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FIA determination of ascorbic acid at low potential using a ruthenium oxide hexacyanoferrate modified carbon electrode

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Abstract

The anodic oxidation of ascorbic acid on a ruthenium oxide hexacyanoferrate modified electrode was characterized by cyclic voltammetry. On this modified surface, the electrocatalytic process allows the determination of ascorbic acid to be performed at 0.0 V and pH 6.9 with a limit of detection of 2.2 μM in a flow injection configuration. Under this experimental condition, no interference from glucose, nitrite and uric acid was noticed. Lower detection limit values were obtained by measuring flow injection analysis (FIA) responses at 0.4 V (0.14 μM), but a concurrent loss of selectivity is expected at this more positive potential. Under optimal FIA operating conditions, the linear response of the method was extended up to 1 mM ascorbic acid. The repeatability of the method for injections of a 1.0 mM ascorbic acid solution was 2.0% ($n = 10$). The usefulness of the method was demonstrated by an addition-recovery experiment with urine samples and the recovered values were in the 98–104% range.

Keywords: Ruthenium oxide hexacyanoferrate; Modified electrode; Ascorbic acid; Urine; Flow injection analysis

1. Introduction

Ascorbic acid is consumed on a large scale as an antioxidant agent in food, drinks and medicines, due to its importance in several enzymatic reactions and in the defence against oxidative stress [1]. In addition, ascorbic acid has been used for the prevention and treatment of common cold, mental illness, infertility, cancer and AIDS [2]. Wilson and Guillan [3] have reported abnormalities related to the ascorbic acid levels in schizophrenic patients. The content of ascorbic acid in biological fluids can be used to evaluate the extent of oxidative stress in human metabolism, this parameter being associated with cancer, diabetes mellitus and other diseases [4].

Different analytical methods have been employed to evaluate the ascorbic acid concentration in pharmaceutical formulations, foods and biological fluids and they include chromatography [5,6], electrochemistry [6] and spectrophotometry [6,7]. Methods involving colour measurement are less convenient because they are based on derivatisation of the analyte to produce a coloured compound, which is time-consuming. Therefore, a more rapid, accurate and simple method is sought, justifying the attention attracted by electrochemical methods because they possess quick response, low cost, simplicity of instrumentation, high sensitivity and possibility of miniaturization.

A major drawback associated with voltammetry is the poor selectivity of the measurements. This is particularly important in the case of ascorbic acid because its direct oxidation on metal (or carbon) electrodes leads to poor sensitivity with large overpotentials and fouling problems by adsorption of oxidation products are also reported [8–10]. Recent works have demonstrated the advantages of using specific unmodified surfaces such as edge plane pyrolytic graphite electrodes to perform electroanalytical determinations as the electron-transfer rate is accelerated at these materials, a good discrimination between concomitant analytes (for instance, dopamine, ascorbic acid and serotonin) being achieved [11,12].

The development of electroanalytical methods for the selective determination of ascorbate at less positive potentials has been a major target, especially in the case of complex matrices. Numerous attempts have been made to reduce the large overpotential by depositing chemical mediators that decrease the energy required in the oxidation of ascorbic acid [13,14]. Brett and co-workers [15] reported a modification of the glassy carbon surface with copper hexacyanoferrate for the determination

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of ascorbic acid in different samples. The potential reported in this work for the maximum current peak for the electrochemical oxidation of ascorbic acid was +0.4 V vs. standard calomel electrode (SCE), but these authors noticed that a better selectivity is attainable with lower sensitivity at +0.05 V vs. SCE with a detection limit value of 2.1 μM.

The preparation of a new hexacyanoferrate films with ruthenium as redox centre has been recently reported [16–18]. These films exhibit electrocatalytic properties related to the oxidation of cysteine, methionine, cystine, ascorbic acid (0.52 V vs. Ag/AgCl), sulphur oxoanions and alcohol in acid medium [16,17]. We have recently reported the electrocatalytic properties of a glassy carbon electrode coated with an electrodeposited film of ruthenium (III) oxide hexacyanoferrate towards the anodic oxidation of 2′-deoxyguanosine [19] and the flow injection analysis (FIA) quantification of the analyte in DNA samples [20]. Voltammetric experiments with rotating disc electrode suggested that the rate of cross-chemical reaction between Ru(IV) centers immobilized onto the film and 2′-deoxyguanosine controls the overall electrode process. A similar principle was envisaged for the detection of ascorbic acid, hence we show in the present work the results of our attempts to employ this amperometric sensor for the analyte determination in a FIA assembly (pH 6.9). Investigations on the electrochemical behaviour of ascorbic acid using the proposed modified electrode indicated improved electrocatalytic response with reduced overpotential, yielding increased sensitivity and low detection limit. The usefulness of the FIA method was evaluated by monitoring ascorbic acid in urine without the necessity of sample pre-treatment.

2. Experimental section

2.1. Chemicals and materials

All solid reagents were of analytical grade and were used without further purification. Potassium hexacyanoferrate, ascorbic acid, potassium dihydrogen phosphate, sodium nitrate and sodium hydroxide were obtained from Merck (Darmstadt, Germany), ruthenium (III) chloride was obtained from Alfa Aesar (Massachusetts, USA). In almost all cases the solutions were prepared by dissolving the reagents in deionized water processed through a water purification system (Nanopure Infinity, Barnstead).

2.2. Electrodes and instrumentation

A PalmSens portable electrochemical analyser (Palmsens BV, Houten, The Netherlands) was used for the electrochemical measurements. A homemade Ag/AgCl (saturated KCl) and a platinum wire were used as reference and counter electrodes, respectively. The working electrode was a glassy carbon (GC) electrode ($d = 3$ mm).

2.3. Modification of the electrode surface

The surface of the GC electrode was polished with alumina suspension (1 μm, Alfa Aesar, Massachusetts, USA) on a micro-cloth polishing pad, rinsed with water and sonicated for 5 min in distilled water. The electrochemical deposition of the ruthenium oxide hexacyanoferrate film was accomplished by cyclic voltammetry at the potential limits of −0.5 and 1.3 V at 100 mV s^{-1} in a solution containing 0.5 M $NaNO_3$ + 0.05 M HCl + 1 mM $K_3Fe(CN)_6$ + 1 mM $RuCl_3$. The formation of the ruthenium oxide hexacyanoferrate film on the glassy carbon disc surface was verified by recording cyclic voltammograms with the modified electrode in a supporting electrolyte solution containing 0.5 M $NaNO_3$ + 0.05 M HCl. The amount of immobilized material on the electrode surface after potentiodynamic experiments was controlled by the number of potential cycles and the surface excess (Γ) was determined from the charge (Q) under the voltammetric peak for the Ru(II/III) redox process as follow:

$$\Gamma = \frac{Q}{nFA} \quad (1)$$

where F is the Faraday's constant, n the number of electrons transferred per molecule of redox active species and A is the area of the electrode.

2.4. Flow injection analysis (FIA)

The flow injection apparatus consisted of a peristaltic pump (Ismatec ISM 828), a homemade rotatory injection valve and an acrylic cell mounted in a wall-jet configuration.

2.5. Urine samples

Human urine samples were collected from the laboratory personnel in 50 ml plastic bottles. The samples were diluted from 20 to 100 times in phosphate buffer pH 6.9 without any further treatment before injection in the FIA system.

3. Results and discussion

3.1. Electrocatalysis of ascorbic acid oxidation

Layers of ruthenium oxide hexacyanoferrate were voltammetrically prepared by repetitive potential cycles in a Ru(III) + $Fe(CN)_6^{3-}$ solution according to the procedure described in Section 2 and reported elsewhere [19,20]. When the modified electrode is thoroughly rinsed with water and a new voltammogram is recorded in the supporting electrolyte solution, the response is retained. A typical voltammogram of the ruthenium oxide hexacyanoferrate modified electrode recorded in a 0.5 M $NaNO_3$ + 0.05 M HCl solution is shown in Fig. 1; three well-defined pairs of anodic and cathodic peaks were observed. Redox activity of ruthenium oxide hexacyanoferrate films are based on electron-transfer processes involving Ru(II)/Ru(III)/Ru(IV) and Fe(II)/Fe(III) species and oxygen uptake [17,19]. Under the experimental conditions shown in Fig. 1A, a film with $\Gamma = 5.22 \times 10^{-9}$ mol cm^{-2} was obtained by measuring the charge under the first process at 0.0 V.

The electrocatalytic feature of ruthenium oxide hexacyanoferrate films towards the anodic oxidation of ascorbic acid is

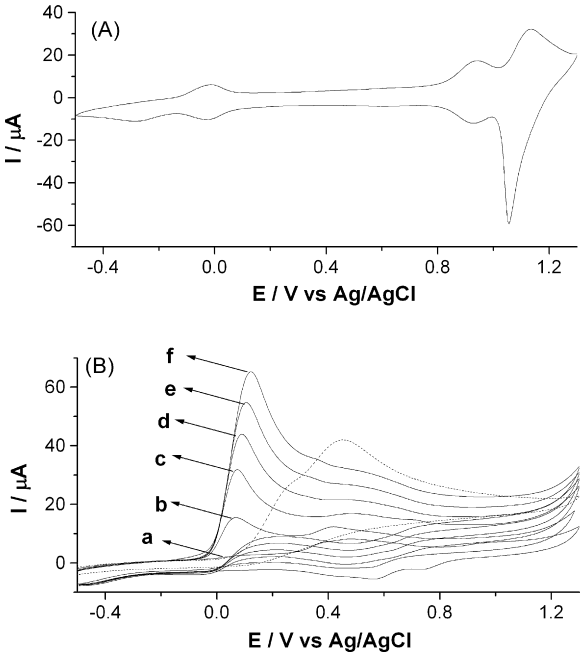

Fig. 1. Fifteenth voltammogram recorded with the ruthenium oxide hexacyanoferrate modified electrode in the supporting electrolyte solution (0.5 M $NaNO_3$ + 0.05 M HCl) (A). Panel B presents cyclic voltammograms recorded in phosphate buffer solution before (a) and after addition of ascorbic acid (final concentration 0.99 mM (b), 1.96 mM (c), 2.91 mM (d), 3.85 mM (e), and 4.76 mM (f)) by using a bare glassy carbon electrode (- - -, final concentration: 4.76 mM) and the ruthenium oxide hexacyanoferrate (—) modified electrode ($\Gamma = 5.22 \times 10^{-9}$ mol cm^{-2}). Scan rate: 100 mV s^{-1}.

demonstrated in Fig. 1B, where the anodic process involving the analyte on a bare GC surface is also shown. Voltammograms were recorded in phosphate buffer (pH 6.9) supporting electrolyte before and after the addition of ascorbic acid resulting in concentrations in the range of 1–5 mM. The significant potential anticipation in comparison to the response obtained with a bare GC electrode (338 mV) and the sharp peaks with enhanced reversibility obtained with the modified electrode clearly confirm that the electron-transfer step involving the oxidation of ascorbic acid is accelerated. The effect of pH on the voltammetric response of the ruthenium oxide hexacyanoferrate modified electrode to ascorbic acid is not considerable regarding the sensitivity, whereas the peak potential shifted towards less positive potentials with increasing pH. This is an important feature of the ruthenium oxide hexacyanoferrate modified electrode considering its use in biofluids as more selective determinations are expected at pH around 7.0.

Fig. 2 shows a hydrodynamic voltammogram in a solution containing ascorbic acid (pH 6.9) while changing the potential at the ruthenium oxide hexacyanoferrate modified electrode. The oxidation of the ascorbic acid on this electrode surface is initiated at around −0.1 V and a continuous current increase is observed up to 0.4 V. Based on this behaviour, FIA determinations demanding high selectivity can be accomplished by working at less positive potentials, even though under these experimental conditions a loss of sensitivity is expected.

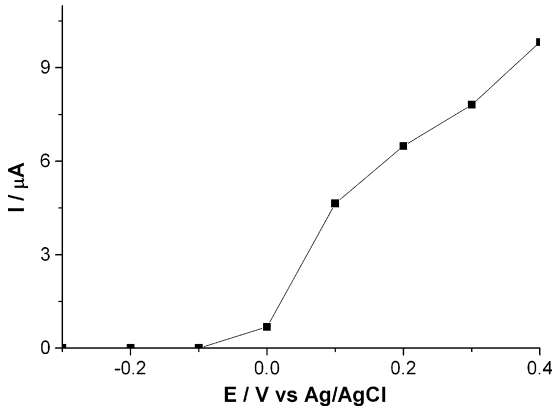

Fig. 2. FIA hydrodynamic voltammogram recorded in a 1 mM ascorbic acid solution (supporting electrolyte = 0.5 M $NaNO_3$ + 0.05 M HCl) with the ruthenium oxide hexacyanoferrate modified electrode. Flow rate: 1.6 ml min^{-1}, sample volume: 100 μl, carrier solution: phosphate buffer (pH 6.9). $\Gamma = 5.22 \times 10^{-9}$ mol cm^{-2}.

3.2. FIA optimization studies and analytical parameters

The influence of FIA parameters such as flow rate and sample volume was also investigated. Fig. 3A presents results on the variation of flow rate and a signal increase is observed in the range 0.26–2.60 ml min^{-1}, indicating that mass-transport exerts control on the overall electrocatalytic process. The flow rate of 2.00 ml min^{-1} was selected as the most favourable, taking into account the sensitivity of the measurements and consumption of the carrier solution. The influence of the sample volume on the analytical signal was also studied and experiments were carried out by using loops of 100, 150, 200 and 250 μl. No noteworthy difference was observed as shown in the results in Fig. 3B, hence a 150 μl sample volume was selected as the most appropriate, taking into consideration the sample consumption. Under these FIA optimized conditions, the throughput was calculated as 385 h^{-1} with negligible carry-over.

Two series of experiments in triplicate were carried out in order to obtain the calibration plot. FIA responses in the 50–1000 μM concentration range were obtained by working at two different potentials, 0.0 and 0.4 V, in order to evaluate the variation in sensitivity. A great improvement in sensitivity was noticed at the more positive potential, as discussed in the previous section. However, even at 0.0 V a calibration plot over the range 100–1000 μM ((I/μA) = 0.0041 + 7.3 × 10^{-4} (C/μM), $R^2 = 0.9999$) was obtained. At this less positive potential the detection limit was calculated as 2.2 μM (S/N = 3), a significant lower value being obtained if measurements were performed at 0.4 V (0.14 μM).

The use of precious metals (ruthenium) to achieve stability and to improve the electrochemical activity of metal hexacyanoferrates is well known in the literature. These features of the ruthenium oxide hexacyanoferrate modified electrode have been demonstrated by measuring the sensor sensitivity as a function of time. The slope of the calibration plot at pH 6.9 was unchanged during a 24 h experiment ((7.27 ± 0.08) × 10^{-4} and (7.18 ± 0.05) × 10^{-4} μA μM), confirming its long-term stability. The modified electrode was stored under dry conditions and

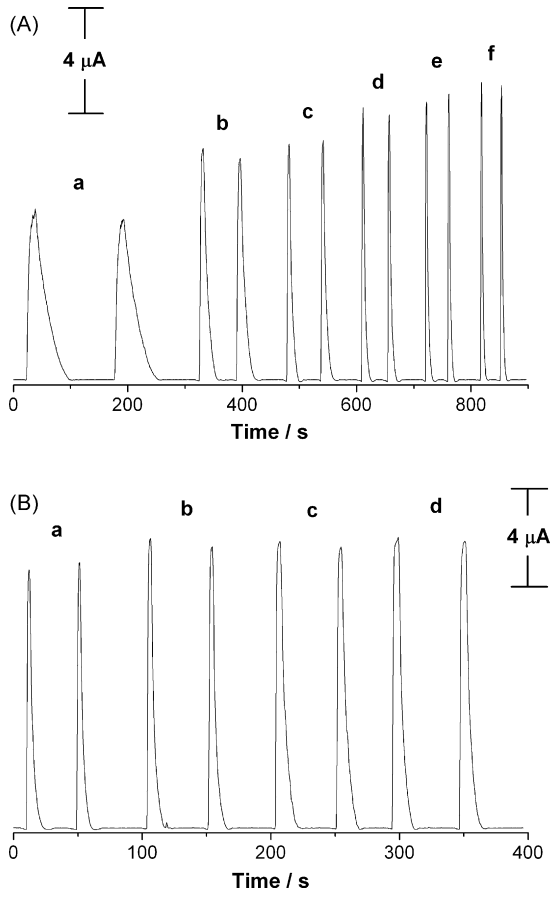

Fig. 3. Flow injection peaks recorded for 1 mM ascorbic acid at various flow rates (A): 0.26 ml min^{-1} (a), 0.70 ml min^{-1} (b), 1.1 ml min^{-1} (c), 1.6 ml min^{-1} (d), 2.0 ml min^{-1} (e) and 2.5 ml min^{-1} (f) (sample volume 100 μl) and various sample volumes (B) 100 μl (a), 150 μl (b), 200 μl (c), and 250 μl (d) (flow rate 2.0 ml min^{-1}). $\Gamma = 5.22 \times 10^{-9}$ mol cm^{-2}, carrier solution: phosphate buffer (pH 6.9), $E = 0.4$ V.

at room temperature between the experiments. The increased stability of the film layer has been attributed to the incorporation of ruthenium and the formation of bonds between Ru and Fe atoms [21,22]. Repeatability of FIA measurements was examined by 10 repetitive injections of a 130 μM ascorbic acid at 0.0 V and the relative standard deviation was found to be 2.0%. This is a very important feature inherent to modified electrodes for ascorbic acid determination, as the oxidation of the analyte on bare surfaces is recognized to be non-reproducible because of fouling effects [8–10].

The sensor response with respect to potentially interfering coexisting species was also examined. Fig. 4 shows FIA responses for injections of ascorbic acid and mixtures containing this analyte and concurrently nitrite, glucose and uric acid at twofold excess. The absence of significant shifts in the peaks recorded in the presence of the interfering species is an unambiguous confirmation that at 0.0 V the ruthenium oxide hexacyanoferrate modified electrode can operate as detector in an FIA apparatus with excellent selectivity. This assumption can be confirmed by the data in Table 1 showing that the working potential of the proposed modified electrode is significantly reduced in comparison with other electrochemical sensors, with

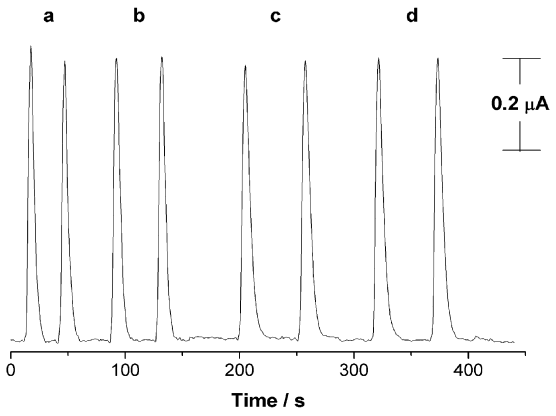

Fig. 4. Peaks recorded for repetitive injections of 1 mM ascorbic acid + 2 mM glucose (a), 1 mM ascorbic acid + 2 mM nitrite (b), 1 mM ascorbic acid + 2 mM uric acid (c), and 1 mM ascorbic acid (d) solutions at $E = 0.0$ V. Carrier solution: phosphate buffer (pH 6.9). Flow rate: 2.0 ml min^{-1} and sample volume: 150 μl.

a detection limit similar or even better than most of the values reported in the literature. It should be remembered that more sensitive determinations can be performed if measurements are carried out at more positive potentials, but in this case the existence of interfering species in complex matrices can restrain the use of the proposed sensor.

3.3. FIA determination of ascorbic acid in urine samples

The applicability of the ruthenium oxide hexacyanoferrate modified electrode for detection of ascorbic acid in real samples was examined by measuring the analyte concentration in urine. Fig. 5 presents FIA responses under optimized conditions for injections of ascorbic acid in the range 50–1000 μM as well as two urine samples. Well defined and sharp peaks with negligible carry-over effect are seen in this figure. Table 2 shows the ascorbic acid concentration of four urine samples and the results were in good agreement with those typically found in this kind of biological fluid, as reported in the literature [45]. The large range of ascorbic acid concentration found in plasma or urine samples

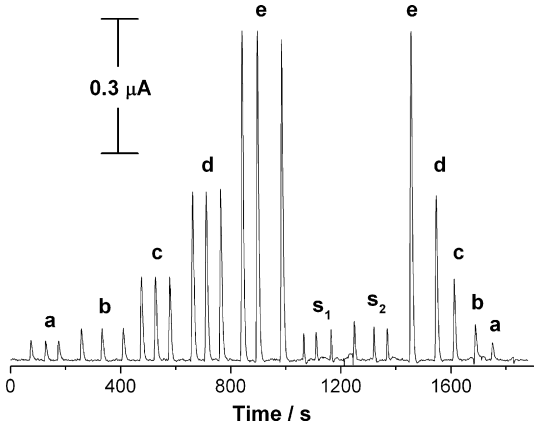

Fig. 5. Peaks recorded for injections of ascorbic acid standard solutions at the optimized FIA conditions (50 μM (a), 100 μM (b), 200 μM (c), 500 μM (d), and 1000 μM (e)) and two urine samples (s_1 and s_2). Carrier solution: phosphate buffer (pH 6.9). $E = 0.0$ V.

Table 1
Limit of detection and working potential values for ascorbic acid determination

Electrode	Reference	Sample	Detection limit (μM)	Application	Working potential (mV)
Cobalt(II) phthalocyanine-doped with iodine	[23]	–	–	Batch	Potentiometric
Fe(II) phthalocyanine modified carbon-paste electrode	[24]	Vitamin preparations	0.5	Batch	Potentiometric
Edge plane pyrolytic graphite electrode	[12]	Laked horse blood (40 μM ascorbic acid spiked)	0.2	Batch	−30 vs. SCE[a]
Ruthenium oxide hexacyanoferrate modified electrode	This work	Urine	2.2	FIA	0 vs. $Ag/AgCl_{(sat.)}$
GC/monolayer of propionylcholina	[25]	Urine	–	Batch	20 vs. SCE[a]
GC[b]/copper hexacyanoferrate	[15]	Wine, tea, fruit juice	2.1	Batch	50 vs. SCE[a]
7,7,8,8-Tetracyanoquinodimethane modified graphite	[26]	Fruit juice	23	Batch	50 vs. $Ag/AgCl_{(sat.)}$
Tetrabromo-*p*-benzoquinone modifier carbon paste electrode	[27]	–	0.62	Batch	60 vs. $Ag/AgCl_{(sat.)}$
Carbon ceramic electrode prepared by sol–gel technique	[28]	Urine and serum	1.7	Batch	100 vs. SCE[a]
GC[b]/5-hydroxytryptophan	[29]	Urine	4.2	Batch	170 vs. SCE[a]
Hg	[30]	Wine	3.1	Batch	200 vs. Ag/AgCl (3 M)
GC[b]/copper hexacyanoferrate	[31]	Pharmaceutical formulation	–	Batch	200 vs. SCE[a]
Ferrocenium-thioglycollate modified electrode	[32]	Urine	0.1	Batch	200 vs. SCE[a]
Pt/fluorosurfactant modifier	[33]	Urine	1.0	Batch	200 vs. SCE[a]
$[Cu(bipy)_2]$/SAM[c] modified electrode	[34]	–	0.081	Batch	200 vs. SCE[a]
Manganese dioxide graphite composite electrode	[35]	–	0.4	Batch	225 vs. SCE[a]
Edge plane pyrolytic graphite electrode	[11]	Comercial drink	71	Batch	230 vs. $Ag/AgCl_{(sat.)}$
GC[b]/sol–gel ceramic film incorporating methylene blue	[36]	Urine	0.005	Batch	240 vs. $Ag/AgCl_{(sat.)}$
GC[b]/[osmium(2,2-bipyridyl)$_2$-(poly-4-vinylpyridine)$_{10}$Cl]Cl	[37]	–	–	Batch	250 vs. SCE[a]
GC[b]/ruthenium oxide modified electrode	[38]	–	–	Batch	300 vs. $Ag/AgCl_{(sat.)}$
Polypyrrole films containing ferrocyanide ions deposited onto thermally pre-treated and untreated iron substrate	[39]	–	15,000	Batch	300 vs. $Ag/AgCl_{(sat.)}$
GC[b]/*N*,*N*-dimethylaniline	[40]	Juice	–	FIA	350 vs. $Ag/AgCl_{(sat.)}$
Al/nickel and nickel hexacyanoferrate films	[41]	–	–	Batch	440 vs. SCE[a]
Arrays of gold microelectrodes modified by electrodeposition of palladium	[42]	Urine	–	FIA	550 vs. $Ag/AgCl_{(sat.)}$
GC[b]/cobalt hexacyanoferrate	[43]	–	–	Batch	600 vs. $Ag/AgCl_{(sat.)}$
MnO_2 bulk modified screen printed electrode	[44]	Pharmaceutical formulation	1.1	Batch	600 vs. $Ag/AgCl_{(sat.)}$

[a] SCE = standard calomel electrode.
[b] GC = glassy carbon.
[c] SAM = self-assembled monolayer.

is influenced by lifestyle or other factors, such as smoking, oral contraceptives, physical exercise, gender, alcohol consumption, age and certain disease states [46].

Recovery studies were also carried out for validating the performance and accuracy of the proposed analytical method. Recovery values were calculated after spiking the urine samples with ascorbic acid. The data shown in Table 2 confirm the usefulness of the FIA amperometric method with the ruthenium oxide hexacyanoferrate detector for the determination of ascorbic acid in urine samples according to the required range stipulated by the Association of Official Analytical Chemists (AOAC) [47].

Table 2
Determination, addition and recovery of ascorbic acid in four different urine samples

Sample	Ascorbic acid concentration[a] (mM)	Ascorbic acid added (μM)	Ascorbic acid found[a] (mM)	Recovery (%)
1	1.64 ± 0.01	500	2.13 ± 0.02	98
2	2.1 ± 0.2	500	2.6 ± 0.1	100
3	3.42 ± 0.07	500	3.90 ± 0.05	96
4	0.98 ± 0.04	500	1.50 ± 0.04	104

[a] Average of three replicates.

4. Conclusions

Layers of ruthenium oxide hexacyanoferrate electrodeposited onto glassy carbon surfaces have proved to facilitate the anodic oxidation of ascorbic acid by reducing the overpotential and increasing the current response. The modified electrode was adapted in a flow injection system and a selective and sensitive amperometric method was proposed for measuring the ascorbic acid content in urine samples. The good selectivity of the method in the presence of possible coexisting interfering species was obtained by working at a significantly lowered potential (0.0 V). Under this experimental condition, the limit of detection was calculated to be sufficiently low to monitor ascorbic acid in the investigated urine samples. However, much lower limits of detection values were found by measuring FIA responses at 0.4 V (0.14 μM).

In order to optimize the response of electrocatalytic sensors and to guarantee that higher flux of the analyte are handled by the film layer containing the catalyst, investigations on the influence of the coating thickness on the overall electrode process are required. Further studies will be directed to investigate the dynamics of the electrocatalytic process and to evaluate the possibility of increasing the sensitivity of the device to monitor ascorbic acid at 0.0 V in cellular environments such as

neuroblastoma cells [48] or in secretion of pancreatic islets [49]. The highly selective determination of ascorbic acid at the ruthenium oxide hexacyanoferrate sensor in such complex systems is critical for online measurements because of the impossibility of using sample separation procedures.

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