ELSEVIER

Analytica Chimica Acta 464 (2002) 123–133

ANALYTICA CHIMICA ACTA

www.elsevier.com/locate/aca

An amperometric method for the determination of trace mercury(II) by formation of complexes with L-tyrosine

Sanaâ Majid [a], Mama El Rhazi [a], Aziz Amine [a,*], Christopher M.A. Brett [b]

[a] *Laboratoire des Analyses Chimiques et des Biocapteurs, Faculté des Sciences et Techniques, B.P. 146, Mohammedia, Morocco*
[b] *Departamento de Quimica, Universidade de Coimbra, 3004-535 Coimbra, Portugal*

Received 8 January 2002; received in revised form 2 May 2002; accepted 14 May 2002

Abstract

A selective and sensitive amperometric method of analysis has been developed for determination of the trace amounts of mercury in waters at a platinum electrode based on the effect of the presence of mercury ions on the current due to oxidation of L-tyrosine. A decrease of signal was observed due to the formation of a complex of tyrosine with the Hg(II) ion adsorbed on the electrode surface. Several parameters were varied, such as applied potential, pH and concentration of tyrosine. The calibration plot was linear in the range from 0.02 to 3 μmol l^{-1} Hg(II) with $r = 0.997$ and the detection limit (3σ) was 0.014 μmol l^{-1}; the relative standard deviation was 2.2%. The study of interferences from other metal ions revealed a good selectivity of this method towards mercury(II). The stoichiometry of the mercury–tyrosine complex was determined to be 1:2 and the formation constant 627 ± 19. Formation of complexes with mercury ions was also demonstrated with several catechol compounds and other amino acids. The method was applied to the analysis of contaminated waters.

Keywords: Mercury; L-Tyrosine; Amperometry; Amino acids

1. Introduction

Mercury is one of the most well known toxic metals. Its toxicity is attributed to its harmful effects on the central nervous system disturbing haemin synthesis as well as causing neuropsychiatric disorders [1]. Reports of such cases have come from many areas of the world, but those from Asia have been the most numerous; the cases in Minamata Bay in Japan in 1953 [2] were particularly disastrous. Mercury is usually present at low concentrations in environmental samples as inorganic, free or complexed with inorganic and organic ligands or as organomercury compounds [3] and, therefore, highly sensitive and selective methods are required for analysis. A number of methods have been used for the determination of total mercury, such as cold vapour atomic fluorescence spectrometry CV-AFS [4], cold vapour atomic absorption spectrometry CV-AAS [5], inductively coupled plasma atomic emission spectrometry ICP-AES [6] and inductively coupled plasma mass spectrometry ICP-MS [7].

It is highly desirable to be able to transfer these measurements from the central laboratory into the field. Spectroscopic techniques, commonly used for trace measurement of mercury in the laboratory, are not suitable for the task of on-site testing and monitoring. The portable nature and excellent sensitivity of electrochemical techniques make them very attractive for field monitoring of trace metals [8].

* Corresponding author. Fax: +212-2331-5353.
E-mail address: azizamine@yahoo.fr (A. Amine).

0003-2670/02/$ – see front matter
PII: S0003-2670(02)00422-1

The use of chemically modified electrodes (CMEs) for the determination of mercury is an area of great interest and complexation reactions with organic reagents as modifiers have been widely applied. Strategies used include the modification of glassy carbon surface by perfluorinated ion exchangers [9] or permselective modified electrodes [10]: low detection limits were reached using anodic stripping voltammetry. Problems associated with possible surface contamination are the main disadvantage of this kind of electrodes.

Determination of Hg(II) has been successfully performed by means of chemically modified carbon paste electrodes (CPE). Preconcentration by complexation was used with CPEs modified with 1,5-diphenylcarbazide [11], humic acid [12], zinc-diethyldithiocarbamate [13] and with ion exchange Amberlite LA2 [14]. The detection limits obtained were 5, 0.5, 0.8 and 50 nM, respectively.

The best electroanalytical performance for measuring low concentrations of mercury ions has been achieved with gold [15] and gold-plated [16–19] electrodes. The main disadvantages of gold electrodes are the well known structural changes of the gold surface, caused by amalgam formation and the time-consuming and complex electrochemical pre-treatments that are needed to achieve reproducibility.

Many metal ions form complexes with amino acids and small peptides and studies of this complexation have been carried out in order to obtain a better understanding of transition metal complexes in proteins. The location of different metal ions in the hydrophobic cavity of a protein depends on the relative intrinsic bond strength between the metal ions and the various possible metal binding sites. Recently, the lithium, sodium and copper [20] and zinc [21] ion affinities of the most common amino acids have been measured. Metal ion–tyrosine complexes have been also widely examined [22,23]. It is evident from all these studies that metal ions can bind to amino acids in several ways.

In the present work, we investigate the use of the amino acid, L-tyrosine, for the determination of mercury in aqueous samples through the decrease of the oxidation current of tyrosine at platinum electrodes caused by the formation of an adsorbed electroinactive complex. Tyrosine is an amino acid with pK_a values of 2.2 (–COOH group) and 9.1 ($–NH_3^+$ group); it is a direct precursor of various hormones, biogenic amines and neurotransmitters [24]. The experimental conditions were optimised and analytical parameters, such as linear range and detection limit were determined. An electrochemical study of the formation of the complex between L-tyrosine with mercury ions was also carried out, concerning which no work exists in the literature. The data obtained allow the deduction of the stoichiometry of the complex and permit the calculation of the formation constant. Additionally, a study of the interferences and a comparison with other molecules with characteristics similar to tyrosine—catechols and other amino acids—was carried out. Finally the technique was applied to the analysis of tap water and waste water samples.

2. Experimental

2.1. Apparatus

Amperometric measurements were carried out with BAS CV-27 instrument coupled to a BAS LC-3D electrochemical detector (Bioanalytical Systems) and a YOKOGAWA 3025 X–Y recorder, under constant stirring conditions (300 rpm). Cyclic voltammetry experiments were performed with an Autolab PGSTAT 10 System (Ecochimie). The three-electrode system consists of a platinum disc as working electrode (2 mm diameter), an Ag/AgCl reference electrode (3 M KCl) and a stainless steel rod auxiliary electrode. The pH was adjusted using a Fisher Scientific Accumet AB15 BASIC pH meter.

The platinum electrode was polished on a polishing cloth with wet alumina powders, starting with 15 μm particle size and then with finer grades down to 1 μm. It was then rinsed with deionized water and sonicated for 5 min.

2.2. Reagents

All chemicals used were analytical reagent grade. Aqueous solutions were prepared with doubly distilled water. A 10^{-3} mol l^{-1} Hg(II) stock solution was prepared from $HgCl_2$ (Fluka). Solutions of concentration 20 μM of the amino acids L-tyrosine, L-cysteine hydrochloride, L-tryptophan, L-dopa (3,4-dihydroxyphenylalanine), dopac (3,4-dihydroxyphenylacetic acid) and dopamine (3-hydroxytyramine) from Aldrich, were

prepared in 0.1 M pH 7.0 phosphate buffer unless otherwise specified. Methylmercury chloride was purchased from JM Alfa Products, Karlsruhe, Germany and phenylmercuric acetate from Sigma.

Tap water samples were collected in the laboratory. Waste water samples were adjusted to pH 2.0 with concentrated nitric acid and filtered through membrane filters of 0.45 μm pore size. The sample tested was diluted 10 times with pH 7.0 phosphate buffer.

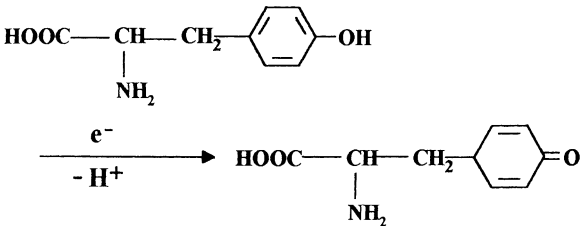
All experiments were carried out at room temperature (25 ± 1 °C).

Each individual experiment was performed at least three times, then the results were averaged.

3. Results and discussions

3.1. Electrochemical oxidation of L-tyrosine

The amino acid L-tyrosine is electro-oxidisable at platinum electrodes at high positive potentials. Cyclic voltammograms for the electro-oxidation of 20 μM L-tyrosine in phosphate buffer solution at pH 7, as shown in Fig. 1, present an irreversible oxidation wave at 0.8 V and a poorly defined electrochemical response, in agreement with the literature data [25–28]. The mechanism of the electrochemical oxidation of tyrosine, in neutral medium, is:



The addition of 5×10^{-5} mol l^{-1} of mercury(II) caused a 20% decrease in peak current at a scan rate of 50 mV s^{-1} and a shift in the peak potential toward more positive values, which could be due to the formation of an electro-inactive complex between tyrosine and mercury ions. In order to exploit the complex formation for analytical purposes amperometric measurements were made under different conditions by maximising the sensitivity and lowering the detection limit.

3.2. Optimisation of tyrosine response

The effect of applied potential on tyrosine oxidation, at neutral pH, was studied in the range of 0–1 V

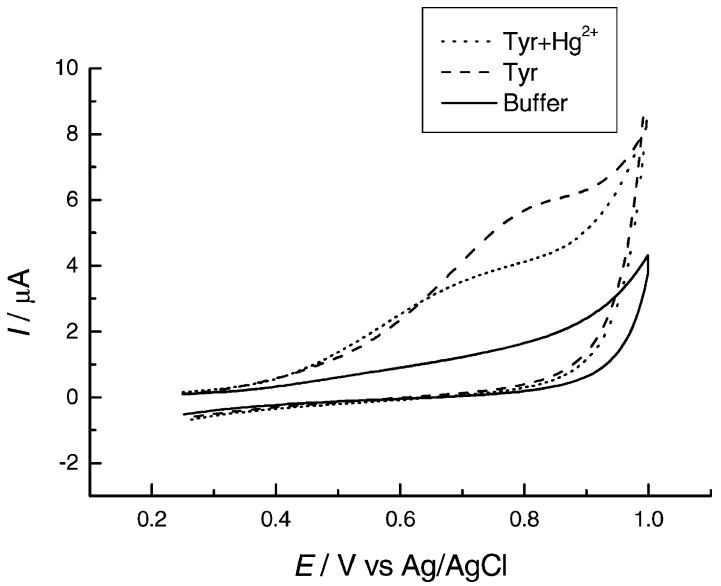


Fig. 1. Cyclic voltammograms for the electro-oxidation of 20 μM L-tyrosine without (dashed line) and with (dotted line) 50 μM Hg^{2+} in pH 7 of 0.1 M phosphate buffer (solid line): scan rate 0.05 V s^{-1}.

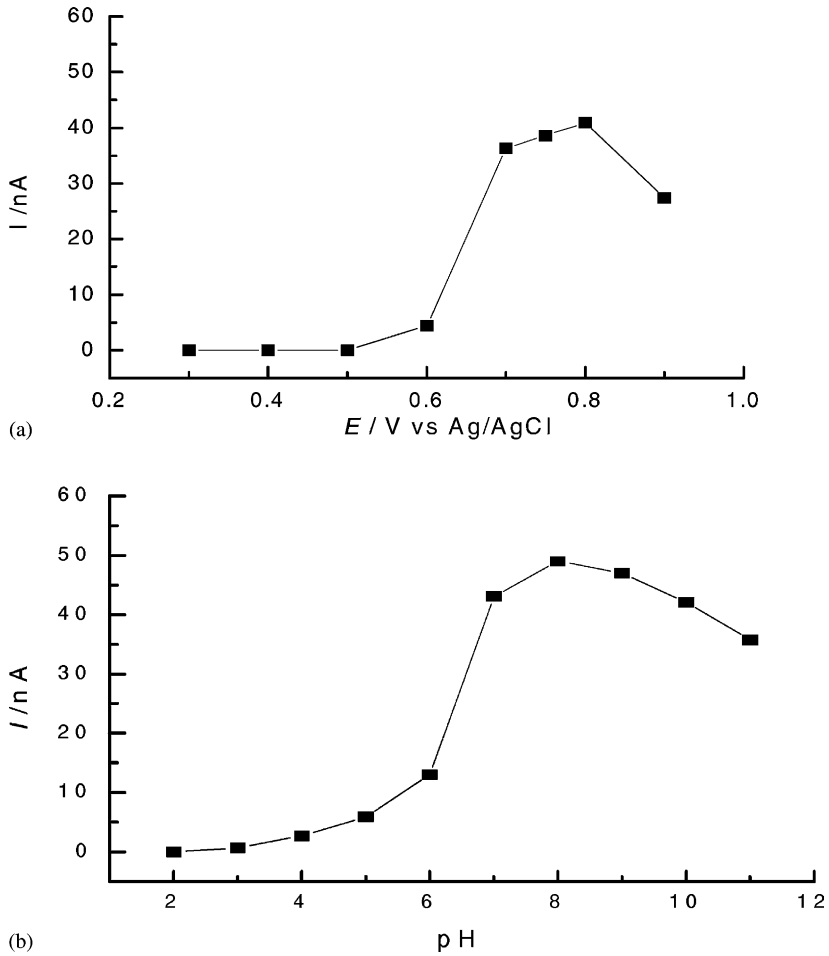


Fig. 2. Effect of the applied potential on the oxidation current for 20 μM L-tyrosine in 0.1 M phosphate buffer of (a) applied potential at pH 7 and (b) pH at +0.75 V vs. Ag/AgCl.

using amperometric measurements. Fig. 2a shows that tyrosine oxidation begins to appear at +0.6 V in the solution with pH 7. This potential value corresponds with the completely flat orientation of the tyrosine's benzene ring to the electrode surface [29]. The adsorption of amino acids onto platinum electrode, in basic solution, is achieved through the terminal carboxyl of fully unprotonated anions from +0.4 V versus Ag/AgCl. It was established in FT-IR studies [29] on the electrochemical oxidation of tyrosine on platinum electrodes that at potentials more negative than +0.1 V, the orientation of tyrosine should be random, and the benzene ring begins to be oriented parallel to the electrode surface from +0.3 V. The terminal carboxyl of tyrosine is adsorbed from +0.4 V, and the flat orientation of the benzene ring is completed at about +0.6 V. Studies were carried out with similar results at silver [30] and gold [31] electrodes.

Under our experimental conditions, no decrease of signal was observed in the amperometric measurement when a low concentration of tyrosine (20 $\mu mol\,l^{-1}$) was used. However, at higher concentration (e.g. 100 $\mu mol\,l^{-1}$) the signal is unstable and starts to decrease slightly. This suggests that the orientation of tyrosine molecules on the surface of the electrode is coverage dependent. This is in agreement with another study in which it was also shown that the

orientation of the adsorbed molecules depends on the surface coverage [32].

The effect of pH was investigated over the range of pH from 2 to 11. Fig. 2b shows that the amperometric signal of tyrosine oxidation is pH dependent and reaches maximum values in the range of 7–9. In further studies, pH 7 was employed.

3.3. Procedure for mercury(II) determination

The bare platinum electrode was immersed in a cell containing 10 ml of pH 7 phosphate buffer; after stabilisation of residual current, tyrosine was added. Fig. 3 shows typical current–time curves. The current response to tyrosine addition was measured as I_1. When mercury ions are added to the solution, they form an electro-inactive complex with tyrosine, a decrease of tyrosine signal was observed and the current I_2 was measured after 11 min which corresponds to 90% of the maximum change in the current.

The percentage decrease in the tyrosine signal due to the addition of Hg(II) ions can be expressed as:

$$I\% = \frac{I_1 - I_2}{I_1} 100$$

The influence of tyrosine concentration was investigated; different concentrations of 4, 20 and 100 μmol l^{-1} were used. Increasing the tyrosine concentration

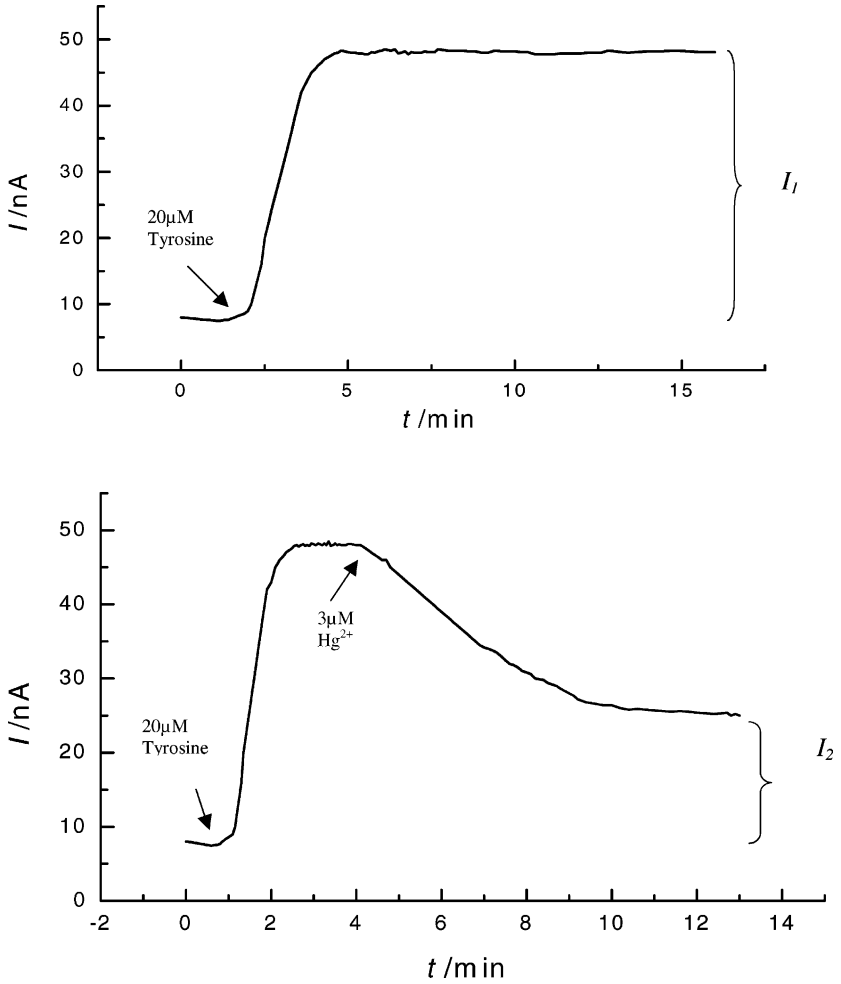


Fig. 3. Typical current–time curves obtained before and after adding mercury ions: applied potential +0.75 V, 0.1 M phosphate buffer at pH 7.

resulted in an increase in the tyrosine signal, I_1, but no significant change in (I%), due to the formation of the mercury–tyrosine complex, was recorded after adding mercury ions. Also, a high concentration of tyrosine, 100 μM, leads to an unstable tyrosine signal. Therefore, a concentration of 20 μM was chosen as the optimised tyrosine concentration.

The effect of the presence of mercury ions on the tyrosine oxidation current was studied in the range of potential within which there is a good response for tyrosine oxidation, i.e. from 0.6 to 0.9 V. Fig. 4a shows that the best potential that gives a significant decrease in the signal, after the addition of 3.0 μmol l^{-1} mercury ions, was +0.75 V.

Regarding the effect of the solution pH and as previously mentioned, the tyrosine signal reaches maximum values in the pH range 7–9. Measurements were carried out at +0.75 V, within this range and the greatest decrease in the signal was obtained at pH 7, as shown in Fig. 4b.

The reason for the large change in the oxidation response for relatively small concentrations of Hg(II) can be traced to the large formation constant of the electro-inactive mercury(II)–tyrosine complex (see Section 3.4). Indeed, the incubation of a solution containing tyrosine (200 μmol l^{-1}) and mercury (30 μmol l^{-1}) for varying periods of time (10–60 min) and its injection into a cell solution (dilution factor of 10) gave the large signal of tyrosine followed by a decrease in oxidation current during about 11 min (similar to Fig. 3) indicating adsorption and that mercury complexation occurs on the surface of the electrode. This suggests that the orientation of tyrosine molecules on the surface of the electrode (see Section 3.2) is favourable for the formation of adsorbed electro-inactive mercury complexes.

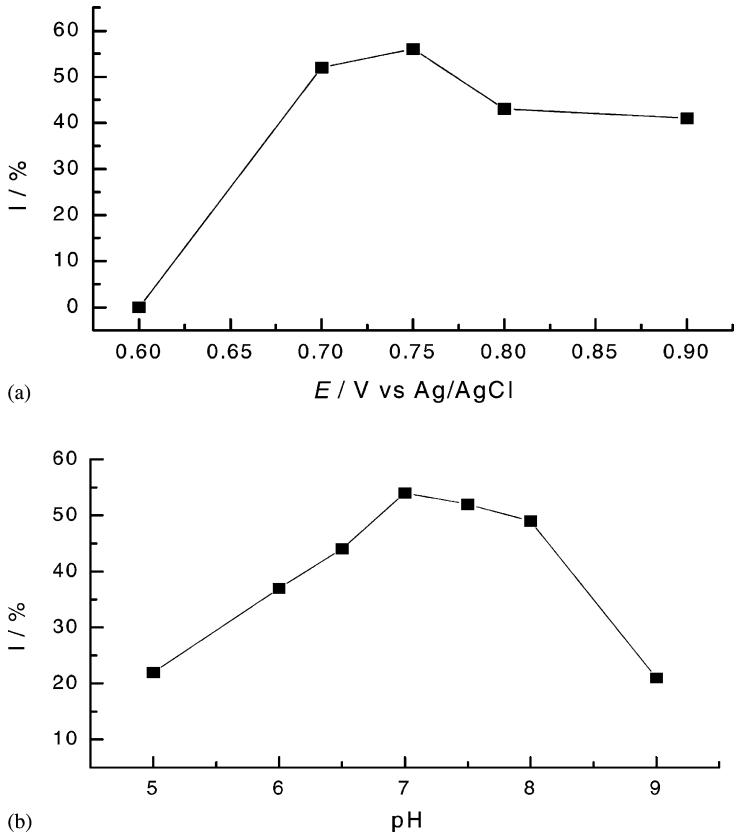


Fig. 4. Effect on the percentage decrease in oxidation current of 20 μM L-tyrosine + 3 μmol l^{-1} Hg^{2+} in 0.1 M phosphate buffer of (a) applied potential at pH 7 and (b) pH at +0.75 V vs. Ag/AgCl.

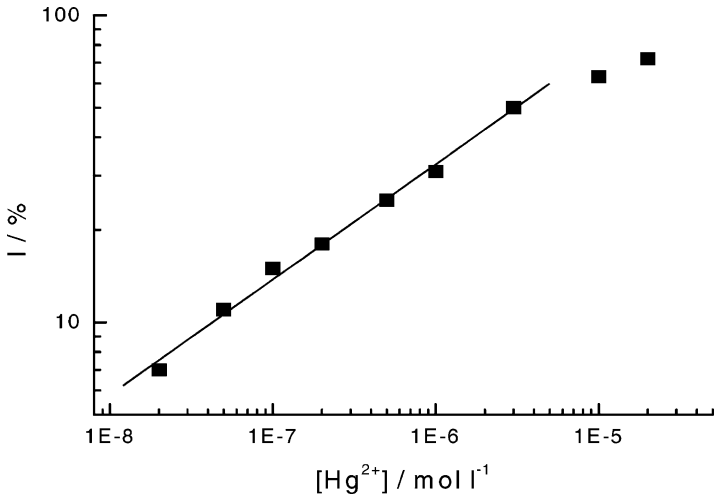


Fig. 5. Calibration plot for mercury ions: 20 μM L-tyrosine; applied potential +0.75 V; 0.1 M phosphate buffer, pH 7.

Under the chosen optimised conditions, the variation of the percentage decrease in the tyrosine signal as a function of the concentration of mercury ion added to the solution at pH 7 and +0.75 V was studied. Fig. 5 shows the calibration plot. Linearity is obtained in the range of 0.02–3 μmol l^{-1} mercury(II), the linear regression equation was $\log I\% = 0.38 \log [Hg^{2+}] + 3.8$ with $r = 0.997$. Six repeated measurements of 3 μM mercury lead to 2.2% relative standard deviation. The detection limit was estimated to be 0.014 μmol l^{-1} based on three times the standard deviation (3σ).

The optimised method was also tested for determination of trace levels of two of the most common organomercury species, methylmercury and phenylmercury, but no change in the oxidation signal of adsorbed tyrosine was found, suggesting that other organomercury compounds would also give no result. It is, thus, deduced that this method is useful for the detection of labile Hg^{2+} ions, either free or in weakly-bound complexes. Regarding application to the measurement of other forms of mercury, organomercury compounds can be oxidized to inorganic Hg by potassium permanganate, persulphate, and heat as reported in the AOAC method [33]. In the case of waste waters, the various mercury species can bind to the particulate matter that may exist, for example [34]. The concentration of mercury bound to such matter could be determined as the difference in analytical test results of total mercury and soluble mercury species, the latter obtained after appropriate filtering of the waste water, normally a 0.45 μm filter, as described in Section 2.

3.4. Stoichiometry of the mercury complex

The measured current for tyrosine oxidation is proportional to the surface concentration of adsorbed tyrosine. Addition of Hg^{2+} ions to the solution, leads to the formation of an electro-inactive complex with tyrosine, and a decrease of the observed current. This decrease is directly related to both the concentration of mercury ions and the formation constant of the complex.

The formation of the complex between tyrosine and Hg^{2+} can be written as:

$$\text{Tyr} + n\,\text{Hg}^{2+} \overset{\beta_n}{=} [\text{Hg}_n\,\text{Tyr}]^{2n+} \quad (1)$$

The formation constant, β_n, is

$$\beta_n = \frac{[\text{Hg}_n\,\text{Tyr}]^{2n+}}{[\text{Tyr}][\text{Hg}^{2+}]^n} \quad (2)$$

During the experiments, the total concentration of tyrosine on the electrode surface, $[\text{Tyr}]_\text{T}$, has a constant value and can be expressed as

$$I_1 = k[\text{Tyr}]_\text{T} \quad (3)$$

Table 1
Concentration of various cations leading to 10% $[\,]_{10}$ and 50% $[\,]_{50}$ reduction in the oxidation current of 20 μmol l^{-1} tyrosine at +0.75 V vs. Ag/AgCl in pH 7, 0.1 M phosphate buffer

Concentration (μmol l^{-1})	Cation									
	Hg^{2+}	Zn^{2+}	Ag^{+}	Cu^{2+}	Ni^{2+}	Cd^{2+}	Pb^{2+}	Fe^{2+}	Fe^{3+}	Co^{2+}
$[\,]_{10}$	0.05	8.0	6.2	0.7	7.9	5.9	12.3	5.6	10.1	8.2
$[\,]_{50}$	3	400	330	40	400	330	600	330	500	400

where k is a constant. Thus, the initial current, I_1, measured in the absence of mercury is directly proportional to $[\mathrm{Tyr}]_T$.

When mercury ions are added to the solution, the measured current is proportional to the concentration of uncomplexed tyrosine:

$$I_2 = k[\mathrm{Tyr}] \tag{4}$$

Additionally, from mass balance:

$$[\mathrm{Tyr}]_T = [\mathrm{Hg}_n\,\mathrm{Tyr}]^{2n+} + [\mathrm{Tyr}] \tag{5}$$

Substitution of Eqs. (3)–(5) in Eq. (2) leads to:

$$\beta_n = \frac{[\mathrm{Tyr}]_T - [\mathrm{Tyr}]}{[\mathrm{Tyr}][\mathrm{Hg}^{2+}]^n} = \frac{I_1 - I_2}{I_2[\mathrm{Hg}^{2+}]^n} \tag{6}$$

Thus, the value of n and, hence, the stoichiometry of the complex can be determined from the slope of the plot of $\log[(I_1 - I_2)/I_2]$ versus $\log[\mathrm{Hg}^{2+}]$. A straight line with a slope of $n = 0.51$ is obtained. This value of n indicates that two molecules of tyrosine are involved in each electro-inactive complex on the electrode surface. Accordingly, Eq. (1) can be written as:

$$\mathrm{Tyr} + 2\mathrm{Hg}^{2+} \overset{\beta_{1/2}}{=} [\mathrm{Hg}_n\,\mathrm{Tyr}]^{2n+}$$

Eq. (6) can be rearranged as:

$$\frac{I_1}{I_2} = 1 + \beta_n[\mathrm{Hg}^{2+}]^n \tag{7}$$

which allows us to determine the value of $\beta_{1/2}$ by plotting the variation of I_1/I_2 as a function of the square root of $[\mathrm{Hg}^{2+}]$. The slope of this curve gives the formation constant of the mercury–tyrosine complex, $\beta_{1/2}$, of 627 ± 19.

3.5. Interferences

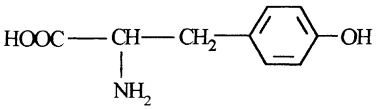
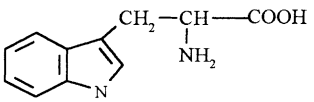
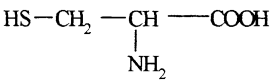
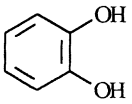
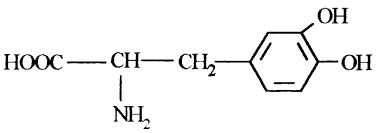
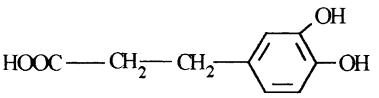
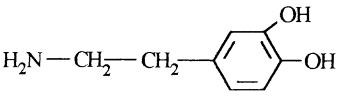
To assess the selectivity of this method, the same experiment was carried out in the presence of different metal cations: Zn^{2+}, Ag^{+}, Cu^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+}, Co^{2+}, $Fe^{2+}$and Fe^{3+} under the optimised conditions given above. The metal ion concentration leading to a 10 and 50% decrease in the current corresponding to oxidation of 20 μM tyrosine was determined. The results, as shown in Table 1, revealed an excellent selectivity towards mercury(II) ions. No significant complexation was found, excepting copper ions, and a 10% decrease was obtained at a concentration more than 100 times the concentration of mercury ion causing the same percentage decrease. In the case of copper ions, a concentration 10 times higher than that of mercury ions leads to a 10% decrease in oxidation current, which could represent a significant interference. For a 50% decrease in the current, only copper ions represented a significant interference, again at a concentration 10 times higher than that of mercury ions.

3.6. Comparison with other complexants

The behaviour of tyrosine was compared with several catechol compounds—catechol, dopa, dopac and dopamine—which have a similar structure to tyrosine. Also, two oxidisable amino acids were studied, cysteine and tryptophan. The structures of these molecules are given in Table 2.

Experiments were carried out under the same optimised conditions as before, and it was found that none of these molecules has the same complexation behaviour as tyrosine. Only cysteine showed some decrease of current after adding mercury: when mercury ions were injected into phosphate buffer containing 20 μM cysteine, the current decreased much faster than with tyrosine. Cysteine reacts through the thiol groups with mercury in solution to form the mercury–cysteinate complex in solution, which explains why 90% of the final response was obtained only 30 s after adding mercury. This contrasts with mercury ions which form a complex with tyrosine on

Table 2
Structures of the mercury ion complexants

Molecule	Structure
Tyrosine	
Tryptophan	
Cysteine	
Catechol	
Dopa	
Dopac	
Dopamine	

the electrode surface, 90% of the final response being obtained only 11 min after adding mercury ions.

The results summarised in Table 3 compare possible interference effects from copper ions. They show that cysteine is three times more sensitive to mercury ions than tyrosine but, on the other hand, is less selective. Indeed, 50% decrease in the signal for cysteine oxidation was obtained with a concentration of copper five times lower than that of mercury. Thus, tyrosine is to be preferred for selectivity towards mercury ions.

Table 3
Concentration corresponding to 50% decrease in the current for amino acid oxidation (20 μM) after adding the metal ion

	Hg^{2+} ($\mu mol\,l^{-1}$)	Cu^{2+} ($\mu mol\,l^{-1}$)
Tyrosine	3.0	40.0
Cysteine	1.0	0.2

3.7. Reversibility of complex formation

To avoid physical polishing of the electrode at the beginning of each measurement, which would preclude any practical analytical benefits of the method, the working surface of the electrode was rinsed with only distilled water and the experiments repeated. A 15% decrease in tyrosine signal was found but no change in percentage current decrease was recorded after adding mercury ions. Sequential experiments can, thus, be performed.

Additionally, the reversibility of adsorption of the mercury–tyrosine complex on the electrode was investigated after a sequence of many experiments in which the electrode surface became completely covered. It was found that 24% of the initial tyrosine signal was restored after adding 20 μM EDTA in pH 7 phosphate buffer containing 20 $\mu mol\,l^{-1}$ tyrosine and 3 $\mu mol\,l^{-1}$ mercury ions, due to the formation of the soluble Hg–EDTA complex. Higher EDTA concentrations gave no further improvement. This regeneration capability is also an important benefit for the analytical applications.

3.8. Analysis of real samples

The analytical performance of the method was first assessed by determination of Hg^{2+} in spiked tap water samples. Water was collected in the laboratory and analysed without any pre-treatment. The effect of three samples on the signal corresponding to oxidation of 20 μM tyrosine was studied under the optimised conditions. The results of the determination of Hg^{2+} in these solutions are given in Table 4. As can be seen, satisfactory recovery was obtained in the spiked water samples. However, if the mercury concentration is lower than 0.2 μM, one must be aware that the recovery may be somewhat lower.

The method was also applied to the three different waste waters, where possible interferences due to the

Table 4
Determination of Hg^{2+} in tap water sample

Sample no.	Spiked ($\mu mol\,l^{-1}$)	Found ($\mu mol\,l^{-1}$)	Recovery (%)
1	0.20	0.17	85 ± 3
2	0.30	0.29	97 ± 2
3	0.40	0.42	105 ± 2

Table 5
Determination of Hg^{2+} in waste water samples

	Sample 1	Sample 2	Sample 3
Detected value in diluted sample (nmol l^{-1})[a]	24 ± 1.5	22 ± 1.3	46 ± 3.4
Detected value after 50 nM spike (nmol l^{-1})	75 ± 4.5	70.4 ± 4.1	93 ± 8
Recovery (%)	102	97	94
Real value (μmol l^{-1})[a]	0.240 ± 0.015	0.220 ± 0.013	0.460 ± 0.034
Coefficient of variation (%)	6	6	7

[a] Real value is obtained by multiplying the detected value by the dilution factor.

complexity of the matrix could appear. For these water samples, the method of standard addition was used. The spiked samples gave low recoveries of mercury standards without sample pre-treatment (<40%). This suggests that the analyte matrix is strongly complexing the mercury ions, so that they are not labile and, thus, unavailable to form complexes with tyrosine. Therefore, the samples were acidified at pH 2.0 to make the mercury ions labile and filtered through membrane filters (0.45 μm pore size) before analysis. Any interference from other metal cations, that can be present in high concentration, was successfully avoided by adding 10^{-3} mol l^{-1} potassium sodium tartrate masking agent to the samples. Under these conditions, good recoveries were obtained (94–102%). The results of the analysis of these three different samples are given in Table 5. For sample 3, that contains some electro-active organic compounds, the signal due to the oxidation of these compounds (<25% of the tyrosine signal) was subtracted from that of tyrosine.

The analysis of real samples shows that although our proposed method is not sufficiently sensitive to allow the determination of mercury in natural water, as in [17], it does represent an extremely useful and robust approach for the analysis of waste waters contaminated with other metal ions and electro-active organic compounds.

4. Conclusions

An amperometric procedure for determination of mercury(II) was developed, based on the formation, on a platinum electrode surface, of a complex of formula HgL_2 between the amino acid, tyrosine, and mercury ions. It has been demonstrated that the amino acid, tyrosine, has a high sensitivity and excellent selectivity towards mercury ions.

This method is very simple, offering a relatively short analysis time of 11 min, is inexpensive, with easy operability, and is applicable for field use. Beside good selectivity, it has a low detection limit of 14 nmol l^{-1} and is applicable with sufficient reliability to the determination of mercury in contaminated water.

Work is in progress for using this proposed method to determine and discriminate between tyrosine and other oxidizable amino acids and catechol compounds in physiological media.

Acknowledgements

The authors gratefully acknowledge Dr. H. Randriamahazaka from University of Cergy, Pontoise and the International Foundation for Science for the financial support of this work (A/2912-1 and A/2780-1). The authors also acknowledge support from the Scientific and Technical Co-operation Agreement Portugal, Morocco (ICCTI-CNCPRST, 2000–2001), and the Portuguese Foundation for Science and Technology—Project POCTI (co-financed by the European Community Fund FEDER).

References

[1] J. Frausto da Silva, R.J.P. Williams, The Biological Chemistry of the Elements, Clarendon Press, Oxford, 1993, p. 539.
[2] T. Tsubaki, K. Irukayama, Minamata Disease: Methylmercury Poisoning, Kodensha, Tokyo, 1977.
[3] A.K. Das, M. de la Guardia, M.L. Cervera, Talanta 55 (2001) 1.
[4] M. Yoshino, H. Tanaka, K. Okamoto, Bunseki-Kagaku 44 (1995) 691.
[5] C. Zarzanini, G. Sacchero, M. Aceto, O. Abollino, E. Mentasti, J. Chromatogr. A 626 (1992) 151.
[6] I.S. Krull, D.S. Bushee, R.G. Shleicher, S.B. Smith, Analyst 111 (1986) 345.

[7] C.F. Harrington, T. Catterick, J. Anal. At. Spectrom. 12 (1997) 1053.
[8] C.M.A. Brett, Electroanalysis 11 (1999) 1013.
[9] L.M. Moretto, G.A. Mozzocchin, P. Ugo, J. Electroanal. Chem. 427 (1997) 113.
[10] I. Turyan, T. Erichsen, W. Schuchmann, D. Mandler, Electroanalysis 1 (2001) 79.
[11] Z. Navratilova, Electroanalysis 3 (1991) 799.
[12] Z. Navratilova, P. Kula, Electroanalysis 4 (1992) 683.
[13] S.B. Khoo, Q. Cai, Electroanalysis 6 (1996) 549.
[14] X. Cai, K. Kalcher, W. Diewald, C. Neuhold, R.J. Magee, Anal. Chem. 345 (1993) 25.
[15] Y. Bonfil, M. Brand, E. Kirowa-Eisner, Anal. Chim. Acta 424 (2000) 65.
[16] E.P. Gil, P. Ostapczuk, Anal. Chim. Acta 293 (1994) 55.
[17] C. Faller, N.Y. Stojko, G. Henze, K.Z. Brainina, Anal. Chim. Acta 396 (1999) 195.
[18] I. Svancara, M. Matousek, E. Sikora, K. Schachl, K. Kalcher, K. Vytras, Electroanalysis 9 (1997) 827.
[19] J. Wang, B. Tian, Anal. Chim. Acta 274 (1993) 1.
[20] T. Marino, N. Russo, M. Toscano, J. Inorg. Biochem. 79 (2000) 179.
[21] R. Andreoli, G. Battistuzzi Gavioli, L. Benedetti, G. Grandi, G. Marcotigiano, L. Menabue, G.C. Pellacani, Inorg. Chim. Acta 46 (1980) 215.
[22] V. Ryzhov, R.C. Dunbar, B. Cedra, C. Wesdemiotis, J. Am. Soc. Mass Spectrom. 11 (2000) 1037.
[23] D. Van Der Helm, C.E. Tatsch, Acta Cryst. 28 (1972) 2307.
[24] L. Stryer, Biochemistry, 4th Edition, Freeman, New York, 1995.
[25] B. Malfoy, J.A. Reynaud, J. Electroanal. Chem. 114 (1980) 213.
[26] G.A. Rivas, V.M. Solis, Anal. Chem. 63 (1991) 2762.
[27] X.-L. Wen, Y. Jia, L. Yang, Z.-L. Liu, Talanta 53 (2001) 1031.
[28] J. Saurina, S. Hernandez-Cassou, E. Fabregas, S. Alegret, Anal. Chim. Acta 405 (2000) 153.
[29] K. Ogura, M. Kobayashi, M. Nakayama, Y. Miho, J. Electroanal. Chem. 463 (1999) 218.
[30] M. Mikowska, M. Jurikiewicz-Herbich, Pol. J. Chem. 72 (1998) 1096.
[31] R. Slojkowska, M. Jurikiewicz-Herbich, Colloids Surf. Part A. Physicochem. Eng. Aspects 178 (2001) 325.
[32] M. Jurikiewicz-Herbich, A. Muszalska, R. Slojkowska, Colloids Surf. Part A. Physicochem. Eng. Aspects 131 (1998) 315.
[33] Official Methods of Analysis of AOAC, 16th Edition, Vol. II, 1995, Chapter 11, p. 17.
[34] H. Biester, G. Müller, H.F. Schöler, Sci. Total Environ. 284 (2002) 191.