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Application of stripping voltammetry to trace lead analysis in intermediates and final products of syntheses of pharmaceuticals

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Abstract

Applications of differential pulse anodic stripping voltammetry using a new pen-type renewed hanging mercury electrode have been investigated for trace analysis of lead in pharmaceutical substances and intermediates of their syntheses, such as procaine hydrochloride, 4-aminobenzoic acid, methyl 4-aminobenzoate, 2-(4-chlor-3-aminobenzoyl)benzoic acid, benzyl 2-naphthyl ether, 5-aminoisophthalic acid, 3-aminobenzoic acid, 5-hydroxyisophthalic acid and *N,N'*-dibenzylethylenediamine diacetate. Samples were dissolved in 1 M HCl or 1 M NaOH and the electrochemical scan was carried out. No sample mineralization was necessary. The method showed a good linearity up to 50–100 ppm Pb with a detection limit less than 10 ppb. The results agreed well, but were more precise than those obtained by atomic absorption spectrometry using air/acetylene flame atomisation.

Keywords: Lead determination; Trace analysis; Stripping voltammetry; Pen-type mercury electrode

1. Introduction

The presence of heavy metals such as lead, cadmium, copper, mercury, thallium, etc., in pharmacological substances is usually undesirable due to their biotoxicity. The metallic ions mentioned are complexed by proteins with subsequent change in their conformation. Trace amounts of heavy metals in biological, environmental, technical, food and pharmaceutical samples are conventionally determined by atomic spectroscopy methods, such as atomic absorption spectrometry (AAS), inductively-coupled plasma (ICP), ICP-mass spectrometry (MS), etc. [1–10]. Electrochemical analysis, based on differential pulse voltammetry (DPV) coupled with a preconcentration technique, i.e. anodic stripping voltammetry (DPASV), exhibits a sufficient detection limit to allow the determination of traces of heavy metals in substances of pharmaceutical interest [11,12]. Different substrates can serve as working electrode, either solid (platinum, gold, glassy carbon), composite (carbon paste) or liquid (mercury film

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or hanging mercury drop (HMDE) electrodes [9]). It should also be mentioned that mercury microelectrodes obtained by deposition of mercury onto a surface of a platinum microelectrode gives numerous advantages [13], mainly because forced convection during the preconcentration stage is not necessary.

In addition, mercury shows high sensitivity to heavy metals when compared to others due to the creation of amalgams. This is used as the first step of the analysis for preconcentration of ions (M^{n+}) into the electrode by bulk electrolysis at a constant potential value in a stirred solution. The second step corresponds to the oxidation of M^0 back to M^{n+} by shifting the working potential to positive values and by recording the resulting current against the applied potential.

$$M^{n+} + ne^- \underset{\text{Analysis}}{\overset{\text{Preconcentration}}{\rightleftharpoons}} M^0$$

However, applications of this electrochemical method for heavy metal analysis in the presence of organic compounds could be limited by participation in metallic ion–ligand equilibria. The accumulation potential (E_{acc}) necessary for electrolysis is affected by the stability of the complex. When the stability constant of such a complex formed is too high, the E_{acc} applied must be sufficiently negative and sometimes it may be out of the electrode potential working range. Then, a total sample mineralization is necessary before the DPASV scan. This procedure is also necessary when the dissolved organic matrix interferes, e.g. in lead determination, because of the presence of some electroactive functional groups (nitro-, nitroso-, etc.) which are reduced in the same potential range. Oxidative UV photolysis has been found to be eminently suitable as a digestion method in the case of voltammetric determination of Pb, Cd, Pt, Ni and Co (HMDE–DPASV) in samples such as water with a high organic load (≈ 500 mg l^{-1}) of EDTA, picrate and humic acid, in beverages (Coca-Cola, fruit juices, wine and whisky) and also in biological samples such as blood and urine [14]. However, the method is rather time-consuming, the decomposition taking ≈ 150 min. Some products of insufficient degradation of the organic matrix (fragments from destroyed aromatic compounds, etc.) can unfavourably affect detection limits more than the matrix itself [15]. When the sample is insoluble in water, it can be derivatized to a soluble compound, such as sulfonate. However, sulfonates and other surfactants may also interfere. It should also be mentioned that both procedures, i.e. mineralization and derivatization, give risks of sample contamination by heavy metals from the chemicals used. The natural water sample pretreatment for voltammetric analysis is discussed in detail by Bott [16]. It is well known that the high amount of organic compounds in the sample solution may cause the fouling of voltammetric trace metal analysis, but not all organic matrices show this phenomenon. DPASV was successfully applied to determination of copper and zinc in multivitamins with mineral tablets [17] using a mercury film electrode. No sample digestion for stripping square wave voltammetric determination of trace lead concentrations in high fructose corn syrup and other corn sweeteners was required [18]. The similarity between both studies mentioned above and the work of this group is the high loading of the organic matrix and no sample mineralization.

In this paper the development is described of a simple, reliable, reproducible, sensitive and fast sample preparation technique for lead determination in intermediates and final products of syntheses of pharmaceuticals. Interferences from high matrix concentrations and other metals possibly present in the samples were also studied.

2. Experimental

2.1. Reagents

Lead(II) nitrate, 98% sulphuric acid, hydrochloric acid, sodium hydroxide, potassium cyanide and iron(II) sulphate heptahydrate (all from Lachema, Brno, Czech Republic) were used as analytical-grade reagents. Procaine hydrochloride, 4-aminobenzoic acid (PAB), 3-aminobenzoic acid (MAB), methyl 4-aminobenzoate (MePAB), 2-(4-chlor-3-aminobenzoyl)benzoic acid (CABB), benzyl 2-naphthyl ether (BNE), 5-aminoisoph-

Table 1
DPASV calibration parameters, peak characteristics and sample solution compositions

Compound	Sensitivity (nA ppm^{-1})	C.S.[a] ($\times 10^{-7}$)	E_{acc} (mV)	E_p[b] (mV)	Solvent	Sample weight (g per 25 ml)	0.3 M KCN (ml per 25 ml)
CABB	2.42	0.62	−1000	−620	1 M NaOH	2.5	
BNE	0.95	1.76	−1700	−687	Satd. NaOH	0.5[c]	
AIP	10.00	3.38	−1500	−780	1 M NaOH	1.5	0.80
HIP	13.75	6.92	−1000	−680	1 M NaOH	1.0	0.25
PAB	28.10	7.70	−1500	−730	1 M NaOH	2.0	0.80
MAB	31.76	12.19	−1500	−750	1 M NaOH	1.5	0.60
Procaine	50.00	12.20	−1000	−420	0.4 M HCl	2.0	
DBEDA	20.00	12.34	−1000	−420	1 M HCl	0.8	
MePAB	76.00	15.92	−1000	−430	1 M HCl	2.5	

[a] Sensitivity recalculated for a ratio $C(Pb)/C(matrix) = 1$ and for a bulk matrix concentration of 1 mol l^{-1} and thus given in nA per mol l^{-1}.
[b] Potential of peak maximum.
[c] Grams per 50 ml; full description is given in text.

thalic acid (AIP), 5-hydroxyisophthalic acid (HIP) and *N,N'*-dibenzylethylenediamine diacetate (DBEDA) were purchased from Synthesia (Pardubice, Czech Republic). Water was purified by a Milli Q$^+$ (Millipore) purification system with output conductivity ≈ 50 nS cm^{-1} before use.

2.2. Apparatus

For voltammetric measurements, an ECO-TRIBO Polarograph (Polaro-Sensors, Prague, Czech Republic) interfaced to an IBM compatible computer was used. All measurements were carried out in a three-electrode system using a platinum plate and a saturated calomel electrode (SCE) as the auxilary and reference electrodes respectively. The high-precision PC-controlled pen-type renewed hanging mercury drop stationary electrode (Polaro-Sensors) [19–21] has been connected as the working electrode (area 0.86 mm^2). All experiments were performed in a 10 ml glass cell. AAS, carried out on a AAS 3 instrument (Zeiss, Jena, Germany) with an air/acetylene flame atomiser, has been evaluated as a reference method. Samples were dissolved using an ultrasonic bath Uc 405 BJ 1 (Tesla) when necessary.

3. Procedures

3.1. Sample preparation

All samples were simply dissolved in HCl or NaOH using an ultrasonic bath, then 0.3 M KCN solution was added when necessary, and the volume was adjusted with solvent as is shown in Table 1. The final volume of the dissolved sample was 25 ml. Only benzyl 2-naphthyl ether was derivatized using sulfonation by heating with 2 ml 98% H_2SO_4 for ≈ 5 min until SO_3 vapours were generated. After cooling, 5 ml of water was added and mixed in a cooling water bath. This mixture was then quantitatively transferred to a 50 ml volumetric flask containing 5 ml of saturated NaOH. After cooling to room temperature, water was finally added to 50 ml.

3.2. Voltammetry

A volume of 10 ml of the sample solution prepared as above was placed in the electrochemical cell and bubbled with argon for at least 5 min in order to remove dissolved oxygen. After bubbling, argon was also admitted above the

sample solution surface to prevent oxygen air diffusion during the experiment. DPASV was used for all measurements. The following general voltammetric parameters were used: pulse amplitude, 50 mV; pulse width, 100 ms; current sampling at 90% (beginning after 90 ms during the last 10 ms of pulse). The scan rate was 20 mV s^{-1} and the valve was opened for 0.2 s. All these parameters were optimised in our previous work [22]. Specific parameters such as accumulation potential (E_{acc}) are given in Table 1 and are different for each sample. Accumulation time (t_{acc}) applied was 80 s. However, as this parameter influences the sensitivity (and detection limit) it was found that 80 s is sufficient time for a lead concentration range comprised of 0.1–50 ppm (mg(Pb) kg^{-1} sample). When higher sensitivity is necessary, t_{acc} must be longer. It should be mentioned that with increasing t_{acc} the relative standard deviation also increases. Similarly, when the sensitivity is too high, decreasing t_{acc} or diluting the sample solution is satisfactory. For lead determination, the multiple standard addition method was used.

3.3. AAS measurements

For AAS validation, sample solutions with the same composition as for voltammetric measurements were used. The wavelength was 217.0 nm, slit 0.3 mm. The lead content was determined using the standard addition method as well.

4. Results and discussion

4.1. Calibration, sensitivity and linearity

As can be seen from Figs. 1 and 2, the baseline around the Pb peak in all samples is clean, allowing good peak readability and a good detection limit. The baseline of BNE–sulfonate solution (Fig. 1e) is probably affected by a strong sorption effect of this molecule which could act as a surfactant. However, the Pb peak is far enough away from those peaks (at ≈ -1400 and ≈ -400 mV vs. SCE, Fig. 1e) to be affected. In the latter Figure the calibration line is not shown, but a good linearity was obtained. In MAB, which is depicted in Fig. 2a, a lead peak is situated on a baseline which consists of three small peaks (between -750 and -500 mV) which were reproducible but could not be ascribed to any other metal. They may possibly be related to sorption/desorption phenomena of the matrix. This interpretation is related to the fact that no dependence on t_{acc} was observed as it would be in the case with an electrolytic mechanism. However, the Pb peak is not affected in our concentration range of interest (1–50 ppm).

The potential of the peak maximum (E_p) is more affected by the pH than by the matrix composition or by E_{acc}, as can be seen from Table 1. In general, using strongly alkaline media, the E_p values have been found in an interval ranging from -780 to -620 mV. Using strongly acid media, these values were shifted to ≈ -420 mV. This shows that the activation energy for oxidation of lead in amalgam $Pb^0(Hg)_x$ is lower in alkaline than in acidic solution, i.e. lead(II) hydroxocomplexes in NaOH solution are more stable than chlorocomplexes in HCl.

The main factor affecting sensitivity is the matrix composition [23]. The lead sensitivity values obtained are given in Table 1. There is a big variation, from 0.95 to 76 nA ppm^{-1}, obtained in sulfonated BNE and MePAB respectively. For better comparison of sensitivities, these values were recalculated assuming the same sample concentration and the same Pb/matrix molar ratio (corrected sensitivity, C.S.). When compounds of similar structure are compared (AIP vs HIP or PAB vs MePAB), the Pb sensitivity (and also the C.S.) is $\approx$twice as high in HIP, which shows the higher stability of the Pb–AIP than the Pb–HIP complex (similarly for PAB vs. MePAB). This was not unexpected as the amino group acts as an electron-pair donor. This result also corresponds well with higher sensitivity for Pb(II) in HCl-containing solutions, where all amino groups are protonated and are not acting in complex formation (procaine, DBEDA and MePAB). The last one, MePAB, shows the highest sensitivity, because there is no free functional group. The matrix with the lowest C.S. value, CABB, can act as a strong complexing ligand, because there are three groups in a favourable conformation ("lob-

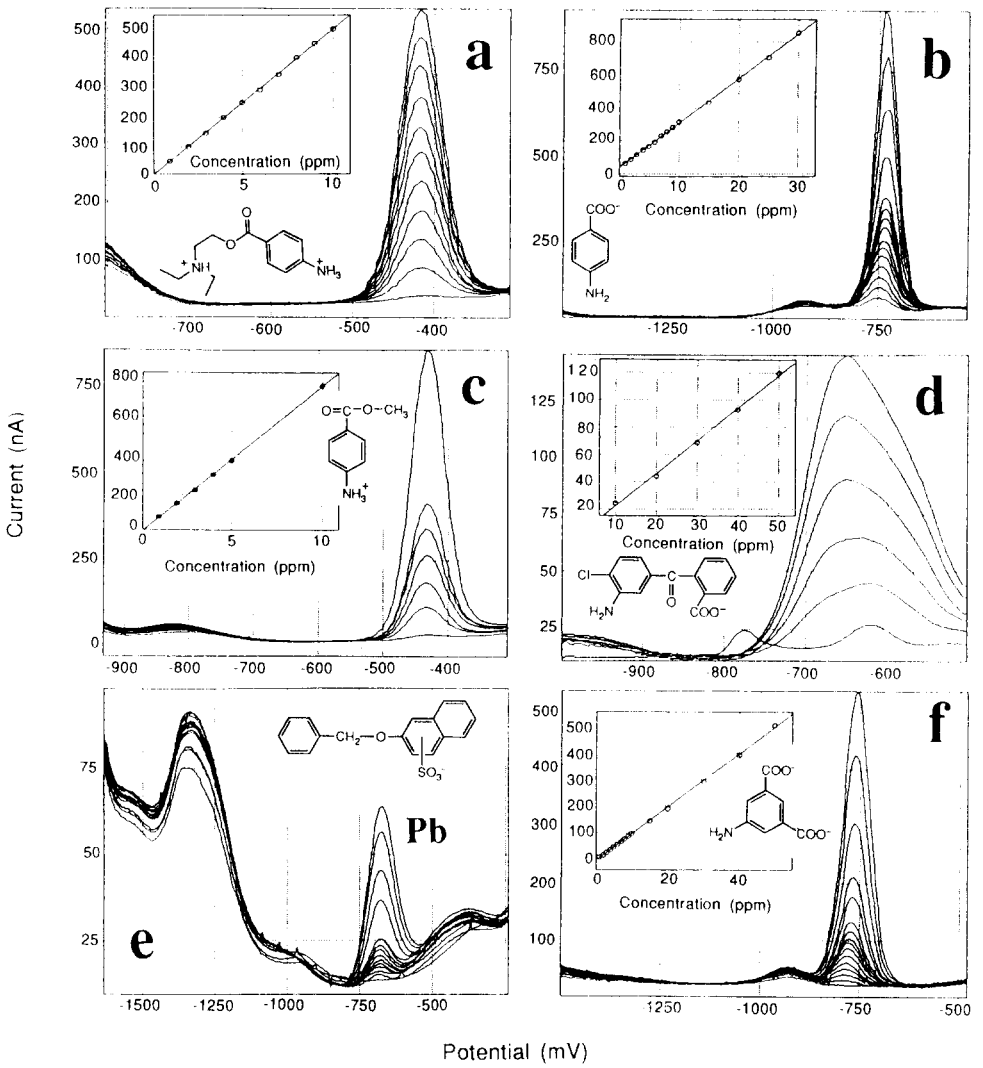

Fig. 1. Calibration of lead in substances: (a) procaine; (b) PAB; (c) MePAB; (d) CABB; (e) BNE; (f) AIP. Each part of a figure contains three things: DPASV voltammograms, calibration curves with points evaluated from Pb peaks and the formulae of the main matrix form presented in the sample solution measured. Pb^{2+} increments are: (a) 0–10 with step 1 ppm; (b) 0–10, 15, 20, 25, 30 ppm; (c) 0–5, 10; (d) 0–50 with step 10 ppm; (e) 0–10, 20, 30, 40, 50 ppm; and (f) as in (b) plus values 40 and 50 ppm. General parameters: pulse amplitude, 50 mV; width, 100 ms; scan rate, 20 mV s^{-1}; valve opening, 0.2 s. E_{acc}, E_p and solution compositions are given in Table 1 in detail.

ster-claw"). In fact, the Pb peak in a solution of this substance is large and probably consists of two overlapping peaks, because by increasing the lead concentration, the E_p values are shifted to more negative potentials (the shift in simple systems is, when it exists, to more positive values; compare Fig. 1d with Figs. 1a, 1f, 2b and 2c). In addition, the calibration plot is not strictly linear. All these facts show that when $C(Pb) \ll C(matrix)$, a different complex formation than in the case of a higher lead concentration is possible (in accordance with the breakpoint of the calibration which occurs at ≈ 20 ppm, where those overlapping peaks are of the same height, see Fig. 1d). It can also be seen from Table 2 that lead in this sample of CABB is determined with the worst

precision. In this case, the sample digestion described by Pisch et al. [14] is recommended.

4.2. Interferences

Except for interferences from matrix and dissolved oxygen discussed above, no interfering effects from other species (Cd^{2+}, Sn^{2+}, Tl^{+}, CrO_4^{2-}) were observed. Only Fe^{3+} in the case of AIP, HIP, PAB and MAB interfered in lead(II) determination (see Figs. 1b, 1f, 2a and 2b). A small peak close to the Pb Peak at ≈ -950 mV unfavourably affects the detection limit. By increasing the amount of iron, this peak also increases, but not in a reproducible way (Fig. 3A). The same effect has been found for all four substances. In the case of MAB and HIP, another effect has been found, as is shown in Fig. 3C; a very sharp non-redox peak is superposed on the main Pb peak at -700 mV. To eliminate these effects, we have used KCN, which is the strongest iron-complexing agent present in the sample solution. As can be seen from Figs. 3B and 3D, both interfering peaks are completely suppressed. The use of cyanide shows that by increasing the amount of KCN, the CN^- oxidation current shifts the positive potential limit to more negative values (Figs. 3B and 3D), and subsequently can totally overlap the Pb peak. Thus, the amount of KCN added had to be low and optimised (see Table 1). Because of the low complexation of lead(II), the addition of KCN decreases the Pb peak itself but the decrease was never more than 5% under the conditions employed here.

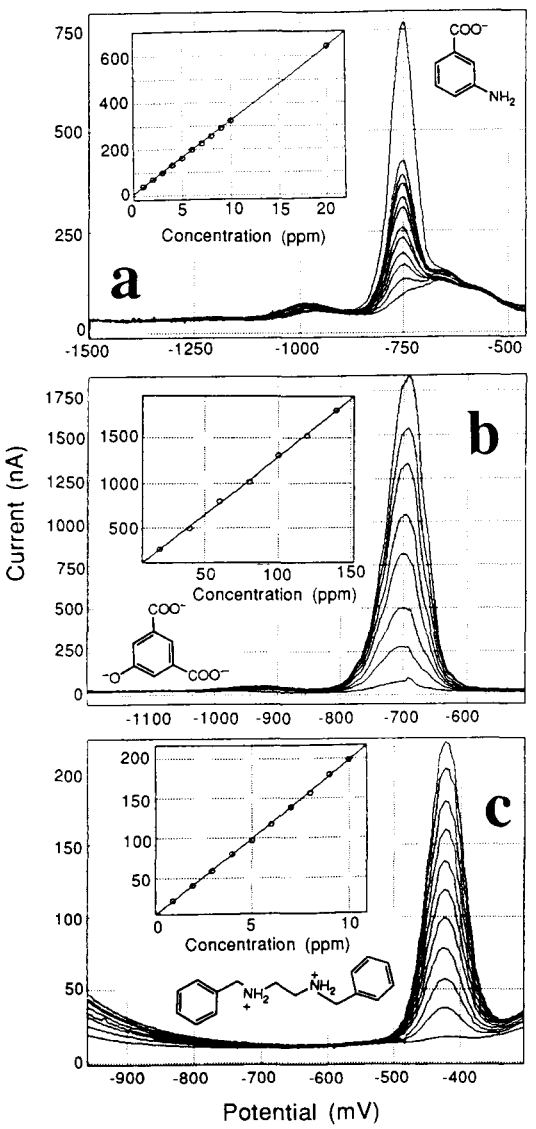

Fig. 2. Calibration of lead in substances; (a) MAB; (b) HIP; (c) DBEDA. Pb^{2+} increments are: (a) 0 10, 20 ppm; (b) 0–140 with step 20 ppm; (c) 0–10 ppm. Other conditions as in Fig. 1.

5. Validation and conclusion

The DPASV procedures described above were applied to determine Pb in real samples and the results were compared with those obtained by flame AAS. Results are given in Table 2. No significant statistical difference ($\alpha = 0.05$) was observed, but the results of DPASV determinations were more precise as indicated by values of relative standard deviation (RSD). The main advantage of the HMDE–DPASV technique is that the electrodeposition time can be increased for very low analyte concentrations. In addition, the surface renewal of the pen-type HMDE used in this study is fully automated by software with high reproducibility (RSD = 1.03%, $n = 15$) compared to solid state electrodes, where the surface renewing procedure may cause problems. Based on the experiments presented, DPASV can be recommended to monitor the lead contamination of

Table 2
Validation of DPASV results to those obtained by AAS, $n = 3$

Compound	DPASV		AAS	
	Mean (ppm)	RSD (%)	Mean (ppm)	RSD (%)
CABB	0.82	50	1.00	100
BNE	< 0.1	20		
AIP	1.0	15	2.9	30
HIP	7.0	10	8.0	50
PAB	3.3	20	1.4	120
MAB	0.66	5		
Procaine	0.002	20	0.003	100
DBEDA	0.73	15		
MePAB	5.2	20	1.2	330

intermediates and final products in the production of pharmaceuticals as a simple and relatively inexpensive electroanalytical method.

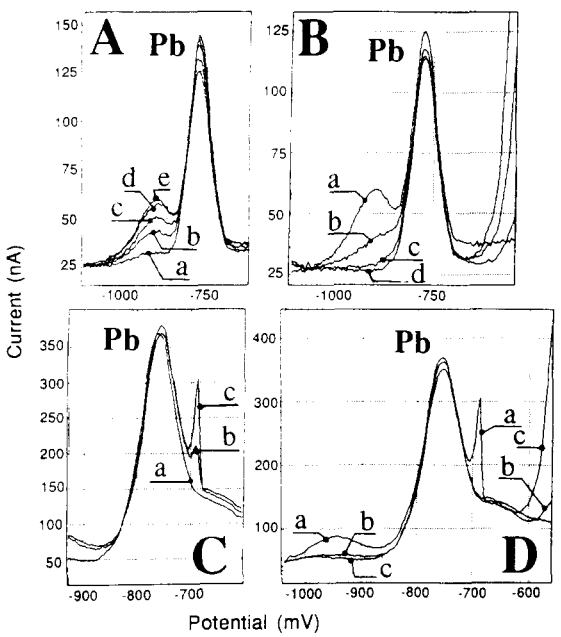

Fig. 3. Interfering effect of iron and its elimination. (A, curve a): AIP, 10 ppm Pb; (A, curves b, c, d, e): additions of 10, 20, 30 and 40 ppm Fe respectively. (B, curve a): identical to A, curve e; (B, curves b, c, d): additions of 100, 200 and 300 μl 0.3 M KCN. Other conditions as in Fig. 1f. (C, curve a): MAB, 10 ppm Pb; (C, curves b, c): addition of 30 and 50 ppm Fe respectively. (D, curve a): identical to C, curve c. (D, curves b, c): addition of 200 and 500 μl 0.3 M KCN respectively. Other conditions as in Fig. 2a.

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References

[1] J. Thomas, B. Anglov and J.M. Christensen, Analyst, 117 (1992) 419–424.
[2] G. Tölg, Anal. Chim. Acta, 283 (1993) 3–18.
[3] T.C. Rains, Anal. Spectrosc. Libr., 5 (1991) 191–226.
[4] T.M. Florence, G.M. Morrison and J.L. Stauber, Sci. Total. Environ., 125 (1992) 1–13.
[5] S.A. Listov, Farm. Zh. (Kiev), 5 (1990) 18–20.
[6] M. Krelowska-Kulas, Nahrung, 36 (1992) 265–268.
[7] B. Gercken and R.M. Barnes, Anal. Chem., 63 (1991) 283–287.
[8] K. Vermeiren, C. Vandeasteele and R. Dams, Analyst, 115 (1990) 17–22.
[9] I. Šestáková, D. Miholová, A. Slámová, P. Mader and J. Záková, Electroanalysis, 6 (1994) 1057–1062.
[10] S.B. Adeloju, A.M. Bond and M.H. Briggs, Anal. Chem., 57 (1985) 1386–1390.
[11] J. Wang, Stripping Analysis: Principles, Instrumentation and Applications, VCH, Deerfield Beach, FL, 1985.
[12] S. Mannino and J. Wang, Electroanalysis, 4 (1992) 835–840.
[13] K.R. Wehmeyer and R.M. Wightman, Anal. Chem., 57 (1985) 1989–1993.
[14] J. Pisch, J. Schäffer and D. Frahne, Int. Labmate, 20 (1995) 12–16.
[15] K. Pratt, H.M. Kingston, W.A. MacCrehan and W.F. Koch, Anal. Chem., 60 (1988) 2024–2027.
[16] A.W. Bott, Curr. Sep., 14 (1995) 24–30.
[17] J. Wang and H.D. Dewald, Anal. Lett., 16 (1983) 925–939.
[18] E.D. Hissong, Curr. Sep., 11 (1992) 43,44.
[19] L. Novotný, U.S. Patent No. 5173101, prior 1990.
[20] L. Novotný, in Book of Abstracts (Addendum), 4th European Conference on Electroanalysis, Noordwijkerhout, The Netherlands, 31 May–3 June, 1992.
[21] L. Novotný, R. Kalvoda, V. Novák and K. Uher, Eur. Pat. Appl. No. 376920, 1990.
[22] M. Pravda, M.Sc. Thesis, University of Pardubice, Pardubice, 1993.
[23] T.M. Florence, Analyst, 117 (1992) 551–553.