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SIMULTANEOUS DETERMINATION OF EPINEPHRINE AND NOREPINEPHRINE BY ELECTROCHEMICAL REDUCTION AT THE PRE-TREATED PENCIL GRAPHITE ELECTRODE

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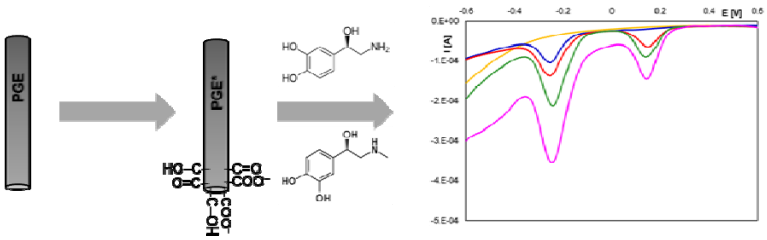
Epinephrine (EP) and norepinephrine (NP) using an electrochemically pre-treated pencil graphite electrode (PGE*) is reported. The analytical performances of the PGE* towards EP and NP determination have been evaluated by using square-wave voltammetry. Under optimized conditions, detection limits of 1.40×10^{-6} M for EP and 1.46×10^{-6} M for NP were attained. Interference studies showed that the PGE* has good selectivity with regard to EP and NP, in the presence of uric and ascorbic acids. The method was successfully applied to EP and NP quantification in human plasma samples.



INTRODUCTION

Epinephrine (EP) and norepinephrine (NP) are two important catecholamines with complementary actions, being essential in the mammalian central nervous system.[1] EP has an important role during stressful periods,[2] while NP increases the conversion of glycogen to glucose in the liver, helps in transforming fats into fatty acids, and relaxing the bronchial muscles.[3] These molecules are widely known as part of "fight or flight" response of biological system.[2] As a medication, they are mainly used to regulate the heart rate and blood pressure. Changes in concentrations of EP and NP can be correlated with neurological and neuropsychiatric disorders. Low levels of EP have been found in patients with Parkinson's disease,[4] while NP is implicated in a number of common neuropsychiatric (*e.g.* depression and drug abuse),[5,6] and neurodegenerative illnesses (*e.g.* Alzheimer and Parkinson).[7] Knowledge of plasma catecholamines concentrations is of great importance in monitoring nerve physiology, doping cases, in biomedical and biopharmaceutical research and in clinical diagnosis of some aforementioned affections.[8] Moreover, this is often useful for the evaluation of therapeutic and pharmacodynamics effects of/in neurological, psychiatric and cardiovascular disorders.[9]

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High-performance liquid chromatography,[10] gas chromatography,[11] chemiluminescence,[12] electrophoresis,[13] fluorimetry[14] and spectrophotometry[15] were used for EP and NP determination. Although these conventional analytical methods produce accurate results, they are time consuming, expensive, and involve tedious procedures for sample preparation.

Electrochemical techniques have been extensively used in the determination of biologically important molecules because of their simplicity, easy miniaturization, high sensitivity and relatively low cost compared to classical methods. The only structural difference between EP and NP is that the amine on the ethyl group of epinephrine is methylated, whereas the amine of norepinephrine is not. Therefore, at bare electrodes the electrooxidation process of EP and NP takes place at almost the same potential, which results in overlapped voltammetric responses, making their discrimination very difficult. As a consequence, the determination of EP and NP by employing modified electrodes and distinct electrochemical techniques has attracted more attention.[16-20] While simultaneous voltammetric determination of EP and NP based on their oxidation reactions at modified electrodes has been presented in literature,[16-22] little attention has been paid to their reduction reactions.[20,23]

The use of disposable pencil graphite electrode (PGE) in electrochemical studies constitutes an advantageous alternative to the conventional solid and modified electrodes, representing an affordable, reliable and facile tool in environmental, health and drug analyses.[24-26] Simultaneous determination of EP and NP through their direct reduction at an electrochemically pre-treated pencil-graphite electrode (PGE*) using a square-wave voltammetry method is reported for the first time. Unlike common voltammetric measurements of catecholamines that rely on their anodic oxidation signals, the new square wave voltammetric (SWV) method is based on their cathodic responses at PGE*. The method was applied for EP and NP quantification in biological samples.

EXPERIMENTAL

Reagents and solutions

Epinephrine hydrochloride (EP), norepinephrine hydrochloride (NP), L-ascorbic acid (AA), and uric acid (UA) were purchased from Sigma Aldrich (Milwaukee, WI). The used chemicals were of analytical grade and all the solutions were prepared in ultrapure water. Britton-Robinson buffer solution (BRBS) and phosphate buffer solution (PBS) were used as supporting electrolytes. For voltammetric measurements 1×10^{-2} M standard stock solutions of EP, NP, AA, and UA were freshly prepared and further diluted with BRB or PB solutions to the desired concentrations just before each experiment. Fresh human plasma samples were provided from local hospitals and were stored at −4 °C when were not used.

Equipment

A PGSTAT 128N (Ecochemie B.V., Netherlands) controlled by Nova 1.8 software was used for voltammetric studies. Pencil graphite (PG) and electrochemically pre-treated pencil graphite (PG*) leads were used as working electrodes in a 10 mL voltammetric cell including a Pt wire and Ag|AgCl (3.0 M KCl) as auxiliary and reference electrodes, respectively. The square-wave voltammetry (SWV) parameters were: frequency 5 Hz, step potential 2 mV, amplitude 50 mV, interval time 0.2 s. To observe reductive behavior of the target molecules, the potential was scanned between +0.70 V and −0.70 V. All working solutions were degassed by bubbling argon for 10 min. Each voltammetric recording was carried out using a new graphite pencil lead.

Electrochemical activation of PGE

PG electrodes (0.5 mm HB Rotring pencil lead) were electrochemically pre-treated by performing 10 cyclic voltammetric scans from −0.20 V to 3.00 V at a scan rate of 0.500 V/s, in BRB solution of pH 2.21, this step assuring the electrode surface activation and stabilization.[27] Surface characterization of PGE and PGE* by atomic force microscopy and their electrochemical behavior in a $1x10^{-3}$ M $K_3[Fe(CN)_6]$ solution prepared in 1.00 M KCl were presented in a previous paper.[28]

Sample analysis

Fresh human plasma samples were 5 fold diluted with PBS pH 7.40 in order to minimize the matrix effects. Appropriate amounts of EP and NP ($1x10^{-5}$ M each) were spiked in 10 mL of diluted plasma samples. Quantifications were performed by means of the standard addition method. Thus, three additions of EP or/and NP standard solutions were done and SW voltammograms were recorded after each addition. The concentrations of EP and NP were determined from their cathodic current responses recorded at +0.15 V and −0.23 V.

RESULTS AND DISCUSSION

Electrochemical behavior of EP and NP by SWV

Variation of the pH of the supporting electrolyte (BRB solutions) over the range 2.21-9.15 showed a significant effect on the SW voltammetric behavior of EP and NP at the PGE* surface (Fig. 1). EP displayed two reduction peaks, as shown in Fig. 1a. At pH 2.21 a first reduction peak appeared at $E_{p1/EP}$ = +0.45 V, its current

decreasing by solution pH increasing and finally disappearing at neutral pH. A second poorly defined peak appeared at a more negative potential ($E_{p2/EP}$ = +0.15 V), its current increasing until pH about 7.00 and then remaining constant (Fig. 1a, inset). In the case of NP, at pH 2.21 a first reduction peak ($E_{p1/NP}$) appeared at +0.44 V (Fig. 1b), presented almost the same current value for pH < 7.00, and drastically decreased in basic solutions (Fig. 1b inset). A second reduction peak ($E_{p2/NP}$) was observed at +0.04 V and its associated current increased with pH increasing, reaching a maximum value at pH 7.96; this reduction peak is better defined starting with pH 5.72.

Similar studies of pH (in a narrower range 5.80-7.80) and ionic strength influences upon the electrochemical reduction peaks of EP and NP were performed in PBS (a widely used buffer in biological studies). In PBS pH 7.40, using SWV, EP showed a single reduction peak at $E_{p1/EP}$ = -0.25 V, whereas NP presented two well defined reduction peaks at $E_{p1/NP}$ = +0.15 V and at $E_{p2/NP}$ = -0.22 V (Fig. 2). These initial observations suggest the possibility to detect simultaneously EP and NP in PBS pH 7.40, at PGE*.

The two neurotransmitters exhibited relatively poor current responses at bare PGE. In the case of PGE* the cathodic peak currents of EP and NP were more than tenfold higher when compared to those obtained at PGE. The enhanced current responses clearly indicate that the activation of the PGE determines an improved conductivity, an increase of its electro-catalytic activity and a better electron transfer rate with regard to the reduction of EP and NP, resulting in a better sensitivity of this electrochemical sensor toward the neurotransmitters.[29] At PGE*, the reduction peaks of both neurotransmitters overlapped at around $E_{p/EP+NP}$ = -0.23 V, while the first reduction peak at $E_{p1/NP}$ = +0.15 V for NP is unchanged (Fig. 2), fact that allows the convenient estimation of NP. EP concentration in a mixture of neurotransmitters can be evaluated after NP determination by exploiting the signal that appears at $E_{p/EP+NP}$ = -0.23 V, as shown below.

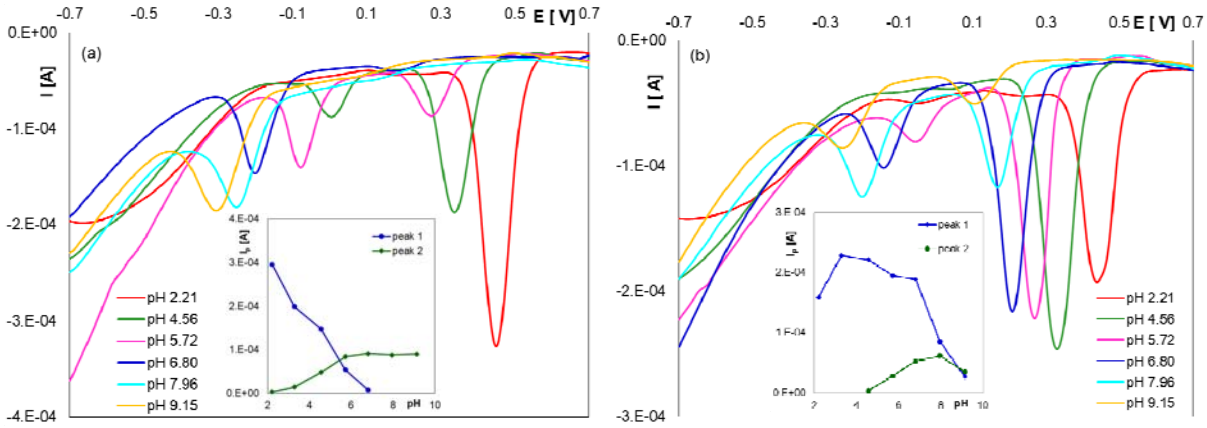

Fig. 1 – SWVs of 5×10^{-5} M EP (a) and 5×10^{-5} M NP (b) in 0.04 M BRBS of different pH values at PGE*; (inset I_p vs. pH graphs).

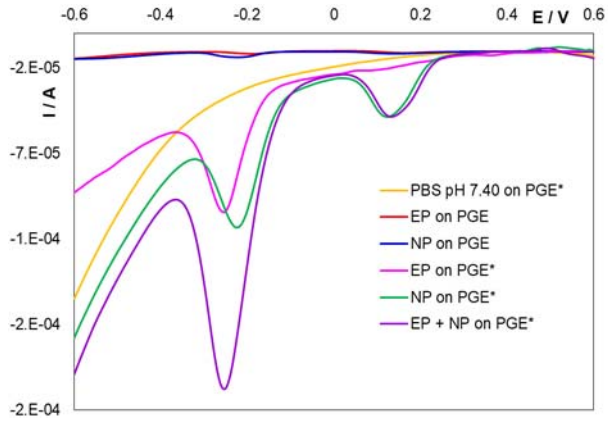

Fig. 2 – SWVs of 5×10^{-5} M EP and NP in separate and equimolecular mixture solutions in 0.1 M PBS pH 7.40 at PGE* and PGE.

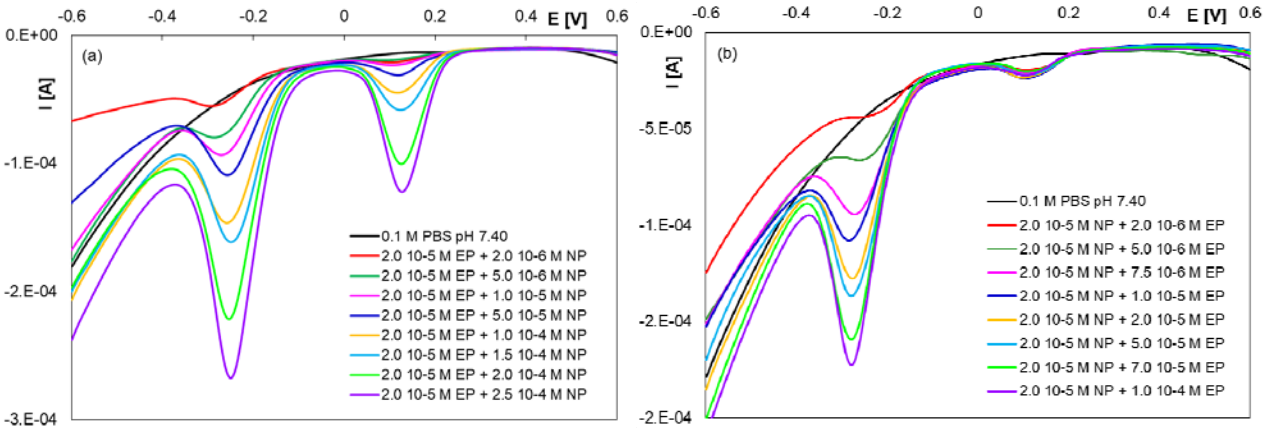

Fig. 3 – SWVs of 2×10^{-5} M EP and different concentrations of NP (a) and of 2×10^{-5} M NP and different concentrations of EP (b) in 0.1 M PBS pH 7.40 at PGE*.

In all further SWV studies 0.1 M PBS with an optimized pH value of 7.40 was selected as supporting electrolyte for the voltammetric determination of EP and NP at PGE*.

Quantitative determination of EP and NP

The main objective of the present investigation was the simultaneous SWV determination of EP and NP at PGE*. This was performed by varying the concentration of one compound, while keeping the other one constant (Fig. 3). Initially, by adding different concentrations of NP (2×10^{-6} to 2.5×10^{-4} M) to a 2×10^{-5} M EP solution in PBS pH 7.40, two peaks, at $E_{p1/NP}$ = +0.15 V and $E_{p/EP+NP}$ = -0.23 V, attributed to NP and to both EP and NP electrochemical reduction reactions, respectively, were observed at PGE*. The reduction currents linearly increased with NP concentration increasing (Fig. 3a).

The linear regression equations were I_{p1} (A) = $0.4088C_{NP}$ (M) + 4.02×10^{-7} (R^2 = 0.9997) and I_{p2} (A) = $0.6255C_{NP}$ (M) + 2.89×10^{-5} (R^2 = 0.9989). Although a better sensitivity is attained at $E_{p/EP+NP}$ = -0.23 V, for practical applications it is advantageous to quantify NP at $E_{p1/NP}$ = +0.15 V because at this potential value, EP was electrochemically inactive.

Similarly, the concentration of NP was kept constant at 2×10^{-5} M in PBS pH 7.40 and EP concentration was varied (2×10^{-6} to 1×10^{-4} M), as shown in Fig. 3b. It can be seen that the peak current at $E_{p1/NP}$ = +0.15 V remains almost constant, while the currents of the overlapped reduction peak at $E_{p/EP+NP}$ = -0.23 V, corresponding to EP and NP electro-reduction, increased with EP concentration. The linear regression equation was I_p (A) = $1.1098C_{EP}$ (M) + 6.10×10^{-5} (R^2 = 0.9992).

Quantitative determination of EP in a mixture of the two neurotransmitters can be done at PGE* only after the subtraction of the NP concentration from the total concentration.

Detection (LOD) and quantification (LOQ) limits for EP and NP were calculated based on the relationships 3SD/b and 10SD/b, respectively, according to IUPAC recommendations,[30] where SD is the standard deviation for the lowest analyte concentration (n = 6) and b is the slope of the linear regression equation. The LOD and LOQ values were 1.40×10^{-6} M and 4.67×10^{-6} M for EP, and 1.46×10^{-6} M and 4.87×10^{-6} M for NP. The performance characteristics of the PGE* are superior in comparison with other electrodes used for the simultaneous determination of EP and NP reported in the literature[20,23] in terms of its sensitivity and simplicity.

Interferences

It is well known that ascorbic acid (AA) and uric acid (UA) are the main biological compounds interfering in the electrochemical determination of EP and NP. The effect of 100 fold concentrations of these species on EP and NP voltammetric determination was evaluated. No reduction peaks were observed for AA and UA over the scanned potential range. The lack of the electrochemical cathodic response of AA and UA at PGE* is due to the well-known irreversibility of their redox processes.[20] Therefore, the PGE* can be used for cathodic measurements of micromolar EP and NP levels in the presence of large excess of ascorbic and uric acids.

Table 1

Determination of EP and NP in human plasma samples (average of six determinations)

EP/NP added ($\times10^{-5}$ M)	NP found ± SD ($\times10^{-5}$ M)	R%	RSD%	EP found ± SD ($\times10^{-5}$ M)	R%	RSD%	EP found ± SD* ($\times10^{-5}$ M)	R%*	RSD%*
Individual determination									
1.00	1.01±0.01	101.13	0.85	1.01±0.01	101.44	1.17			
2.00	2.02±0.06	100.55	2.87	2.01±0.08	99.97	3.74			
3.00	3.06±0.03	101.49	0.86	3.00±0.10	99.57	3.42			
4.00	3.98±0.03	99.16	0.85	4.02±0.06	100.22	1.57			
Simultaneous determination									
1.00	0.99±0.01	99.44	0.79	1.00±0.01	99.71	0.72	1.00±0.01	99.71	1.43
2.00	1.98±0.08	99.05	4.04	1.97±0.08	98.78	0.74	1.95±0.03	97.57	1.49
3.00	3.03±0.02	101.29	0.72	3.09±0.04	102.93	1.37	3.18±0.08	105.96	2.65
4.00	3.97±0.03	99.51	0.68	3.95±0.01	98.74	0.23	3.90±0.02	97.62	0.46

*EP determination after NP subtraction.

Analytical application

The SWV method was applied for the simultaneous determination of the two catecholamines in human plasma samples which is of great importance to monitor nerve physiology and doping cases. The SW voltammograms of the spiked plasma samples and after each of four additions of EP or/and NP were recorded. In a mixture of neurotransmitters, NP was quantified directly at +0.15 V, while quantitative determination of EP was done by using the peak at -0.23 V, attributed both to EP and NP reduction, two approaches being considered: firstly, EP concentration was estimated based on the EP standard addition, and secondly, was calculated after the subtraction of NP concentration evaluated at +0.15 V. The results for separate and simultaneous determination of the two compounds in the spiked plasma samples are given in Table 1. The RSD% values revealed a good precision of the electrochemical method. The average recovery (R%) values of the spiked samples between 97.57-105.96% for EP and 99.05-101.49% for NP indicated that there were not any significant matrix interferences for the analyzed samples.

CONCLUSIONS

A new SW voltammetric method for the simultaneous determination of EP and NP in PBS pH 7.40 using an electrochemically activated PGE is presented. The results showed that PGE* presented a more sensitive response towards EP and NP compared with the non-pretreated PGE. The study revealed the possibility of exploiting the reduction peaks of EP and NP in mixtures of analytes solution in the presence of some possible interfering biological compounds (AA and UA), which represents a very difficult problem to avoid within clinical and biomedical investigations. The new SWV method demonstrated the feasibility of PGE* for simultaneous determination of EP and NP in spiked human plasma samples, with satisfactory recoveries. EP and NP detection in real samples was done in a single measurement using their electro-reduction signals. The conductance and surface area characteristics of PGE* together with the electro-catalytic activity of the electrode surface offer significant advantages for improving electrochemical detection of EP and NP. Compared to conventional methods, the electrochemical method is simple, rapid and low cost, being a valuable tool for sensing applications.

REFERENCES

1. W. G. Meijer, S. C. V. M. Copray, H. Hollema, I. P. Kema, N. Zwart, I. Mantingh-Otter, T. P. Links, P. H. B. Willemse and E. G. E. de Vries, *Clin. Chem*., **2003**, *49*, 586-593.
2. D. L. Wong, *Cell. Mol. Neurobiol*., **2003**, *26*, 891-898.
3. M. E. Gibbs and R. J. Summers, *Prog. Neurobiol*., **2002**, *67*, 345-391.
4. W. P. Gai, L. B Geffen, L. Denoroy and W. W. Blessing, *Ann. Neurol*., **1993**, *33*, 357-367.
5. D. Weinshenker and J. P. Schroeder, *Neuropsychopharmacol*., **2007**, *2*, 1433-1451.
6. P. Remy, M. Doder, A. Lees, N. Turjanski and D. Brooks, *Brain*, **2005**, *128*, 1314-1322.
7. K. Del Tredici and H. Braak, *J. Neurolog. Neurosurg. Psych*., **2013**, *84*, 774-783.
8. D. J. Michael and R. M. Wightman, *J. Pharm. Biomed. Anal*., **1999**, *19*, 33-46.
9. M. E. Fox and R. M. Wightman, *Pharmacol. Rev*., **2017**, *69*, 12-32.
10. V. Carrera, E. Sabater, E. Vilanova and M. A. Sogorb, *J. Chromatogr. B*, **2007**, *847*, 88-94.
11. D. G. Ferrer, A. G. García, J. Peris-Vicente, J. V. Gimeno-Adelantado and J. Esteve-Romero, *Anal. Bioanal. Chem.*, **2015**, *407*, 9009-9018.

12. Z. Lin, X. Wu, X. Lin and Z. Xie, *J. Chromatogr. A*, **2007**, *1170*, 118-121.
13. M. C. Puyana, R. Drewnowska, V. P. Fernandez, M. A. Garcia Antonio, L. M. L. Marina, *Electrophor*., **2009**, *30*, 2947-2954.
14. X. Zhu, P. N. Shaw and D. A. Barrett, *Anal. Chim. Acta*, **2003**, *478*, 259-269.
15. M. Zhu, X. Huang, J. Li and H. Shen, *Anal. Chim. Acta*, **1997**, *357*, 261-267.
16. R. N. Goyal and S. Bishnoi, *Talanta*, **2011**, *84*, 78-83.
17. H. Beitollahi, H Karimi-Maleh and H. Khabazzadeh, *Anal. Chem*., **2008**, *80*, 9848-9851.
18. S. Yuanxi, Y. Baoxian, W. Yu, T. Xiaorong and Z. Xingyao, *Microchem. J*., **1998**, *58*, 182-191.
19. S. S. M. Hassan and G. A. Rechnitz, *Anal. Chem*., **1986**, *58*, 1052-1054.
20. K. Pihel, T. J. Schroeder and R. M. Wightman, *Anal. Chem*., **1994**, *66*, 4532-4537.
21. Y. Ni, Y. Gui and S. Kokot, *Anal. Meth*., **2011**, *3*, 385-392.
22. N. Lavanya and C. Sekar, *J. Electroanal. Chem*., **2017**, *801*, 503-510.
23. T. Cho and J. Wang, *Electroanal.*, **2018**, *30*, 1-6.
24. A. Özcan and Y. Şahin, *Biosens. Bioelectron*., **2010**, *25*, 2497-2502.
25. M. R. Akanda, M. Sohail, M. A. Aziz and A. N. Kawde, *Electroanal*., **2016**, *28*, 408–424.
26. I. G. David, D. E. Popa and M. Buleandra, *J. Anal. Methods. Chem*., **2017**, 1905968.
27. D. Patrascu, I. David, V. David, C. Mihailciuc, I. Stamatin, J. Ciurea, L. Nagy, G. Nagy and A. A. Ciucu, *Sens. Actuat. B: Chem*., **2011**, *156*, 731-736.
28. M. Buleandra, A. A. Rabinca, I. A. Badea, A. Balan, I. Stamatin, C. Mihailciuc and A. A. Ciucu, *Microchim. Acta*, **2017**, *184*, 1481-1488.
29. D. Pletcher, R. Greff, R. Peat, L. M. Peter and J. Robinson, "Instrumental methods in electrochemistry", Woodhead Pub., 2001.
30. M. Thompson, H. M. Bee, R. V. Cheeseman, W. H. Evans, D. W. Lord, B. D. Ripleyand, R. Wood and J. J. Wilson, *Analyst*, **1987**, *112*, 199-204.