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Liquid chromatography with electrocatalytic detection of oxalic acid by a palladium-based glassy carbon electrode

Innocenzo G. Casella[a,*], Carlo G. Zambonin[a], Fabrizio Prete[b]

[a]*Dipartimento di Chimica, Universita' degli Studi della Basilicata, Via N. Sauro 85, 85100 Potenza, Italy*
[b]*Laboratorio di Analisi, ASL N2, 85100 Potenza, Italy*

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Abstract

Cyclic voltammetry, flow injection analysis and liquid chromatography experiments were performed to characterise a palladium-modified glassy carbon electrode as an amperometric sensor for the determination of oxalic acid in perchloric acid solutions. The effects of several common interferents on the amperometric signal were also evaluated. The electrode stability, precision, limit of detection and linear range were evaluated at a constant applied potential of 1.1 V vs. Ag/AgCl. Calibration plots, obtained using liquid chromatography, were linear from 0.2 μ*M* to 1.2 m*M* (r^2=0.9986) and the detection limit was 0.15 μ*M* for a 50-μl injection. An example of analytical application, which includes the chromatographic separation and detection of oxalic acid in human urine, is given.

Keywords: Detection, LC; Electrochemical detection; Oxalic acid

1. Introduction

Numerous reports confirm that a high oxalate content in human urine or blood accompanies a number of renal metabolic disorders and renal diseases [1]. Most of this oxalate excess is excreted unchanged in the urine, but an unknown part of its contributes directly to the formation of nephrocalcinosis and renal calculi. In addition, oxalic acid (OXA) determinations in aluminium anodizing solutions for producing high quality metal coatings with increased corrosion, wear and abrasion resistance is a very important step [2]. Thus, precise and sensitive methods for the determination of oxalate in urines of stone formers (urolithic patients), foodstuffs and industry is of great importance.

The measurement of oxalate in biological matrices has traditionally proven difficult due to the much higher concentrations of other anions, cations and proteinaceous substances. Many analytical techniques as spectrophotometric [3], isotope dilution mass spectrometric [4], gas chromatographic [5] and enzymatic methods have been proposed to quantify oxalic acid in several real matrices. In addition, liquid chromatographic and capillary electrophoresis separations combined with suppressed conductivity detectors [6] and indirect UV absorbance detection mode [7,8] are also successful employed for the determination of oxalate species. However, because of the high concentrations of other biological components in real matrices, many of the later techniques often require extraction, derivatization or other sample clean-up procedures.

Liquid chromatography with electrochemical de-

*Corresponding author. Fax: +39-971-474-223.

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tection (LC–ED) is a very sensitive and selective technique for the determination of many important analytes in biological systems [9,10]. However, many important solutes undergo very slow heterogeneous electron transfer at the common substrate electrodes and consequently, exhibit irreversible electrochemical behavior. A promising approach for enhancing the electrochemical reversibility is through the use of chemically modified electrodes (CMEs). Electrochemically or alumina pre-treated glassy carbon electrodes have shown interesting catalytic properties towards the electrooxidation of hydrazine compounds [11] and oxalate ions [12]. Metallophthalocyanines films used as electrocatalysts, were shown to reduce overpotentials for the oxidation of oxalic acid by several hundred millivolt [13]. Dispersing metallic particles into organic polymers or simply on inert surface provides a good physical dispersion of the catalytic centres leading to a highly active electrode surface suitable for efficient electrocatalysis [14–18]. Ultrafine palladium particles supported on graphitic substrates [19,20], dispersed in carbon paste matrices [21] or electrodeposited in multimetallic alloy catalysts [22,23] have been proposed as electrode materials in electroanalysis.

In this paper a palladium-modified glassy carbon electrode (Pd-GCE) was tested as an amperometric sensor for the detection of oxalic acid at a low pH (0.1 *M* $HClO_4$). Cyclic voltammetry (CV) was used to characterize the electrochemical behavior of the Pd-GCE towards the OXA oxidation, while flow injection analysis (FIA) and ion-chromatography were used to characterize the sensor for analytical determination of OXA. Examples of determination of OXA by LC–ED in real matrices (such biological fluids) are also given.

2. Experimental

2.1. Reagents

All solutions were prepared from analytical-reagent grade chemicals (Aldrich) without further purification and using double distilled and deionized water. Sodium dodecyl sulfate (SDS) and bovine serum albumin (BSA) were purchased from Sigma and Fluka, respectively, and used as received. Individual 0.5 *M* oxalic acid solutions were daily prepared in distilled water. Unless otherwise specified, experiments were performed using 0.1 *M* $HClO_4$ as background electrolyte. In voltammetric experiments the solutions were protected from oxygen by purging with high-purity nitrogen. Deproteinization of the urine samples was carried out by mixing urine (5.0 ml) with about 240 mg sulfosalicylic acid and filtering the mixture through a 0.45-μm Millipore membrane. All experiments were carried out at ambient temperature.

2.2. Apparatus

A potentiostat/galvanostat Model 273 Princeton Applied Research (PAR EG&G) was used for electrochemical measurements. CV was done in a three-electrode cell using the Pd-GCE electrode, a SCE (saturated KCl calomel electrode) reference electrode and a platinum foil counter-electrode. The glassy carbon electrode used in CV (geometric area, 0.125 cm^2) was purchased from PAR. All current densities in this paper are quoted in terms of mA/cm^2 of apparent geometric area.

Amperometric measurements in flowing streams were performed using a PAR Model 400 electrochemical detector and a flow-through thin-layer electrochemical cell consisting of a Pd-GCE as working electrode, a Ag/AgCl (4 *M* KCl) reference electrode, and a stainless steel counter electrode. A Servogor 120 BBC was used to record the output signal. Flow injection experiments were carried out with a Varian 2510 pump equipped with a Model 7125 Rheodyne injector using a 50-μl sample loop. The mobile phase was purged from oxygen with an on-line degassing system (Hewlett-Packard Series 1050).

Chromatographic separations were performed by using an Aminex HPX-87H (300×7.8 mm I.D., Bio-Rad) ion-exclusion column.

2.3. Electrode preparation

Before each electrode modification electrodic surfaces were polished using a polishing cloth and 0.05 μm α-alumina slurry and rinsed with copious amount of distilled water. The electrodeposition of

palladium on glassy carbon was accomplished by cycling the potential continuously between 0.0 V and −0.4 V for five cycles at 50 mV/s in 5 m*M* PdO and 2 *M* HCl solution. Subsequently, the modified electrode was rinsed thoroughly with distilled water and conditioned by continuous CV cycling between the

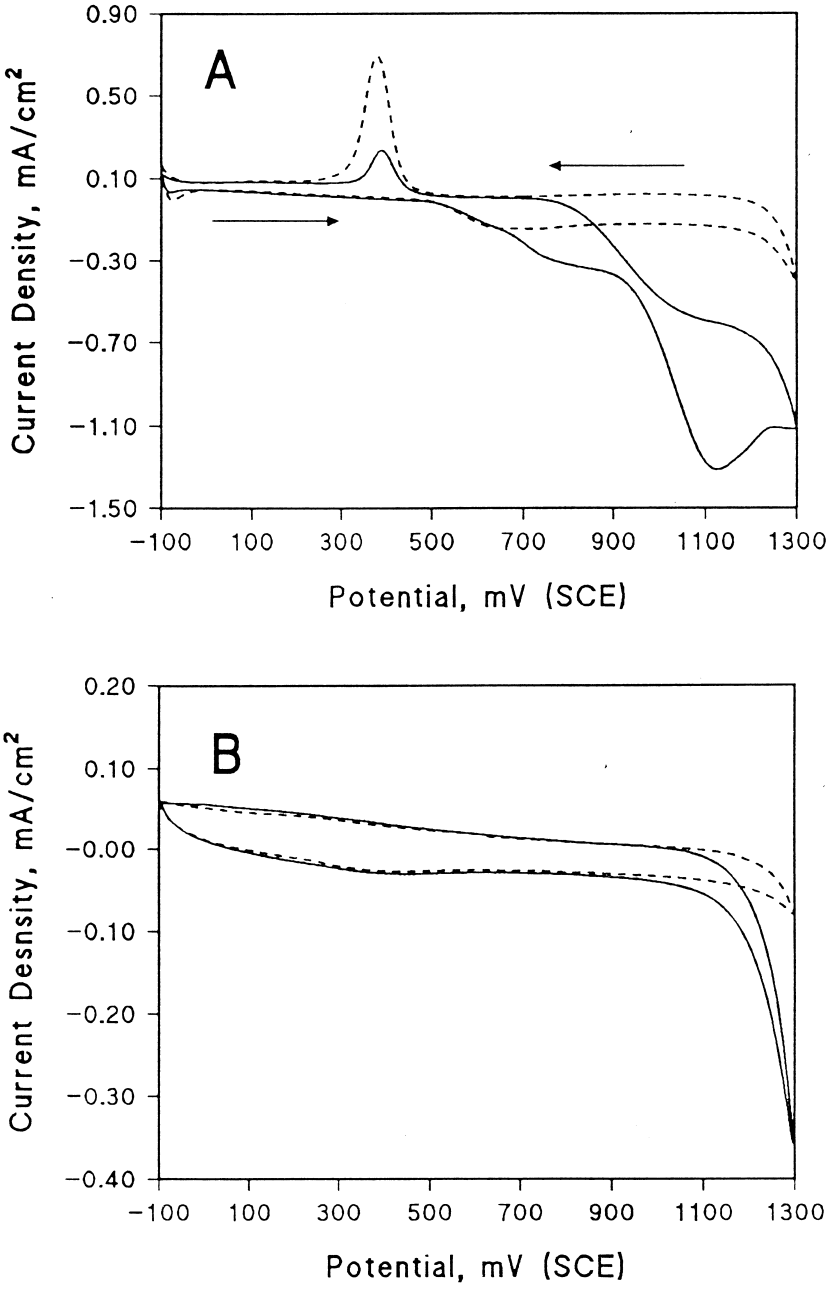

Fig. 1. (A) Cyclic voltammograms at a Pd-GCE electrode in 0.1 *M* $HClO_4$ degassed solution (dashed curve) and in presence of 5.0 m*M* oxalic acid (solid curve); (B) voltammograms at a glassy carbon electrode in absence (dashed curve) and presence of 5.0 m*M* oxalic acid (solid curve). Sweep rate, 50 mV/s.

scan limits −0.1 and 1.3 V for 5 min at a scan rate of 50 mV/s in 0.1 *M* $HClO_4$ electrolyte. The surface concentration of palladium electrocatalytic sites (Γ_{Pd}), was calculated by oxidizing the palladium species at 1.3 V for 30 s in 0.1 *M* $HClO_4$, and then determining the charge under the cathodic peak when the potential was switched in the cathodic direction at 10 mV/s.

3. Results and discussion

3.1. Electrochemical measurements

Representative cyclic voltammograms in 0.1 *M* $HClO_4$ (dashed curve) and in solutions containing 5.0 m*M* OXA (solid curves) of a Pd-GCE electrode, having about 6 μg/cm^2 palladium loading are reported in Fig. 1A. In presence of OXA (see Fig. 1A, solid curve), two news oxidation peaks appeared at about 0.75 V and 1.1 V vs. SCE. The peak current at 1.1 V increased linearly on increasing the OXA concentration up to 55 m*M* (correlation coefficient> 0.9987). In comparison, the unmodified glassy carbon electrode (see Fig. 1B) shows very low catalytic activity toward OXA oxidation and significant currents were observed only for potential higher than 1.3 V. The influence of palladium loading on catalytic activity was examined in voltammetric experiments. In the presence of 3.5 m*M* OXA, the current density of the peak at 1.1 V increases proportionally with the palladium loading up to 7–8 μg/cm^2, then it remains practically constant up to 16 μg/cm^2. Therefore, to obtain the maximum sensitivity and amperometric stability of the modified electrode, all experiments were carried out with a loading of about 6–8 μg/cm^2.

3.2. Amperometric flow measurements

The Pd-GCE electrode was tested as an amperometric detector in a flowing stream of 0.1 *M* $HClO_4$ solution by using a conventional thin-layer electrochemical cell. To optimize the applied potential, the hydrodynamic voltammogram for the oxidation of 3.5 μ*M* OXA was studied at 50-mV increments between 0.6 and 1.4 V vs. Ag/AgCl. Fig. 2 (curve A) shows the resulting hydrodynamic voltammogram obtained under flow injection conditions. The amperometric signal increased signifi-

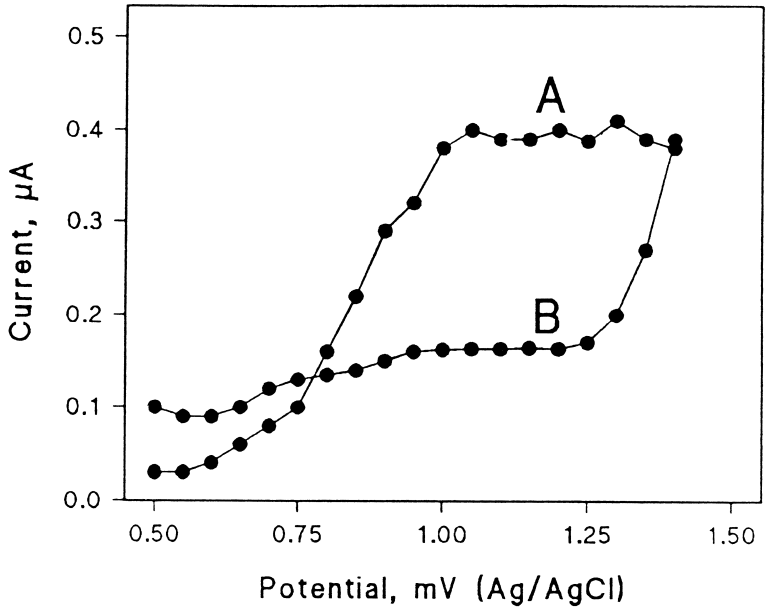

Fig. 2. (A) Hydrodynamic voltammogram of 3.5 μ*M* OXA in FIA at the Pd-GCE electrode. Applied potential, 1.1 V vs. Ag/AgCl; carrier, 0.1 *M* $HClO_4$; flow-rate 1.0 ml/min; sample loop 50 μl; (B) background current.

cantly between 0.7 V and 1.1 V, and remain nearly constant for high potentials. Curve B, relevant to the background current, shows a nearly constant value of about 0.12–0.16 μA in the range of potentials between 0.75–1.3 V. It is interesting to underline that at relatively low applied potentials (i.e., 0.7–1.0 V) the amperometric signal decreases with successive injections, whereas at high potentials (i.e., >1.0 V) the electrode response remains essentially constant after repetitive injections of OXA. The observed trend at low applied potentials, may be due to the slow desorption of reactants and/or intermediates of reaction on the electrode surface, with subsequent continuous diminution of electrode response. At high applied potentials, the absence of adsorption/desorption processes explains the integrity of the catalytic activity during the repetitive injections of OXA.

The effect of flow-rate on detector response was also tested by FIA using injections of 40 μ*M* OXA solutions at an applied potential of 1.1 V and at 0.3 ml/min increments between 0.1 and 4.0 ml/min. The amperometric response was found to increase with increasing flow-rate in the range 0.1–1.5 ml/min, while remains practically constant for high flow-rates. The observed flow-rate behavior is characteristic of electrooxidation processes based under simple mass transport control.

Representative responses of 10 μ*M* (a), 20 μ*M* (b) and 40 μ*M* (c) OXA solutions, obtained with a Pd-GCE electrode using 0.1 *M* $HClO_4$ as mobile phase at 1.0 ml/min flow-rate and a constant potential of 1.1 V vs. Ag/AgCl are shown in Fig. 3. The calibration graph, obtained for OXA, had a linear range comprised between 0.1 μ*M* and 0.5 m*M* (correlation coefficient 0.9998) and the detection limit evaluated at a signal-to-noise ratio of 3 from the lowest injected concentration was 55 n*M* (≅2.8 pmol injected). A linear least-squares analysis of the points obtained in the linear range yielded a slope of 110±5 nA/μ*M*. Further, the relative standard deviation (R.S.D.) of 85 consecutive injections (about 2 h of operation time) of 10 μ*M* OXA solution was 3.1%.

The catalytic response is very enduring; injection measurements periodically performed over a 1.5-month period were sufficiently reproducible: a maximum 25% variation in electrode response was observed.

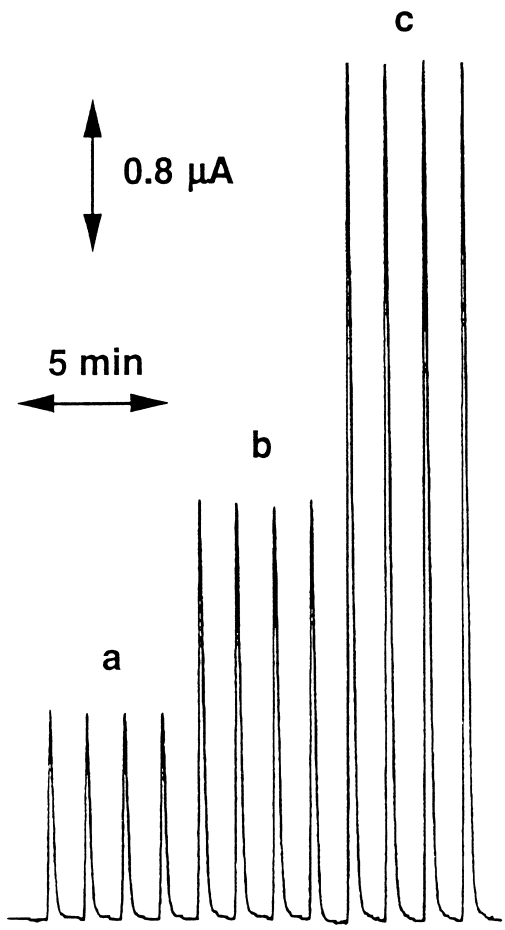

Fig. 3. Multiple flow-injection peaks of (a) 10 μ*M*, (b) 20 μ*M*, (c) 40 μ*M* of oxalic acid at a Pd-GCE electrode. Applied potential, 1.1 V vs. Ag/AgCl; Other experimental conditions as in Fig. 2.

3.3. Liquid chromatography with electrochemical detection of OXA: calibration, detection limit and reproducibility

The most interesting features of the Pd-GCE electrode is the possibility of using them for amperometric monitoring of OXA in complex samples following chromatographic separations. Considering that the modified electrode enables the electrooxidation of OXA to proceed in acidic media, a hydrogen sulfonated divinyl benzene–styrene copolymer as stationary phase able to separate this compound was used. Accordingly, the chromatograms were obtained using 0.1 *M* $HClO_4$ as mobile phase with an Aminex

HPX 87-H ion-exclusion column. The retention time of the chromatographic peak of OXA, obtained at 1.0 ml/min, is generally of about 5.4 min.

A calibration graph (three replicates analysis at each different concentration) was obtained for the OXA with a linear range between 0.2 μ*M* and 1.2 m*M* with a correlation coefficient of 0.9986. The detection limit ($S/N=3$) was 0.15 μ*M* with a sample loop of 50 μl. Further, the precision expressed as R.S.D. of six chromatographic experiments (about 1 h of operation time) was 3.2% for 10 μ*M* OXA. It is interesting to observe that, the analytical performance expressed in terms of limit of detection (LOD) and linear range obtained here, are better than those obtained with other analytical procedures based on spectrophotometric, enzymatic or gas chromatographic techniques [3,7,8,24].

Long-term electrode stability was studied during a continuous chromatographic injections of 10 μ*M* OXA. The peak heights for this test yielded an average 3.8% variation in the electrode response after more than 7 h of operating time.

3.4. Interference studies

The effects of several potential interferents such chloride, ascorbic acid, pyruvic acid, uric acid, sulfite and thiocyanate were evaluated by LC, whereas electroinactive molecules such as glucose, BSA, SDS, glycine, urea and gluconic acid were studied by flow injection analysis. Table 1 shows the relevant results. Very small interference effects were observed for ascorbic acid, pyruvic acid, uric acid, gluconic acid, sulfite, glucose, BSA, SDS, glycine, and urea:

Table 1
Influence of several substances on oxalic acid response

Substance	*D* (%)	t_R (min)	Substance	*D* (%)	t_R (min)
LC experiments					
Ascorbic acid			Pyruvic Acid		
3.0 μ*M*	0.0	6.6	0.40 m*M*	0.0	10.4
0.45 m*M*	+3.1		1.0 m*M*	+0.8	
1.20 m*M*	+4.3		2.0 m*M*	+1.2	
Uric acid			Thiocyanate		
0.10 m*M*	−0.2	10.4	10 μ*M*	−9.6	4.1
0.18 m*M*	−0.9		42 μ*M*	−31	
0.25 m*M*	−1.7		80 μ*M*	−55	
Chloride			Sulfite		
4.0 m*M*	+2.0	4.0	0.4 m*M*	0.0	11.4
8.0 m*M*	+6.4		1.0 m*M*	−1.2	
90 m*M*	+15		1.3 m*M*	−1.2	
Flow injection experiments					
Gluconic acid			Glycine		
4.0 m*M*	0.0		4.5 m*M*	0.0	
8.0 m*M*	+0.6		9.0 m*M*	−1.2	
16 m*M*	+1.4				
BSA			Urea		
80 mg/l	−1.7		4.3 m*M*	−1.2	
430 mg/l	−2.9		9.0 m*M*	−2.7	
SDS			Glucose		
0.6 m*M*	−1.3		2.5 m*M*	−0.5	
1.2 m*M*	−3.1		35 m*M*	+0.2	

The experiments were performed in a flowing stream of 0.1 *M* $HClO_4$ solution at 1.0 ml/min and an applied potential of 1.1 V vs. Ag/AgCl. Chromatographic separations were performed by using an Aminex HPX-87H column. *D* (%) represents the percent differences between the average current values ($n=5$) of 50 μ*M* OXA in the presence and absence of interferent. t_R represents the retention time. The retention time of OXA is about 5.4 min.

variations of the amperometric signal of less than 5% were generally obtained. However, a significant interference effect for chloride and thiocyanate ions was observed: variations of +15% and −55% on the amperometric signal were obtained in presence of chloride (90 m*M*) and thiocyanate (80 μ*M*), respectively. The diminution of the amperometric signal of OXA in presence of SCN^-, is likely due to the stronger adsorption effects of this ion on the catalytic sites. It is interesting to observe that the activity loss observed is not irreversible and the initial activity is restored after few injections of free thiocyanate solutions. In this respect, when are analysed real samples containing thiocyanate or large contents of chloride, a standard addition method is necessary to overcome these matrix effects.

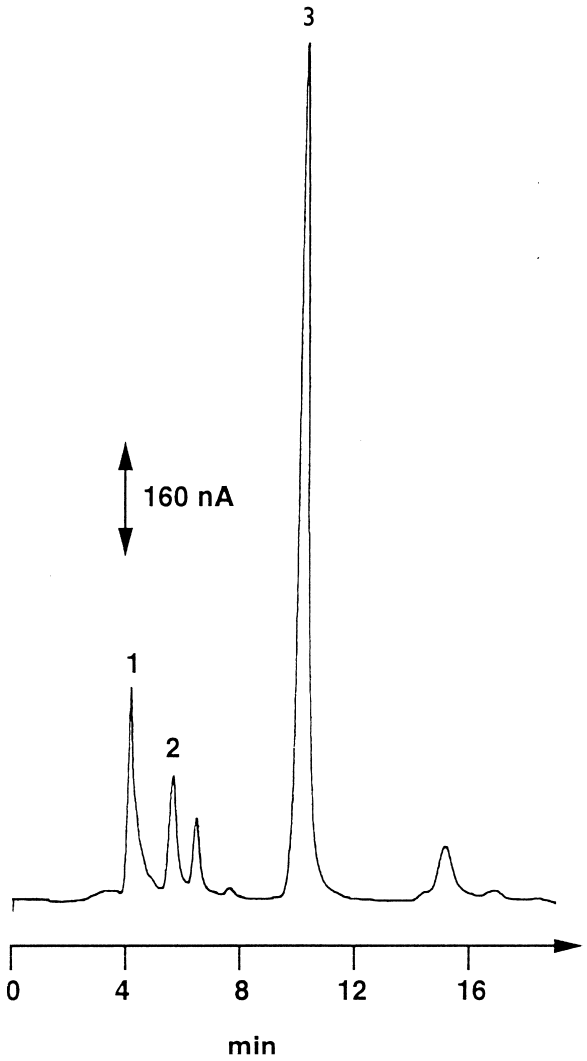

Fig. 4. Liquid chromatogram of human urine deproteinized, filtered and successively diluted 1:50 with mobile phase. Column, Aminex HPX-87H 300×7.8 mm (Bio-Rad). Peaks: 1=Chloride+ thiocyanate, 2=oxalic acid, 3=uric acid. Experimental conditions as in Fig. 3.

3.5. Analytical applications

To demonstrate the usefulness of the proposed modified electrode and to show the simplicity of the LC–ED method, a determination of oxalic acid in human urine was performed. The original samples were deproteinized after treatment with sulfosalicylic acid and filtered through filter membrane, then diluted 1:50 with mobile phase prior the injection in column. Fig. 4 shows the relevant chromatogram. The analytical figures of merit are summarized in Table 2. The concentration of OXA was determined by a standard addition method. In order to ascertain the influence of proteins, chloride and thiocyanate concentration on the accuracy of the oxalate determination, an urine sample not deproteinized and fortified with a large excess of these anions (155 m*M* and 43.7 m*M* of chloride and thiocyanate, respectively) was analysed. As can be seen, these results shown that the proposed analytical method is accurate and give good recoveries (95–98%) of added oxalate independently by the presence of potential interfering species.

4. Conclusions

Palladium catalytic particles dispersed on a graphitic substrates were characterized in an acidic medium towards the electrooxidation of oxalic acid. The suitability of the Pd-GCE modified electrode as an amperometric detector for the OXA determination in flowing streams, including LC has explored. The effects of several common interferents on the amperometric responses in FIA and LC were estimated. A simple, rapid, reproducible and sensitive analytical method for oxalic acid determination is proposed. The method appears to be an appropriate analytical procedure for the routine determination of oxalate in

Table 2
Determination and recovery of OXA in human urine analysed by LC–ED at a Pd-GCE electrode

Sample	Added (μM)	Found (μM)	Recovery (%)	R.S.D. (%) ($n=3$)	r^2 ($n=5$)
A	–	4.22	–	3.9	0.9984
	7.84	11.87	98.4	3.2	0.9978
	15.6	19.36	97.8	2.9	0.9985
B	–	4.13	–	4.1	0.9986
	6.12	9.75	95.1	3.8	0.9988
	12.33	16.17	98.2	3.5	0.9990

Experimental conditions: liquid chromatographic analysis with an Aminex HPX-87H column; flow-rate 1.0 ml/min; mobile phase 0.1 *M* $HClO_4$; sample loop 50 μl; applied potential 1.1 V (Ag/AgCl). The original deproteinized sample (A) was diluted 1:50 with mobile phase; sample (B): original sample not deproteinized and fortified with 155 m*M* Cl^- and 43.7 m*M* SCN^-. The concentrations were evaluated by the standard addition method (four additions).

biological matrices, avoiding tedious or complex pre-treatment of the real samples.

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