Simultaneous Determination of Acetaminophen (Paracetamol) and Ascorbic Acid in Pharmaceutical Formulations by LC Coupled to a Screen Printed Carbon Based Amperometric Detector

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Abstract
The assay of acetaminophen (APAP) and ascorbic acid (AA) was performed by liquid chromatography (LC) with amperometric detection at a screen printed carbon based electrode poised at +0.6 V versus Ag pseudoreference electrode. A microparticle based conventional RP-C18 and a monolithic RP-C18 column were studied comparatively for the analytes separation using a mobile phase based on 0. 1 M phosphate buffer-methanol 91 : 9 (v/v) at pH 6.5. The linear calibration range for both compounds was comprised within 2×10^{-7}–1×10^{-4} M. Limit of detection (*LOD*) for APAP and AA was 2×10^{-7} and 5×10^{-7} M, respectively and the limit of quantification (*LOQ*) was 5. $10^{-7}$and 1×10^{-6} M, respectively. Square wave voltammetry at a Nafion modified glassy carbon electrode was developed and applied to the determination of APAP in order to validate the results obtained by the monolithic LC method for drug formulations.

Keywords: Acetaminophen, Screen printed electrodes, Monolithic column, Nafion modified electrodes

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1 Introduction

Acetaminophen (APAP) is a very popular analgesic and a nonsteroidal moderate anti-inflammatory drug. It is a non carcinogenic and an effective substitute of aspirin for patients who cannot tolerate aspirin. At normal therapeutic doses, APAP is rapidly and extensively metabolized by undergoing glucuronidation and sulfation to inactive metabolites which are eliminated in urine along with 5% of APAP being eliminated unchanged [1–3]. Overdoses of APAP may cause several severe disorders such as hepatic and renal necrosis [1–4]. The hydrolysis of APAP principally generates *p*-aminophenol (PAP) which could be present in pharmaceutical preparations as a degradation product of APAP or as a synthetic intermediate. PAP has been reported to exhibit serious side effects and teratogenic problems as well as nephrotoxicity [1] The acceptable limit of PAP in the drug raw material is 0.005% and 0.1% w/w in tablet formulations [5,6].

A very large number of analytical techniques for the determination of APAP in biological fluids and pharmaceutical formulations have been published such as direct redox titrimetry, UV-vis spectrophotometry, fluorimetry, infrared spectrophotometry, electrochemistry (EC), liquid chromatography or capillary electrophoresis coupled to UV-vis, amperometric and mass spectrometric detectors, flow injection analysis using different methods of detection such as UV-vis and EC detectors. A thorough review on APAP determination in pharmaceutical and biological samples has recently been published [7]. Electroanalytical methods are well suited for APAP determination since the molecule is readily oxidized at a low applied potential. Differential pulse or square wave voltammetry at bare [8,9] or modified electrodes [10–33] and amperometry coupled to liquid chromatography [34,35] or capillary electrophoresis have been described [36].

Ascorbic acid (AA) is an essential human nutrient and an important compound from the clinical and the food industrial points of view. It is well known that it is one of the major interfering species in electroanalysis which may often be encountered in biological fluids and in drug formulations. AA can be present in some APAP formulations since it has been reported to have a protective role in vivo with respect to APAP hepatotoxicity [37] although some controversy exist on this matter [38]. The oxidation potential of AA is rather close to the oxidation potential

of APAP at a bare electrode in physiological pH [9,19,22], precluding its determination without any previous separation step or electrode surface modification [19,22]. The determination of AA by electrooxidation with conventional electrodes, such as Hg, Au, Pt, and glassy carbon electrode (GCE), is hampered due to a sluggish electron transfer giving rise to low response currents spread over a large potential domain. This problem can eventually be solved by electrode surface modification with a redox mediator [19,22], surface activation or by dispersion of metal oxide particles onto the surface [39].

Some recent methods have utilized screen printed carbon based electrodes for the individual determination of *p*-aminophenol [40], acetaminophen [41] and ascorbic acid [42,43] in a flow injection analysis set up. The technology based on screen-printing of carbon or metallic based pastes onto various solid substrates allows for mass production of EC detectors in a reproducible manner [44,45] opening new horizons in LC-EC. The SPE_s have, however, seldom been applied to LC chromatography and monolithic LC columns have till now seen limited coupling to electrochemical detectors [46]. Such columns exhibit a lower back-pressure and separations are performed with a shorter analysis time compared to conventional microparticles based columns as reported in thorough recent review articles [47,48].

The aim of the present work was the development of a rapid and sensitive method for simultaneous quantification of acetaminophen and ascorbic acid by a monolithic based LC coupled to a single wall carbon nanotube based SPE housed in a wall-jet flow cell configuration. The analytical usefulness of the proposed electrochemical methodology was demonstrated by the determination of these two molecules, single or combined, in some pharmaceutical formulations.

2 Experimental

2.1 Chemicals and Reagents

Acetaminophen was supplied by SERVA (Heidelberg, Germany) and p-aminophenol, ascorbic acid and sodium hydroxide by ACROS organics (Geel, Belgium). Sodium di-hydrogen phosphate di-hydrate was from Fluka/chemika. Formic acid 85%, di-sodium hydrogen phosphate dihydrate, sodium nitrate were of analytical grade from Merck (Darmstadt, Germany). Methanol HPLC-grade and $EDTANa_2H_2$, were of analytical grade from VWR Prolabo (Belgium). Nafion perfluorinated ion-exchange resin 5% wt was from (Aldrich, Germany) and used after dilution 2.5% with ethanol.

EfferalganVitamine C drug was from UPSA (France) and contained: APAP 500 mg, AA 200 mg, citric acid anhydrous, sodium bicarbonate, sodium carbonate anhydrous, sorbitol, sodium benzoate, citrus aroma, saccharin sodium, povidone, docusate sodium.

Dafalgan was from Bristol-Myers Squibb (Belgium) and contained: APAP 500 mg, hepromellose, povidone, croscarmellose sodium, microcristalline cellulose, glycerol behenate, magnesium stearate.

2.2 Apparatus

A BASi EPSILON potentiostat with a C3 Cell Stand served for cyclic voltammetric experiments at the SPE and glassy carbon electrode (GCE). Square wave parameters were: quite time 30 s, frequency 15 Hz, SW amplitude 25 mV. The pH of the solution was controlled with a Metrohm 827 pH Lab system. All experiments were performed at room temperature (23 °C). The GCE was from Metrohm, (Switzerland).

The LC system consisted of a Gilson 307 pump (USA) connected to a 20 μL sample loop injector (Rheodyne N° 7125, USA), a flow-cell (DRP-FLWCL, Dropsens, Spain) for housing the single wall carbon nanotube based SPE (DropSens, Spain) coupled to a Powerchrom 280 (eDAQ Europe) integrator. The LC mobile phase was kept overnight at a low flow rate (50 μL/min) with the EC detector at off position.

A reversed-phase column, Chromolith FastGradient RP-18 endcapped 50-2 packed with 2 mm C18 monolithic silica, and a 125-4 endcapped conventional column packed with 5 μm C18 silica reversed-phase particles (Lichrospher 100 RP-18-5 μm) equipped with a guard column packed with the same C18 material, were used. These two columns were obtained from Merck (Darmstadt, Germany). Drug formulation assays were performed using a mobile phase based on 0.1 M phosphate buffer (PB) pH 6.5 and methanol in the ratio of 91:9 (v/v). The mobile phase was degassed by nitrogen bubbling.

Stock solutions were prepared daily by dissolving and ultrasonicating aliquots of APAP and AA in buffer solution to obtain a 4×10^{-3} M stock solution of each. The standard solutions were prepared by dilution of stock solutions of APAP and AA to reach a concentration range within 2×10^{-7}–1×10^{-4} M for each substrate for the LC–EC experiments and within 2×10^{-5}–1×10^{-3} M of APAP for the SWV-GCE experiments. AA solutions were degassed by nitrogen bubbling.

Sample preparation was carried out by mixing and homogenizing the content of 8 tablets in mortar. An accurately weighed amount was dispersed in buffer solution and ultrasonicated for 2 min. A portion of the resulting solution was filtered through a 0.45 μm disposable syringe filter and diluted with the mobile phase prior analysis by LC. Peak asymmetry factors, and resolution were calculated by the Powerchrom 280 incorporated software and statistic data were obtained using the Statistix program (Analytical Software).

The preparation of the Nafion modified GCE consisted in manually smoothing the GCE with a 0.5 μm alumina slurry, rinsing and sonicating in HNO_3- ethanol 1:1 (v/v), then rinsing with deionized water and allowing air-drying.

The electrode surface was spiked with 10 μL of the 2.5 % Nafion solution and allowed to air-dry at room temperature, then it was rinsed with water and air-dried at room temperature.

3 Results and Discussion

3.1 LC Determination

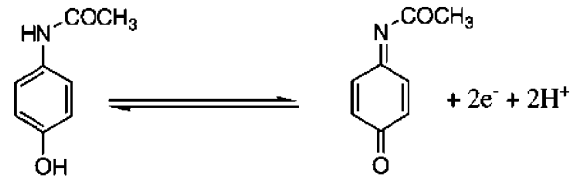
3.1.1 Mobile Phase and pH Effect

Retention times of APAP and AA in formic acid buffer pH 3.5 were very close to each other at the conventional C18 column with retention times (t_r) of 3.95 and 4.16 min, respectively. At pH values above the pK_{a1} (4.1) of AA, good separation was achieved using phosphate buffer of pH 7.4 or 6.5 with retention times of APAP equal to 15. 5 and 13. 5 min, respectively, whereas ascorbate was eluted within one minute. Methanol at a percentage of 9 % (v/v) was added in the PB solution pH 6.5 allowing short retention times to be obtained for APAP with $t_r = 3.6$ and 0.81 min for APAP and AA, respectively. Under such conditions, the peak asymmetry factors for APAP were 2.2 and 1.1, and for AA the values were 2.2 and 2.6 at the monolithic and conventional columns, respectively.

3.1.2 Electrochemical Behavior

The cyclic voltammetry (CV) of APAP and AA at the SPE was studied in PB-methanol 91 : 9 (v:v) at pH 6. 5. One single oxidation peak was obtained for both molecules at 0. 198 and 0. 120 V vs. Ag /AgCl electrode, respectively (Figure 1 A and B).

The voltammograms ($v = 25$ mV s^{-1}) exhibited an irreversible pattern for AA, and a quasireversible behavior for APAP ($E_{pa} = 0.198$ V, $E_{pc} = 0.045$ V) in agreement with literature [40–42]. The APAP electrooxidation has been extensively investigated in the literature pointing out an oxidation mechanism very much function of the solution pH [41,49–51]. In neutral media, a two-electron and two-proton transfer process occurs to produce the relatively stable ($t_{1/2} = 47$ min.) oxidized product *N*-acetyl-*p*-quinoneimine NAPQI [49–51] (Scheme 1).



acetaminophen *N*-acetyl-*p*-quinoneimine

Scheme 1.

It is important to point out that, in neutral media, part of the formed NAPQI reacts with excess of parent APAP in solution to form an APAP dimer which may precipitate onto the electrode surface [50,51].

3.1.3 Amperometric Detection

Liquid chromatography using the conventional C18 or the monolithic C18 column was studied comparatively in terms of separation and EC performances. The coupling with the wall-jet flow cell housing the screen printed electrode was expected to offer several advantages comparing to a GCE detector, such as wide selection of SPEs and modified SPEs and reproducible sensor renewing. The flow cell based on transparent polymethacrylate material allowed to check for the presence of bubbles in the electrochemical cell. Also an innovative open–close system based on magnetic bars avoids the use of screws and facilitates the replacement of the screen printed electrodes [41].

Hydrodynamic curves of APAP (1 μM) and AA (1 μM) were studied in the range from 0 to 0.7 V. (Figure 2). The current plateau (both using the conventional and the monolithic columns) was obtained at 0.55 V and 0.20 V vs. pseudo Ag for APAP and AA, respectively.

The flow rate effect on the analytical signal magnitude was studied in the range 0.3–1.4 mL/min. The peak current (both for APAP and AA) increased by raising the flow rate (due to lower band broadening and higher flow velocity at the electrode surface) with a leveling off at the monolithic and conventional columns beyond 0.5 and 1.4 mL/min, respectively (figure not shown). It should be

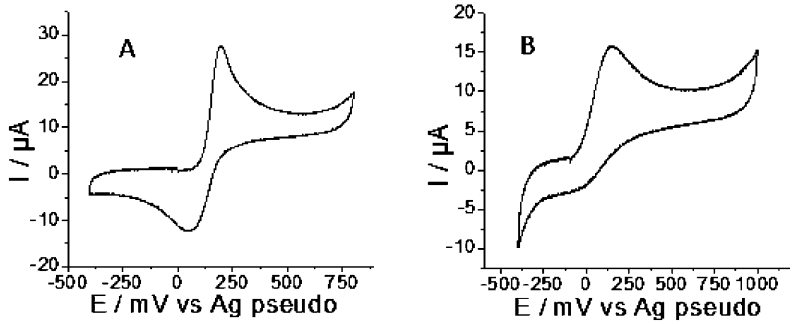


Fig. 1. Cyclic voltammograms of 1×10^{-3} M; A) APAP, B) AA, PB (0.1 M) pH 6. 5 – methanol 91 : 9 (v:v), scan rate 25 mV/s.

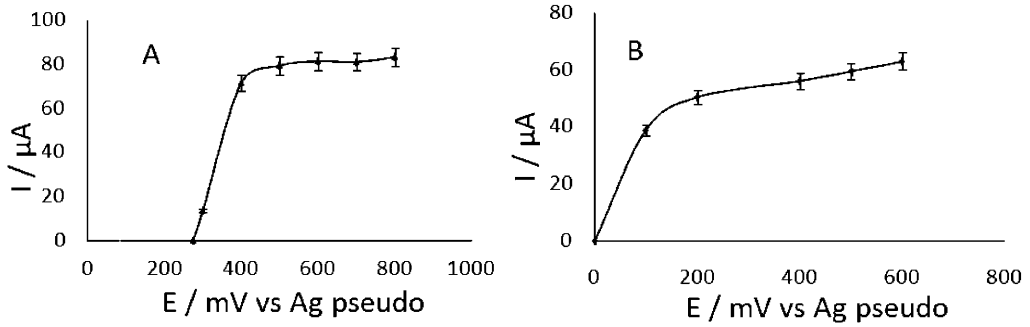


Fig. 2. Hydrodynamic voltammograms of 1×10^{-6} M; A) APAP B) AA at the SWCNT-SPE, monolithic column, flow rate 0.8 mL/min, PB (0. 1 M) pH 6.5 – methanol 91:9 (v/v), $N=5$.

noted that similar results were obtained by plotting peak area as a function of the flow rate.

Based on these data, an applied potential of 0.6 V and a flow rate of 0.5 mL/min were selected when using the monolithic column and 0.6 V and 1.4 mL/min when using the conventional column. Both molecules eluted within less than 1 min from the monolithic column and four minutes from the conventional column with resolution R_s of 1.91 and 5.93 for monolithic and conventional column, respectively (Figure 3). Since the LC separation of APAP and AA was successfully realized with good resolution, no further optimization was needed.

The selectivity of the assay towards APAP degradation product, in particular PAP, was studied as well. The latter was readily oxidized at the studied SPE with a peak potential in CV at 0.090 V and with the same signal magnitude (sensitivity) as for APAP in PB pH 6.5. The three compounds (APAP, PAP, and AA) were successfully separated with good resolution using the conventional C18 column with (t_r) 3.6, 1.55, 0.81 min, respectively. Lower mobile phase consumption and lower retention times with $t_{r1}=0.41$ and $t_{r2}=0.91$ compared to the conventional column with $t_{r1}=0.81$ and $t_{r2}=3.6$ for AA and APAP, respectively prompted us to use the monolithic column in subsequent work.

Using the latter, the retention time of PAP and AA was identical while APAP was well separated and no difference between the peak intensity of APAP in the presence or in the absence of PAP or AA was observed. It is worth to mention that, caffeine and codeine, which may be present in APAP drug formulations, were not interfering both in the LC-EC and voltammetric assays because being not electroactive at the selected potential at the SPE. Calibration curves were obtained by plotting the peak height of oxidation current of APAP and AA versus the corresponding concentrations. The correlation coefficient (r) of all calibration curves were consistently greater than 0.99. Linear regression equations obtained by least square regression method were y (nA) $=8\times10^{7}\ x$(M) $+1.003$ and y (nA) $=6\times10^{7}\ x$(M) -5.830 for APAP and AA, respectively.

The repeatability (intra-day), as indicated by the value of the relative standard deviation (*RSD*) of 3 replicates of the slope of the calibration curve, was less than 1.0 and 3.9 % for APAP and AA, respectively. The inter-day precision was evaluated by recording calibration curves in the range of 2×10^{-7}–5×10^{-6} M, for a time period of 5 consecutive days. The *RSD* of the slopes ($N=5$) was less than 1. 4 and 4.0 % for APAP and AA, respectively.

The limit of detection (*LOD*) and limit of quantification (*LOQ*) were calculated according to the IUPAC recommendations, as 3. $3\times s_b/m$ and $10\times s_b/m$, respectively, where s_b is the standard deviation of the blank measurements, and m is the slope of the calibration curve [52]. On this basis, the *LOD* and *LOQ* of the proposed method were 2. 10^{-7} and 5. 10^{-7} M for APAP, respectively and 5. $10^{-7}$and 1. 10^{-6} M for AA, respectively. These results comply very well with the concentration ranges gen-

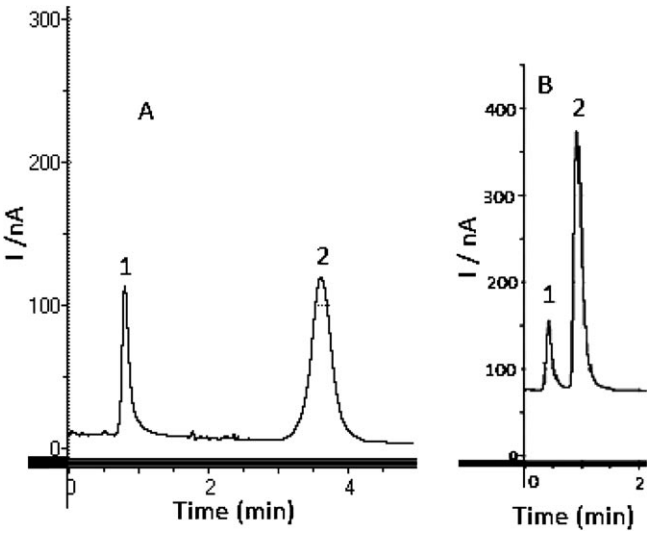


Fig. 3. LC chromatograms of AA and APAP: A) classic column, flow rate 1.4 mL min^{-1}; B) monolithic column, flow rate 0.5 mL min^{-1}. (1) 1×10^{-6} and 5×10^{-6} M AA at monolithic and conventional column, respectively (2) 1.5×10^{-6} and 5×10^{-6} APAP at monolithic and conventional column. Mobile phase PB (0.1 M) pH 6.5 + methanol 91:9 (v/v), $E_{app}=600$ mV

erally reported in the literature for APAP determination by electroanalytical methods is reported in Table 1.

In terms of electrode stability, it was noticed that the SPE provided repeatable injections for a 2. 5 μM APAP solution ($RSD = 0.97$, $N = 6$). The SPE operational stability was one week, then, the signal started to decrease progressively and a very long time was needed before achieving a good baseline. The signal decrease was attributed to progressive surface fouling by the APAP insoluble dimer. The SPE was disgarded after one week of use since at-

Table 1. Survey of electroanalytical methods for APAP determination. CPE: carbon paste electrode, MWCNT: multiwall carbon nanotubes, DPV: differential pulse voltammetry, FI: flow injection analysis, AD: amperometry, MIP: molecular imprinted polymer, SWAdSV: square wave adsorptive stripping voltammetry, CE: capillary electrophoresis.

Working electrode	Modifier	Detection mode	Application	*LOD* / *LOQ* (M)	Linear range	Ref
CPE (APAP)	–	DPV	Plasma, tablets	–	10^{-6}–10^{-4}	[8]
CPE (AA + APAP)	Stearic acid and nonmodified CPE	CV	Tablets	–	10^{-3}–10^{-2} (AA) 3×10^{-6}–$7.5.10^{-3}$ (APAP)	[9]
Pencil graphite	MIP	DPV	Tablets	7.9×10^{-7}	5×10^{-6}–5×10^{-4}	[10]
	–	DPV	Tablets	–	5×10^{-4}–5×10^{-2}	
Platinum (APAP)	Cellulose acetate-polycarbonate membrane	AA	Plasma	1×10^{-4}	–	[11]
CPE(APAP)	Pumice	DPV	Urine, tablets	2×10^{-8}	6×10^{-8}–1×10^{-6} 2×10^{-6}–$9.8.10^{-5}$	[12]
GCE(APAP+Caffeine)	Nafion/ruthenium oxide pyrochlore	SWV	Tablets	1.2×10^{-6}	5×10^{-6}–2.5×10^{-8}	[13]
GCE (APAP, AA, and DA)	Carbon-nickel magnetic nanoparticles	DPV	Tablets	2.3×10^{-6} 4.1×10^{-5} (AA)	7.8×10^{-6}–1.1×10^{-4} (APAP) 1.4×10^{-4}–1.3×10^{-3} (AA)	[14]
Nanogold	Indium tin oxide	DPV	Tablets	1.8×10^{-7}	2×10^{-7}–1.5×10^{-3}	[15]
GC tubular electrode	Nafion–enzymatic reaction	LSV	Serum, tablets	1.7×10^{-5}	5×10^{-5}–5×10^{-4}	[16]
Diamond	Boron	SWV and DPV	Tablets	4.9×10^{-7}	5×10^{-7}–8.3×10^{-5}	[17]
GCE	5-Amino-1,3,4-thiadiazole-2thiol	AD	Plasma	3.4×10^{-10}	5×10^{-8}–5×10^{-5}	[18]
CPE	Thionine-MWCNT	DPV	Plasma, tablets	5×10^{-8}	1×10^{-7}–1×10^{-4}	[19]
Graphite	MWCNT-basal plane pyrolytic	SWAdSV	Tablets	1×10^{-8}	1×10^{-8}–2×10^{-6} 2×10^{-6}–2×10^{-5}	[20]
SPE	Poly 3,4 ethylene-dioxythiophene	CV DPV AD(FI)	Tablets	3.6×10^{-6} 1.39×10^{-6} 1.6×10^{-7}	1×10^{-5}–1×10^{-3} 4×10^{-6}–4×10^{-4} 5×10^{-7}–6×10^{-4}	[21]
Gold	Tetraoctyl ammonium Br^-Au nanoparticles-1,6 hexanedithiol	AD	Plasma, tablets	2.6×10^{-9}	1.5×10^{-7}–1.34×10^{-5}	[22]
GC	Cadmium pentacyano nitrosylferrate	AD	Serum, tablets	2.04×10^{-6}	1.6×10^{-6}–5.3×10^{-5}	[23]
Pyrolytic graphite	SWNT	SWV	Urine, tablets	2.9×10^{-9}	5×10^{-9}–1×10^{-6}	[24]
	MWCNT	SWV	Urine, tablets	1.8×10^{-8}	2×10^{-8}–1×10^{-6}	
GC	Graphene	SWV	Tablets	3.2×10^{-8}	1×10^{-7}–2×10^{-5}	[26]
Diamond	Boron	FI/AD	Syrup	2×10^{-9}	$0.5^{-5}0\ 10^{-6}$	[27]
GCE	Cu/Polymer	CV/ AD	Tablets	5×10^{-6}	2×10^{-5}–5×10^{-3}	[28]
GCE	C60	CV	Urine, tablets	–	5×10^{-5}–1.5×10^{-3}	[29]
GCE	PANI-MWCNT	CV/SWV	Tablets	2.5×10^{-7}	1×10^{-6}–1×10^{-4}	[30]
C/film	–	FI/AD	Tablets	1.4×10^{-7}	8×10^{-7}–5×10^{-4}	[31]
5H Pencil/lead	–	FI-CV	Tablets	–	0.1×10^{-3}–5×10^{-3}	[32]
Pyrolytic graphite	–	AD(FI)	Tablets	1.42×10^{-8}	1×10^{-6}–8×10^{-6} 2×10^{-5}–1×10^{-4}	[33]
CPE	–	LC	Serum, tablets	6.6×10^{-6} (*LOQ*)	6.6×10^{-7}–6.6×10^{-5}	[34]
LCA	–	LC	Plasma	6.6×10^{-8} (*LOQ*)	1.2×10^{-7}–2.4×10^{-7}	[35]
Carbon disk	–	AD(CE)	Tablets	5.9×10^{-7}	5×10^{-6}–1×10^{-3}	[36]

Table 2. Results obtained for APAP and AA determination in pharmaceutical formulation EfferalganVitamine C ($N=5$).

Analyte	Nominal (mg)	Determined (mg)	*RSD* (%)
AA	200	204.4	2.6
APAP	500	541.7	1.4

tempts to clean (organic solvents, diluted acids) and reuse of the SPE were unsuccessful.

3.1.4 Assay of a Pharmaceutical Formulation

The utility of the proposed method was demonstrated by determining the concentration of APAP and AA jointly present in a commercial drug formulation (EfferalganVitamine C). The experimental results are presented in Table 2. The percent recoveries were between 107.3–109.1 % for APAP, and 99.9–106.5 % for AA.

3.2 Square Wave Voltammetry

Square wave voltammetry (SWV) at a Nafion modified GCE was carried out to validate the APAP determination by LC-EC. The modified electrode, thanks to the presence of the thin Nafion permselective membrane, allowed for the direct SWV determination of APAP in the presence of ascorbate in 0. 1 M PB solution pH 6.5. CV and SWV measurements were performed to examine the response of the Nafion modified and bare GCE toward the detection of APAP individually and in the presence of AA. The CV of 1×10^{-4} M APAP at bare and Nafion-GCE in 0.1 M PB solution pH 6.5 are displayed in Figure 4A. Oxidation and reduction peaks of APAP where at 0.510 (a1) and 0.067 (b1) V, respectively at the bare electrode and at 0.420 (a2) and 0.178 (b2) V, respectively at the Nafion-GCE. At the Nafion-GCE, a shift of 90 mV towards less positive potentials was observed for APAP oxidation current compared to the bare GCE (Figure 4a2). The observed slightly facilitated oxidation of APAP at Nafion-GCE was likely due to the hydrogen bonding between heteroatoms of Nafion film and APAP molecule, similar hypothesis was reported using nanostructure film of functionalized thiadiazol modified electrode [18]. The presence of the Nafion thin film, however, limited APAP diffusion and a lower signal was obtained at the Nafion-GCE (Figure 4Aa2) compared to the GCE (Figure 4Aa1).

The electrochemical behavior of APAP and AA in a mixture at the bare and Nafion-GCE electrodes was studied in 0. 1 M PB solution pH 6.5. The oxidation peak of both AA and APAP at the bare GC clearly gave a substantial signal overlapping (Figure 4B). The signal of AA, however, was totally eliminated at the Nafion-GCE due to electrostatic repulsion between ascorbate and Nafion (Figure 4C). This effect was exploited for the analysis of APAP by SWV. The latter provided a response linearly related to the concentration of APAP in the range 2×10^{-5}–1×10^{-3} M (Figure 4D) with a regression equation: $y(\mu A)=755\,x(M)-9\times10^{-5}$. The precision, inter-(12 days) and intra-days, of the assays and the stability of the Nafion-GCE were studied by realizing assays within the linear range using the same electrode. No significant difference was observed between the peak intensity for each

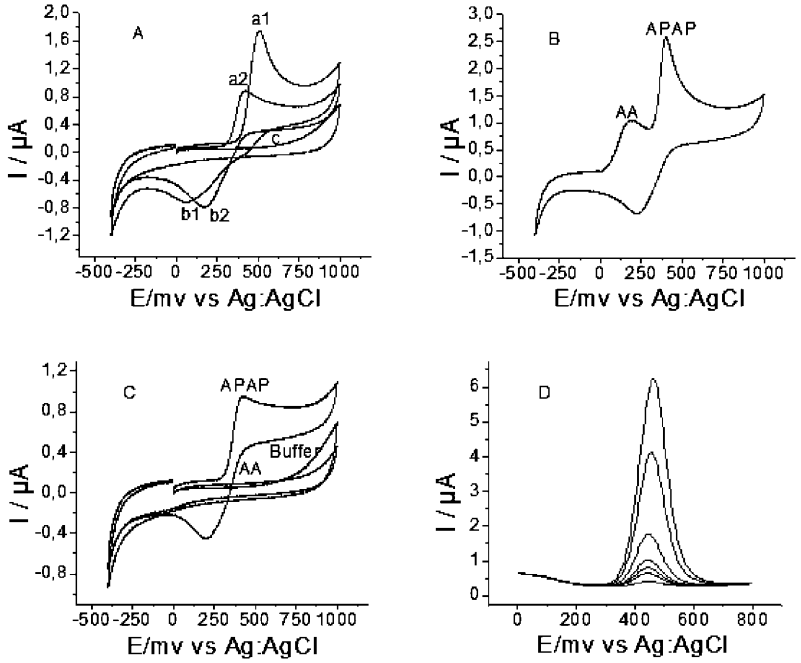


Fig. 4. CVs of : (A) 1×10^{-4} M APAP at bare (a1,b1) and Nafion-GCE (a2,b2), background current (c); (B) mixture of 1×10^{-4} APAP and AA at bare GCE; (C) mixture of 1×10^{-4} M of APAP and AA at Nafion-GCE; SWV of: (D) APAP 2×10^{-5}, 5×10^{-5}, 7×10^{-5}, 1×10^{-4}, 2×10^{-4}, 5×10^{-4}, 1×10^{-3} M at Nafion-GCE. PB (0.1 M) pH 6.5.

Table 3. Results obtained for APAP determination alone [a] and in the presence of AA [b] in pharmaceutical formulations ($N=5$).

Analyte	Nominal value (mg)	Determined (mg)	*RSD* (%)	Recovery (%)
APAP	[a] 500	495.8	0.47	99.2
	[b] 500	528.9	0.40	105.8

[a] Daflagan 500 mg APAP. [b] EfferalganVitamine C 200 mg AA and 500 mg APAP.

studied APAP concentration with a *RSD* lower than 0.8 % ($N=5$) for all the daily realized calibration curves. The intra- and inter-day *RSD* ($N=5$) of the slope of the calibration curve, was less than 0.5 and 1 %, respectively. The *LOD* and *LOQ* (*RSD* = 0.5 %) was equal to 2×10^{-5} and 5×10^{-5} M, respectively. SWV at the Nafion-GCE was applied to the determination of APAP in the presence of AA (EfferalganVitamine C) and APAP alone (Dafalgan) in drug formulations as reported in Table 3. Student-*t*-test applied to the assay of APAP in EfferalganVitamine C permitted to state that both techniques (LC and SWV) provided results for APAP that were not statistically different at a confidence level of 95 % (calculated *t* value: 1.70, theoretical *t* value: 2.14).

4 Conclusions

Screen printed electrodes may advantageously be combined as amperometric detector to LC as illustrated by the present application for the selective assay of paracetamol in drug formulations. Such electrodes are readily replaced in a reproducible manner and they are available in variable shapes, sizes and compositions. The commercially available used detector was, however, not designed for a coupling to LC and future technical modification should be realized in order to minimize the post column dead volume for improved sensitivity to be obtained. Monolithic columns appear to be well suited for coupling with EC detectors for high assay throughput and lower mobile phase consumption compared to conventional microparticles based columns.

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References

[1] G. G. Graham, K. F. Scott, R. O. Day, *Drug. Saf.* **2005**, *28*, 227.
[2] B. Ward, J. M. Alexander-Williams, *Acute Pain* **1999**, *2*, 139.
[3] P. I. Dargan, A. L. Jones, *Trends Pharm. Sci.* **2003**, *24*, 154.
[4] S. T. Stern, M. K. Bruno, G. E. Hennig, R. A. Horton, J. C. Roberts, S. D. Cohen, *Tox. Appl. Pharmacol.* **2005**, *202*, 151.
[5] M. S. Bloomfield, *Talanta* **2002**, *580*, 1301.
[6] B. Dejaegher, M. S. Bloomfield, J. Smeyers-Verbeke, Y. Vander Heyden, *Talanta* **2008**, *75*, 258.
[7] M. Espinosa Bosch, A. J. Ruiz Sanchez, F. Sanchez Rojas, C. Bosch Ojeda, *J. Pharm. Biomed. Anal.* **2006**, *42*, 291.
[8] I. Navarro, D. Gonzalez-Arjona, E. Roldan, M. Rueda, *J. Pharm. Biomed. Anal.* **1988**, *6*, 969.
[9] R. Sandulescu, S. Mirel, R. Oprean, *J. Pharm. Biomed. Anal.* **2000**, *23*, 77.
[10] L. Özcan, Y. Sahin, *Sens. Actuators B, Chem.* **2007**, *127*, 362.
[11] I. Christie, S. Leeds, M. Baker, F. Keedy, P. Vadgama, *Anal. Chim. Acta* **1993**, *272*, 145.
[12] C. Wang, X. Hu, Z. Leng, G. Yang, G. Jin, *Anal. Lett.* **2001**, http://www.informaworld.com/smpp/title~db=all~content=t713597227~tab=issueslist~branches=34, 2747.
[13] J-M. Zen, Y-S. Ting, *Anal. Chim. Acta* **1997**, *342*, 175.
[14] S-F. Wang, F. Xie, R-F. Hu, *Sens. Actuators B* **2007**, *123*, 495.
[15] R. Goyal, V. Gupta, M. Oyama, N. Bachheti, *Electrochem. Commun.* **2005**, *7*, 803.
[16] M. L. S. Silva, M. B. Q. Garcia, J. L. F. C. Lima, E. Barrado, *Anal. Chim. Acta* **2006**, *573–574*, 383.
[17] B. C. Lourenção, R. A. Medeiros, R. C. Rocha-Filho, L. H. Mazo, O. Fatibello-Filho, *Talanta* **2009**, *78*, 748.
[18] P. Kalimuthu, S. A. John, *Electroanalysis* **2010**, *22*, 303.
[19] S. Shahrokhian, E. Asadian, *Electrochim. Acta* **2010**, *55*, 666.
[20] R. T. Kachoosangi, G. G. Wildgoose, R. G. Compton, *Anal. Chim. Acta* **2008**, *618*, 54.
[21] W-S. Su, S-H. Cheng, *Electroanalysis* **2010**, *22*, 707.
[22] S. S. Nair, S. A. John, T. Sagara, *Electrochim. Acta* **2009**, *54*, 6837.
[23] H. Ramzi, M. Harasi, *J. Iran. Chem. Soc.* **2008**, *5*, 296.
[24] R. N. Goyal, V. Gupta, S. Chatterjee, *Sens. Actuators B* **2010**, *149*, 252.
[25] X. Kang, J. Wang, H. Wu, J. Liu, I. A. Aksay, Y. Lin, *Talanta* **2010**, *81*, 754.
[26] M. C. Q. Oliveira, M. R. V. Lanza, A. A. Tanala, M. D. P. T. Sotomayor, *Anal. Meth.* **2010**, *2*, 507.
[27] N. Wangfuengkanagul, O. Chailapakul, *J. Pharm. Biomed. Anal.* **2002**, *28*, 841.
[28] M. Boopathi, M-S. Won, Y-B. Shim, *Anal. Chim. Acta* **2004**, *512*, 191.
[29] R. N. Goyal, S. P. Singh, *Eletrochim. Acta* **2006**, *51*, 3008.
[30] M. Li, L. Jing, *Electrochim. Acta* **2007**, *52*, 3250.
[31] F. S. Felix, C. M. A. Brett, L. Angnes *J. Pharm. Biomed. Anal.* **2007**, *43*, 1622.
[32] P. Masawat, S. Liawruangrath, Y. Vaneesorn, B. Liawruangrath, *Talanta* **2002**, *58*, 1221.
[33] R. M. de Carvalho, R. S. Freire, S. Rath, L. T. Kubota, *J. Pharm. Biomed. Anal.* **2004**, *34*, 871.
[34] R. M. Riggin, A. L. Schmidt, P. T. Kissinger, *J. Pharm. Sci.* **1975**, *64*, 680.
[35] J. L. Palmer, *J. Chromatogr.* **1986**, *382*, 338.
[36] Q. Chiu, L. Jiang, X. Tian, J. Ye, *Anal. Chim. Acta* **2008**, *606*, 246.
[37] T. C. Raguram, D. Krishnamurthi, R. Kalamegham, *Toxicol. Lett.* **1978**, *2*, 175.
[38] C. Romero-Ferret, G. Mottot, J. Legros, G. Margetts, *Toxicol. Lett.* **1983**, *18*, 153.
[39] R. Thangamuthu, S. M. S. Kumar, K. C. Pillai, *Sens. Actuators B, Chem.* **2007**, *120*, 745.
[40] P. J. Lamas-Ardisana, P. Queipo, P. Fanjul-Bolado, A. Costa-García, *Anal. Chim. Acta* **2008**, *615*, 30.

[41] P. Fanjul-Bolado, P. J. Lamas-Ardisana, D. Hernández-Santos, A. Costa-García, *Anal. Chim. Acta* **2009**, *638*, 133.
[42] Y. Sha, L. Qian, Y. Ma, H. Bai, X Yang, *Talanta* **2006**, *70*, 556.
[43] S.-H. Huang, H-H. Liao, D-H. Chen, *Biosens. Bioelectron.* **2010**, *25*, 2351.
[44] K. C. Honeychurch, J. P. Hart, TrAC *Trends Anal. Chem.* **2003**, *22*, 456.
[45] O. Dominguez Renedo, M. A. Alonso-Lomillo, M. J. Arcos Martinez, *Talanta* **2007**, *73*, 202.
[46] H. Sangjarusvichai, W. Dungchai, W. Siangproh, O. Chailapakul, *Talanta* **2009**, *79*, 1036.
[47] G. Guiochon, *J. Chromatogr. A* **2007**, *1168*, 101.
[48] K. K. Unger, R. Skudas, M. M. Schulte, *J. Chromatogr.* **2008**, *1184*, 393.
[49] D. J. Milner, J. R. Rice, R. M. Riggin, P. T. Kissinger, *Anal. Chem.* **1981**, *53*, 2258.
[50] K. G. Madsen, J. Olsen, C. Skonberg, S. H. Hansen, U. Jurva, *Chem. Res. Toxicol.* **2007**, *20*, 821.
[51] D. Nematollahi, H. Shayani-Jam, M. Alimoradi, S. Niroomand, *Electrochim. Acta* **2009**, *54*, 7407.
[52] J. Mocak, A. M. Bond, S. Mitchell, G. Scollary, *Pure Appl. Chem.* **1997**, *69*, 298.

