Simultaneous determination of copper, lead, cadmium, zinc, and selenium in cow liver by differential pulse polarography

Güler Somer, Gülbeniz Guliyeva, Güler Ekmekci, and Olcay Sendil

Abstract: A fast and simple method was established for the determination of trace elements in liver. DP polarograms of wet digested liver samples were taken in acetate buffer, pH about 4, for lead, cadmium, and zinc determinations. For copper, addition of EDTA at pH 4 was needed for a better separation from the iron peak. Selenite ion was determined using the hydrogen catalytic peak after the addition of Mo(VI) to the same solution. Trace element levels were different for two separate sections of liver. For the first section (S_1) the quantities were found to be 8.12 ± 0.21 mg g^{-1} Cu, 1.16 ± 0.12 mg g^{-1} Zn, 1.09 ± 0.11 mg g^{-1} Cd, 0.59 ± 0.07 mg g^{-1} Pb, and 2.05 ± 0.22 mg g^{-1} Se, in dry liver. For the second section (S_2) the results were the same for selenium, but Cd was too small to be detected. The other trace element quantities were 0.48 mg g^{-1} Cu, 0.22 mg g^{-1} Pb, and 0.29 mg g^{-1} zinc. The validity of the method was demonstrated with a synthetic sample resembling liver composition. This method enabled the simultaneous determination of heavy trace elements such as copper, lead, cadmium, molybdenum, selenium, and zinc by using an inexpensive instrument and without any separation or pre-concentration procedures.

Key words: cow liver, determination, differential pulse polarography, trace elements.

Résumé : On a développé une méthode simple et rapide pour déterminer les éléments à l'état de trace dans le foie. Afin d'évaluer les concentrations de plomb, de cadmium et de zinc, on a mesuré les polarogrammes à pulsation différentielle d'échantillons frais de foie dans un tampon d'acétate, à un pH d'environ 4. Pour le cuivre, à un pH de 4, il est nécessaire d'ajouter de l'EDTA afin d'obtenir une meilleure séparation du pic du fer. On a déterminé l'ion sélénite en faisant appel au pic catalytique de l'hydrogène après addition de Mo(VI) à la solution. Les concentrations des éléments à l'état de trace sont différentes pour deux sections différentes du foie. Dans la première section (S_1) de foie sec, on a trouvé les quantités suivantes: 8,21 ± 0,21 mg g^{-1} de Cu, 1,16 ± 0,12 mg g^{-1} de Zn, 1,09 ± 0,11 mg g^{-1} de Cd, 0,59 ± 0,07 mg g^{-1} de Pb et 2,05 ± 0,22 mg g^{-1} de Se. Dans la deuxième section (S_2) les résultats sont les mêmes pour le sélénium, mais les quantités de Cd sont trop faibles pour être détecter. Les quantités des autres éléments présents à l'état de trace sont de 0,48 mg g^{-1} de Cu, 0,22 mg g^{-1} de Pb et 0,29 mg g^{-1} de zinc. On a démontré la validité de la méthode à l'aide d'un échantillon synthétique dont la composition ressemble à celle du foie. Cette méthode permet de déterminer de façon simultanée des traces d'éléments de métaux lourds, tels le cuivre, le plomb, le cadmium, le molybdène, le sélénium et le zinc en faisant appel à des instruments peu coûteux et sans nécessité de procéder à des séparations ou à des préconcentrations.

Mots clés: foie de vache, polarographie à pulsation différentielle, éléments à l'état de trace.

[Traduit par la Rédaction]

Introduction

The total trace element levels have become of prime importance to aid both the clinician in the diagnosis and treatment of a variety of diseases and the life science researcher in the investigation of the role of trace elements in health and disease. Considering biological research, the role of some trace and ultra-trace elements in the body is very rich and varied. Some of them are essential to life while others are toxic even at very low concentrations (1–3). Since these elements are taken in mostly by human diet, the determination of their concentration in food is very important. Liver is known to deposit many trace elements; thus, cow liver is a potent source of heavy elements. Unfortunately, very few investigations have been attempted relating to the determination of the concentration of heavy elements. Most of the work has been concentrated on their effect on liver and physiologic body functions (4–6). The effect of ingestion of heliotrope (homeopathic medicine) and copper on the concentration of zinc, selenium, and molybdenum in the liver of the sheep has been studied (4); the ingestion of heliotrope was found to reduce liver concentrations of zinc and molybdenum. The concentration of selenium in the liver was found to increase only when heliotrope was given with copper. The depletion of glutathion in selenium-deficient liver and kid-

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ney of rats lead to necrosis in these organs, which is associated with evidence of lipid peroxidation (7). Some elements such as Co, Se, Mo, and Cu in liver have been analyzed by flame and electrothermal AAS after extraction of their complexes with APDC into chloroform (8). These analyses have shown the analytical details and instrument parameters for the determination of these four elements in National Institute of Standards and Technology standard reference material (NIST SRM) bovine liver. In another study, it was found that the quantitative distribution of trace elements (P, Fe, Zn, Cu, Mn, Mo, Co) in the liver lobes of cattle and of pigs was not uniform, as a result of different metabolism in different parts of the organ (9).

Since many elements important in the biological life of the body deposit in the liver, their concentrations have to be determined very accurately. Determination of trace elements in biological samples is usually difficult because of long and tedious digestion procedures and risks of interference problems. The interfering ions have to be separated by preconcentration techniques such as solvent extraction, ion exchange, or hydride generation. These are all time-consuming procedures, and losses of elements are also possible. It is therefore very important to accomplish the determination with minimum interference problems. With electrochemical methods, the interference problems can be solved by changing only either the supporting electrolyte or the pH (10, 11). These methods require relatively inexpensive instrumentation, are capable of determining elements accurately at trace and ultra-trace levels, and have demonstrated the ability for multi-element determination.

This paper describes a simple polarographic method for the determination of trace elements in liver. In this proposed method there is no need for sophisticated instruments or tedious separation procedures.

Experimental

Materials

A polarographic analyzer (PAR 174 A) equipped with a PAR mercury drop timer was used. The drop time of the electrode was in the range 2 to 3 s (2.37 mg s^{-1}). A Kalusek electrolytic cell with a reference saturated calomel electrode (SCE), separated by a liquid junction, was used in the three-electrode configuration. The counter electrode was platinum wire. The polarograms were recorded with a Linseis (LY 1600) X-Y recorder under the conditions of a drop life of 1 s, a scan rate of 5 mVs^{-1}, and a pulse amplitude of 50 mV.

Reagents

All reagents used were of analytical reagent grade (pro-analysis). Triply-distilled water was used in the preparation of all solutions and at all stages of analysis. The mercury used in the dropping mercury electrode was obtained from Merck (Darmstadt, Germany). Contaminated mercury was cleaned by passing it successively through dilute HNO_3 and water columns, in the form of fine droplets, using a platinum wire gauze. The collected mercury was dried between sheets of filter paper. A polarogram of this mercury was taken before use to ensure the absence of impurities.

Stock standard solutions (0.1 M) of Pb, Cu, Cd, and Zn were prepared with triply distilled water from their nitrate and sulphate salts. Selenite stock solution was prepared from SeO_2 by dissolving it in hot water. The stock solution of molybdenum was prepared from $(NH_4)_2Mo_7O_{24}\cdot 4H_2O$. Dilute solutions were prepared before every use to avoid solution degradation.

A 1.0 M acetic acid buffer, pH 4, was prepared as follows: a 1.0 M NaOH solution was added to 1.0 M acetic acid until the chosen pH was reached, according to a pH meter.

Methods

Digestion of the liver sample

According to former studies (9), the deposition of trace elements vary in different sections of the liver. Thus two different sections (S_1 and S_2) were taken as two separate samples from one cow liver. They were dried for 48 h in an oven at 120°C to remove the water content and to obtain a constant weight. The water content was found to be 72.2%. Samples of dried liver (3.02 g (S_1) and 2.90g (S_2)) were transferred into a 100 ml long-necked glass flask. For the digestion of the sample, a concentrated acid mixture of 0.5 ml H_2SO_4, 5 ml $HClO_4$, and 5 ml HNO_3 was added and left to stand overnight. A glass funnel was inserted into the digestion flask to prevent rapid evaporation. The solution was kept in an oil bath at 50°C until the foaming stopped. Then the temperature was increased to 150°C and heating was continued until the evolution of brown fumes of nitrogen oxides ceased. When the digestive sample turned yellowish to deep dark brown, there was a danger of explosion, so 5 ml of nitric acid had to be added; the flask was cooled for about 2 min before addition. Heating was continued until nitrogen oxide fumes were no longer given off. The digestion was completed, with the appearance of white fumes of perchloric acid, when approximately 1.0 ml solution remained. Finally, 2.0 ml of hydrochloric acid was added and heated for at least 20 min to convert all selenium to selenium(IV). The final solution was evaporated to approximately 1.0 mL and cooled to room temperature. The funnel was rinsed with water into the flask and the contents transferred into a 10.0 ml teflon flask, which was made up to the mark with triply-distilled water. Depending on the concentration of each element in the liver, the digests had to be diluted 10, 100, or 500 times before use.

To check the recovery of the elements during digestion, the same digestion procedure was applied to a synthetic sample containing the same elements. The recovery efficiencies were found to be between 95 and 97%.

Voltammetric determination

For the determination of lead, cadmium, and zinc in liver, 10.0 mL of a 1.0 M acetic acid – acetate buffer (about pH 4) in a polarographic cell was deoxygenated by a stream of high-purity nitrogen for 5 min. The DP polarogram was recorded by scanning the potential in the negative direction from 0.0 V to –1.5 V at a scan rate of 5 mV s^{-1}. Then, according to the need, 0.05–0.1 mL digested sample of liver was added, and once more the DP polarogram was recorded. Standard additions were made according to the corresponding element. For the determination of copper, EDTA solution was added so that the final concentration was 0.02 M. The selenium content, on the other hand, was determined using the catalytic hydrogen wave. For this purpose, Mo(VI) had

to be added in 50–100 times larger amounts than the concentration of selenite present.

Results and discussion

Preliminary experiments

Various supporting electrolytes including KCl, HCl, HAc–NaOAc, and HAc buffer with and without EDTA were used over a wide range of pH. The peak potential values of elements that may be present in liver such as Fe, Cu, Pb, Cd, Zn, Ti, Mo, and Se were determined in these supporting electrolytes. Acetate buffer was found to be the most suitable electrolyte because of its ability to function at various pH values and thus enable the separability of the peaks.

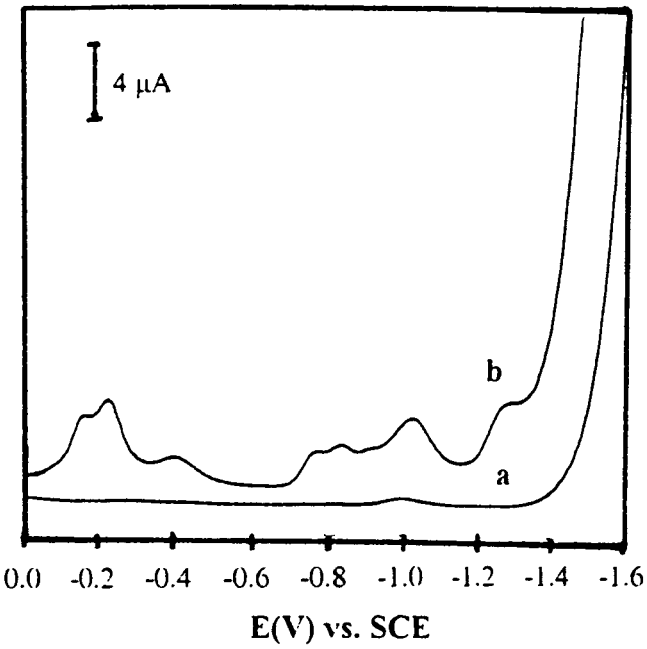
Determination of trace elements in liver

The DP polarograms of digested liver samples in acetate buffer at pH 4.2 have peaks at –0.23 V, –0.40 V, –0.60 V, and –0.98 V. Figure 1 is given as an example where copper, lead, selenium, and zinc peaks are observable. The polarogram of the S_1 sample had similar peaks, but the copper peak was off the scale because of its high concentration. By the additions of Pb, Cd, and Zn standard solutions, the peaks at –0.40 V, –0.60 V, and –0.98 V increased, respectively, indicating the presence of these ions, as expected. For the determination of these elements a 10-times dilution was sufficient, but for the determination of copper in the S_1 sample, because of its high concentration (the peak was too large to observe), either a 100-times dilution or a change in current density was required. On the other hand, the solution either had to be diluted or the current range had to be adjusted accordingly for selenium, since the very sensitive method of catalytic polarography had been used in that case.

Determination of selenium

In our earlier polarographic work (12) we observed a hydrogen catalytic peak when Se(IV) and Mo(VI) were present together in a solution. These kinds of catalytic currents are obtained with many organic compounds including amines, proteins, thiols, and phosphines. They are also observed with some inorganic substances, for example, complexes of tungsten (VI) with certain carboxylic acids (13). The catalytic peak obtained with Mo(VI) and Se(IV) has been used for the determination of very low concentrations of both ions in blood (14). The detection limit (signal to noise ratio, S/N = 3) of the method for Mo(VI) and for Se(IV) was 1.5×10^{-9} M. For the determination of one of these ions, the second ion concentration had to be about 10^2–10^3 times higher than the other ion that was being investigated. However, at concentrations higher than 10^{-6} M, this ratio may be 1:1. Although it is not possible to see a peak for selenite at a concentration lower than 10^{-6} M with DPP, by the addition of Mo(VI) a peak at –1.1 V becomes observable, and by standard additions of selenite the amount of it can be determined. The DP polarogram of a 500-times diluted liver sample in acetate buffer, pH 4, is given in Fig. 2(*a*). The sample had to be diluted 500 times since at lower dilution the catalytic peak was off the scale. As can be seen in Fig. 2 there was no peak for selenium, but by the addition of Mo(VI) a hydrogen catalytic peak at about –1.1 V appeared. At first with further additions of molybdenum it increased, but then it became

Fig. 1. DP polarogram of liver sample S_2: 10.0 mL of a 1.0 M HAc–Ac buffer (pH = 4.2) and 0.1 mL liver sample (10 times diluted).



constant. At that point standard additions of selenite were made for the determination of selenium (Fig. 2(*b*)). Because of overlapping peaks, zinc usually interferes with the hydrogen catalytic peak; thus, EDTA addition is needed. However, although zinc was present in this sample, because of the 500-times dilution for selenite determination no peak corresponding to zinc was visible. The Se(IV) content for six different samples were determined with the above method, and the following concentrations were found: 2.05 ± 0.22 mg g^{-1} Se in the S_1 sample and 2.08 ± 0.21 mg g^{-1} Se in the S_2 sample.

Determination of copper

According to our preliminary experiments, copper and iron peaks may overlap in acetate buffer with pH about 4. Addition of EDTA, however, enabled their separation: the copper peak was found at –0.23 V and the iron peak at –0.13 V. In the liver sample that was diluted only ten times, the polarogram had a very large copper peak at –0.23 V; thus, the sample solution had to be diluted 100 times. The polarogram for this liver sample, S_1, is given in Fig. 3. As can be seen from the figure, the iron peak, which may appear at –0.13 V, was not observable because of low sensitivity in this medium. According to our previous studies, the current obtained for iron in this medium was ten times smaller than the one that was observed for copper for the same concentration. The copper content was determined with standard additions and was found to be 8.12 ± 0.21 mg g^{-1} in the S_1 sample and 0.48 ± 0.02 mg g^{-1} in the S_2 sample.

Determination of zinc, cadmium, and lead

As mentioned earlier, the polarogram (Fig. 2) of the liver sample had peaks belonging to lead, cadmium, and zinc. Standard addition was used for the determination of their concentrations. The polarograms are given in Figs. 4, 5, and

Fig. 2. Determination of selenium in liver. Panel (*a*): Formation of the hydrogen catalytic peak: (*a*) 10.0 mL of a 1.0 M Hac–Ac buffer, pH = 4.3, (*b*) a + 0.1 mL S_1 sample (500 times diluted), (*c*) b + 0.2 mL of a 10^{-3} M solution of Mo(VI), (*d*) c + 0.1 mL of a 10^{-3} M solution of Mo(VI), (*e*) d + 0.05 mL of a 10^{-3} M solution of Mo(VI); Panel (*b*): Standard additions of Se (IV): (*f*) e, taken once more, (*g*) f + 0.1 mL of a 10^{-4} M solution of Se(IV), (*h*) g + 0.05 mL of a 10^{-4} M solution of Se(IV), and (*i*) h + 0.05 mL of a 10^{-4} M solution of Se(IV).

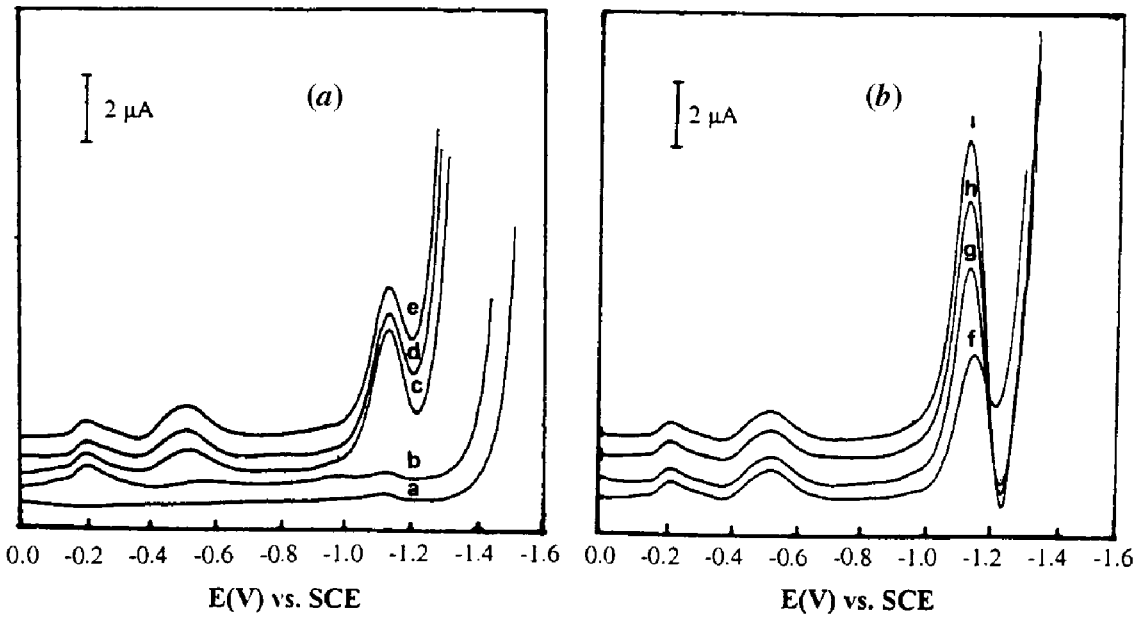


Fig. 3. Determination of copper in liver: (*a*) 2.0 mL of a 1.0 M solution of HAc–Ac buffer + 5.0 mL of a 0.1 M solution of EDTA + 3.0 mL water, pH = 4.3; (*b*) a + 0.1 mL of the S_1 sample (100 times diluted); (*c*) b + 0.3 mL of a 10^{-3} M solution of Cu^{2+}; (*d*) c + 0.3 mL of a 10^{-3} M solution of Cu^{2+}; and (*e*) d + 0.3 mL of a 10^{-3} M solution of Cu^{2+}.

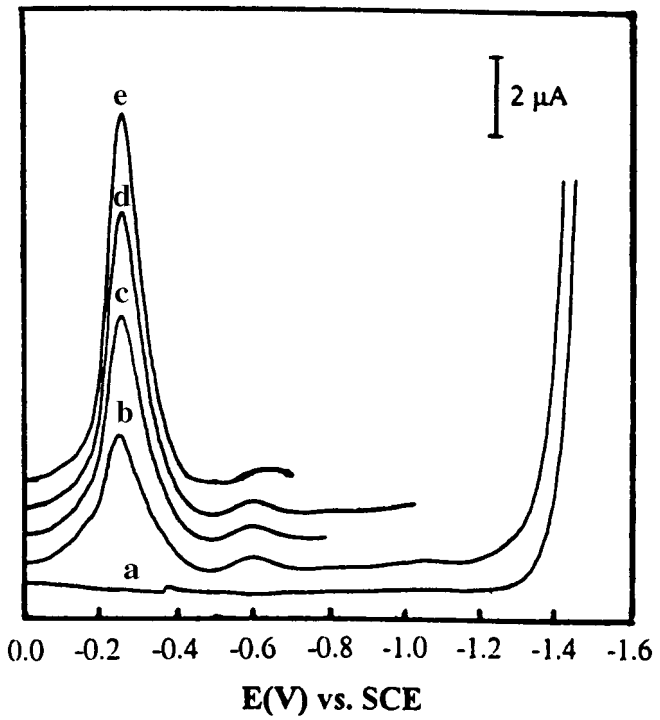
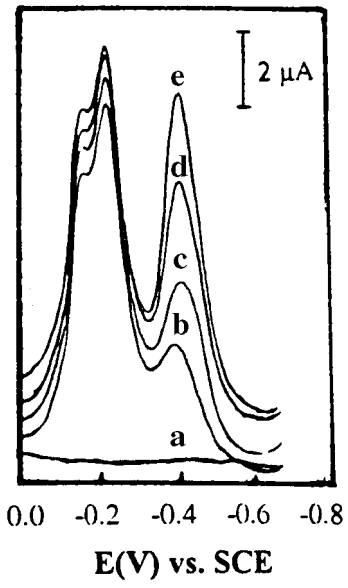


Fig. 4. Determination of lead in liver: (*a*) 10 mL of a 1.0 M HAc–Ac buffer, pH = 4.3; (*b*) a + 0.1 mL of the S_2 sample (10 times diluted); (*c*) b + 0.05 mL of a 10^{-3} M solution of Pb^{2+}; (*d*) c + 0.05 mL of a 10^{-3} M solution of Pb^{2+}; and (*e*) d + 0.05 mL of a 10^{-3} M solution of Pb^{2+}.



6. In the S_1 sample, lead, cadmium, and zinc concentrations were found to be 0.59 ± 0.04 mg g^{-1}, 1.09 ± 0.11 mg g^{-1}, and 1.16 ± 0.12 mg g^{-1}, respectively. The S_1 sample was also analyzed by flame AAS for zinc content, and it was found to be 1.01 ± 0.24 mg g^{-1}, which was consistent with the result obtained with polarography. In the S_2 sample, however, while no detectable amount of cadmium was observed, lead content was found to be 0.22 ± 0.03 mg g^{-1}, and zinc content was found to be 0.29 ± 0.03 mg g^{-1}. These results are summarized in Table 1. The trace element quanti-

Table 1. Trace elements in dry liver, mg g^{-1} ($x \pm (t\text{xs})/\sqrt{N}$).

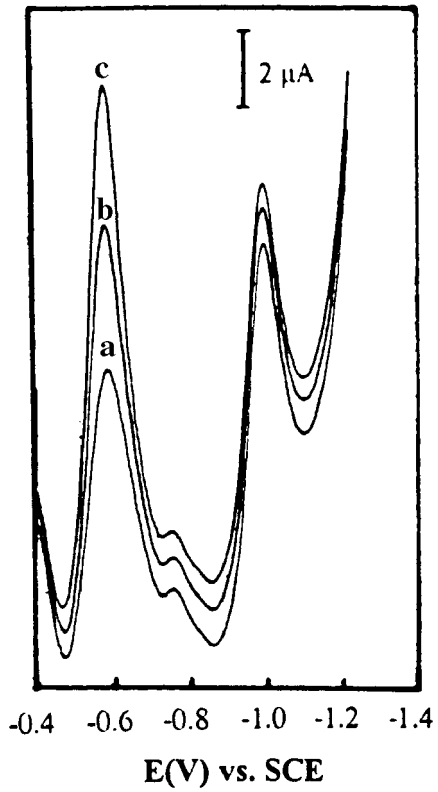
Sample	Cu(II)	Pb(II)	Cd(II)	Zn(II)	Se (IV)
S_1	8.12 ± 0.21	0.59 ± 0.14	1.09 ± 0.11	1.16 ± 0.12	2.05 ± 0.22
S_2	0.48 ± 0.21	0.22 ± 0.03	—	0.29 ± 0.03	2.08 ± 0.21

Note: t = confidence interval (90%); (N = 6).

Table 2. Determination of trace elements in a synthetic sample.

Elements	Cu ($M \times 10^{-6}$)	Pb ($M \times 10^{-7}$)	Cd ($M \times 10^{-7}$)	Zn ($M \times 10^{-7}$)	Se ($M \times 10^{-7}$)
Present	1.00	1.00	1.00	1.00	1.00
Found	1.07 ± 0.09	1.00 ± 0.11	0.57 ± 0.12	1.21 ± 0.11	1.05 ± 0.09

Fig. 5. Determination of cadmium in liver: (*a*) 10.0 mL of a 1 M HAc–Ac buffer, pH 4.3 + 0.1 mL of the S_1 sample (10 times diluted); (*b*) a + 0.1 mL of a 10^{-3} M solution of Cd^{+2}; and (*c*) b + 0.1 mL of a 10^{-3} M solution of Cd^{+2}.

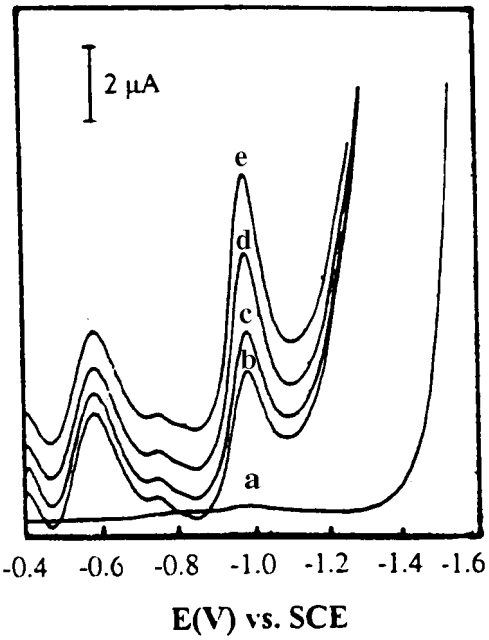


ties found in different sections of liver proves that their distribution is not uniform, as mentioned (9) earlier for cattle and pigs.

Proposed procedure

About 1–2 g of dried liver was digested and then diluted to 10 or 100 mL. An aliquot (0.1 ml) was added to an acetate buffer (pH 4) solution, and the DP polarogram was taken. Lead, cadmium, and zinc were determined by standard additions. Then EDTA solution was added, so that its final concentration was 0.05 M, and copper content was determined. For selenite, Mo (VI) was added to obtain the hydrogen catalytic peak, and the selenite content was determined by standard additions of selenite.

Fig. 6. Determination of zinc in liver: (*a*) 10.0 mL HAc–Ac, pH = 4.3; (*b*) a + 0.1 mL of the S_1 sample; (*c*) b + 0.1 mL of a 10^{-3} M solution of Zn^{2+}; (*d*) c + 0.2 mL of a 10^{-3} M solution of Zn^{2+}; and (*e*) d + 0.2 mL of a 10^{-3} M solution of Zn^{2+}.



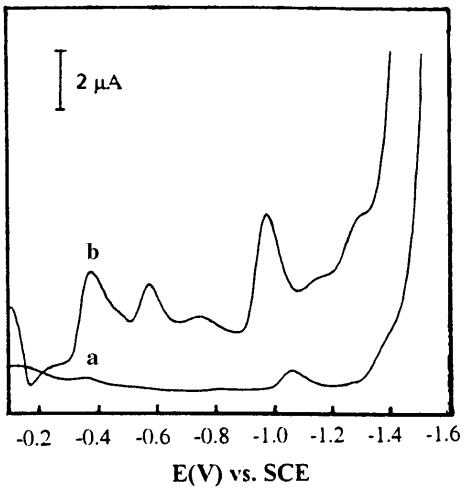
Synthetic sample

To check the validity of the suggested method, a synthetic sample containing similar amounts of metal ions present in liver was used. The polarogram of the metal ions in acetate buffer at pH 4 is shown in Fig. 7. The elements present were determined with the proposed method. The results are given in Table 2 with their standard deviations. As can be seen, a good consistency was observed for most of the ions, except cadmium. Only 55% of the quantity of cadmium could be determined. This can be explained by the interference of selenium with cadmium (15), which was further investigated by us.

Conclusions

The present DP polarographic method, which was used for the first time for the determination of heavy elements in liver, enabled the direct determination of their concentration without any separation or pre-concentration techniques.

Fig. 7. DP polarogram of a synthetic sample: (*a*) 9.5 mL of a 1.0 M HAc–Ac buffer; (*b*) 0.1 ml of a 10^{-2} M solution of Cu^{2+}; 0.1 ml of a 10^{-3} M solution of Pb^{2+}; 0.1 ml of a 10^{-3} M solution of Cd^{2+}; 0.1 ml of a 10^{-3} M solution of Se(IV); 0.1 ml of a 10^{-2} M solution of Zn^{2+}.



Electroanalytical methods are quite simple, their sensitivity and selectivity are high, and the instruments used are inexpensive. By digestion of about 1–2 g of sample, dilution to 10–100 ml, and by using 0.1 ml aliquots it is possible to determine the concentration of at least five to six elements simultaneously. This method can be applied to other biological samples containing complex mixtures of metal ions.

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