Pergamon

Journal of Pharmaceutical & Biomedical Analysis, Vol. 12, No. 9, pp. 1069–1074, 1994

0731-7085/94 $7.00 + 0.00

0731-7085(94)00055-7

Application of a Nafion-modified carbon paste electrode for the adsorptive stripping voltammetric determination of fenoterol in pharmaceutical preparations and biological fluids

DAMIEN BOYD, JOSÉ RAMÓN BARREIRA RODRÍGUEZ, PAULINO TUÑÓN BLANCO* and MALCOLM R. SMYTH†

Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Asturias, Spain
†School of Chemical Sciences, Dublin City University, Dublin 9, Ireland

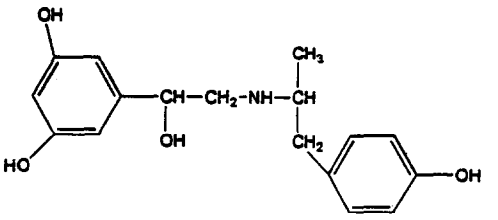
Abstract: The preconcentration of fenoterol on a Nafion-modified carbon paste electrode and its subsequent determination using differential pulse voltammetry is described. The effect of pH and percentage Nafion concentration on the accumulation behaviour of fenoterol was studied, and accumulation curves, calibration graphs and reproducibility studies at two different Nafion concentrations have been carried out in the range 2.5×10^{-8}–5.0×10^{-7} M fenoterol. A limit of detection in aqueous solutions, calculated using a signal-to-noise ratio (S/N) of 3, was 9.0×10^{-9} M. Application of the electrode to pharmaceutical preparations, without sample pretreatment, resulted in acceptable deviation from the stated concentration (RSD = ± 3.81%, n = 4). For more complex matrices, a suitable extraction procedure was developed, resulting in recoveries of >90% (urine) and >75% (serum).

Keywords: *Fenoterol; Nafion-modified carbon paste electrode; complex matrices.*

Introduction

Fenoterol is one of many selective β_2-adrenergic agonist drugs currently being administered for the treatment of chronic obstructive airway diseases such as bronchial asthma and chronic obstructive bronchitis. It is also used for the inhibition of premature delivery in pregnant women [1]. The recent interest in β_2-agonists has stemmed from their illegal use as growth promoters in animal production. This has resulted in a wide variety of analytical methods available for their determination in animal biological fluids and tissues. Clenbuterol and salbutamol are the two most notorious compounds in this class, having been implicated as growth promoters (or 'repartitioning agents') on a wide-scale in the European Community [2–4].

Fenoterol has not, as yet, been implicated as a repartitioning agent and, in general, analytical procedures for its determination in complex matrices are scarce. Rominger *et al.* reported a very sensitive radioimmunological method for levels in human urine and plasma samples [5]. Ackermans *et al.* described a method of analysis for fenoterol and other β_2-agonists using various separation techniques [6].



Fenoterol

The method developed in this paper is based on the preconcentration of positively-charged fenoterol molecules onto a negatively-charged Nafion film. The advantages derived from using Nafion-modified electrodes have been well-documented. Nafion is chemically very inert, non-electroactive, hydrophilic, thermally stable and insoluble in water [7]. Many different variations of the Nafion-modified electrode have appeared in the literature. The Nafion-modified glassy carbon electrode has been used in flow analysis for the determination of

* Author to whom correspondence should be addressed.

cationic drugs and neurotransmitters [8, 9]. Further modifications of the electrode has resulted in the combination of Nafion with other materials; mercury thin films [10], platinum particles [11], methylene blue dye [12] and solvents (for example tributylphosphate) [13]. A Nafion-modified graphite electrode incorporated with viologens has been developed for catalytic applications [14]. A recent development is the use of a polyaniline–Nafion thin film on a platinum electrode for the determination of alkali and alkali–earth metal ions [15]. Nafion-modified carbon fibre ultramicroelectrodes have been applied to the analysis of neurotransmitters and metal ions [16, 17]. A recent article by Litong *et al.*, reports a chronopotentiometric stripping analysis method for adenosine with a Nafion-modified glassy carbon electrode [18]. Surprisingly, little work has been reported on the use of Nafion-modified carbon paste electrodes. Advantages already derived from using carbon paste electrodes include ease and speed of fabrication and low background currents. Gao *et al.*, reported the use of a Nafion-1,10-phenanthroline-modified carbon paste electrode for the preconcentration of Fe(II), prior to its determination by differential pulse voltammetry [19]. The design of the electrode is quite different to that of the Nafion-modified CPE reported in this paper. In the paper of Gao *et al.* the Nafion is incorporated into the graphite–nujol paste mixture. For the fabrication of the electrode described in this paper, an aliquot of Nafion solution is simply added to the surface of a prepared carbon paste electrode. A thin film is formed after drying. The performance of the electrode is evaluated in optimum pH and Nafion film conditions and analysis of fenoterol in real samples is reported.

Experimental

Reagents and materials

A Britton–Robinson (BR) buffer solution was prepared, containing 11.48 ml acetic acid (99.7%), 13.5 ml phosphoric acid (85%) and 12.44 g boric acid per litre. The pH was adjusted using 2 M sodium hydroxide. Nafion stock solution (5% in lower alphatic alcohols and 10% water) was purchased from Aldrich Chemicals (Gillingham, Dorset, UK) and diluted with 1:1 water: isopropanol. Fenoterol hydrobromide was purchased from Sigma (St Louis, MO, USA). The pharmaceutical preparation (Berotec, Boehringer Ingelheim, Barcelona, Spain) was purchased locally. All standard solutions were prepared using deionized water (obtained by passing distilled water through a Milli-Q water purification system) and stored in the dark at 5°C. The extraction solvent mixture consisted of a 9:1 ratio of ethyl acetate (Farmitalia Carlo Erba, Milan, Italy) and amyl alcohol (Panreac, Barcelona, Spain).

Apparatus

The experiments were carried out in the usual all-glass cell designed for a three electrode potentiostatic circuit. A voltammetric 663-VA stand (Metrohm) equipped with a rotating carbon paste disk electrode (18 mm^2) was connected to a Polarecord differential pulse stripping analyser (Metrohm). The Nafion-modified electrode was used as a working electrode; the reference was a Ag/AgCl electrode; the counter electrode was a platinum electrode.

Methods

Preparation of modified electrode. The Nafion electrode was prepared by pipetting 10 μl of an appropriate concentration of Nafion solution onto the surface of a previously prepared carbon paste electrode. The film was then air-dried using a domestic hair-dryer.

Differential pulse voltammetric procedure. All stripping analyses were carried out in 20 ml of BR buffer, pH 3.0. In operation, the modified electrode was immersed in the buffer solution and rotated at a constant speed (preconcentration times typically 30–45 s). After a 5 s rest period the compound was removed by stripping anodically from 0 to +1.5 V and the peak current measured in the usual manner.

Analysis of pharmaceutical preparation. The pharmaceutical preparation (Berotec, Boehringer Ingelheim, Barcelona, Spain) was diluted 1:500 with deionized water and a 50-μl aliquot was injected into the cell. Fifty-μl aliquots of standard solution (2×10^{-5} M) were added and a 30 s accumulation time was employed.

Extraction methodology. Urine and serum samples, obtained from healthy individuals, were stored frozen until assay. After gentle

thawing, 5-ml aliquots of urine were fortified with an appropriate fenoterol concentration and treated with 2.0 ml of 0.2 M sodium hydroxide. After vortexing for 10 s, 20 ml of solvent mixture was added and the flask shook by hand for 7 min. The layers were then allowed to separate for 15 min. Eighteen ml of the upper organic layer was removed using a glass pipette, evaporated to dryness under a stream of nitrogen at 70°C and redissolved in BR buffer, pH 3.0. For serum a similar procedure was adopted, but using only 1.0-ml aliquots of sample and appropriately-reduced volumes of extraction solvent and sodium hydroxide solution. In this case, for improved sensitivity, the extract was injected into a reduced volume of background electrolyte (10 ml). A 45 s accumulation time was employed throughout.

Results and Discussion

Effect of pH of supporting electrolyte

The electrochemical behaviour of fenoterol at unmodified carbon paste electrodes has recently been discussed [20]. The compound is irreversibly oxidized on carbon paste electrodes in the pH range 2–12 yielding one main irreversible process which shifts towards more negative potentials as the pH increases. A secondary process was also observed but was less distinct and appeared only in the pH range 6–11. A linear response of half-peak potential against pH was observed in the pH range 1–10, giving a negative slope of 59.2 mV pH^{-1} (r = 0.996, n = 9).

The use of Nafion as a modifier on carbon electrodes for the accumulation of cationic compounds has been previously demonstrated [9, 13]. The pH plays an important role, as fenoterol is positively-charged at low pH values, allowing retention on the negatively-charged polymer film. A pH study in the lower pH range (BR buffer, pH 3–8) was carried out. Figure 1 shows the electrochemical response of 5×10^{-7} M fenoterol at various pH values using a 0.2% Nafion-modified CPE. Accumulation times of up to 3 min were employed. From this one can see that the response increases dramatically as the pH decreases. A pH value of 3.0 was found to be optimum in this study. A BR buffer of pH 2.0 gave rise to higher peak current (results not shown) but the effect of background current becomes more pronounced at low pH values. A BR buffer of pH 3.0 combined good analyte response with satisfactory separation from the background current and hence was used for all further work.

Optimization of Nafion concentration

The effect of increasing the concentration of Nafion on the CPE surface was then investigated. Figure 2 shows the accumulation profile of 5×10^{-7} M fenoterol on unmodified and modified electrode surfaces. No preconcen-

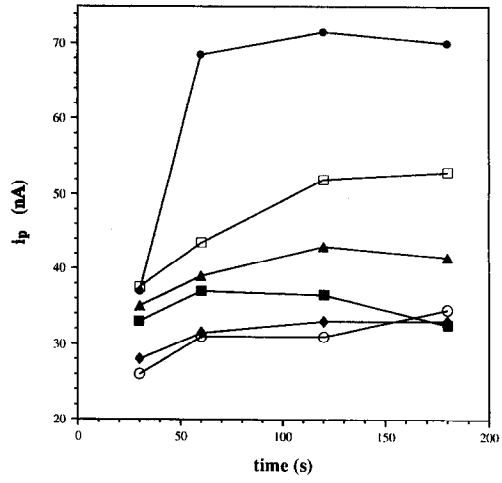


Figure 1
Influence of pH on the accumulation of fenoterol (concentration 5.0×10^{-7} M) using a 0.2% Nafion film and differential pulse voltammetry with an accumulation potential of 0 V (ΔE = 50 mV; Scan rate = 10 mV s^{-1}): pH 3 (●), 4 (□), 5 (▲), 6 (■), 7 (◆) and 8 (○).

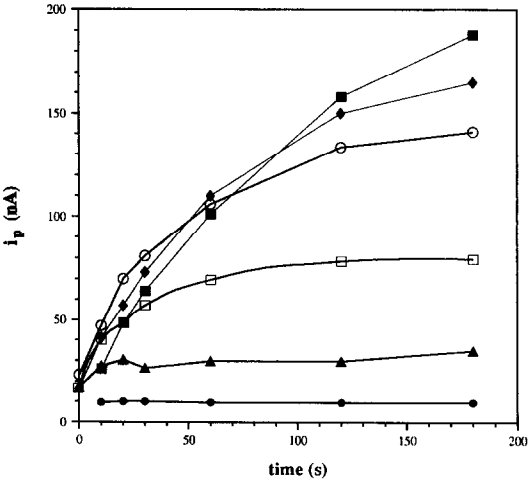


Figure 2
Comparison of the differential pulse voltammetric response of fenoterol at a bare carbon paste electrode and at various Nafion-modified CPEs for 5.0×10^{-7} M fenoterol in pH 3 BR buffer: unmodified CPE (●); 0.1% Nafion electrode (▲); 0.2% Nafion electrode (□); 0.5% Nafion electrode (○); 0.8% Nafion electrode (◆); 2.0% Nafion electrode (■). Experimental conditions as previous Figure.

tration of fenoterol occurs with the unmodified electrode. Using Nafion films, however, a preconcentration process is observed and larger increases in current are observed up until 0.5% Nafion. Further increases of the Nafion concentration (up to 2.0%) results in insignificant increases in peak current, suggesting that 0.5% may be the optimum concentration of Nafion. Moreover, a lower current was obtained for higher percentage Nafion films at low accumulation times, most likely due to resistance of the diffusion of the compound by a thicker layer. However, two Nafion concentrations, 0.5% and 2.0%, were evaluated for their capacity to accumulate fenoterol. Accumulation studies, calibration curves and reproducibility studies were carried out to investigate whether there is any advantage in using thicker Nafion films to enhance the preconcentration process. These will be explained in a later section of the paper.

Influence of pulse height, scan rate and deposition potential

The pulse height was varied over the range 10–100 mV using the optimum buffer and a 0.5% Nafion-modified electrode. The analyte peak current increased significantly with pulse height up to 50 mV, after which further increase in pulse height resulted only in a small increase in peak current. Hence a pulse height of 50 mV was used for all further work.

The influence of scan rate on the peak current was studied in the range 2–25 mV s^{-1}. The optimum scan rate was either 5 or 10 mVs^{-1}, both of which gave similar peak currents. However, 10 mVs^{-1} was chosen for all further work due to the shorter analysis times achieved.

The response of the stripping peak at various initial accumulation potentials was also carried out in the range −0.2 V to +0.7 V. No real difference in peak current was recorded in the range −0.2 V to +0.1 V but from +0.2 V onwards a significant decrease in the response was observed. An electrolysis potential of 0 V was chosen as the optimum accumulation potential.

Fenoterol accumulation studies

Accumulation studies, on electrodes fortified with 0.5% and 2.0% Nafion, were carried out at four concentration levels of fenoterol. Figure 3 (0.5% Nafion) shows the preconcentration of the compound with time. This figure

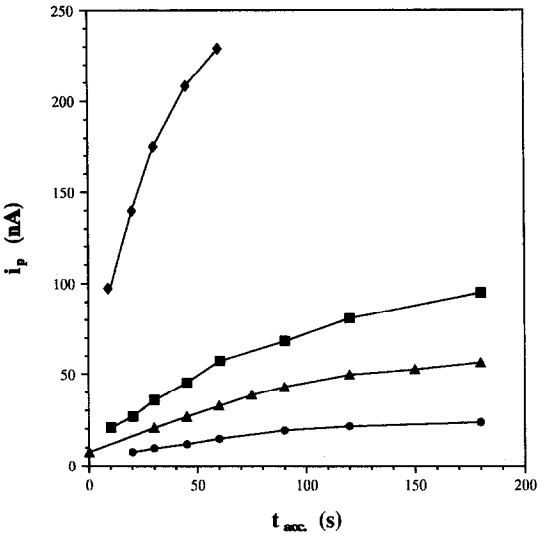


Figure 3
Accumulation curves of fenoterol obtained with a 0.5% Nafion electrode using differential pulse voltammetry at pH = 3 and using the same experimental conditions as in Fig. 1: 2.5×10^{-8} (●); 5.0×10^{-8} (▲); 1.0×10^{-7} (■) and 5.0×10^{-7} M (◆).

demonstrates that at low concentrations of fenoterol (2.5×10^{-8} and 5.0×10^{-8} M) there is a linear response up to 90 s after which the slope changes. For higher concentrations of fenoterol, i.e. 1.0×10^{-7} and 5.0×10^{-7} M linearity is observed up to 60 s and 30 s, respectively. For all concentrations studied there are non-zero intercepts, indicating accumulation of fenoterol during scanning. Accumulation studies with 2% Nafion films (results not shown) show that at low concentrations of fenoterol, i.e. 2.5×10^{-8} M, linearity is observed up to 180 s. For concentrations above this level the linear profile is the same as for 0.5% Nafion. Overall the currents obtained using the higher Nafion concentration were greater but only by a relatively small amount. Non-zero intercepts are obtained as for 0.5% Nafion.

Calibration curves

For both Nafion concentrations one can see that there is linearity, for all concentrations studied, up to the including 30 s. Using this preconcentration time and a 0.5% Nafion film a calibration curve covering one order of magnitude, 5.0×10^{-8} to 5.0×10^{-7} mol dm^{-3}, was obtained. The following calibration equation was obtained:

$$i/nA = 4.08 \times 10^{+8}\, C/M + 6.61 \quad (1)$$
$$(n = 9;\ r = 0.9990)$$

Using the same preconcentration time and a 2.0% Nafion film a linear calibration curve covering over one order of magnitude, 2.5×10^{-8} to 6.0×10^{-7} M, was obtained. The following equation was obtained:

$$i/nA = 3.45 \times 10^{+8}\, C/M + 3.33 \quad (2)$$
$$(n = 9;\ r = 0.9994)$$

It was possible to measure concentrations of this drug compound below 2.5×10^{-8} M by applying longer accumulation times, but adsorption of interferences was observed at long accumulation times. A limit of detection of fenoterol using 45 s preconcentration time (calculated using a signal-to-noise ratio of 3) was 9×10^{-9} M.

Reproducibility

The reproducibility of the Nafion-modified electrode was evaluated at two concentration levels of fenoterol (1.0×10^{-7} and 5.0×10^{-7} M) resulting in acceptable relative standard deviations, for 10 consecutive runs, of ± 2.21 and ± 5.25% (0.5% Nafion film) and ± 3.52 and ± 4.95% (2.0% Nafion film). The higher variation observed at higher concentrations of compound may be due to modification of the electrode surface by a higher proportion of adsorption products. A fresh Nafion-modified electrode was fabricated for each study. The electrode may be prepared reproducibly in a very short time (typically 20 min).

Choice of Nafion concentration for complex matrices

Apart from the slightly higher faradaic current obtained with a 2% Nafion film, no real advantages are derived from its use over 0.5% Nafion. Results from cyclic voltammetry on Nafion-modified electrodes show that high capacitance currents are present for thicker Nafion films [21]. Moreover, other authors have reported that thicker films retain a more powerful barrier to the diffusion of the analyte [8, 19]. Hence, on this basis, a 0.5% Nafion film was chosen for application to complex matrices.

Analysis of fenoterol in complex matrices

The direct determination of fenoterol in a commercially-available product resulted in a level of 4.78 mg ml^{-1} fenoterol hydrobromide being obtained ($n = 4$, RSD (%) = ± 3.81%). This compares reasonably well with the stated level of 5.00 mg ml^{-1}. A *t*-test was carried out on the data to statistically examine the validity of the obtained result. At the 95% confidence level, the value of *t* (experimental) was less than that of *t* (theory) showing that the method has no systematic error.

For biological matrices fenoterol was added in the following manner: urine samples were fortified with fenoterol to achieve final concentrations of 2.5×10^{-7} and 5.0×10^{-7} M, respectively. Serum samples were fortified with fenoterol to achieve final concentrations of 1.0×10^{-6} and 5.0×10^{-6} M, respectively. Figure 4 shows typical voltammograms of fenoterol in a serum extract and subsequent standard additions. The results of these analyses are summarized in Table 1. Good recovery of fenoterol was achieved from both types of matrix.

A paper by Rominger *et al.* indicates that urine samples containing incurred residues of fenoterol (the availability of which were outside the scope of this project) are almost

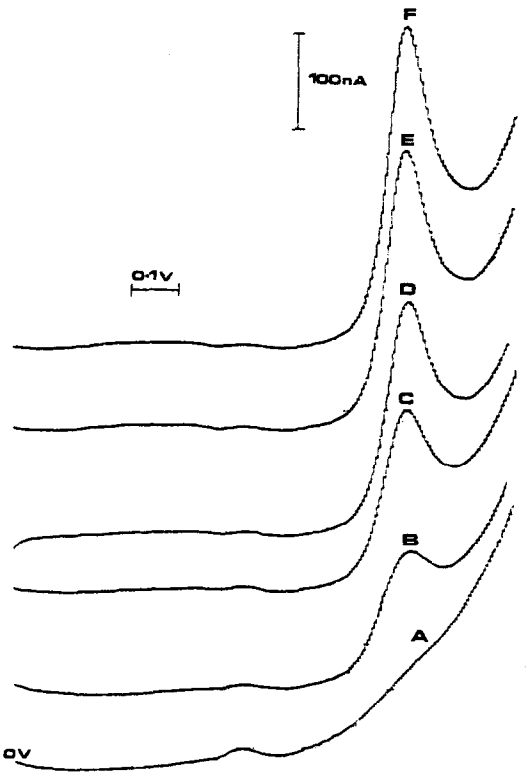


Figure 4
Differential pulse voltammograms of fenoterol at pH = 3 using a 0.5% Nafion electrode and the same experimental conditions as Fig. 1: (A) blank serum extract, (B) extract containing 5×10^{-6} M fenoterol and (C)–(F) additions of 6 μl of 10^{-4} M fenoterol (cell volume = 20 ml).

Table 1
Results obtained for fenoterol-fortified urine and serum samples after using the extraction procedure and differential pulse voltammetry (conditions explained in text)

Sample	Fenoterol added (M)	*n*	Level determined (M)	RSD (%)	Average % recovery
Urine	2.5×10^{-7}	3	2.30×10^{-7}	11.3	91.8
	5.0×10^{-7}	3	4.62×10^{-7}	2.95	92.4
Serum	1.0×10^{-6}	3	8.72×10^{-7}	3.05	87.2
	5.0×10^{-6}	3	3.92×10^{-6}	6.41	78.3

completely conjugated [5]. To this end, the authors recommend a deconjugation procedure. A paper by Van Ginkel *et al.* uses suc d'helix Pomatia juice (solution of enzymes) for the hydrolysis of glucuronide and sulphate conjugates in urine samples [2]. Such a procedure may be carried out in fenoterol-incurred urine prior to extraction and the free form of the compound analysed using the technique developed in this paper.

Conclusions

The development and application of a Nafion-modified carbon paste electrode to the analysis of fenoterol has been reported for the first time. Fabrication of the electrode is both straight-forward and rapid. Optimum accumulation of fenoterol was achieved at low pH values where the amino group on the molecule is positively charged. Investigations into the Nafion film thickness revealed that an optimum is reached at around 0.5% Nafion.

Application of the electrode to pharmaceutical preparations was possible after a suitable dilution of the sample was prepared. The liquid extraction procedure, developed for biological samples, proved to be very selective for fenoterol with good recoveries obtained at the levels tested. For future studies and to provide a good validation of the developed procedure the analysis of certified reference materials and/or incurred samples is recommended.

Acknowledgements — The authors acknowledge the kind assistance of Dr A.J. Miranda Ordieres of the University of Oviedo, Spain and Dr Michael O'Keeffe of The National Food Centre, Ireland. The ERASMUS programme and the Spanish Ministerio de Educacion y Ciencia (DGICYT) project PB90-0381 are thanked for their financial support of this work.

References

[1] B. Boenisch and J.F. Quirke, Safety Assessment of β-Agonists in Proceedings of the EC Workshop, Thessaloniki, Greece, pp. 102–124 (1992).
[2] L.A. Van Ginkel, R.W. Stephany and H.J. Van Rossum, *J. Assoc. Off. Anal. Chem.* **75**, 554–560 (1992).
[3] D. Boyd, P. Shearan, J.P. Hopkins, M. O'Keeffe and M.R. Smyth, *Anal. Chim. Acta* **275**, 221–226 (1993).
[4] M.P. Montrade, S. Riverain, B. Le Bizec and F. Andre, GC/MS Analysis of β-Agonists in Urine and Edible tissues: *in vivo* Study of Salbutamol Metabolism in Calves in Proceedings of the EC Workshop, Thessaloniki, Greece, pp. 143–148 (1992).
[5] K.L. Rominger, A. Mentrup and M. Stiasni, *Arzneim.-Forsch Drug Res.* **40**, 887–895 (1990).
[6] M.T. Ackermans, J.L. Beckers, F.M. Everaerts and I.G.J.A. Seelen, *J. Chromatogr.* **590**, 341–353 (1992).
[7] M.N. Szentirmay and C.R. Martin, *Anal. Chem.* **56**, 1898–1902 (1984).
[8] J. Zhou and E. Wang, *Anal. Chim. Acta* **249**, 489–489 (1991).
[9] J. Wang, P. Tuzhi and T. Golden, *Anal. Chim. Acta* **194**, 129–138.
[10] B. Hoyer, T.M. Florence and G.E. Batley, *Anal. Chem.* **59**, 1608–1614 (1987).
[11] K. Itaya, H. Takahashi and I. Uchida, *J. Electroanal. Chem.* **208**, 373–382 (1986).
[12] Z. Lu and S. Dong, *J. Chem. Soc. Faraday Trans. 1* **84**, 2979–2986 (1988).
[13] P. Audebert, B. Divisia-Blohorn, P. Aldebert and F. Michalak, *J. Electroanal. Chem.* **322**, 301–309 (1992).
[14] K. Shigehara, E. Tsuchida and F.C. Anson, *J. Electronal. Chem.* **175**, 291–298 (1984).
[15] J.-Y. Sung and H.-J. Huang, *Anal. Chim. Acta* **246**, 275–281 (1991).
[16] P. Capella, B. Ghasemzadeh, K. Mitchell and R.N. Adams, *Electroanalysis* **2**, 175–182 (1990).
[17] H. Huiliang, D. Jagner and L. Renman, *Anal. Chim. Acta* **207**, 17–26 (1988).
[18] J. Litong, L. Shenghui, B. Zhuping and F. Yuzhi, *Electroanalysis* **5**, 611–614 (1993).
[19] Z. Gao, P. Li, G. Wang and Z. Zhao, *Anal. Chim. Acta* **241**, 137–146 (1990).
[20] D. Boyd, J.R. Barreira Rodriguez, A.J. Miranda Ordieres, P. Tuñon Blanco and M.R. Smyth, *Analyst* (in press).

[Received for review 23 December 1993; revised manuscript received 21 March 1994]