

Chemically Modified Carbon Paste Electrodes for Ascorbic Acid Determination in Soft Drinks by Flow Injection Amperometric Analysis

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Abstract

Simple, rapid and precise amperometric methods for quantification of ascorbic acid (AA) are presented. Glassy carbon (GC), carbon paste (CP) and modified carbon paste (MCP) electrodes are used for this purpose. MCP electrodes are of CP with 10 % vanadate (V). All electrodes are inserted in a wall-jet device with an Ag/AgCl reference electrode and a platinum auxiliary electrode. This device is coupled to a flow injection analysis (FIA) set-up. Hydrodynamic and amperometric parameters are optimized unvaryingly.

GC electrodes present slopes of 4.75×10^5 nA L mol⁻¹ (± 6.4 %) under optimum conditions. CP and MCP electrodes show higher sensitivity, with slopes of 6.37×10^5 nA L mol⁻¹ (± 6.6 %) and 7.32×10^5 nA L mol⁻¹ (± 4.4 %). Linear responses range $(1.0-2.0) \times 10^{-6}$ to $(0.8-1.0) \times 10^{-5}$ mol L⁻¹. Correlation coefficients were > 0.994 and about 48 samples are analysed *per* hour. Application of the proposed method to the analysis of soft drinks is presented.

Keywords: ascorbic acid, chemically modified electrodes, flow-injection analysis, amperometry.

Introduction

Antioxidant defences are extremely important for biological sites when under oxidative stress [1], a process in which the dynamic redox balance between oxidants and antioxidants is intensely shifted towards oxidative potentials. Antioxidants play here an important role because of their ability to scavenge

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radicals, neutralizing the highly instable free radical molecules by supplying them with electrons and preventing, or at least limiting, the drain reactions that cause tissue damage [2]. The intake of dietary antioxidants such as vitamin C may provide higher antioxidant protection. The small stability of this antioxidant when in aqueous media advises the rigorous control of its levels in commercial beverages.

For this purpose, a wide variety of methods are presented in literature. Methods based on spectrophotometric readings of UV [3-5] and Vis [6-13] radiations, fluorimetric [14-21] and HPLC [22-32] are frequently reported. They regard optical and separative techniques, and may require more or less sophisticated equipment and extensive sample preparation procedures.

Electroanalytical based methods are currently widely used in many fields because they meet the need of the analytical chemist for rapid, low-cost and accurate analysis. Several voltammetric [33-36] and amperometric [37-46] methods are also reported for AA determination. These are concerned with the interplay between electricity and chemistry, namely the measurements of electrical quantities, such as current, potential and charge, and their relationship to chemical parameters. Several modifications have been tried out to enhance the analytical features of current devices. Mediators of osmium [39], phthalocyanine cobalt (II) [40], platinum [41], dichlorophenolindophenol [42], ferrocene and β -cyclodextrin [43], ruthenium oxide [44], dihidroxibenzaldehyde [45], and tetracyano-p-quinodimet [46] are described to enhance the analytical features of the devices.

The present work describes the electrochemical determination of vitamin C by means of several carbon based electrodes. Electrodes are made of GC, CP or MCP, and are inserted in a wall-jet device coupled to a FIA. Operating conditions of the flow system are optimized unvaryingly and operational features evaluated.

Experimental

Apparatus

The flow injection assembly had a Gilson Minipuls 3 peristaltic pump equipped with pumping tube of the same brand. Samples and standards were introduced into the carrier stream through a six-port Rheodyne[®] 5020 injection valve of exchangeable injection volumes. Omnifit PTFE tubes (0.8 mm i.d.) connected by Gilson[®] end-fittings and joints were used in the construction of the manifold. Confluence points were made in Perspex[®] as described elsewhere [47].

Amperometric detection was carried out in a Metrohm 656 wall-jet cell. Commercial working, reference and auxiliary electrodes were of glassy carbon (Metrohm 6.0805.01), Ag/AgCl (KCl 3.00 mol L⁻¹, Metrohm 6.0727.000), and of gold (Metrohm 6.530.320), respectively. A Metrohm 641 VA-detector was used as amperometric detector and its output signals were recorded in a Kipp & Zonnen BD 111 strip chart recorder.

When required, the glassy carbon electrode was cleaned mechanically by polishing its surface in a special kit (Metrohm 6.2802.010) with α -Al₂O₃ (0.3 μ m). Before use, the electrode was washed with water and dried on tissue paper.

Reagents and solutions

All reagents used were of analytical grade and water was purified with a Milli-Q Millipore system. L-(+)-ascorbic acid (AA) was purchased from Riedel-de-Häen. Ionic strength and pH adjustments required sodium hydroxide (Merck), sodium hydrogen phosphate (Merck), potassium dihydrogen phosphate (Riedel-de-Häen), acetic acid (Merck), phosphoric acid (Merck), boric acid (Riedel-de-Häen) and potassium nitrate (Vaz Pereira). Epoxy resin (from local stores), graphite (Sigma), mineral oil (Fluka), and ammonium vanadate (V) (AMV, Riedel-de-Häen) were used to prepare the electrodes.

Stock solutions of AA ($1.00 \times 10^{-3} \text{ mol L}^{-1}$) were prepared daily by dissolving in water an accurate amount of solid. The resulting solution was kept in the dark and in a refrigerator at $+4 \text{ }^{\circ}\text{C}$. Working standards were prepared by accurate dilution of the previous ones in deionised water. Soft drinks were purchased in local stores.

Procedures

MCP electrodes were prepared by dispersing in a mortar 0.1 g of ammonium vanadate (V) in about 1.0 g of graphite powder. This mixture was added of 300 μL of mineral oil and 0.4 g of epoxy resin. The final paste was packed before hardening into an electrode body, consisting of plastic tube with a copper wire serving as an external electric contact. CP electrodes were prepared similarly by removing the redox mediator from the graphite paste.

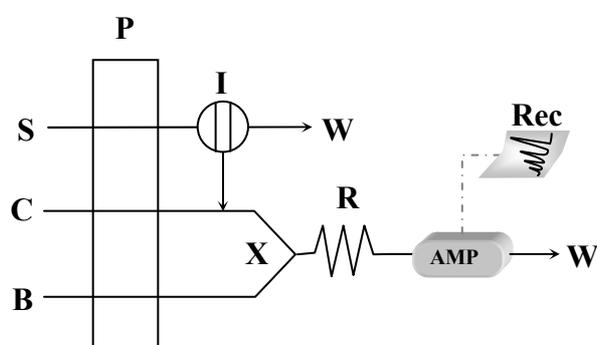


Figure 1. Schematic view of flow set-up. S: sample; C: carrier stream of deionised water; B: buffer solution; X: confluence point; R: coiled reactor; P: peristaltic pump; I: injection unit; W: waste; AMP: amperometric detector; Rec: recorder.

The diagram of the flow assembly is depicted in Fig. 1. Standard or sample solutions (S) were injected into a deionised water carrier stream (C) that merged in confluence X with a buffer solution (B). A coiled reactor (R) ensured convenient mixture between solutions of confluent channels. The detection device combined the working electrode (of GC, CP or MCP), an Ag/AgCl reference electrode, and an auxiliary electrode made of gold. The signal output was recorded in a strip-chart recorder. FI signals were evaluated in terms of peak

height, and after transformed in current (μA). Samples were analysed after suitable dilution with water (to fit the linear range), and results were compared with the FAO procedure [48].

Results and discussion

To provide AA determinations of enhanced sensitivity, several electrodes were investigated. One detector was a commercial device made of GC. The other electrodes were home-made, presenting a conventional configuration filled with CP or MCP having vanadate as redox mediator.

All electrodes were evaluated in flow media, having as carrier a phosphate buffer of pH 6.5. This buffer was selected in agreement with previous trial experiments conducted with all units. Main hydrodynamic variables of the flow set-up were optimized to enable sensitive and reproducible measurements with high sampling throughputs. Selection of optimum conditions followed univariant procedures.

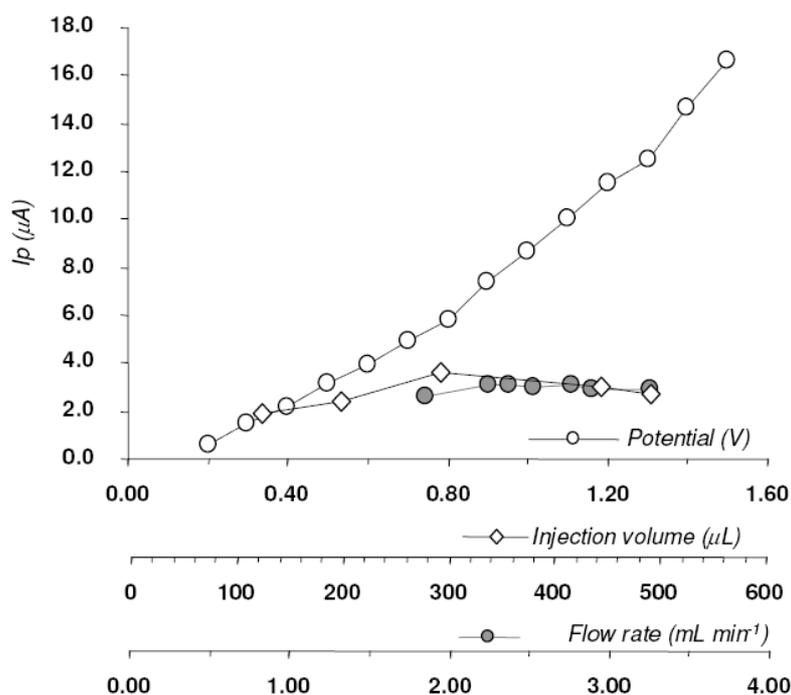


Figure 2. Values of i_p for $5.0 \times 10^{-6} \text{ mol L}^{-1}$ AA solutions measured with GC electrodes at several potentials, injection volumes and flow-rates.

Glassy carbon electrode

Selection of electrode potential was made after recording the peak heights of $5.0 \times 10^{-6} \text{ mol L}^{-1}$ AA solutions for different potential values (E), varying within 0.2 and 1.5 V. Plot of E (in V) against i_p (Fig. 2) showed that an increase in potential corresponded to a proportional increase in current. Considering that the higher potentials would induce interfering effects of coexisting compounds, and the lower ones would provide reduced sensitivity, the detection potential was set to +0.8 V versus an Ag/AgCl (3 mol L^{-1} KCl).

Injection volume was varied between 130 and 510 μL (table 1). Amperometric signals increased with sampling volumes (Fig. 2), until 370 μL was injected. The higher sampling volumes produced a slight decrease in i_p , thus suggesting that long residence time of the sample plug would lead to a memory effect at the detection unit. In addition, the cell washout process took longer, and conducted to decreased sampling rates. Results pointed out the selection of 300 μL at further experiments.

Table 1. Operating variables under study for the flow injection amperometric system.

<i>Parameter</i>	<i>Condition</i>	<i>Studied range</i>	<i>GC</i>	<i>CP</i>	<i>MCP</i>
Hydrodynamic variables	Flow rate (mL min^{-1})	1.8 – 5.2	2.3	2.3	2.3
	Injection volume (μL)	130 – 510	300	250	250
Amperometric analyser	Electrode potential ^{a)} (V)	0.4 – 1.6	+ 0.8 V	+ 0.8 V	+ 0.8 V
	Sensitivity ($\mu\text{A V}^{-1}$)	—	10	10	10
	Measurement mode	—	(peak height, time based)		

^{a)} versus Ag/AgCl, 3 mol L^{-1} KCl.

Total flow-rate was varied from 1.8 to 3.2 mL min^{-1} (table 1). This interval considered limitations of flow pressure exerted upon the wall-jet cell [49], with a dead volume of about 1 μL . No significant i_p variations were observed within this range (Fig. 2). Flow rates higher than 2.3 mL min^{-1} were not satisfactory since they produced irreproducible signals in result of the high pressure exerted by flowing solutions. Lower flow rates gave reproducible signals but provided reduced sampling rates. As a compromise, results pointed out the selection of a 2.3 mL min^{-1} flow rate (table 1).

Carbon paste electrode

Effect of amperometric and hydrodynamic variables at the CP electrode response was evaluated for 5.0×10^{-6} mol L^{-1} AA solutions. This study was carried out similarly to that previously described for GC electrodes.

Potential increase from 0.2 up to 1.5 V provided higher output signals. This behavior is similar to the one presented by the GC electrode. Thus, and due to the previously mentioned reasons, the detection potential was set to +0.8 V.

Injection volume was changed from 130 to 510 μL (table 1). Amperometric signals were increased for increasing sampling volumes, as can be seen in Fig. 3. Higher injection volumes required more sample volume and provided longer residence times at the detection unit, conducting to longer cell washout periods, and to decreased sampling rates. Small injection volumes were also responsible for decreased analytical signals, conducting to decreased sensitivities. As a

compromise, an injection volume of 250 μL was selected in subsequent experiments.

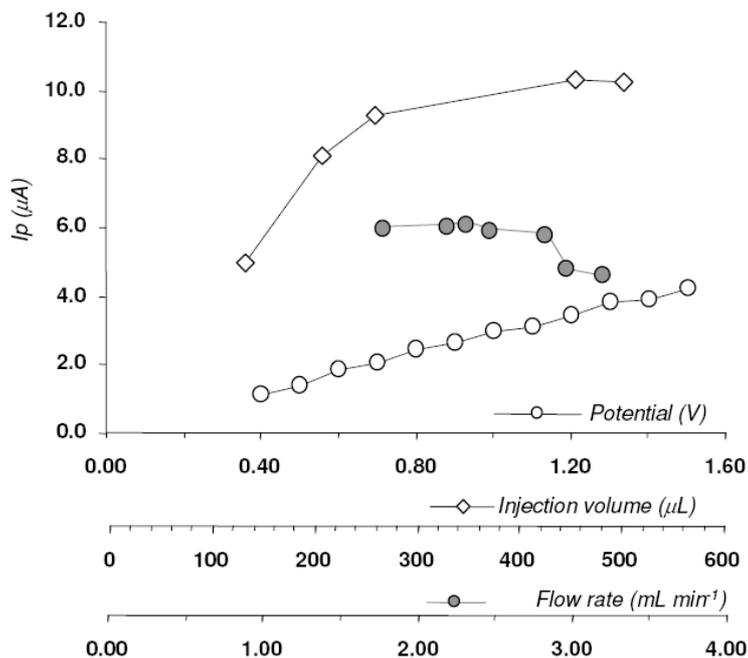


Figure 3. Values of i_p for $5.0 \times 10^{-6} \text{ mol L}^{-1}$ AA solutions measured with CP electrodes at several potentials, injection volumes and flow-rates.

Flow-rates $> 2.8 \text{ mL min}^{-1}$ conducted to a slight decrease in i_p (Fig. 4). This result was coupled to an increase of sampling rate, as solution reached and left the detector more quickly. As a compromise between sensitivity and sampling rates, the flow-rate was set to 2.3 mL min^{-1} (table 1), the same value as the one selected previously for the GC electrode.

Modified carbon past electrode

Selection of amperometric and hydrodynamic conditions followed similar optimization steps to those of previous studies. Optimum potential was selected after injection of $250 \mu\text{L}$ of $1.0 \times 10^{-5} \text{ mol L}^{-1}$ AA solution. A similar behaviour to that recorded for previously studied electrodes was observed, conducting to the selection of $+0.8 \text{ V}$.

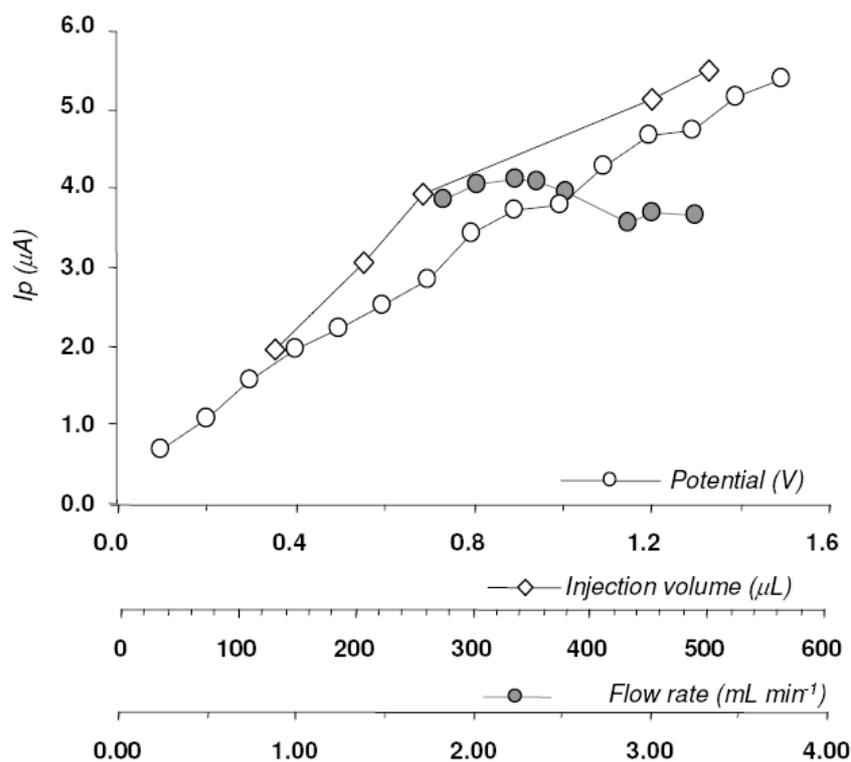


Figure 4. Values of i_p for $1.0 \times 10^{-5} \text{ mol L}^{-1}$ AA solutions measured with MCP electrodes at several potentials, injection volumes and flow-rates.

Table 2. Main analytical features of the proposed amperometric system.

<i>Analytical features</i>	<i>GC</i>	<i>CP</i>	<i>MCP</i>
<i>Slope ($L \text{ nA}^{-1} \text{ mol}^{-1}$)</i>	$4.75 \times 10^5 (\pm 6.4 \%)$	$6.37 \times 10^5 (\pm 6.6 \%)$	$7.32 \times 10^5 (\pm 4.4 \%)$
<i>Intercept (nA)</i>	0.295 ± 0.038	1.816 ± 0.249	2.440 ± 0.665
<i>Correlation coefficient</i>	> 0.9987	> 0.9986	> 0.9981
<i>LLLR (mol L^{-1})</i>	1×10^{-6}	2×10^{-6}	2×10^{-6}
<i>SLLR (mol L^{-1})</i>	1×10^{-4}	8×10^{-5}	1×10^{-4}
<i>SR (samples h^{-1})</i>	48	47	48

LLLR: Lower Limit of Linear Range; SLLR: Superior Limit of Linear Range; SR: Sampling Rate.

Effect of injection volume in i_p values was more evident for MCP electrodes. The analytical signal increased as sampling volumes increased (Fig. 3), and did not tend to steady state within the studied range. The injection volume of $250 \mu\text{L}$ was selected, as it would allow straight comparison between the analytical features of CP and MCP electrodes.

The effect of flow-rate within 1.8 to 3.2 mL min⁻¹ was similar to that recorded for CP electrodes (Fig. 2 and 3). Only a slight decrease was recorded for solutions flowing above 2.8 mL min⁻¹. Following a compromise between sampling rate and sensitivity, and in search of comparable conditions within all electrodes, 2.3 mL min⁻¹ was selected for flow rate. Average sampling rates were 48 samples *per* hour by using this condition.

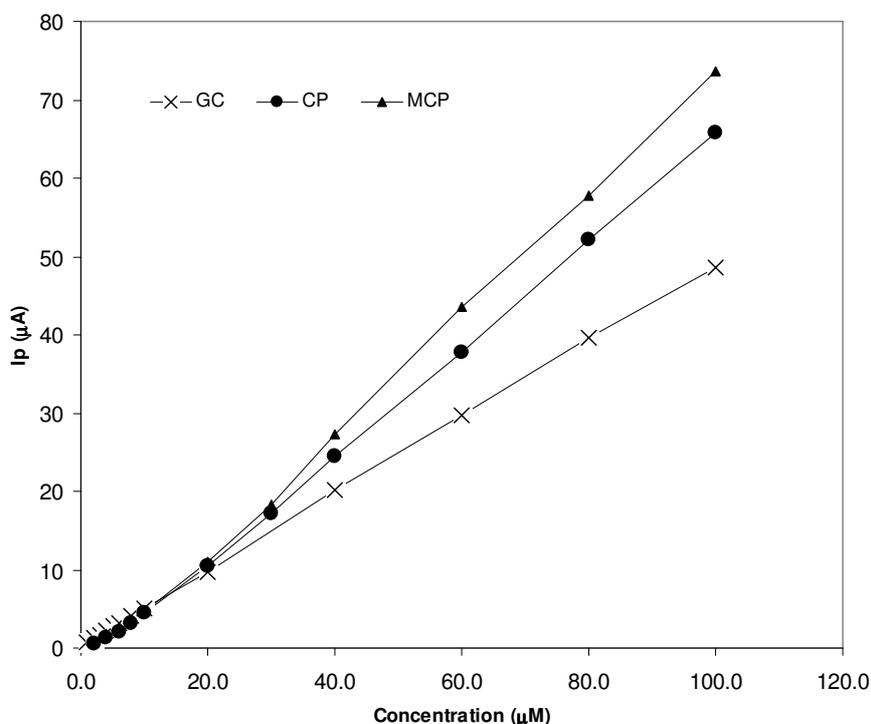


Figure 5. Calibration curves of GC, CP and MCP electrodes recorder under optimum conditions.

Main analytical features

Table 2 shows the main features from calibration plots obtained with the selected experimental conditions. Amperometric responses showed a linear behaviour for a series of AA standard solutions ranging $(1.0-2.0) \times 10^{-6}$ to $(0.8-1.0) \times 10^{-5}$ mol L⁻¹, with correlation coefficients > 0.994 . GC electrodes were those of smaller sensitivity (Fig. 5), with average slopes of 4.75×10^5 nA L mol⁻¹ (± 6.4 %). A 34 % increase was attained by using the CP electrodes (table 2). This increase is most probably correlated to the higher carbon surface of the home-made CP electrodes. Addition of vanadate (V) to the carbon matrix provided a 54 % increase in sensitivity when compared to GC and a 15 % increase when compared to the CP. Thus, the chemical modification of vanadate (V) improved the analytical response of the electrode. The number of readings *per* hour was about 48 when standard solutions were injected (Fig. 6).

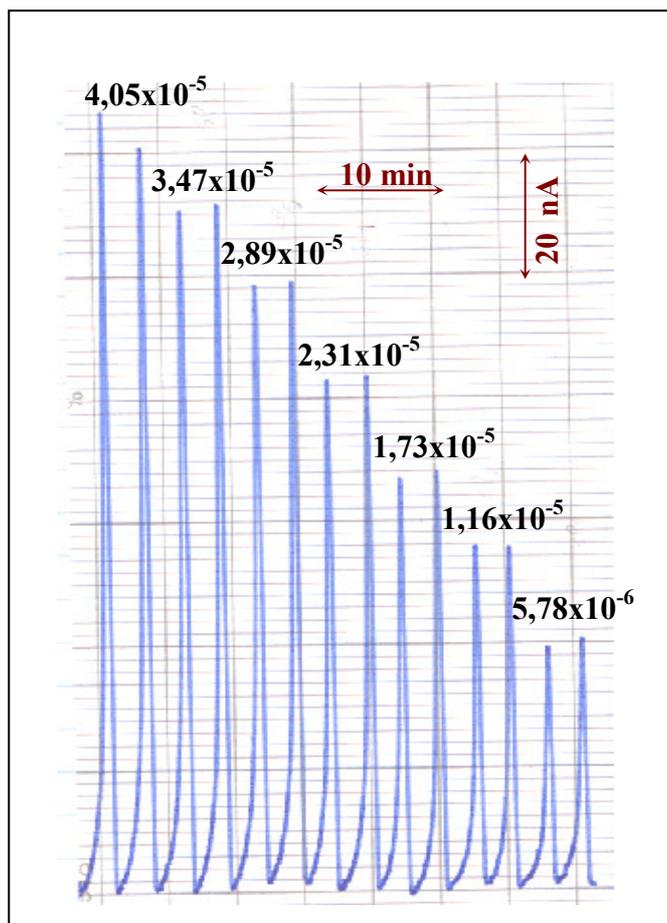


Figure 6. Diagram of CP electrodes after the injection of a set of a standards of a AA samples. Injection volume: 250 μL ; flow rate: 2.3 mL min^{-1} ; and pH 6.5.

Table 3. AA content in commercial soft drinks determined by the proposed amperometric method (AMP) and the comparison method (COMP).

<i>Sample</i>	AA (mg L^{-1})		<i>Relative error (%)</i>
	AMP	COMP	
<i>I</i>	214 ($\pm 9\%$)	210 ($\pm 6\%$)	+1.9
<i>II</i>	206 ($\pm 8\%$)	215 ($\pm 6\%$)	-4.2
<i>III</i>	209 ($\pm 8\%$)	221 ($\pm 4\%$)	-5.4

Analysis of samples

To evaluate the suitability of the amperometric method for the analysis of beverages, several commercial soft drinks were analysed by the proposed method. The MCP electrode was used as detection unit. Amperometric analysis was carried out after injecting samples that were previous diluted with water. Concentrations of AA were calculated from preceding calibration procedures. Mean results of three determinations are given in table 3. Comparison to an independent method confirms the accuracy of the results provided by the

amperometric method, with relative errors below 6%. Precision of the results is also confirmed after the small relative standard deviations.

Conclusions

All electrodes are suitable devices to monitor the electrochemical oxidation of AA. They present wide linear response ranges, with lower limits around 1×10^{-6} mol L⁻¹, and MCP electrodes offer the higher sensitivity. Overall, the analytical procedure is fast and simple, of low cost and of small environmental impact. All these are suitable features when routine measurements are meant. The method seems precise and accurate, and is inexpensive regarding reagent consumption and equipment involved. Several soft drinks are analyzed by the proposed system and results compare well to an independent method.

References

1. R. Kohen, A. Nyska, *Toxicol. Pathol.* 30 (2002) 620–650.
2. T. Ueki, Y. Noda, Y. Teramoto, R. Ohba, S. Ueda, *J. Ferment. Bioeng.* 78 (1994) 262–264.
3. M. Surmeian, *Drug Develop. Ind. Pharm.* 24 (1998) 691–696.
4. M.I. Toral, N. Lara, P. Richtu, A. Tassara, A.E. Tapia, C. Rodriguez, *J. AOAC Int.* 84 (2001) 37–42.
5. A.R. Medina, M.L.F. Cordora, A.M. Diaz, *J. Pharm. Biomed. Anal.* 20 (1999) 247–254.
6. M. Karatepe, *LC GC North America Suppl. S* (2004) 104–106.
7. J.H. Khorasani, M.K. Amini, H. Ghanei, S. Tangestaninejad, *Iranian J. Chem. & Chem. Eng. Int.* 20 (2001) 66–74.
8. M. Noroozifar, M. Khorasani-Motlagh, *Turkish J. Chem.* 26 (2003) 717–722.
9. M. Noroozifar, M. Khorasani-Motlagh, *Talanta* 61 (2003) 173–179.
10. N. Lenghor, J. Jakmunee, M. Vilen, R. Sara, G.D. Christian, K. Grupdon, *Talanta* 58 (2002) 1139–1144.
11. A.A. Ensafi, B. Rezaei, M. Beglari, *Anal. Lett.* 35 (2002) 909–920.
12. T. Lenarezuk, S. Glab, R. Koncki, *J. Pharm. Biomed. Anal.* 26 (2001) 163–169.
13. M. Du, F.Z. Liang, B. Tang, Y.J. Luo, H.X. Shen, B. Teng, Y. Liu, *Chin. Chem. Lett.* 11 (2000) 23–26.
14. X. Wu, Y.X. Diao, C.X. Sun, J.H. Yang, Y.B. Wang, *Talanta* 59 (2003) 95–99.
15. R.Y. Wang, Z.H. Lin, R.X. Cai, X.Y. Li, *Anal. Sci.* 18 (2002) 977–980.
16. T.P. Ruiz, C.M. Lozano, V. Tomas, J. Fenol, *Analyst* 126 (2001) 1436–1439.
17. J.H. Yang, C.X. Sun, X. Wu, Y.X. Diao, *Anal. Lett.* 34 (2001) 1331–1339.
18. Z.H. Lin, Q. Wang, L.Y. Mao, R.X. Cai, *Anal. Chim. Acta* 413 (2000) 167–173.
19. J.H. Yang, Q.L. Ma, F. Huang, L.M. Sun, J.A. Dong, *Anal. Lett.* 31 (1998) 2757–2766.

20. A.A. Enfasi, B. Rezaei, *Anal. Lett.* 31 (1998) 333–342.
21. T.P. Ruiz, C.M. Lozano, V. Tomas, C. Sidrach, *Analyst* 122 (1997) 115–118.
22. M. Karatepe, *LCGC North America Suppl. S* (2004) 104–106.
23. H. Iwase, *Talanta* 60 (2003) 1011–1021.
24. A.R.B. Quiros, J.L. Hernandez, J.S. Lozano, *Chromatogr.* 53 (2001) S246–S249.
25. L.D. Muller, *J. Pharm. Biomed. Anal.* 25 (2001) 985–994.
26. R.N. Castro, L.C. Azevedo, M.A.A. Azevedo, C.S.T. Sampaio, *J. Liq. Chromatogr. & Rel. Techn.* 27 (2001) 1015–1020.
27. N. Kaid, J. Potus, J. Nicolas, *Sciences Des Aliments* 20 (2000) 237–251.
28. H. Iwase, *J. Chromatogr. A* 881 (2000) 317–326.
29. A. Rumelin, U. Fauth, M. Halmagyi, *Clin. Chem. Lab. Med.* 37 (1999) 533–536.
30. M.A. Kall, C. Andersen, *J. Chromatogr. B* 730 (1999) 101–111.
31. M.A. Moser, H.H. Dorfner, B. Kniel, *Deutsche Lebensmittel – Rundschau*, 95 (1999) 180–185.
32. H. Iwase, I. Ono, *J. Chromatogr. A* 806 (1998) 361–364.
33. Y.S. Fung, S.Y. Mo, *Anal. Chim. Acta* 261 (1992) 375–380.
34. G.R. Zhang, X.L. Wang, X.W. Shi, T.L. Sun, *Talanta* 51 (2000) 1019–1025.
35. J. Wu, J. Suls, W. Sansen, *Electrochem. Commun.* 2 (2000) 90–93.
36. Z.Q. Gao, K.S. Siow, A. Ng, Y.M. Zhang, *Anal. Chim. Acta* 343 (1997) 49–57.
37. Y.S. Fung, S.Y. Mo, *Anal. Chim. Acta* 261 (1992) 375–380.
38. A.M. Almubeil, A. Townshend, *Talanta* 39 (1992) 1459–1462.
39. J.J. Fei, L.M. Luo, S.S. Hu, Z.Q. Gao, *Electroanalysis* 16 (2004) 319–323.
40. J.H. Khorasani, M.K. Amini, H. Ghanei, S. Tangestaninejad, *Iranian J. Chem. & Chem. Eng.* 20 (2001) 66–74.
41. R.A.A. Munoz, R.C. Matos, L. Angnes, *Talanta*, 55 (2001) 855–860.
42. A.B. Florou, M.I. Prodromidis, S.M.T. Karayanni, M.I. Karayanis, *Anal. Chim. Acta* 423 (2000) 107–114.
43. G.R. Zhang, X.L. Wang, X.W. Shi, T.L. Sun, *Talanta* 51 (2000) 1019–1025.
44. J. Wu, J. Suls, W. Sansen, *Electrochem. Commun.* 2 (2000) 90–93.
45. Z.Q. Gao, K.S. Siow, A. Ng, Y.M. Zhang, *Anal. Chim. Acta* 343 (1997) 49–57.
46. J. Kulys, E.J. Dcosta, *Anal. Chim. Acta* 243 (1991) 173–178.
47. S. Alegret, J. Alonso, J. Bartroli, A.A.S.C. Machado, J.L.F.C. Lima, J.M. Paulis, *Quim. Anal.* 6 (1987) 278.
48. <http://www.fao.org/ag/agn/jecfa-additives/specs/Monograph1/Additive-043.pdf>, accessed on the 30th of August of 2007.
49. Metrohm, Electrochemical detection in HPLC, Metrohm AG CH-9100, Herisau, (1984).