was treated for 35 min in 150 ml of boiling distilled water, filtered through a 0.45-μm Millipore membrane and before injection in the column was diluted 1:100 with distilled water.

2.2.1.2. Balsamic vinegar of Modena and cider vinegar

Vinegar samples were appropriately diluted with distilled water, passed through filter paper and injected onto the column.

2.2.1.3. Orange honey

A 0.2-g amount of sample was dissolved in 200 ml of distilled water and filtered through filter paper and injected onto the column.

Tea powder from Unilever Italia (Milan, Italy), cider vinegar from Ponti Ghemme (Cuneo, Italy) and balsamic vinegar from Levoni (Mantova, Italy) were purchased from a local retailer. Orange honey samples from Apium Villa (Potenza, Italy) were courteously provided directly by the honey grower. All samples were analyzed in triplicate.

2.2.2. Electrode preparation

Prior to each electrode modification, previous traces of copper species were removed from the electrode surface by soaking in hydrochloric acid (37%, w/w) for a few minutes. Successively, the electrode was polished with 0.05-μm α-alumina powder on a polishing microcloth, for improving the uniformity and adherence of the copper film, and washed with doubly distilled water. Films of copper oxide were deposited by potentiostatic conditions at 0.55 V vs. SCE or by voltage cycling (50 mV/s) between −0.1 V and 0.65 V. The modified electrode used in the flowing stream were prepared using not deaerated 0.1 *M* NaOH solutions containing 0.5 g/l of $KCu(CN)_2+K_3Cu(CN)_4$ directly under flow-through conditions at 0.5 ml/min. Similarly, a non-deaerated electrolytic solution composed of 0.1 *M* NaOH plus 0.5 g/l of $KCu(CN)_2+K_3Cu(CN)_4$ was employed to prepare the modified electrodes used in batch experiments.

The surface concentration of copper sites (Γ_{Cu})

was estimated by CV in alkaline medium (0.2 *M* NaOH) by integrating the cathodic wave spanning from 0.15 to 0.65 V, originating from the $Cu^{III} \rightarrow Cu^{II}$ reduction process. Unless otherwise specified, a copper surface concentration of about 2.5–4.0 μg/cm^2 was used.

3. Results and discussion

3.1. Electrochemical behaviour of organic acids in alkaline medium

Representative CVs in 0.1 *M* NaOH (dashed curves) and in solutions containing galacturonic (A), gallic (B) and lactobionic acid (C) of a GG–Cu modified electrode are reported in Fig. 1. In presence of galacturonic acid (see Fig. 1A) a new oxidation peak at about 0.48 V vs. SCE during the anodic sweep is observed. The oxidation peak of galacturonic acid increased linearly on increasing the analyte concentration, while the peak potential shifted towards more positive values. Analogous electrochemical behaviour is seen for other investigated acids such as salicylic, vanillic, 3-hydroxybenzoic, 2-hydroxyphenylacetic, gluconic and glucuronic acid. In the presence of gallic acid, the Cu–GC modified electrode shows a broad oxidation wave in the 0.0–0.65 V potential region with current magnitude proportional to analyte concentration. A similar behaviour was observed for other examined compounds including ascorbic, caffeic and uric acid. A different electrochemical behaviour is observed for the lactobionic oxidation process (Fig. 1C) a significant electrooxidation current is observed only for potentials higher than 0.55 V. The same behaviour was observed for the electrooxidation of glutamic, mandelic and malic acids. Therefore, it seems most likely that the electrooxidation process seen in Fig. 1A and C, in agreement with the literature data [22,26–28,33,34], is electrocatalytic sustained by Cu(III) oxyhydroxide species formed on the electrode surface. On the contrary, for gallic acid and its related compounds, the enhancements in the anodic current, observed at much lower potentials (0.0–0.4 V) could be attributed to the complexation of the Cu(II) species with electroactive analytes with subsequent electrooxidation of the relevant complexes

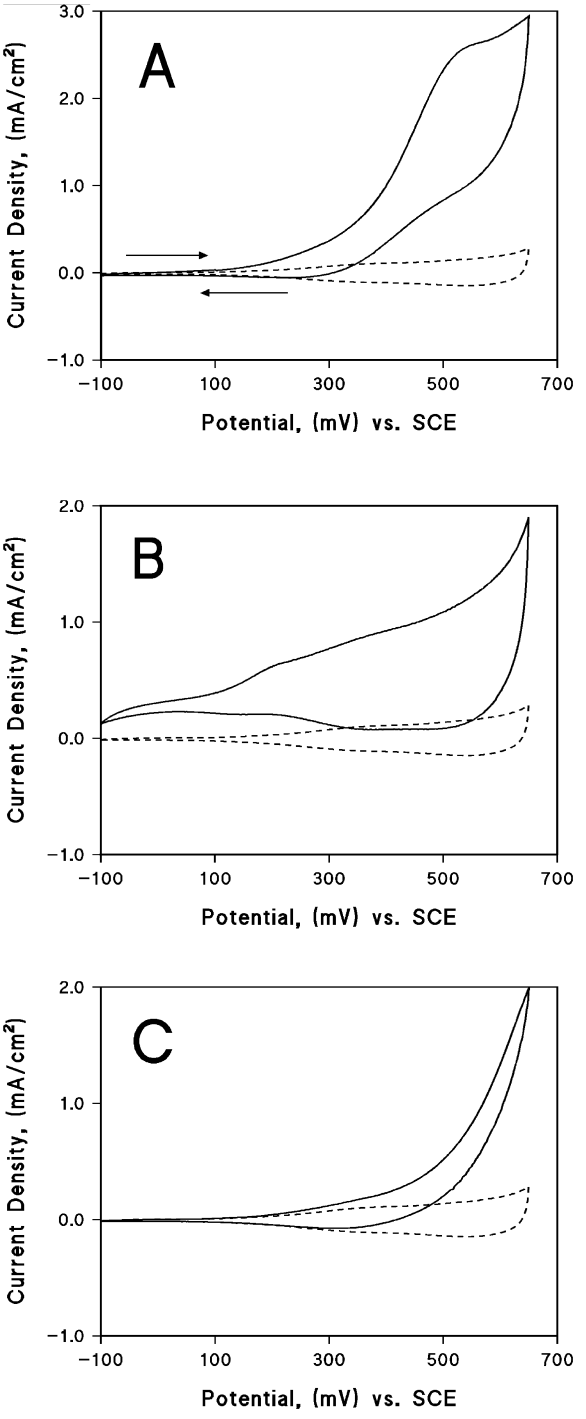

Fig. 1. Cyclic voltammograms at a Cu–GC modified electrode in 0.1 *M* NaOH solutions (dashed curves) and in the presence of 2.0 m*M* galacturonic acid (A), 2.0 m*M* gallic acid (B) and 2.0 m*M* lactobionic acid (C). Scan rate, 50 mV/s.

[35,36]. Thus, although electrochemical processes involved oxidative detection at copper active electrode surfaces, the different electrochemical behaviour observed in Fig. 1 for various molecules, is consistent with the assumption that different electrooxidative mechanisms were operating in each case. The electrochemical performance in terms of potential window in which are observed oxidation currents, sensitivity and linear range, obtained in cyclic voltammograms at the Cu–GC electrode in alkaline medium, for several representative compounds, are summarized in Table 1.

3.2. Effect of applied potential in flow injection analysis

Using a flow-through thin-layer cell with a Cu–GC modified working electrode, the electrochemical oxidation of gallic, gluconic, ascorbic, galacturonic, glucuronic and lactobionic acid (0.1 m*M* of each investigated compound) in 0.1 *M* NaOH solution used as carrier electrolyte was studied as a function of applied potential. Fig. 2 shows the resulting hydrodynamic voltammograms obtained under flow injection conditions (0.5 ml/min) and at 50 mV increments of potential between 0.2 and 0.8 V vs. Ag–AgCl for (A) gallic acid, (B) gluconic acid and (C) glucuronic acid. All examined acids exhibited almost the same behaviour as a function of the applied potential except that the gallic acid. In general, the maximum amperometric responses were observed between 0.5 and 0.7 V, where the presence of active Cu(III) species sustain the electrooxidation process. On the contrary, the gallic acid shows a

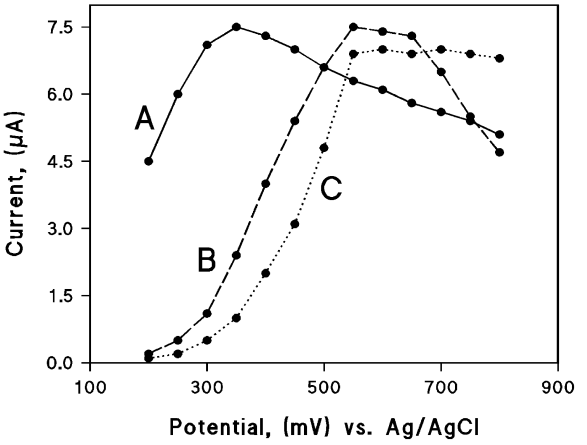

Fig. 2. Hydrodynamic voltammograms obtained in flow injection analysis (FIA) at a Cu–GC electrode for 0.1 m*M* gallic acid (A), 0.1 m*M* gluconic acid (B), 0.1 m*M* glucuronic acid (C). Experimental conditions: carrier electrolyte: 0.1 *M* NaOH; flow-rate: 0.5 ml/min; injection volume: 50 μl.

Table 1
Quantitative responses obtained in CVs of Cu–GC modified electrode to various electroactive organic acids in 0.1 *M* NaOH solutions[a]

Compounds	Potential window (V)	Sensitivity (mA/m*M* cm^2)	Linear range (m*M*)
Ascorbic acid	0.0–0.7	1.1	0.1–10
Caffeic acid	0.0–0.7	0.8	0.1–15
Gallic acid	0.0–0.7	0.7	0.1–12
Galacturonic acid	0.3–0.7	1.5	0.1–10
Gluconic acid	0.3–0.7	1.4	0.2–15
Glucuronic acid	0.3–0.7	1.3	0.1–15
Glutamic acid	0.5–0.7	1.0	0.1–15
3-Hydroxybenzoic acid	0.3–0.7	0.9	0.1–10
2-Hydroxyphenylacetic acid	0.3–0.7	0.7	0.1–10
Lactobionic acid	0.5–0.7	0.7	0.2–12
Malic acid	0.5–0.7	0.4	0.1–10
Mandelic acid	0.5–0.7	0.6	0.1–8
Salicylic acid	0.3–0.7	0.9	0.2–15
Uric acid	0.0–0.7	1.1	0.2–8
Vanillic acid	0.3–0.7	0.8	0.1–15

[a] The fifth scan was recorded at each tabulated CV experiment. Scan rate, 50 mV/s. The currents measured at the potential of 0.55 V vs. SCE during the anodic sweep were corrected by subtracting the background currents (at 0.55 V) obtained at the modified electrode in the supporting electrolyte (0.1 *M* NaOH). Correlation coefficients were always greater than 0.99.

good electrochemical response in the range of potentials comprised between 0.2 and 0.8 V vs. Ag–AgCl with a maximum response at about 0.3 V. The complexation reaction between Cu(II) species and chelating electroactive molecules leads to a significant extension of the potentials where the modified electrode can be used as an amperometric sensor. An operating potential of 0.55 V vs. Ag–AgCl was chosen in the following flow-through measurements as it represents the best compromise between maximum current response for all investigated compounds and minimum residual current noise (generally the values were comprised between 0.2 and 0.6 μA).

3.3. Optimization of separation conditions: effect of hydroxide and acetate ions on the chromatographic performance

In an alkaline medium copper-based electrodes show powerful electrochemical activity for the anodic oxidation of hydroxyl-containing organic compounds [17–22,26–30]. Therefore under such experimental alkaline conditions, an anion-exchange column was employed for the separation of several common electroactive organic acids. Owing to the high affinity of organic acids toward the stationary phase, the chromatographic separation by the anion-exchange mode requires stronger solution conditions than those employed with carbohydrates and alditols. Thus, the addition in mobile phase of nitrate or acetate ions to the alkaline medium is generally required to reduce the retention time of those compounds having high affinity with the stationary phase. In the initial phase of our investigation, we were concerned with optimizing eluent composition in order to achieve maximum separation of acids compounds in a reasonable time and with good amperometric performance. In order to evaluate the effect of the hydroxide concentration on the retention times, a standard mixture of acids was eluted isocratically with mobile phases containing 80 m*M* CH_3COONa and various concentrations of NaOH ranging from 50 to 400 m*M* using a CarboPac PA1 analytical column. The retention times, capacity factors and peak area evaluated from each chromatographic peak are summarised in Table 2. As expected, the retention of each investigated compound is decreased, and the signal response (expressed as peak area) of all acids is decreased with sodium hydroxide concentration higher than 0.3 *M*. Mobile phases with hydroxide concentration lower than 0.1 *M* were not recommended because the retention times were considerably longer, the peaks were large and accompanied by a pronounced tailing. On the contrary, mobile phases with hydroxide concentration higher than 0.3 *M* led to a poor resolution of gallic–ascorbic–gluconic acids and a partial overlapping of the lactobionic–galacturonic peaks. In addition, a considerable increase in the background currents of the detector is observed when the mobile phase contains NaOH solutions with concentrations higher than 0.3 *M*. Therefore, for the analytical applications of the Cu–GC modified electrode, which forms the focus of this work, an alkaline eluent with a concentration ranging from 0.1 to 0.2 *M* NaOH was chosen as being a good compromise between reasonable retention times, resolution, magnitude of the amperometric response and minimum residual current noise.

In order to improve the peak shape and to accelerate the elution of organic acids, the effect of addition of a ‘pusher’ species as mobile phase modifiers was investigated. In this respect, sodium acetate solutions ranging from 40 to 80 m*M* were added at 0.1 *M* NaOH carrier electrolyte. The retention order of the investigated analytes was always the same, but as expected, the retention times were significantly reduced with increasing acetate ions concentration in the mobile phase. In addition, the signal response of all organic compounds is enhanced with increasing acetate concentration. The relevant results were summarized in Table 3. Thus, the employment of 0.1 *M* NaOH plus 80 m*M* CH_3COONa as eluent solution allowed the isocratic separation of several common organic acids in less than 20 min with good peak resolution and analytical sensitivity. Under such experimental chromatographic conditions other electroactive molecules such as glucose, fructose, lactose, sucrose, serine, lysine, histidine, cadaverine and alkylamines (methylamine and ethylamine) are co-eluted close to the solvent front. Thus, the presence of a relatively high concentration of other electroactive compounds (i.e. carbohydrates) did not produce visible effects on the

Table 2
Effect of hydroxide concentration on the retention times (t_R), capacity factors (k) and peak area (Pa) of various organic acids separated by an anion-exchange chromatography with a Cu–GC modified electrode[a]

	Gallic	Ascorbic	Gluconic	Lactobionic	Galacturonic	Glucuronic
50 m*M* NaOH + 80 m*M* CH_3COONa						
k	1.1	1.8	2.2	3.1	8.4	14.4
t_R (min)	2.9	4.0	4.5	5.7	16.4	21.55
Pa	11.2	9.5	17.0	11.2	14.0	15.0
100 m*M* NaOH + 80 m*M* CH_3COONa						
k	0.9	1.6	2.3	3.7	8.5	11.0
t_R	2.6	3.7	4.6	6.5	13.3	16.9
Pa	10.3	8.4	16.0	9.1	11.5	12.8
200 m*M* NaOH + 80 m*M* CH_3COONa						
k	0.8	1.3	2.0	3.3	4.9	6.8
t_R	2.3	3.2	4.1	6.0	8.3	10.5
Pa	11.0	8.6	15.8	9.2	9.3	10.7
300 m*M* NaOH + 80 m*M* CH_3COONa						
k	0.5	1.1	1.6	2.6	3.1	4.1
t_R	2.1	2.9	3.6	5.0	5.7	7.2
Pa	10.8	8.1	15.0	9.1	9.3	10.1
400 m*M* NaOH + 80 m*M* CH_3COONa						
k	0.4	0.9	1.3	2.0	2.0	2.8
t_R	2.0	2.6	3.2	4.1	4.1	5.3
Pa	10.2	7.8	14.3	9.0	8.9	9.9

[a] Experimental conditions: Dionex CarboPac PA1 anion-exchange column plus guard column; flow-rate: 1.0 ml/min; column dead time: 1.4 min; concentration: 0.1 m*M* of each injected compound; sample loop: 50 μl; applied potential: 0.55 V vs. Ag–AgCl. The peak area (Pa) is expressed in arbitrary units.

Table 3
Effect of the acetate concentration on the retention times (t_R), capacity factors (k) and peak area (Pa) of various organic acids[a]

	Ascorbic	Gluconic	Lactobionic	Galacturonic	Glucuronic
100 m*M* NaOH + 40 m*M* CH_3COONa					
k	2.9	4.7	9.3	22.5	27.8
t_R (min)	5.5	8.0	14.4	32.9	40.3
Pa	7.4	14.0	8.1	10.5	11.8
100 m*M* NaOH + 60 m*M* CH_3COONa					
k	2.3	3.3	5.8	13.8	17.5
t_R	4.7	6.0	9.6	20.7	26.0
Pa	8.1	15.7	8.6	10.9	12.3
100 m*M* NaOH + 80 m*M* CH_3COONa					
k	1.6	2.3	3.7	8.5	11.0
t_R	3.7	4.6	6.5	13.3	16.9
Pa	8.4	16.0	9.1	11.5	12.8

[a] Experimental conditions as in Table 2.

chromatographic peak of the organic acids investigated.

3.4. Calibration graphs, detection limits and reproducibility

Under the optimum chromatographic conditions described above (0.1 *M* NaOH plus 80 m*M* CH_3COONa), a standard mixture of aliphatic organic acids, using a CarboPac PA1 anion-exchange column, was chromatographed. A typical isocratic chromatogram obtained with a Cu–GC electrode at a constant applied potential of 0.55 V vs. Ag–AgCl and operating under a flow-rate of 1.0 ml/min is illustrated in Fig. 3. The limits of detection (LOD), linear range and reproducibility (RSD%) are summarized in Table 4. For most of these compounds the peak heights varied linearly with the concentration over three order of magnitude range with correlation coefficients of at least 0.99. The detection limits, determined at the lowest injected concentration as a signal-to-noise ratio of 3 are, for all investigated compounds, in the pmol level. The precision expressed as RSD of four repetitive chromatographic experiments (about 2 h of operation time), ranged from 3 to 5%. The Cu–GC modified electrode used here shows a good temporal stability: a freshly prepared electrode after more than 6 h of continuous injections of 0.1 m*M* gluconic acid in flow injection analysis (about 60 injections), exhibited a signal decrease of less than 5%. In addition, the variation of the baseline was quite small: during 6 h of operation time a maximum variation of 10–12% was observed. The retention time reproducibility expressed as the RSD was lower than 4% ($n=4$) for all investigated compounds. In addition, no differences were observed in the retention time and capacity factors of the analysed organic acids during several days of operation with this mobile phase (0.1 *M* NaOH plus 80 m*M* CH_3COONa).

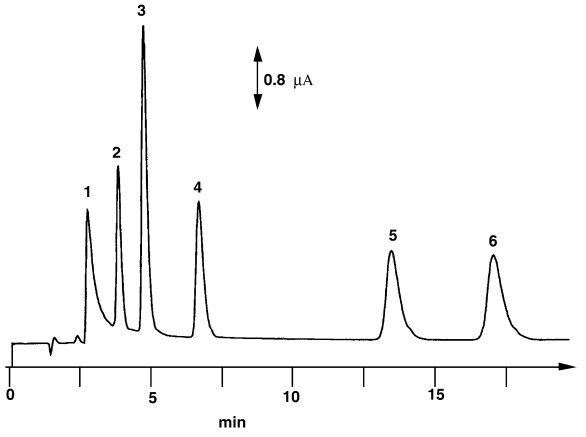

Fig. 3. Isocratic separation of a standard mixture of organic acids containing (1) gallic acid, (2) ascorbic acid, (3) gluconic acid, (4) lactobionic acid, (5) galacturonic acid, (6) glucuronic acid. Experimental conditions: Column: CarboPac PA1 (250×4 mm I.D.) coupled with a guard CarboPac PA1 column (50×4 mm I.D.); mobile phase: 0.1 *M* NaOH plus 80 m*M* CH_3COONa; flow-rate: 1.0 ml/min; amperometric conditions: Cu–GC modified electrode operating at constant applied potential of 0.55 V vs. Ag–AgCl; analyte concentration: about 100 μ*M* of each injected compound; sample loop: 50 μl.

Table 4
Quantitative parameters of some organic acids determined by anion-exchange chromatography using a Cu–GC modified electrode[a]

Compound	LOD (pmol)	Linear range (μ*M*)	Repeatability (%)
Gallic acid	2	0.2–800	5
Ascorbic acid	5	0.5–800	4
Gluconic acid	3	0.5–700	3
Lactobionic acid	3	0.4–500	4
Galacturonic acid	5	0.5–700	5
Glucuronic acid	5	0.4–800	5

[a] Mobile phase: 0.1 *M* NaOH plus 80 m*M* CH_3COONa; other experimental conditions as in Table 2. The repeatability was expressed as relative standard deviation (%) of four repetitive chromatographic experiments at 0.1 m*M* concentration of each analyte considered. The correlation coefficients were always >0.99.

3.5. Analysis of real samples

As anticipated above, the separation and detection of sugar acids is an interesting and challenging topic in analytical biochemistry because these compounds are present in large amounts in foods, beverages, body fluids etc., and are involved in many biochemical, pathological and physiological processes. In this section, in order to demonstrate the easy and simple use of the present analytical method for practical applications, some selected applications of the determination of ascorbic, gluconic and galacturonic acids in commercially available foods and beverages

are reported. Peak identification was based on the retention time of the specified analytes and was confirmed by spiking authentic standard solutions to the sample extract. Recoveries were evaluated for each constituent by spiking the extraction solutions with pure compounds and performing triplicate assays after each addition.

3.6. Modena balsamic vinegar and cider vinegar

Commercial samples of Modena balsamic (MBV) and cider vinegars have been analysed with regard to gluconic and galacturonic acid. Generally, the amount of gluconic acid observed in MBV samples, comprised between 0.1 and 7 g/100 ml of product and is always higher than that observed in other vinegars (i.e. wine, cider, etc.) [37]. The high values of gluconic acid observed in the MBV samples, are due to the catabolism of the glucose by the acetic acid bacteria. It is thus suggested that the gluconic acid may be considered as a parameter of the genuineness of MBV [38]. A typical chromatogram of a commercially available MBV is shown in Fig. 4. As comparison, a chromatogram of a cider vinegar sample is shown in Fig. 5. In this last sample, the chromatographic peak of ascorbic acid was also identified and quantified. The relevant amount and recovery data of analyzed sugar acids are summarized in Table 5. In agreement with literature data [37,38], the analyses of vinegars extracts revealed a higher concentration level of gluconic acid in the MBV sample than in the cider vinegar. Thus, the present method can be used to check the quality level of various vinegars samples.

3.7. Orange honey

Natural enzyme activity in honey leads to the production of free sugar acids so that a high acidity is indicative of prolonged storage of the product [39]. Thus, the direct analytical determination of these compounds, represents an important step to ascertain the quality level of the honey samples. The relevant chromatogram of a diluted 1:500 orange honey is illustrated in Fig. 6 and in Table 5 are collected the results obtained for this sample.

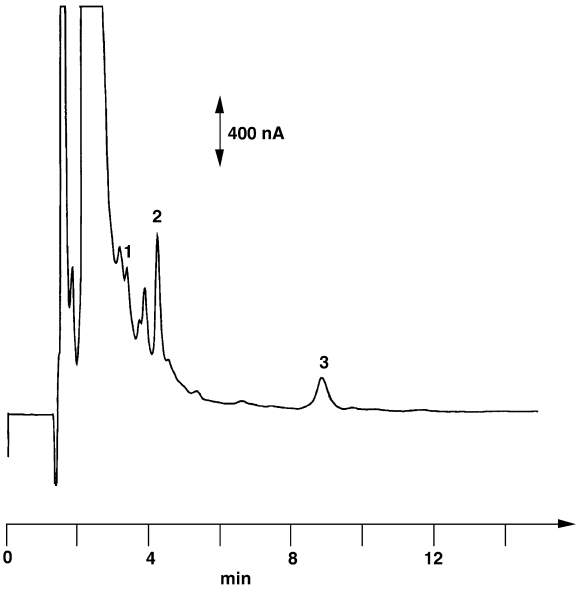

Fig. 4. Typical chromatogram of a balsamic vinegar of Modena (BMV) (diluted 1:200 with distilled water). Peaks: 1 = ascorbic acid, 2 = gluconic acid, 3 = galacturonic acid. Unlabeled peaks are unknown compounds. Experimental conditions: Mobile phase: 0.15 *M* NaOH plus 80 m*M* CH_3COONa; other experimental conditions as in Fig. 3.

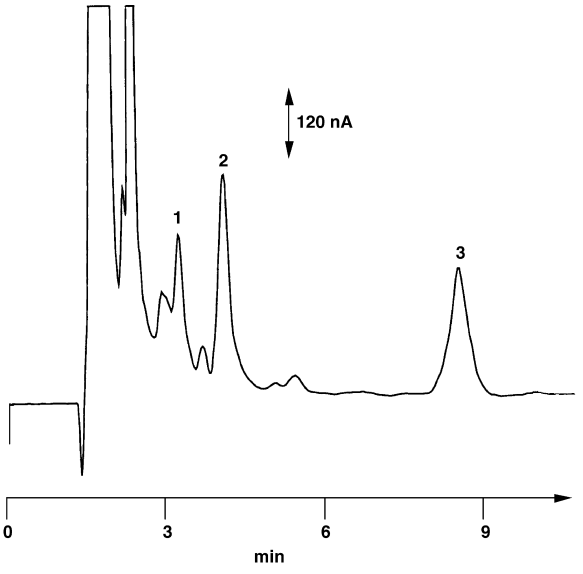

Fig. 5. Chromatogram of a cider vinegar sample (diluted 1:25 with distilled water). Peaks: 1 = ascorbic acid, 2 = gluconic acid, 3 = galacturonic acid. Experimental conditions as in Fig. 4.

Table 5
Analyses of some organic acids in food samples by anion-exchange chromatography with a Cu–GC modified electrode used as amperometric sensor[a]

	MBV	Cider vinegar	Honey	Tea
Ascorbic acid				
Found	–	14.9 mg/100 g	–	3.6 mg/g
Recovery (%)	–	91	–	95
Gluconic acid				
Found	122 mg/100 g	7.3 mg/100 g	2.9 mg/g	–
Recovery (%)	97	95	98	–
Galacturonic acid				
Found	47.7 mg/100 g	9.5 mg/100 g	–	–
Recovery (%)	96	95	–	–

[a] The real samples were treated as reported in the experimental section. Recoveries were evaluated for each analyte determined by spiking the extraction solutions with the relevant organic acid component at the level of about 30–50% of the measured content. The concentrations were evaluated by the standard addition method (four addition). The precision, expressed as RSD by three repetitive injections of the sample extract, was between 6 and 9% for all determined molecules.

3.8. Tea powder

Non-enzymatic browning has been considered one of major causes of quality and color loss during the processing and storage of food products. In this respect, ascorbic acid is widely used in foods as antioxidant for the stabilization of color and aroma with subsequent extension of the storage time of the products. Thus, a simple and inexpensive analytical method for the determination of ascorbic acid during production, transformation and storage of food products is of particular importance in industrial context. Fig. 7 shows a typical chromatogram of an extract of tea powder sample; a good separated peak of ascorbic acid was observed and its relevant amount is reported in Table 5.

4. Conclusions

A simple, sensitive and reproducible chromatographic–amperometric method for the determination

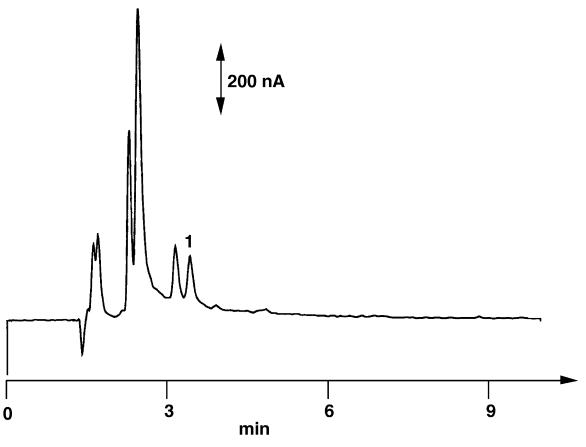

Fig. 6. Anion-exchange chromatographic profile of a orange honey (diluted 1:500 with distilled water). Peaks: 1 = gluconic acid. Experimental conditions as in Fig. 4.

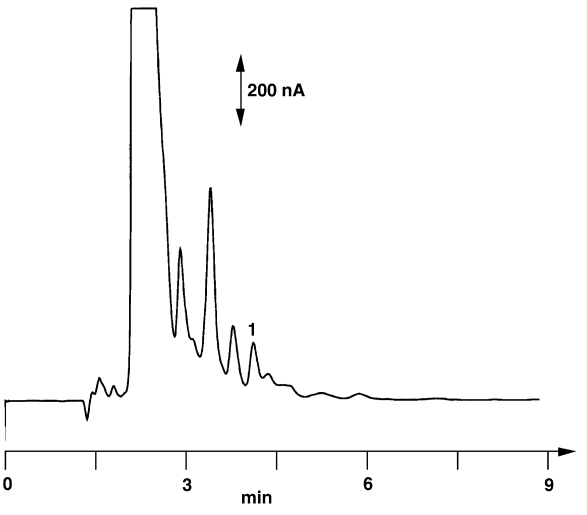

Fig. 7. Anion-exchange chromatographic profile of a tea powder sample extract (diluted 1:100 with distilled water). Peaks: 1 = ascorbic acid. Experimental conditions as in Fig. 4.

of some organic acids in several real samples has been developed. The proposed method combines, under isocratic chromatographic conditions, simple, rapid analysis times with ease of sample preparation. In addition, the Cu–GC modified electrode employed here represents a very effective sensing probe for constant-potential amperometric detection in flowing alkaline solutions. The employment of alkaline mobile phases (i.e. 0.1 or 0.15 *M* NaOH plus 80 Mm CH_3COONa) as eluent solution allowed the isocratic separation of several common organic acids in less than 20 min with good peak resolution and analytical sensitivity. The present method appears to be very useful for the practical determination of several common organic acids in real matrices without any complicated extraction or derivatization procedures and with a good level of sensitivity and recoveries.

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