Journal of Chromatography, 619 (1993) 103–109
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 7012

Electrochemical detection of cobalt in hair following on-column derivatization with 1-(2-pyridylazo)-2-naphthol-6-sulphonic acid and reversed-phase liquid chromatography

Jianxun Zhou, Lijing Zhang☆ and Erkang Wang*

Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022 (People's Republic of China)

(First received January 26th, 1993; revised manuscript received May 28th, 1993)

ABSTRACT

A method for the specific determination of cobalt based on reversed-phase liquid chromatography with amperometric detection via on-column complex formation has been developed. A water-soluble chelating agent, 1-(2-pyridylazo)-2-naphthol-6-sulphonic acid (PAN-6S), is added to the mobile phase and aqueous cobalt solutions are injected directly into the column to form *in situ* the cobalt–PAN-6S chelate, which is then separated from other metal PAN-6S chelates and subjected to reductive amperometric detection at a moderate potential of -0.3 V. Because the procedure eliminates the interference of oxygen and depresses the electrochemical reduction of the mobile phase-containing ligand PAN-6S, by virtue of the quasi-reversible electrode process of the cobalt–PAN-6S complex, a low detection limit of 0.06 ng can be readily obtained. Interference effects were examined for sixteen common metal species, and at a 5- to 8000-fold excess by mass no obvious interference was observed. The feasibility of the method as an approach to the specific analysis of cobalt in a hair sample has been demonstrated.

INTRODUCTION

In recent years, numerous reports have demonstrated that liquid chromatography (LC) possesses excellent capability for the determination of metal ions at low concentrations, by the formation of metal complexes with suitable ligands and combined with spectrophotometric, electrochemical or atomic absorption detection [1–4].

Although the determination of metal ions via polarographic, voltammetric and other electrochemical techniques is extremely sensitive [5,6], many determinations are prone to interference from overlapping waves [5–8]. It is not surprising that LC coupled with electrochemical detection (LC–ED) via chemical derivatization is a rapidly developing field of multi-element analysis [9–20]. Various chelating agents have been employed and evaluated for this purpose. These include dithiocarbamic acids (DTCs) [9–16], 8-hydroxyquinoline [17], 4-(2-pyridylazo)resorcinol (PAR) [18,19] and tiron [20]. Nearly all these methods involved oxidative amperometric detection at anodic potentials in the range 0.6–1.2 V, with the exception of 8-hydroxyquinoline [17], where reductive amperometric detection at -0.6 V was applied for the determination of several transition metal ions.

Reductive amperometric detection partially overcomes problems due to the presence of oxygen and its removal when LC–ED is conducted at negative potentials (-0.6 V or below). Three

* Corresponding author.
☆ Present address: Centre for Physical and Chemical Testing, Xingjiang University, Wulumuqi, Xingjiang 830046, People's Republic of China.

0378-4347/93/$06.00

kinds of methodology have been used: precolumn derivatization [9,10,16–20], *in situ* complex formation [9–13] and postcolumn on-line mixing [14,15]. Precolumn derivatization yields a low background current, because of the absence of the electroactive ligand from the mobile phase [9,18]. On-column complex formation leads to inherently increased sensitivity owing to the elimination of the dilution factor in external complex preparation [9–13]. Postcolumn mixing has been successfully applied in the automated monitoring of metal ions in industrial effluents by microprocessor-assisted LC–ED [14,15], and seven-day operation without operator intervention has been achieved.

The essential role of cobalt in ruminant nutrition, its function as a key element in vitamin B_{12}, and the extensive evidence on the beneficial effects of small amounts of this metal on the growth and development of plants, animals, microorganisms and, in particular, of human beings, place cobalt in a unique position among the rarer heavy metals which are found in biological systems [21]. Therefore, it is highly desirable that low concentrations of cobalt in biological samples, for example, in human hair (since it was found that cobalt content did not decline with age [22]) be effectively determined.

This paper describes the use of the water-soluble complexing agent, 1-(2-pyridylazo)-2-naphthol-6-sulphonic acid (PAN-6S), as an alternative to the all other ligands reported previously for the specific determination of cobalt. The PAN-6S was added to the mobile phase and complex formed *in situ* with ions injected and separated with an ODS column, followed by reductive amperometric detection. As demonstrated in the following sections, the advantages of the established LC–ED method are several. First, PAN-6S and its metal complexes are water-soluble and highly stable. This eliminates the difficulties in both the solvent extraction in the complex preparation and the necessity of using extensively the toxic mobile phase containing a high percentage of acetonitrile in the LC–ED programme, as frequently encountered with the DTC derivatives [9–12]. Second, based on the quasi-reversible voltammetric behaviour of the Co–PAN-6S complex, occurring within a moderate potential range, reductive amperometric detection can be readily performed at a moderate potential, from which at least two benefits can be expected: compared with the oxidative detection mode, interferences can be greatly minimized for the specific monitoring of cobalt in real samples, especially in environmental species and physiological fluids; oxygen interference and its removal, as typically seen in the reductive LC–ED [17], are also naturally eliminated. Third, the ligand PAN-6S itself is electroinactive at the operating potential, contributing little to the background current, therefore it does not need to be removed from the solution by ion chromatography prior to the amperometric detection. This significantly simplifies the instrumentation and operation, and yet the detectability is comparable with precolumn derivatization via oxidative LC–ED [18] and apparently superior to *in situ* DTC complex formation coupled with oxidative LC–ED [11–15] for the specific cobalt determination. And last, these advantages have been illustrated in and consolidated by the application of the methodology in the determination of cobalt in hair samples.

EXPERIMENTAL

Reagents and solutions

The cobalt stock solution (1 mg/ml in 0.1 *M* HCl) was prepared by nitration of the pure metal. Standard working solutions were obtained by serial dilution of this stock solution with the mobile phase. The analytical reagent 1-(2-pyridylazo)-2-naphthol (PAN) was from Beijing Pharm (Beijing, China).

PAN-6S was prepared from PAN by sulphonation [23]. The 1 m*M* PAN-6S stock solution was prepared with doubly distilled water.

All other chemicals were of analytical grade. Doubly distilled water was used for the preparation of all solutions.

The mobile phase was 40:60 (v/v) methanol–0.1 *M* KH_2PO_4 containing 0.5 m*M* PAN-6S. The flow-rate was 1.0 ml/min.

Procedures

Preparation of complexes. External complex formation for electrochemical investigation was made by mixing appropriate small amounts of aqueous cobalt and other stock metal solutions and PAN-6S solution in 10 ml of 0.1 *M* KH_2PO_4 containing different percentages of methanol. Chelates of Co(II)–PAN-6S were formed immediately at room temperature and were stable for at least 8 h.

Preparation of the hair sample solution. A sample of hair was soaked in a detergent solution for 0.5 h and washed with water repeatedly until no foam could be observed. It was then rinsed three times with deionized water to remove the oily coating of the hair. The cleaned hair was dried at 110°C for 1 h and was then kept in a dry container for use. Weigh accurately 1–2 g of the dried hair, add 45 ml of 4 *M* HNO_3 to dissolve the hair completely by nitration at a moderate temperature. Concentrated perchloric acid was added dropwise to the solution, and temperature slowly raised until a large amount of white smoke was produced. Further drops of $HClO_4$ were added until the solution was finally discoloured. The temperature was kept high until no more smoke was given off. After the solution had cooled slowly to room temperature, a small amount of water was added and the solution was heated to dissolve completely the resultant residue. The pH was adjusted to weakly acid (pH *ca.* 6) with 1 *M* NaOH. The solution was finally diluted to 10 ml with mobile phase.

A 20-μl volume of the hair sample solution and the Co(II) solution were injected directly into the LC system for the determination, calibration and characterization.

Instrumentation

Liquid chromatography was performed with a Model 510 pump, a U6K injector, and a 481 UV–VIS spectrophotometer (Waters Assoc., USA). The analytical column was Spherisorb C_{18} (5 μm, 200 mm × 4.0 mm I.D.). A TL-5A thin-layer amperometric detector was used (Bioanalytical Systems, USA), with a laboratory-made bipotentiostat monitoring the applied potentials and measuring the currents. Cyclic voltammetry was conducted with a locally built potentiostat in a conventional three-electrode system: glassy-carbon-disk working electrode, Ag/AgCl (saturated KCl) reference electrode, and platinum wire counter-electrode. The solutions were deaerated thorougly by high-purity nitrogen when the cathodic potential scan exceeded −0.4 V.

RESULTS AND DISCUSSION

Cyclic voltammetry

Although PAN (Fig. 1) is traditionally a widely used unselective chelating reagent [25], studies on complex formation of PAN-6S(I) with metal ions are fewer [23,26].

PAN-6S, considered to be a tridentate ligand, forms chelates with ligand/metal ion ratios of 1:2 over a wide pH range for Co(II) and most common metals, with only a few exceptions [26]. Since the PAN-6S molecule bears the naphthol moiety, which is oxidizable at solid electrodes [27], and the azo linkage, which is reducible at mercury electrodes [28,29], chelation with PAN-6S labels the metal ion with electroactive groups and makes LC–ED monitoring possible. The electrochemical behaviour of PAN-6S and its Co(II) complex were investigated by cyclic voltammetry (CV) as shown in Fig. 2. PAN-6S exhibited an irreversible anodic wave with a peak potential (E_p) of *ca.* 0.95 V, corresponding to the electrochemical oxidation of naphthol group [27]. Chelated PAN-6S is more difficult to oxidize than the unchelated complex, with the anodic wave overlapping that of the parent PAN-6S and the E_p shifted more anodic to 1.1 V. Extending the potential scanning cathodically to −0.8 V, PAN-6S yielded another couple of quasi-reversible

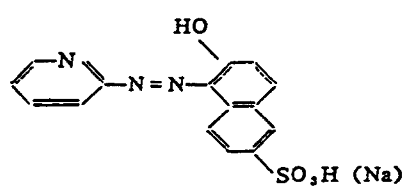

Fig. 1. Structure of PAN-6S.

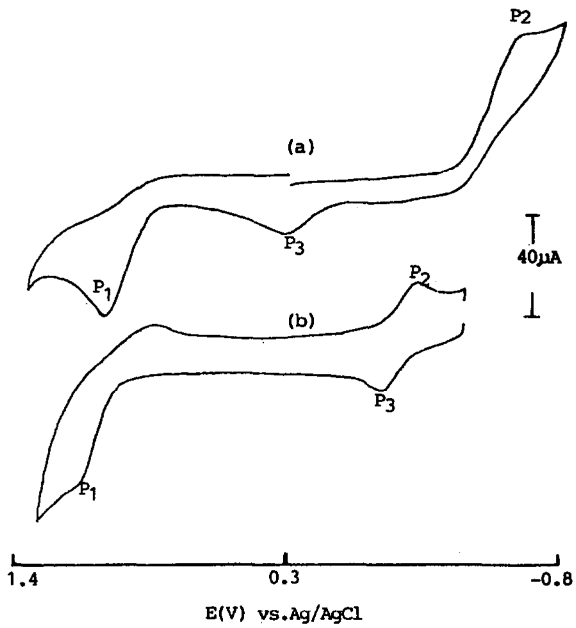

Fig. 2. Cyclic voltammograms of 1 mM PAN-6S (a) and 1 mM Co(PAN-6S)$_2$ (b) in 0.1 M KH_2PO_4 (pH 6.5). Scan-rate, 100 mV/s.

redox waves, with an oxidation peak potential (E_{pa}) and a reduction peak potential (E_{pc}) of *ca.* 0.25 V and -0.6 V, respectively. This apparently resulted from the quasi-reversible reduction of the azo linkage [28,29]. In the case of the Co(II)–PAN-6S complex, however, the reversibility of the azo linkage reduction was greatly improved. The peak-to-peak potential difference, ΔE_p, was reduced from 0.85 V for unchelated PAN-6S to 0.11 V ($E_{pa} = -0.1$ V, $E_{pc} = -0.21$ V), and the peak current ratio, i_{pa}/i_{pc}, was much closer to 1.0. Both i_{pa} and i_{pc} increased linearly with the square root of the potential scan-rate in the range 0–100 mV/s, while the E_p values remained virtually the same (Fig. 3), indicating typically an electrode process of diffusion control. The peak currents of all these peaks markedly decreased with increasing methanol content in the supporting electrolyte, and the magnitude for the anodic wave at E_p of 1.0 V (P1) was much greater than those for the quasi-reversible redox couple (P2, P3). Similar phenomena were previously observed for other azo complexes at mercury electrodes [28]. Since the P1 wave of the Co(II)-chelated PAN-6S severely overlapped that of the parent PAN-6S, on-column derivatization LC–ED would be impractical if the surplus PAN-6S is not removed prior to detection. Our attention was therefore focused on the investigation and utilization of reductive LC–ED for specific cobalt determination. A total of seventeen common metals were surveyed by CV. It was found that the reversibility of the azo linkage reduction for PAN-6S chelated with all but cobalt metal ions deteriorated compared with the parent PAN-6S. Subsequently, when the cathodic potential sweep was limited to -0.4 V (a value beyond which reductive LC–ED would become unfeasible owing to oxygen interference and its removal), no obvious redox waves (P2, P3) were observed in the range from 0.2 V to -0.4 V for all but Co(II)–PAN-6S complexes. From this, practical reductive LC–ED via on-column complex formation could be expected for the specific cobalt determination.

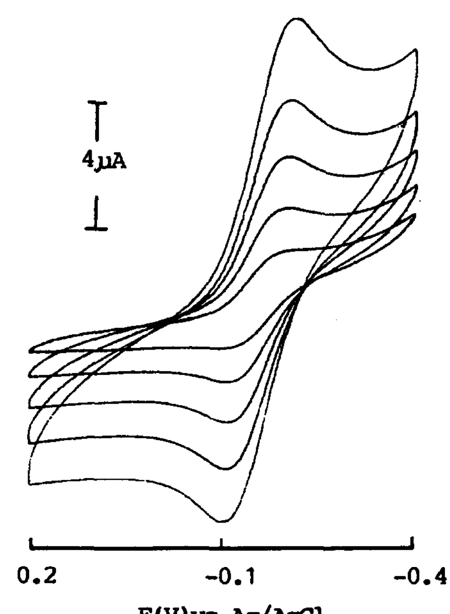

Fig. 3. Cyclic voltammograms of 1 mM Co(PAN-6S)$_2$ at scan-rates of 3, 10, 25, 50 and 100 mV/s. Supporting electrolyte as in Fig. 2.

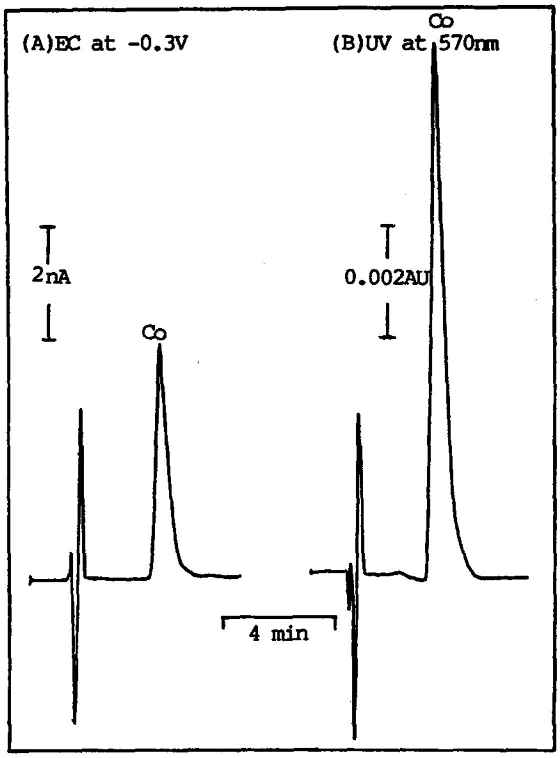

Fig. 4. Chromatograms of 1 ppm Co(II) with (A) ED at −0.3 V *vs.* SCE and (B) UV at 570 nm. Mobile phase, 40% methanol in 0.1 M KH_2PO_4 (pH 6.5) containing 0.5 mM PAN-6S at 1.0 ml/min. Column, Spherisorb C_{18} (5 μm, 200 mm × 4.0 mm I.D.); injection volume, 20 μl.

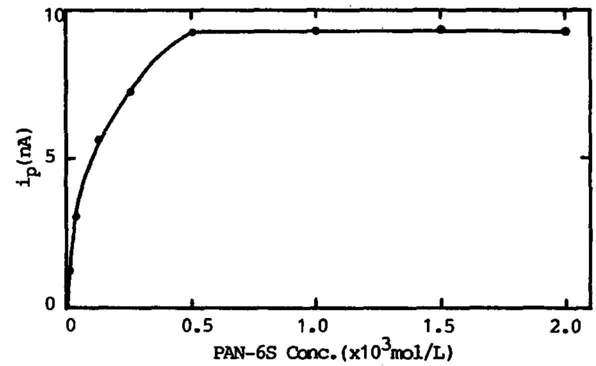

Fig. 5. Effect of PAN-6S concentration on the Co(II) LC–ED peak current. Other conditions as in Fig. 4.

Liquid chromatography–electrochemical detection

Fig. 4 shows chromatograms of Co(II) recorded with the amperometric detector at a potential of −0.3 V and the spectrophotometer at a wavelength of 570 nm. Under the separation conditions mentioned above, well defined and symmetric peaks were obtained. The chromatographic conditions can be varied more for amperometric detection, since PAN-6S forms complexes with many metal ions which have maximal absorption wavelengths of *ca.* 570 nm [26], and the selectivity of the detector response is therefore not comparable with that of amperometric detector. $Co(PAN\text{-}6S)_2$ is not retained on the ODS column under the mobile phase composition of pH <4.0 or methanol content >65%. Using the mobile phase of pH >7.0 or methanol content <35% gave rise to severe peak tailing and lower effective column efficiency. A mobile phase composition of 40:60 (v/v) methanol–0.1 M KH_2PO_4 at pH 6.5 was selected.

The effect of the PAN-6S concentration in the mobile phase on the Co(II) current response was investigated and shown in Fig. 5. It is clear that the Co(II) peak current increased almost linearly with PAN-6S concentration up to 0.5 mM, whereafter a maximal and constant response level was achieved. A PAN-6S concentration of 0.5 mM

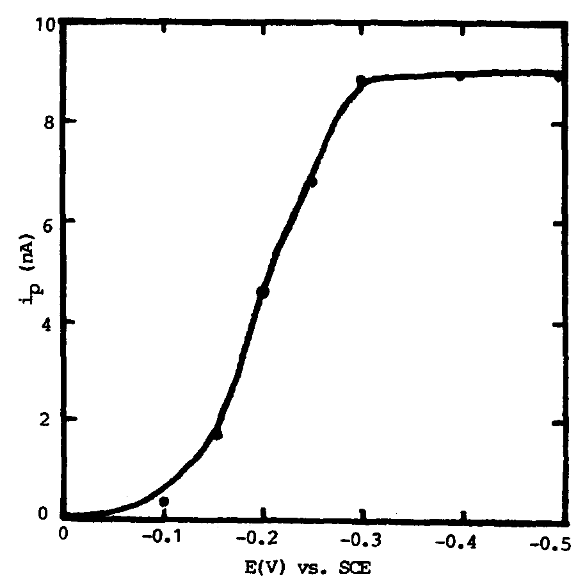

Fig. 6. Hydrodynamic voltammograms of 1 ppm Co(II). Chromatographic parameters as in Fig. 4.

was used for the adequate current response sensitivity and low background current. Hydrodynamic voltammetry was performed to determine the proper working potential (Fig. 6). Maximal and constant current response arose at −0.3 V and beyond. A working potential of −0.3 V was therefore selected to obtain high sensitivity of detector response and to eliminate the necessity of oxygen removal.

Under the working conditions established, LC–ED via on-column complex formation for Co(II) analysis gave a detection limit of 0.06 ng (20 μl) and a dynamic linear response range of 0.1–20 ng, with a correlation coefficient $r > 0.99$ and a y-intercept of 0.00053. The small y-intercept is indicative of both the low background current (mainly from PAN-6S reduction) and the absence of on-column hydrolysis of the Co(II)–PAN-6S chelates.

A precision study by eight repetitive injections of 1 ppm of Co(II) produced a relative standard deviation (R.S.D.) of 8% for the peak current measurements.

Effect of foreign ions

It has been reported that PAN-6S, similar to PAN, can form complexes with many metals of adequate stability [25]. Seventeen common metal ions were selected to determine the interference of foreign ions and to evaluate the selectivity of the method for Co(II) analysis. Results showed that the presence of 50 ng each of Fe(II), Ni(II) and Cu(II), 10 μg each of Cd(II), Zn(II), Mo(V), W(V), As(III), Al(III), Sn(II), Mn(II), Hg(II) and Pb(II), 24 μg of Mg(II), 40 μg of Ca(II) and 25 ng of Cr(III) had virtually no effect on the peak current of 5 ng Co(II) injected. It can be concluded that this procedure is reliable for the specific monitoring of Co(II) at low concentrations.

Sample analysis

The feasibility of performing specific analysis of cobalt in a hair sample with the described LC–ED method is demonstrated in Fig. 7A. Fig. 7B shows the chromatogram obtained by LC–UV. Well defined Co(II) peaks arose from the background, making the specification and quantitation of Co(II) in the matrix easy and precise. Estimation of the Co(II) content by standard addition is *ca.* 0.14 and 0.15 ppm by LC–ED and LC–UV, respectively. Recovery by standard addition was found *ca.* 86 ± 0.5%. The reproducibility for the LC–ED response of Co(II) in hair samples was *ca.* 8.5% (R.S.D. $n = 6$).

Finally, it should be noted that the detection limit achieved by the LC–ED approach is quite satisfactory, considering the simplicity of the analytical procedure and instrumentation.

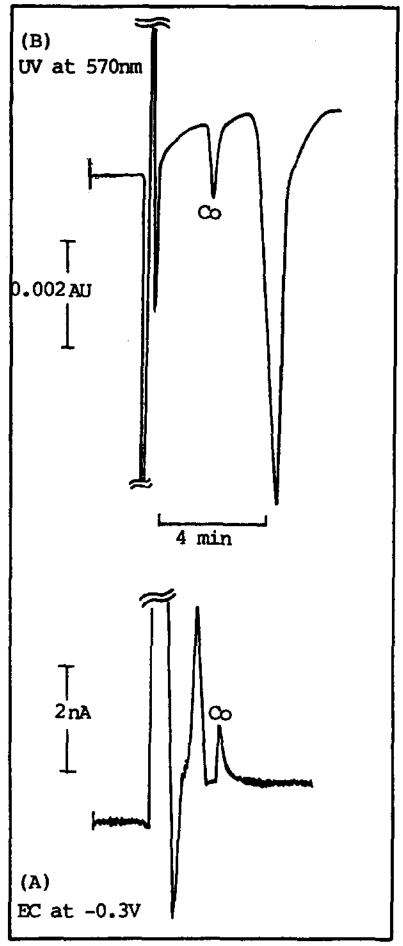

Fig. 7. Chromatograms of a hair sample detected by (A) ED at −0.3 V and (B) UV at 570 nm. The amount of Co found was *ca.* 0.14 ppm and 0.15 ppm, respectively. Conditions as in Fig. 4.

ACKNOWLEDGEMENTS

The support of the National Natural Science Foundation of China and the Basic and High-Tech Research Division of the State Commission of Science and Technology is gratefully appreciated.

REFERENCES

1 G. Schwedt, *Chromatographia*, 12 (1979) 613.
2 J. S. Fritz, *Pure Appl. Chem.*, 49 (1977) 1547.
3 B. R. Willeford and H. Veening, *J. Chromatogr.*, 251 (1983) 61.
4 A. R. Timerbaev, O. M. Petrukhin and Y. A. Zolotov, *Fresenius' Z. Anal. Chem.*, 327 (1987) 87.
5 A. M. Bond, *Modern Polarographic Methods in Analytical Chemistry*, Marcel Dekker, New York, 1980.
6 J. B. Flato, *Anal. Chem.*, 44 (1972) 75A.
7 E. Barendrecht, in A. J. Bard (Editor), *Electroanalytical Chemistry*, Vol. 2, Marcel Dekker, New York, 1967, pp. 53–103.
8 T. R. Copeland, R. A. Osteryoung and R. K. Skogerboe, *Anal. Chem.*, 46 (1974) 2093.
9 A. M. Bond and G. G. Wallace, *Anal. Chem.*, 53 (1981) 1209.
10 A. M. Bond and G. G. Wallace, *Anal. Chem.*, 54 (1982) 1706.
11 A. M. Bond and G. G. Wallace, *Anal. Chem.*, 55 (1983) 718.
12 A. M. Bond and G. G. Wallace, *Anal. Chem.*, 56 (1984) 2085.
13 H. Ge and G. G. Wallace, *Anal. Chem.*, 60 (1988) 830.
14 A. M. Bond, W. N. C. Garrard, I. D. Heritage, T. P. Majewski, G. G. Wallace, M. J. P. McBurney, E. T. Crosher and L. S. McLachlan, *Anal. Chem.*, 60 (1988) 1357.
15 A. M. Bond and T. P. Majewski, *Anal. Chem.*, 61 (1989) 1494.
16 E. Wang and A. Liu, *Fenxi Huaxue*, 19 (1991) 869.
17 A. M. Bond and Y. Nagosa, *Anal. Chim. Acta*, 178 (1985) 197.
18 D. A. Roston, *Anal. Chem.*, 56 (1984) 241.
19 H. Ji and E. Wang, *Sepu*, 6 (1988) 36.
20 E. Wang and A. Liu, *Microchem. J.*, 43 (1991) 191.
21 R. S. Young, *Cobalt in Biology and Biochemistry*, Academic Press, New York, 1979.
22 H. A. Schroeder and A. P. Nason, *J. Invest. Dermatol.*, 53 (1969) 71.
23 F. Tang, X. Mao, J. Cheng and J. Zhang, *Fenxi Huaxue*, 15 (1987) 216.
24 R. G. Anderson and G. Nickless, *Analyst*, 92 (1967) 207.
25 S. Shibata, *Bunseki Kagaku*, 21 (1972) 551.
26 Z. Zhou and Z. Liang, *Chem. J. Chin. Univ.*, 7 (1986) 595.
27 V. D. Parker, in M. M. Braizer (Editor), *Organic Electrochemistry*, Marcel Dekker, New York, 1973, p. 531.
28 T. M. Florence, *J. Electroanal. Chem.*, 52 (1974) 115.
29 G. W. Latimer, *Talanta*, 15 (1968) 1.