



ELSEVIER

Journal of Chromatography B, 693 (1997) 43–49

JOURNAL OF  
CHROMATOGRAPHY B

# Ion chromatographic study of sodium, potassium and ammonium in human body fluids with bulk acoustic wave detection

Bing-Sheng Yu, Li-Hua Nie, Shou-Zhuo Yao\*

*Chemistry and Chemical Engineering College, Hunan University, Changsha 410082, China*

Received 5 February 1996; revised 22 November 1996; accepted 5 December 1996

## Abstract

In the present paper, determination of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{NH}_4^+$  in saliva and serum was carried out using an ion chromatography (IC) method with a bulk acoustic wave (BAW) sensor as detector and 2.0 mmol/l nitric acid as mobile phase. The IC–BAW method is simple, rapid and accurate. Comparison between the BAW detector and the conventional conductivity detector (CDD) was discussed. The IC–BAW showed agreement with the commonly used flame photometric method for  $\text{Na}^+$  and  $\text{K}^+$  and enzymatic method for  $\text{NH}_4^+$ , as well as IC–CDD for those three cations.

**Keywords:** Bulk acoustic wave detection; Sodium; Potassium; Ammonium

## 1. Introduction

Assay of sodium, potassium and ammonium in human fluids is clinically important. The reference values for serum sodium and potassium are 136–145 mmol/l and 3.8–5.4 mmol/l, respectively [1]. Under pathological conditions, serum sodium and potassium levels probably deviate from the corresponding normal value. Therefore monitoring of serum sodium and potassium is helpful for diagnosis and therapy of some diseases [1]. Salivary metal ion concentration can also reflect ailment of oral organs, such as salivary glands [2]. Flame photometry, ion-selective electrode (ISE) methods, and colorimetric methods are commonly used for clinical determination of sodium and potassium [1,3,4], and colorimetric and enzymatic methods for ammonium [5]. By flame

photometry sodium and potassium can be simultaneously determined, but the determination is readily influenced by the operating conditions (such as, gas pressure, etc.). The ISE and colorimetric methods are subject to the problem of selectivity [4]. Ion strength is another adverse factor for ISE. Ion chromatography (IC), as a versatile separation technique, can circumvent most of those problems and has been used in determination of salivary sodium and potassium [6]. IC has achieved wide application in many areas since it was developed [7,8]. It underscores its appealing potency in separation and determination of ionic species. In IC, conductometric detection is widely used because of its simplicity and specificity for ionized components. In the present paper, an ion chromatographic method with a bulk acoustic wave (BAW) detector is proposed. The BAW detection system for IC was designed and constructed in this laboratory.

\*Corresponding author.

## 2. Experimental

### 2.1. Reagents and materials

The chemicals were of analytical grade or above. Distilled deionized water was used throughout. The mobile phase and sample solutions were filtered through a 0.45  $\mu\text{m}$  filter membrane (Millipore, USA) to protect the flow line of the chromatography system from logging.

### 2.2. The BAW detector

The BAW detector was composed of a serial piezoelectric quartz crystal (SPQC) oscillator and a conductivity detection cell. The SPQC device was assembled by connecting a 9 MHz quartz crystal to a TTL-IC oscillating circuit. The detection cell was made as follows: two platinum (Pt) wires ( $\varnothing$  1 mm, pre-treated with 6 M  $\text{HNO}_3$ , then by water and acetone), as conductivity electrodes, were inserted oppositely in the detection cell, with a distance of 0.5 mm between them. The body of the detector was made of two plexiglass plates with a thickness of 5 mm, within which a cylindrical trough (diameter 2 mm and length 8 mm) was used as detection cell. Two stainless steel tubes ( $\varnothing$  0.5 mm) were, respectively, used as the inlet and outlet of the cell for mobile phase. The distance between the electrode and the inlet was 5 mm.

### 2.3. F/V convertor

A universal frequency counter (Model SC-7201, Iwatsu, Japan) was first used to investigate the performance of the BAW sensor with respect of frequency shift. As the frequency data must be transformed into a format which could be used by the chromatographic workstation, a frequency-to-voltage convertor (made in this laboratory [9]) was used to transform the frequency signal of the BAW detector to a C-R4A Chromatopac data processor (Shimadzu, Japan), which was used to record chromatograms in real time and integrate peak areas.

### 2.4. Chromatographic system

Chromatographic separation was carried out using a Shimadzu IC-6A ion chromatography system,

consisting of a LP-6A liquid delivery pump, a SLC-6B system controller, a SIL-6B auto injector, a CTO-6AS column oven and a CDD-6A conductivity detector, which was used in this work to testify the feasibility of the BAW detector. The column used was a Shim-pack IC-C1 (15 cm $\times$ 5 mm I.D. stainless column), packed with a surface functional cation-exchange resin on polystyrenedivinylbenzene support incorporating a sulfonic acid base as a functional group. A Shim-pack IC-GC1 guard column (10 mm $\times$ 4.0 mm) and a Shim-pack IC-PC1 pre-column (50 mm $\times$ 8.0 mm I.D.) were used. Mobile phase was 2.0 mmol/l nitric acid at a flow-rate of 1.2 ml/min, which was degassed prior to use. The temperature of column and detector was set at 40°C.

### 2.5. Experimental procedure

The mobile phase was pumped into the chromatographic column to stabilize the chromatography system for about 2 h until injections of the cation standard solutions gave reproducible retention times and peak areas. The sample solutions were then introduced, the elutes were detected by the BAW and/or CDD-6A detectors, and chromatograms were recorded by the aforementioned method.

### 2.6. Sample processing

Serum samples were centrifuged at 2000 g for 5 min, the supernatants were then passed through a 0.45  $\mu\text{m}$  filter membrane, and a 100- $\mu\text{l}$  aliquot of filtrate was injected onto the IC system. Unstimulated saliva was collected and the same process was performed for saliva samples but without centrifugation. The filtrate was diluted, if necessary, prior to injection.

## 3. Results and discussion

The BAW sensor was first developed in 1964 by King [10] and since then has been heavily researched by analytical chemists.

Considering a solution subject to BAW research, we have

$$G_0 = k \cdot \kappa \quad (1)$$

$$C_s = k \cdot \varepsilon + C' \quad (2)$$

where  $G_0$  is conductance of the solution,  $\kappa$  the specific conductivity of the solution, and  $C_s$  the capacitance of the solution,  $\varepsilon$  the dielectric constant of the solution,  $k$  the cell constant of the conductivity detector and  $C'$  the parasitic capacitance of the resonant circuit.

If the solution is used as reference, and the conductance change of the solution ( $\Delta G$ ) is much less than  $G_0$ , the frequency shift ( $\Delta F$ ) in SPQC was expressed as [11]:

$$\Delta F = \Delta G \pi F_0^2 C_q (2G_0 \omega_0 C_s + A \omega_0^2 C_s^2 - A G_0^2) / [G_0^2 - \omega_0 C_0 G_0 A + \omega_0^2 C_s (C_0 + C_s)]^2 \quad (3)$$

wherein  $F_0$  is the fundamental frequency of the quartz crystal,  $C_q$  is the motional capacitance of the SPQC,  $\omega_0 = 2\pi F_0$  and  $C_0$  is the parallel capacitance,  $A$  is tangent of the phase shift angle of the oscillator. It follows thus that, there is linear relationship between frequency shift and conductance change of the solution provided other conditions are kept constant. Therefore BAW sensor can be used as conductivity detector in IC. In the 1970s it was researched as a universal mass detector in liquid chromatography [12,13]. Recently applications of BAW sensors as conductivity detectors in LC [14,15] and IC [16,17] were reported.

### 3.1. Influence of mobile phase concentration

Mobile phase concentration was an important influential factor in chromatographic resolution and detection in IC. Although low concentration mobile phase could result in acceptable resolution, the separation became time-consuming because of the long retention time or even worse, the chromatographic peak became broad and skew, and hence, the sensitivities decreased. Contrarily, high concentration mobile phase rendered rapid elution, but the high background conductivity might also lead to low sensitivity. When the BAW detector was used, it responded to both the conductivity and permittivity of solution, it was rather sensitive over the range of 150 to 1200  $\mu\text{S}/\text{cm}$  background conductivity, while the dilute solution used as mobile phase maintained the permittivity constant. In this work, experimental results showed that 2.0 mmol/l nitric acid solution

was suitable mobile phase (background conductivity about 1100  $\mu\text{S}/\text{cm}$ ).

### 3.2. Effects of the detector temperature

The baseline, noise, and hence, sensitivity of the BAW detector were influenced by temperature [11]. Thus it was necessary to understand the effect of temperature on the BAW detector. It was observed from experimental data (Table 1) that the noise and drift of the BAW detector increased with elevated temperature difference between the BAW detector and the column. The noise level and drift were minimal when the BAW detector temperature was similar to or adjacent to that of the column. Consequently the temperatures of both the BAW detector and the column were kept at 40°C.

### 3.3. Dependence of sensitivity on cell constant

From Eq. (3), it could be seen that frequency shift was strongly relative to  $C_s$ , whereas  $C_s$  was dependent on the cell constant,  $k$ . Thus the response of the BAW detector was concerned with the cell constant to some extent. Experimental results (Table 2) show that the cell constant 3.2  $\text{cm}^{-1}$  gives the maximal response.

### 3.4. Comparison between the BAW and the conventional conductivity detectors

In this work the BAW detector was simple to construct, and in the viewpoint of measuring principle, it also had substantial advantages in many aspects over the conventional conductivity detector (CDD). A main drawback in CDD was the double electric layer capacitance and Faradaic impedance, which could change the effective potential applied to electrodes of the detector cell and produce confused results [18]. Although this limitation could be reducible by a multi-electrode technique or by applying

Table 1  
Effect of temperature on noise and response drift of the BAW detector (column temperature 40°C)

T (°C)	20	25	35	35	40	45	50	55	60
Noise (Hz)	4	3	2	1	1	1	2	3	5
Drift (Hz/h)	5	3	4	2	2	3	3	5	6

Table 2  
Effect of cell constant on sensitivity of the BAW detector

Cell constant ( $\text{cm}^{-1}$ )	1.04	2.56	3.20	4.05	5.22	6.10
Sensitivity (Hz/ppm $\text{Na}^+$ )	10	13	15	12	10	8

electrodes with alternating potential, the apparatus tended to be more complex. In the BAW detector, the double electric layer did not occur, because the resonator applied the electrode couple a high frequency (9 MHz) alternative electric field. Furthermore, the small potential difference between the electrode couple was not enough to cause electrolysis. These characteristics made the BAW detection less subject to error.

In this work, the BAW detector was compared with the conventional CDD for the determination of sodium, potassium and ammonium ions. Under the previously chosen conditions, the frequency shift responding sensitivity of the BAW detector to sodium, potassium and ammonium ions were 15, 9 and 11 Hz/ppm (or 345, 351 and 198 Hz/mM), respectively. Table 3 shows the performance of the BAW and CDD for the determination of sodium, potassium

Table 3  
Comparison between performance of the BAW and CDD detectors for the determination of sodium, potassium and ammonium

		Linear range (mmol/l)	Regression equation (A: peak area ( $\times 10^5 \mu\text{V/s}$ , C: concentration (mmol/L))	Correction coefficient (r)	Detection limit <sup>a</sup> ( $\mu\text{mol/l}$ )
BAW	$\text{Na}^+$	0.040-5.0	$A = -0.0642 + 5.476 \cdot C$	0.9993	8.7
	$\text{K}^+$	0.040-3.0	$A = -0.0055 + 1.141 \cdot C$	0.9996	8.9
	$\text{NH}_4^+$	0.025-1.2	$A = -0.0073 + 4.130 \cdot C$	0.9994	13.9
CDD	$\text{Na}^+$	0.010-5.5	$A = -0.0247 + 11.85 \cdot C$	0.9999	5.2
	$\text{K}^+$	0.012-3.8	$A = -0.0054 + 2.406 \cdot C$	0.9996	5.8
	$\text{NH}_4^+$	0.016-1.5	$A = -0.0098 + 9.010 \cdot C$	0.9994	8.6

<sup>a</sup> Defined as signal-to-noise ratio of 3.

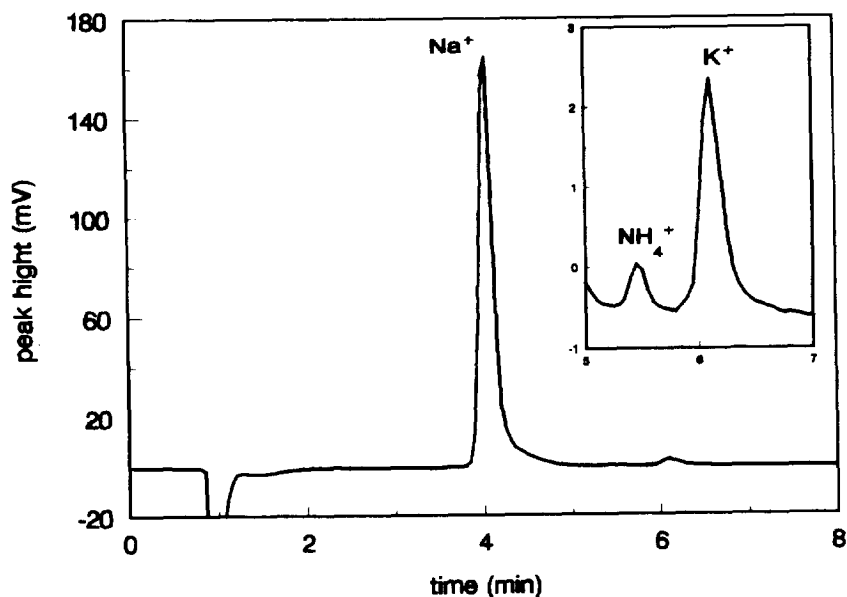


Fig. 1. Chromatogram of a serum sample detected by BAW.

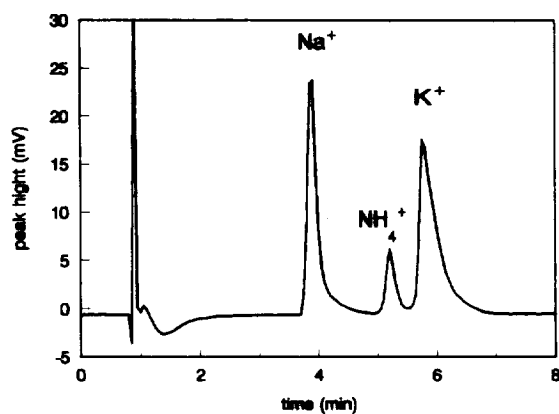


Fig. 2. Chromatogram of a saliva sample detected by BAW.

and ammonium ions with 2 mmol/l  $\text{HNO}_3$  as mobile phase. It could be seen that the two methods had the same magnitude order of detection limits for every cation. The reproducibilities of the methods were tested and illustrated by coefficient of variation (C.V.). Standard solutions of 1.40 mmol/l  $\text{Na}^+$ , 1.0 mmol/l  $\text{K}^+$  and 0.50 mmol/l  $\text{NH}_4^+$  were repeatedly determined for 10 times, the obtained C.V.s for IC–BAW were 0.4%, 0.5% and 0.8%, and 0.4%, 0.4% and 0.6% for IC–CDD, respectively. Moreover, some of the practical samples were determined using both the IC–BAW and IC–CDD methods, the results will be pursued in the following sections.

### 3.5. Practical analysis of human body fluid samples

The present IC–BAW method was used to determination of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{NH}_4^+$  in saliva samples

and serum samples from control and patients. Protein, which was detrimental to chromatographic column and impaired column performance, was isolated from the samples by centrifugation and filtering through micropore filter membrane. Divalent cations (mainly  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ) were not eluted by 2 mM  $\text{HNO}_3$  but trapped in the separation column, disturbing the reliable analysis, therefore a Shim-pack IC-PC1 pre-column was used to solve this problem. Under the chosen conditions, typical chromatograms of serum sample and saliva sample are given in Figs. 1 and 2, where no significant differences in the peak shapes are observed, regardless of whether the BAW or CDD was used. The analytical results are given in Tables 4 and 5, from which some information probably can contribute to medical study.

To validate this method, some of the saliva and serum samples were also determined by flame photometric method for sodium and potassium [1] and glutamate dehydrogenase (GDH) method for ammonium [5], as well as IC–CDD for those three cations. Table 6 gives the results, which show agreement among those methods. Table 7 lists the quantitative recoveries of spiked sodium, potassium and ammonium by the IC–BAW method.

## 4. Conclusion

The proposed ion chromatographic method with bulk acoustic wave (BAW) detection was successfully applied to the determination of sodium, potassium and ammonium ions in human body fluids (saliva and serum). The method is rather simple, rapid and

Table 4  
Results of serum sodium, potassium and ammonium determination<sup>a</sup>

Sample	Sodium (mmol/l)	Potassium (mmol/l)	Ammonium (mmol/l)	Notes
0-1	135.7±5.9	4.2±0.24	25.9±2.7	Health adult
0-2	140.3±5.4	5.1±0.33	31.8±3.0	Health adult
0-3	143.1±5.2	3.7±0.22	27.2±2.5	Health child
0-4	135.6±6.1	5.6±0.30	68.7±6.4	New-born
1-1	148.9±7.3	3.1±0.15	43.0±3.3	Cushing's syndrome
1-2	146.5±8.7	3.1±0.13	65.4±5.6	Cushing's syndrome
1-3	133.2±6.8	6.0±0.28	59.0±5.5	Addison's disease
1-4	135.0±6.1	5.8±0.31	80.0±4.4	Addison's disease
1-5	96.7±4.6	2.9±0.18	26.2±1.8	Ovary cancer

<sup>a</sup> Mean±S.D. [ $n=5$  except for samples 0-4 ( $n=3$ ) and 1-4 ( $n=4$ )].

Table 5  
Results of salivary sodium, potassium and ammonium determination<sup>a</sup>

Sample	Sodium (mmol/l)	Potassium (mmol/l)	Ammonium (mmol/l)	Na/K ratio	Notes
2-1	24.8±1.1	12.2±0.80	4.1±0.35	2.07	25-year adult
2-2	9.2±0.56	9.1±0.42	4.6±0.32	1.01	Periodontitis
2-3	5.4±0.43	10.1±0.55	5.8±0.46	0.53	Hungry
2-4	5.6±0.46	7.8±0.64	8.4±0.65	0.70	9-month infant
2-5	10.9±0.81	9.5±0.84	9.7±0.72	1.15	5-year child
2-6	14.4±1.0	11.5±0.68	3.0±0.32	1.26	13-year juvenile
2-7	21.0±1.4	13.7±1.2	5.5±0.57	1.53	17-year adult
2-8	19.3±0.85	13.3±0.91	3.2±0.25	1.45	40-year adult
2-9	16.1±1.1	17.0±1.3	7.0±0.41	0.95	30-year adult

<sup>a</sup> Mean±S.D. *n*=5, except for samples 2-4, 2-5 (*n*=3) and 2-9 (*n*=4).

Table 6  
Comparison of the IC–BAW and flame photometric method for sodium, potassium and enzymatic method for ammonium<sup>a</sup>

Sample	IC–BAW			IC–CDD			Flame photometric method		GHD method
	Na <sup>+</sup>	K <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>	Na <sup>+</sup>	K <sup>+</sup>	NH <sub>4</sub> <sup>+</sup>
0-2	140.3±5.4	5.1±0.35	31.8±3.0	141.8±5.4	5.1±0.35	31.5±2.7	139.2±8.2	4.9±0.35	32.7±3.3
1-1	150.2±7.3	3.2±0.15	43.0±3.4	152.0±7.4	3.2±0.12	42.3±3.5	138.5±8.2	3.1±0.29	39.4±4.5
1-5	96.7±4.6	2.9±0.18	26.2±1.8	97.5±4.6	3.0±0.16	27.1±1.8	98.8±5.8	2.9±0.2	27.4±1.8
2-1	24.8±1.1	12.0±0.78	4.1±0.34	25.5±1.0	12.3±0.80	4.1±0.33	23.7±1.3	11.1±1.0	4.8±0.47
2-2	9.2±0.56	9.1±0.42	4.6±0.31	9.0±0.61	9.1±0.40	4.8±0.35	9.5±0.68	8.9±0.76	5.7±0.65

<sup>a</sup> Mean±S.D. (*n*=5), unit was mmol/l except for serum ammonium (in samples 0-2, 1-1 and 1-5), which was μmol/l.

accurate. Even though the BAW detector could not completely match with the conventional conductivity detector in performance, it tided over some vital drawbacks which were inevitable with application of the latter one. The IC–BAW method was demon-

strated not only by IC with the conventional conductivity detector but also by flame photometric method for sodium and potassium and enzymatic method for ammonium. Agreement among those methods was observed.

Table 7  
Recoveries of sodium, potassium and ammonium by the IC–BAW

Na <sup>+</sup> /K <sup>+</sup> /NH <sub>4</sub> <sup>+</sup> content (mmol/l)	Na <sup>+</sup> /K <sup>+</sup> /NH <sub>4</sub> <sup>+</sup> added (mmol/l)	Na <sup>+</sup> /K <sup>+</sup> /NH <sub>4</sub> <sup>+</sup> found (mmol/l)	Recovery (%)
135.7 Na <sup>+</sup>	100.0 Na <sup>+</sup>	240.1 Na <sup>+</sup>	104
4.2 K <sup>+</sup>	5.0 K <sup>+</sup>	9.2 K <sup>+</sup>	100
25.9 NH <sub>4</sub> <sup>+</sup>	25.0 NH <sub>4</sub> <sup>+</sup>	51.6 NH <sub>4</sub> <sup>+</sup>	104 <sup>a</sup>
148.9 Na <sup>+</sup>	150.0 Na <sup>+</sup>	303.0 Na <sup>+</sup>	102
3.1 K <sup>+</sup>	3.0 K <sup>+</sup>	6.0 K <sup>+</sup>	97
43.0 NH <sub>4</sub> <sup>+</sup>	50.0 NH <sub>4</sub> <sup>+</sup>	94.1 NH <sub>4</sub> <sup>+</sup>	102 <sup>a</sup>
9.2 Na <sup>+</sup>	10.0 Na <sup>+</sup>	19.2 Na <sup>+</sup>	100
9.1 K <sup>+</sup>	10.0 K <sup>+</sup>	19.3 K <sup>+</sup>	102
4.6 NH <sub>4</sub> <sup>+</sup>	5.0 NH <sub>4</sub> <sup>+</sup>	9.3 NH <sub>4</sub> <sup>+</sup>	94

<sup>a</sup> Unit of concentration for ammonium was μmol/l.

## Acknowledgments

This work was supported by the Natural Science Foundation and the Education Commission Foundation of China.

## References

- [1] A. Kaplan and L.L. Szabo, *Clinical Chemistry: Interpretation and Techniques*, Lea and Febiger, Philadelphia, PA, 1979, p. 131.
- [2] C. Dawes, *Caries Res.*, 1 (1967) 333.
- [3] J. Koryta, *Anal. Chim. Acta*, 233 (1990) 1.
- [4] G. Fawaz and K.V. Dahl, *Lebanese Med. J.*, 16 (1963) 169.
- [5] H.J. Southgate, J.S. Colliss and S.M. Short, *Ann. Clin. Biochem.*, 28 (1991) 412.
- [6] W. Hu and H. Haraguchi, *Anal. Chim. Acta*, 289 (1994) 231.
- [7] H. Small, T.S. Stervens and W.C. Bauman, *Anal. Chem.*, 47 (1975) 1801.
- [8] H. Small, *Ion Chromatography*, Plenum Press, New York, NY, 1989.
- [9] P. Chen, L. Nie and S. Yao, *Instr. Sci. Tech.*, 23 (1995) 137.
- [10] W.H. King Jr., *Anal. Chem.*, 36 (1964) 1735.
- [11] D.Z. Shen, Z.Y. Li, L.H. Nie and S.Z. Yao, *Anal. Chim. Acta*, 276 (1993) 87.
- [12] W.W. Schuly and W.H. King, Jr., *J. Chromatogr. Sci.*, 11 (1973) 343.
- [13] P.L. Konash and G.J. Bastiaans, *Anal. Chem.*, 52 (1980) 1929.
- [14] T. Nomura, T. Yanagihara and T. Mitsui, *Anal. Chim. Acta*, 248 (1991) 329.
- [15] T. Nomura, K. Takada and T. Mitsui, *Bunseki Kagaku*, 41 (1992) 309.
- [16] P. Chen, L. Nie and S. Yao, *J. Chromatogr. Sci.*, 33 (1995) 268.
- [17] B.S. Yu, P. Chen, L.H. Nie and S.Z. Yao, *Anal. Lett.*, 29 (1996) 43.
- [18] P.H. Rieger, *Electrochemistry*, Prentice-Hall, Englewood Cliffs, NJ, 1987, Ch. 3.