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Comparison of silver, gold and modified platinum electrodes for the electrochemical detection of iodide in urine samples following ion chromatography

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Abstract

The electrochemical (EC) detection of iodide at gold, silver and platinum electrodes under similar experimental conditions was evaluated. To achieve optimal amperometric detection, the electrode sensitivity, selectivity, and stability was compared. Isocratic separation of iodide was attained by ion chromatography (IC) using an anion-exchange column with nitrate as an eluent ion (25 mM HNO_3 + 50 mM $NaNO_3$). Although the Ag electrode showed the highest selectivity due to the relatively low applied potential (+0.10 V versus Ag|AgCl), it requires continuous surface polishing upon injection of standard solutions or real samples; in addition, the chromatographic peak of iodide exhibited a pronounced dip-tailing. The limit of detection (LoD) of iodide was estimated to be 3.5 μg/L (S/N = 3) with an injection volume of 50 μL. Likewise, pulsed electrochemical detection at the silver electrode did not demonstrate the expected results in terms of peak shape and low detection limit. Using the same chromatographic conditions, iodide detection at the Au electrode (E_{app} = +0.80 V versus Ag|AgCl) exhibited a regular peak shape accompanied by a sensitivity comparable to the silver one. Yet, upon continuous injections the signal intensity displayed a progressive lowering up to ca. 40% in 6 h. Best results in terms of signal stability, peak shape and analytical response were obtained with a modified platinum electrode which allowed to achieve a LoD of 0.5 μg/L (S/N = 3). The present IC–EC detection method using a modified Pt electrode (E_{app} = +0.85 V versus Ag|AgCl) was successfully applied to determine low contents of iodide in human urine with solid phase extraction as pretreatment. Such a developed method correlated very well with the reference colorimetric method in urine (r = 0.95273), and it is specifically suggested when the iodide content is relatively low, i.e., <20 μg/L.

Keywords: Iodine; Ion chromatography; Electrochemical detection; Human urine

1. Introduction

Iodine is an essential micronutrient for the synthesis of thyroxine which is responsible for growth and development in humans [1]. Because of the difficulty of measuring dietary iodine intake directly and considering moreover that most iodide is excreted in the urine, urinary iodine excretion is currently the most convenient laboratory marker of iodine deficiency [2]. There is a wide range of published methods for the determination of iodide in foodstuff and biological samples. With respect to low detection limits, gas chromatography–mass spectrometry (GC–MS), neutron activation analysis (NAA) and inductively coupled plasma mass spectrometry (ICP-MS) are suitable analytical techniques [3–8]. Although direct quantitative iodine determination of trace amounts by either NAA or ICP-MS is possible, these instrumental techniques are expensive and there is the need of experienced personnel. The most commonly used method for urinary iodine determination is based on the Sandell and Kolthoff reaction [9] in which iodide has a catalytic effect on the reaction between cerium(IV) and arsenic(III) [10,11]. Many laboratories have adopted an automated analyzer program to perform this reaction after sample acid digestion necessary to eliminate the interfering substances occurring in urine before the colorimetric assay [12].

An alternative to the above approaches is afforded by ion chromatography (IC) which allows good selectivity when coupled to electrochemical (EC) detection. Iodide can be easily separated from common anions using an anion exchange column

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[13–15]. Commonly used are carbon-based and noble metal electrodes, such as platinum, gold and silver. Glassy carbon is more resistant to fouling than metal electrodes, and, as a consequence, it is the most popular electrode material in constant-potential EC detection. However, there are only a few applications of iodide determination by using carbon-based or porous graphite electrodes [16,17]. Electrochemical methods for the determination of iodide usually take advantage of the formation of an insoluble salt at the working electrode, e.g., AgI at the silver metal electrode. Indeed, several papers have been published on the use of ion chromatography with amperometric detection at silver electrodes [18–22]. Rocklin and Johnson [18] focused their efforts on the use of silver as electrochemical sensor at relatively low applied potential (i.e., $\leq$+0.2 V versus Ag|AgCl). Major emphasis by these workers was placed upon the ability of this working electrode to provide great selectivity as well as increased sensitivity. As silver is directly involved in the electrode reaction, it acts as a sacrificial anode. Unfortunately, determination of analytes using sacrificial electrodes usually exhibits poor reproducibility, not to mention the occurrence of electrode recession. Iodide detection at gold and platinum electrodes involves its spontaneous oxidative chemisorption to form an overlayer whose structure and composition have been extensively investigated [23–25]. To the best of our knowledge, a sole example of iodide detection at the gold working electrode in liquid chromatography has been reported [26]. Most likely, the limited utilization of gold is perhaps due to the electrode surface dissolution which gradually occurs in the presence of chloride ions. As biological fluids contains relatively high levels of chloride [27], the platinum electrode has been suggested as the best choice to amperometrically detect iodide. Although this electrochemical sensor provides high sensitivity, it is difficult to maintain an acceptable signal stability upon injection both of real and standard solutions. A valuable advancement was reported by Han et al. [28], whereby a platinum electrode modified by an electroactive iodine-based layer was proposed to reduce the decrease in sensitivity during EC detection in flowing stream solutions. In that procedure the platinum working electrode assembly is immersed into a saturated KI aqueous solution for 1 h. As pointed out recently by our group [29], certain drawbacks of such procedure have been examined since the practical application is cumbersome.

Taking into account the good sensitivity of the platinum electrode and to address the above-cited shortcomings of both electrodes, a much more effective electrochemical procedure of platinum modification has been proposed [29]. The key to success was to replace the earlier off-line electrode modification [28] with a simple in-line arrangement in which the platinum working electrode is modified while the electrochemical cell is properly assembled. A syringe pump is used to flush a concentrated solution of KI into the electrochemical cell for ca. 15 min at a constant flow-rate. A very reproducible iodine-based film is formed on the electrode surface at an applied potential of +0.85 V. As the electrochemical sensor is almost ready to use, this electrode modification procedure is compatible with the subsequent use in flowing streams without discontinuing the applied potential. The focus of this paper is thus to compare the performances of the iodine-based platinum electrode with those of silver and gold electrodes at the aim to test the hypothesis that the modified electrode represents the best choice for iodide detection when applied to urine samples.

2. Materials and methods

2.1. Chemicals

Nitric acid 70% (99.999%; 1.400 mg/L), sodium nitrate (99%), sodium bromide (99.0%), potassium thiocyanate (99.0%), sodium thiosulphate (99.5%) and potassium iodide (99.8%) were purchased from Sigma–Aldrich (Steinheim, Germany). Stock solutions were prepared with pure water supplied by Milli-Q RG unit from Millipore (Bedford, MA, USA). All the reagents used in this study were of the highest purity available. Stock solutions of iodide, bromide, thiocyanate and thiosulfate were prepared in water at a concentration of approximately 1000 mg/L, stored in a refrigerator (4 °C) and opportunely diluted before use. As iodide is light-sensitive, exposure to light should be minimized.

2.2. Instrumentation

The HPLC system used throughout this study was a Dionex system (Sunnyvale, CA, USA), consisting of a metal-free isocratic pump (Model IP 20), a Rheodyne injection valve (model RH9125, Cotati, CA, USA) with a 50 μL loop and an electrochemical detector (model ED40). The flow–through detection cell is made of a 1.0-mm diameter platinum, silver or gold working electrode and a pH-Ag|AgCl combination reference electrode; the titanium body of the cell served as the counter electrode. The cell volume is ca. 0.2 μL. A Microinjection Pump CMA/100 Microdialysis was used during the procedure of conditioning the working electrode. Separations were accomplished on a Dionex column, IonPac AS11 (250 mm × 4 mm i.d.) coupled with a guard column (50 mm × 4 mm i.d.) of the same filling. Such a column contains a hydrophilic, anion-exchange resin that is well suited to the chromatography of the relatively hydrophobic iodide anion. The column temperature was regulated by using a homemade water jacket coupled with a circulating water bath model WK4DS from Colora (Colora, Messtechnik GmbH, Germany). As recommended by column manufacturer [30], the analytical and guard columns were periodically regenerated (usually every 4–5 weeks) by washing with water for 15 min., with 200 mM HCl prepared in 80% acetonitrile for ca. 60 min, and then with water for 15 min. The mobile phase solution consisted of 25 mM HNO_3 and 50 mM $NaNO_3$ isocratically eluted at a flow rate of 1.5 mL/min. Briefly, pure water for the eluent preparation was degassed before use by flushing nitrogen for about 20 min. The plastic reservoir bottles (DX 500 2 l bottles, Dionex) were closed and pressurized with pure nitrogen to 0.8 MPa. The system was interfaced, via proprietary network chromatographic software (PeakNet^{TM}), to a personal computer, for instrumentation control, data acquisition and processing (Dionex). A centrifuge model ALC Refrigerated

Centrifuge PK 120 R (ALC International, Milan, Italy) with a rotor ALC T540 was used for sample preparation.

2.3. Electrode preparation

Silver, gold and platinum working electrodes were polished with alumina slurries of decreasing particle size (5 and 0.5 μm), rinsed with deionized water and wiped with a damp paper towel. After this initial polishing, electrodes were polished only if they had not been used for a month or longer or if their performances became worse during a prolonged working session. Moreover, before each working session electrodes were polished by eraser mechanical abrasion. Such a polishing procedure was necessary especially for silver electrode on whose surface a coating formed during electrochemical detection with consequent loss of sensitivity. Platinum electrode, after the above cleaning procedure, underwent to a conditioning step necessary to allow signal stability. The surface electrode modification was carried out using an in-line procedure wherein the electrochemical cell was properly arranged and a potential of +0.85 V versus Ag|AgCl was applied. A concentrated solution of iodide (300 mg/L) was flushed into the cell using a syringe pump at a constant flow-rate of 50 μL/min for 15–20 min. Every 2–3 weeks the platinum electrode was cleaned by electrochemical cycling between −0.25 V and +1.25 V versus Ag|AgCl in 0.5 M H_2SO_4 at a scan rate of 100 mV/s.

2.4. Urine sample preparation

Urine samples (24 h collection) were stored at −20 °C and thawed out immediately before analysis. Their pH value was comprised in the range 5.2–6.3. Before injection, samples were centrifuged at 6000 rpm for 20 min at 4 °C, properly diluted and then passed through a Sep-Pak C_{18} Cartridge (Waters Corporation, Milford, Massachusetts, USA) extraction column (360 mg, 55–105 μm). Before use the extraction columns were rinsed with 5 mL of methanol and 10 mL of deionized water. After this, 5 mL of urine samples were passed through the cartridge, discarding the first 3 mL of eluate while collecting the last two. The eluate was filtered through a 0.2 μm nylon membrane (Nylaflo Aldrich) and injected into the chromatographic system. Each sample was prepared in duplicate for each experiment.

3. Results and discussion

3.1. Iodide detection at the silver working electrode

Generally, a silver electrode is used for the electrochemical detection of iodide in liquid chromatography [13–15,18–22]. The most common applied potentials using silver working electrode are comprised between −35 and +100 mV [19–21]. According to Rendl et al. [21] we used here an applied potential of +0.1 V versus Ag|AgCl which assured the highest signal-to-noise ratio. A series of experiments was carried out employing the experimental conditions listed in Table 1 [29]. The eluent consisted of an admixture of 50 mmol/L $NaNO_3$ and 25 mmol/L HNO_3 passed at a flow rate of 1.5 mL/min. Such an eluent with strong eluting power is effective for this purpose, due to the high affinity of iodide to the columns (guard and analytical) through both hydrophobic and electrostatic interactions.

Table 1
Experimental conditions for the separation of iodide by ion chromatography with electrochemical detection

Eluent	25 mM HNO_3 50 mM $NaNO_3$
Column[a]	IonPac AS11 250 mm × 4 mm i.d.
Guard column[a]	IonPac AG11 50 mm × 4 mm i.d.
Flow rate	1.5 mL/min
Injection volume	50 μL
Column temperature[b]	30 ± 1 °C
Retention time of iodide	2.71 ± 0.05 min

[a] Dionex anion-exchange columns containing a well suited hydrophilic resin for iodide ion separation.
[b] Optimized value for urine samples.

A typical chromatogram of a mixture of bromide, iodide, thiocyanate and thiosulfate is shown in Fig. 1. The experimental conditions allowed a good resolution among these electroactive species. While bromide elutes upon the void volume, thiocyanate and thiosulfate eluted well after iodide whose retention time is 2.7 min. As can be seen the iodide ion presented clear evidence of peak shape distortion, in which the first half of the peak has a Gaussian profile, followed by a tail-end descending below the baseline. Such a large negative dip can be ascribed to the reduction of the AgI deposited on the electrode surface. Since there is no longer iodide in the solution next to the electrode surface as it has all eluted, AgI reduction is favoured in order to satisfy the Nernst equation. A similar negative dip was also observed for bromide and thiocyanate peaks. Attempts to eliminate these tailing effects by changing the chromatographic conditions, such as eluent composition, flow rate and applied potential were unsuccessful. Besides the pronounced dip which complicates the peak signal computation both in terms of peak area and peak height,

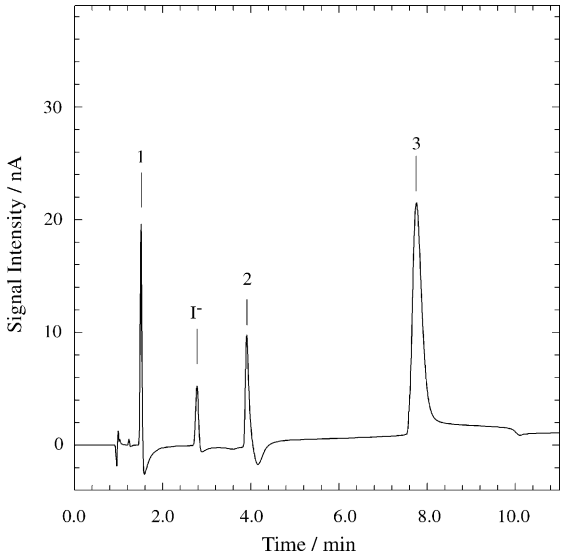

Fig. 1. Electrochemical detection at a silver working electrode following chromatographic separation of a mixture of 30 mg/L bromide (1); 100 μg/L iodide, 50 mg/L thiocyanate (2) and 10 mg/L thiosulfate (3). Detection conditions: constant applied potential, +0.1 V vs. Ag|AgCl.

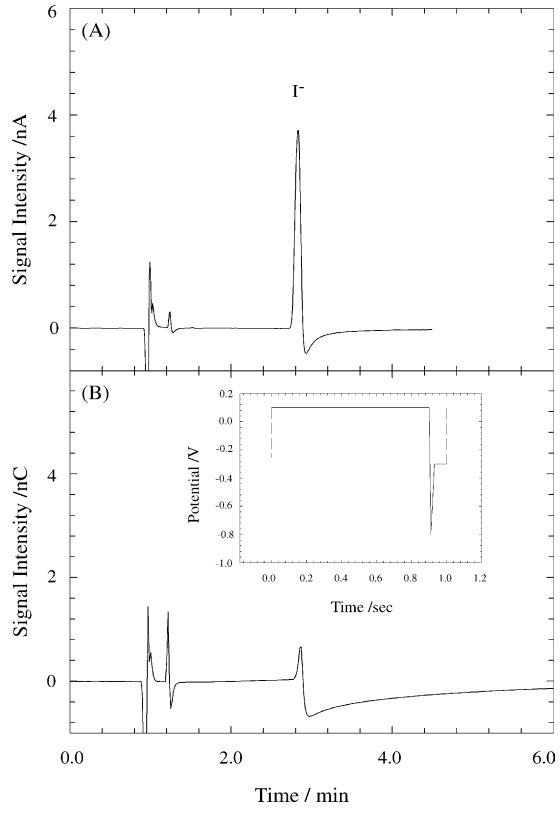

Fig. 2. Comparison between constant and pulsed electrochemical detection of 80 μg/L iodide detected at a silver working electrode: (A) constant potential, +0.1 V vs. Ag|AgCl; (B) pulsed potential waveform, see inset. The chromatographic conditions are those reported in Fig. 1.

the main problem in the use of silver as a working electrode is its oxidation, leading to electrode recession, loss of sensitivity and surface coating. After approximately 4 h of continuous analysis of iodide, if a mechanical or electrochemical regeneration processes were not employed, the electrode surface was darkened, and the electrode characteristics altered. Effects include increased noise, reduced response and low signal repeatability. The use of a silver electrode requires indeed a frequent mechanical polishing.

The Dionex Corporation [13–15], which is the manufacturer of the pulsed electrochemical detector, recommended an optimized potential waveform for iodide analysis. Such a potential waveform is characterized by a detection potential of +0.1 V for 0.91 s with current integration done between 0.2 and 0.9 s. Then a cleaning pulse of −0.8 V is applied for 0.02 s. Finally, a rest potential of −0.3 V is applied for 0.07 s before commencing a new cycle (see insert of Fig. 2B). The authors found that this pulse waveform was satisfactory for iodide analysis in real samples: milk, urine and brines. Fig. 2 shows a comparison of the chromatographic peak relevant to a standard solution of iodide (80 μg/L) obtained by applying at the silver electrode a constant potential of +0.1 V (part A) and the pulsed potential waveform (part B). As can be seen the negative post-peak dip is more pronounced when pulsed amperometric detection was employed. Similar findings, herein not shown, were obtained for bromide and thiocyanate peaks. Further attempts to inhibit peak tailing using pulsed electrochemical detection were found ineffective both in terms of sensitivity, linearity at lower iodide concentrations and signal-to-noise ratio. With a concentration of 80 μg/L iodide the amperometric detection allowed a signal to noise ratio of 870 which resulted more than 8-fold higher than the value obtained employing the pulsed electrochemical waveform, 101. Under the present experimental conditions, the estimated LoDs at a S/N = 3 and 50 μL injection were 35 μg/L and 3.5 μg/L, using pulsed and constant EC detection, respectively. For the calculation of the detection limits the average peak-to-peak noise was used. Obviously, these findings limit the employment of the pulsed waveform for the quantification of trace iodide in real samples (e.g., biological fluids, seawater). Additional experiments were also performed using a series of pulsed potential waveforms with the intent to reduce the dip effect on the chromatographic peak of iodide. Overall, no good results were obtained as a significant tailing of iodide still existed.

3.2. Iodide detection at the gold working electrode

The employment of gold rather than silver electrode for iodide detection has found rare applications in literature. Below and Kahlert [26] analyzed iodide by ion-pair chromatography with EC detection at a gold working electrode (E_{app} = 0 mV versus the palladium reference system)[1] using CH_3CN/buffer solution at pH 6.5 (10/90, v/v). Particular attention by these workers was placed upon the use of a calibration standard to compensate the continuous lowering in sensitivity. They concluded that a decline in sensitivity is invariably to be expected from the deposition of contaminants on the gold electrode surface. Here, the gold electrode was employed as electrochemical sensor under the same experimental conditions adopted for silver, i.e., an acidic nitrate eluent. First of all, the optimum operating potential for iodide detection was established. Upon a comprehensive set of injections using a solution of 100 μg/L iodide the hydrodynamic voltammogram indicated a potential value of +0.80 V versus Ag|AgCl which allowed the highest signal-to-noise ratio. The separation of a standard solution of four anions is shown in Fig. 3. Bromide, iodide, thiocyanate and thiosulphate are detected at the gold electrode with comparable sensitivity than the silver one. Note also the disappearance of the negative post-peak dip. However, to be fully successful as an amperometric sensor, the gold electrode must ensure signal stability by examination of repetitive measurements. Thus, a standard solution of bromide, iodide and thiocyanate was analyzed. The solution was injected throughout the course of the day, and the results are shown in Fig. 4. In this figure the normalized responses evaluated upon consecutive injections of 1 mg/L iodide, 90 mg/L bromide and 2 mg/L thiocyanate are reported. As can be seen, a progressive decrease in the signal response was obtained. Compared to iodide peak, which shows a continuous decline in signal response up to 40% upon ca. 6 h, bromide and

[1] There is a difference of approximately 300 mV between Ag|AgCl and the Pd reference electrode.

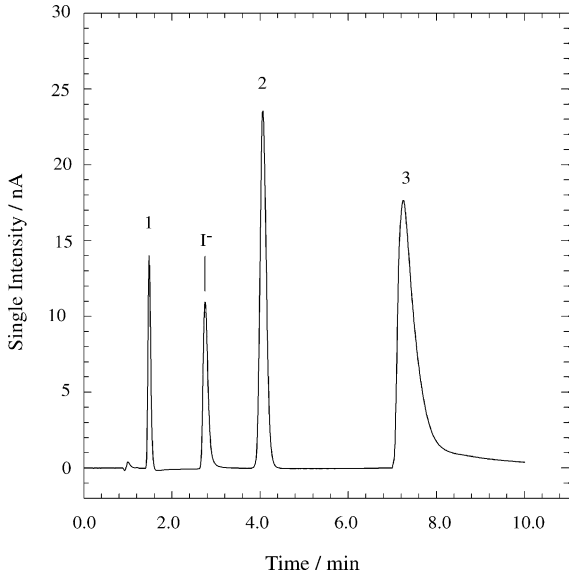

Fig. 3. Electrochemical detection at a gold working electrode following chromatographic separation of a mixture of 90 mg/L bromide (1); 100 μg/L iodide, 2 mg/L thiocyanate (2) and 50 mg/L thiosulfate (3). Constant applied potential, +0.80 V vs. Ag|AgCl. Chromatographic conditions are those reported in Fig. 1.

thiocyanate signals exhibited a more pronounced lowering of 75% and 70%, respectively. The effect was most striking within the initial 4 h of operation. Then the signal intensity lowers less sharply. Attempts to inhibit electrode contamination using a pulsed detection waveform were found ineffective. The same waveform reported by Vandeberg and Johnson for a gold electrode in acidic solutions was employed [31].

3.3. Iodide detection at the modified platinum electrode

In conformity with the results reported by a very recently published work [29] an excellent amperometric sensor for iodide

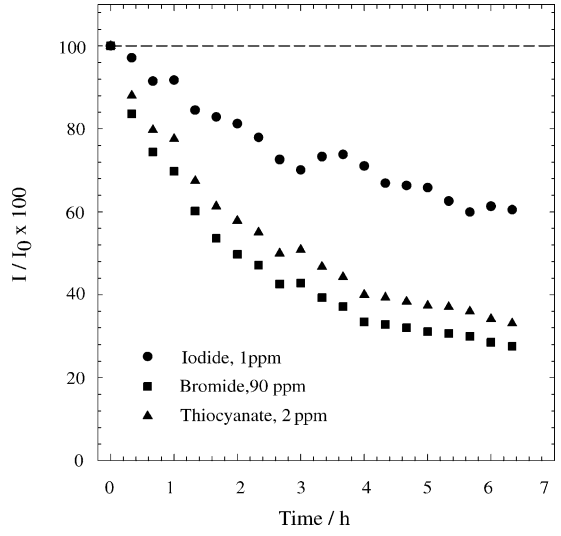

Fig. 4. Amperometric response of a gold working electrode to a standard solution containing 1 mg/L iodide, 90 mg/L bromide and 2 mg/L thiocyanate. The solution was injected for approximately six consecutive hours. Experimental conditions are those reported in Fig. 3.

Table 2
In-line platinum electrode modification using a conventional electrochemical cell and a syringe pump

Pt electrode polarization[a]	+0.85 V vs. Ag|AgCl
KI solution	100–300 mg/L in 25 mM HNO_3 and 50 mM $NaNO_3$
Flow-rate	50 μL/min using a syringe pump
Modification time[b]	ca. 15–20 min
Routine modification[c]	A daily electrode conditioning is recommended
Signal response	22.8 ± 0.5 nA (n = 7) using a standard solution of 100 μg/L iodide (50 μL injected)
Response stability	Expected lowering signal during a typical working daily session: ca. 6%.

[a] Dionex thin-layer cross-flow electrochemical cell with a 1.0-mm diameter platinum electrode. Note that the applied potential for electrode modification is the same value used for iodide detection.
[b] The working electrode remains assembled into the electrochemical cell both during the electrode modification and detection session.
[c] When the electrode modification was performed in this manner very reproducible results were obtained.

detection is given by a modified platinum electrode, which offers several advantages over silver and gold electrodes. We have confirmed the importance of modifying the platinum electrode for detecting iodide with high stability and reproducibility. The key innovation was to perform the electrode surface modification based on a simple in-line procedure using the optimized experimental conditions summarized in Table 2. In acidic solutions, the working electrode is polarized to the limiting current leading to formation of a iodine-based film. This new approach offers a number of advantages over "classical" off-line modification. In the first place, the electrochemical deposition conditions can be carefully controlled. In addition, both contamination and light exposure of the iodide conditioning solution are greatly reduced. Finally, this procedure has the further advantages of a modified electrode which exhibits high analytical response for iodide electrooxidation with good stability and very moderate signal lowering (i.e., <6% in 8 h). Range, linearity, precision, accuracy and response stability were already reported in our previous work [29]. A representative chromatogram is shown in Fig. 5. The constant applied potential at the modified platinum electrode was +0.85 V versus Ag|AgCl. Again, the chromatographic experimental conditions are those illustrated in Table 1. All peaks exhibit symmetrical shapes with no evidence of tailing. The LoD was 25 pg of iodide injected on-column, which translated to a concentration of 0.5 ppb (S/N = 3) in a 50 μL sample. Such a LoD is enough suitable for urine samples as well as other low-content-iodide matrices [32,33].

In order to confirm the modified platinum electrode as the best amperometric sensor for iodide detection, the electrochemical response was compared with that of Ag and Au employing the same chromatographic conditions. An injected concentration of 20 μg/L iodide yielded the analytical results summarized in Table 3. One important feature which needs to be noted is that the response of iodide yields an higher signal-to-noise ratio (1200 ± 110) compared to silver and gold electrodes, 710 ± 140 and 630 ± 100, respectively. The modified Pt electrode is therefore superior to silver and gold electrodes with respect to the

Table 3
Comparison of signal to noise ratio of 20 μg/L iodide at Ag, Au and Pt-modified electrodes

Working electrode[a]	Applied potential, *V* vs. Ag|AgCl	Peak signal (nA ± S.D.)[b]	Noise (peak-to-peak) (nA ± S.D.)	S/N ± S.D.
Ag	+0.10	1.40 ± 0.25[c]	0.00198 ± 0.00016	710 ± 140
Au	+0.80	2.36 ± 0.35[c]	0.00375 ± 0.00027	630 ± 100
Pt-modified[d]	+0.85	5.68 ± 0.21	0.00472 ± 0.00041	1200 ± 110

[a] Thin-layer electrochemical cell with a 1.0-mm diameter working electrode.
[b] Average value of three consecutive injections in ion chromatography with electrochemical detection.
[c] Data obtained at the beginning of the system operation.
[d] Iodine-based electrode prepared following the experimental conditions of Table 2.

peak signal of iodide on the extent and quality of peak shape, sensitivity and stability.

3.4. Determination of iodide in urine samples

Analytical potentialities of the modified platinum electrode with constant EC detection coupled to ion-chromatographic separation of iodide in urine samples were investigated. By applying the optimal conditions described above, the same urine sample was examined using both silver, gold and the modified platinum electrode. The results are illustrated in Fig. 6. An urine sample of an healthy volunteer was injected. As can be seen, the selectivity of silver electrode offers a simple elution profile, as expected [15]. In many respects, gold and the modified platinum electrodes exhibit similar chromatographic profiles, see Fig. 6B and C, respectively. Note that before injection into the chromatographic system, the urine sample was diluted and passed through a solid phase cartridge. Although, the matrix urine is relatively complex and contains a wide variety of organic and inorganic substances, the separation of iodide peak is not subject to interferences in the separation from early-eluting and after-eluting compounds, thus the iodide peak is easy to quantify using the modified Pt electrode. The peak at approximately 4.0 min is due to elution of thiocyanate. Thiosulfate eluted at approximately 7.8 min. Moreover, the resolution among the ions is optimum and the peak shapes are symmetrical, with no evidence of tailing, with the exception of the thiosulfate peak. It is worthwhile mentioning that the run time was of approximately 10 min, but the elution of an endogenous unknown compound occurs at ca. 45 min. A series of injections was made with solutions containing uric acid, ascorbic acid, creatinine and sugar acids for a possible peak identification.

In order to validate the proposed method of iodide detection at the modified platinum electrode, the quantification of such an analyte in urine samples was carried out. As illustrated in Fig. 7, where the chromatographic separation of an urine sample from a

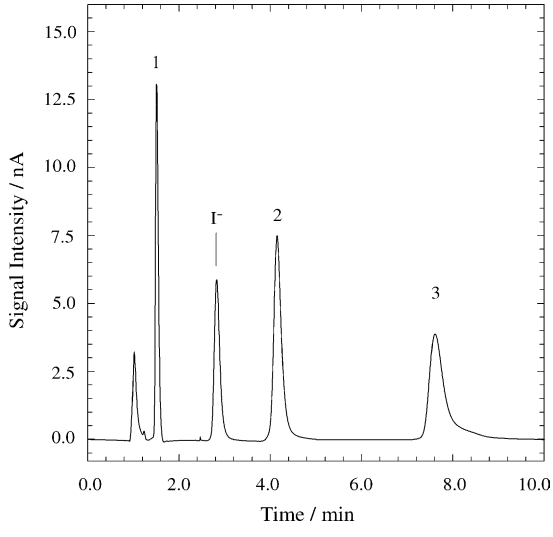

Fig. 5. Electrochemical detection at a modified Pt electrode following ion chromatography of a mixture containing 20 ppm bromide (1); 25 ppb iodide, 50 ppb thiocyanate (2) and 625 ppb thiosulfate (3). Detection conditions: iodine-based Pt modified electrode; constant applied potential, +0.85 V vs. Ag|AgCl. Chromatographic conditions are those reported in Fig. 1.

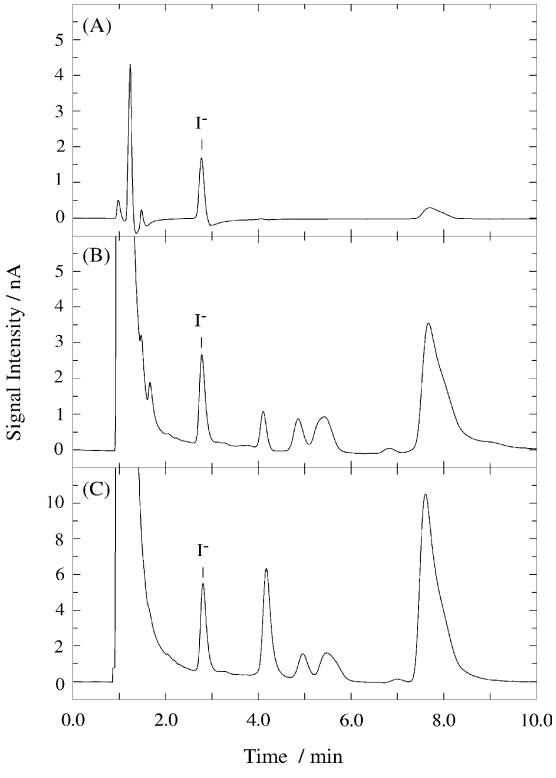

Fig. 6. Chromatographic separation of an urine sample from a healthy women diluted 1:20 with water. Constant potential amperometric detection at (A) a Ag electrode, E_{app} = +0.10 V vs. Ag|AgCl; (B) a Au electrode, E_{app} = +0.80 V vs. Ag|AgCl and (C) a modified Pt electrode, E_{app} = +0.85 V vs. Ag|AgCl. Column temperature, 30 °C. Chromatographic conditions are those reported in Fig. 1. Note that in plot (C) the scale of the signal intensity is doubled.

male healthy subject is reported, the iodide content was obtained by standard addition. As expected in the case of healthy subjects with normal iodine intake [34], the measured concentration of iodide was 127 ± 7 μg/L. At the aim to demonstrate the capability of the proposed method to distinguish various levels of iodine deficiency disorders (severe <20 μg/L, moderate 20–49 μg/L, mild 50–99 μg/L and no deficiency >100 μg/L) [34] fifty urine samples, collected from a mixed population affected by various thyroid pathologies, were analyzed in triplicate. Approximately 10% of such samples were spiked with iodide standard solutions. The measured values for the spiked and unspiked samples were obtained by external calibration using the same calibration curve. An average recovery of (98.1 ± 3.0)% was estimated thus demonstrating that the loss of iodide during sample treatment is negligible. Accuracy was tested by comparing the mean iodide concentration values evaluated in the urine samples by employing the proposed method with the values obtained by a reference colorimetric method [35]. This last method involves ammonium persulfate digestion in test tubes for 30 min at a temperature of 91–95 °C before the automated colorimetric analysis with an autoanalyzer is accomplished. In Fig. 8, the comparison between the two analytical methods on 40 human urine samples is reported. Indeed among the 50 samples analyzed it was not possible to correlate the results relevant to urine from patients affected by severe iodine deficiency because the colorimetric method is not enough sensitive and accurate for such samples and a generic value lower than 20 μg/L is given. Pearson's correlation was applied to the analytical results of these determinations. The regression equation was $y = 0.89\ x + 2.80$, $r = 0.95273$, $p < 0.0001$, where x is the value measured by the colorimetric assay and y is the value determined by the present IC–EC method. The individual confidence intervals of the slope ($p = 0.995$, CI: 0.76–1.01) and the intercept ($p = 0.995$, CI: −13.2 to 18.8) include the ideal values 1 and 0, respectively, and thus there is no statistically significant deviation between the two sets of results. Furthermore, when the paired t-test was used for each sample, it was found that the differences were not significant at a confidence level of 99.5%. As these two methods are not biased and closely correlated, the proposed separation and detection system configuration can be adopted as a valid routine analytical methodology for the determination of trace iodide. The data presented thus far suggest that the present method has less interferences and is more sensitive compared to the colorimetric one. Moreover, it is more easily accessible with respect to NAA and ICP-MS for trace iodide analysis, while the levels of other constituents (e.g., Cl^-) can be several order of magnitude higher.

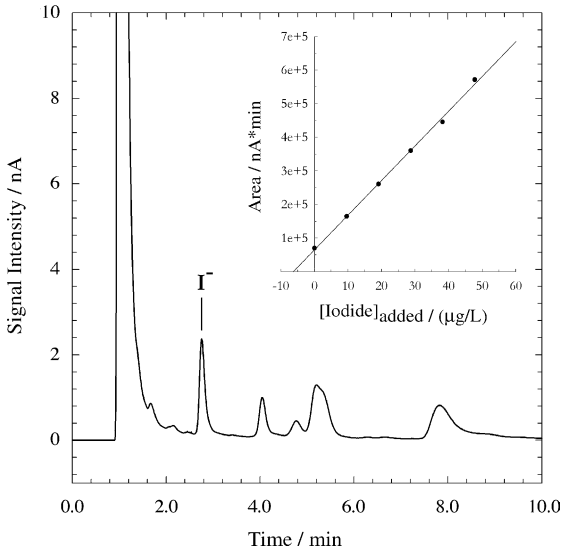

Fig. 7. Chromatographic separation of an urine sample from a healthy man diluted 1:20 with water showing the corresponding quantification curve in inset. Constant potential amperometric detection at a modified Pt electrode (E_{app} = +0.85 V vs. Ag|AgCl). See chromatographic conditions of Fig. 1. Column temperature, 30 °C.

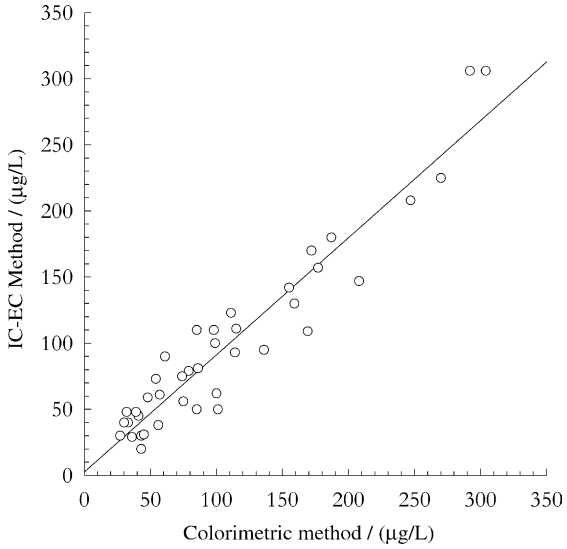

Fig. 8. Correlation between iodide concentration in human urine ($n = 40$) measured by the classic colorimetric method (x) and the present IC–EC detection method using a modified platinum electrode (y).

4. Conclusions

In the present study, we have examined and compared the electrochemical detection of iodide ion at Ag, Au and Pt electrodes in acidic aqueous solutions. Constant applied potential at a modified Pt electrode is recommended as it provides significant advantages over conventional electrochemical sensors. Even though the silver electrode selectivity towards iodide detection is very high, the superiority of the iodine-based platinum electrode in terms of sensitivity, response stability and low detectable levels is demonstrated. The excellent sensitivity of detection at the modified platinum electrode coupled with the good separation capabilities of the ion-exchange column employed represents a very useful combination to analyze complex samples. This is particularly interesting in the prospect of using this technique as a tool for iodide analysis in urine and environmental samples. Results on human urine demonstrated that the method, due to its easy applicability and high accuracy, can be regarded as a valid alternative to conventional assays for urinary iodide detection.

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