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Interference elimination studies during the determination of trace elements in cow liver using differential pulse polarography

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The liver is known to accumulate many trace elements; thus it is very important to determine their level in a sensitive way. The objective of this study was to develop a simple method for the simultaneous determination of trace elements in cow liver in which the elimination of interference can be accomplished. It was observed that Cd, Pb, Zn, and Cu ions could not be determined correctly in the presence of Se(IV), because of interference of Se. It was shown that this interference could be eliminated by using pH 8.5 NaAc electrolyte. In this medium first Cd and Zn were determined; then, after addition of EDTA, Pb and Cu ions were determined by standard addition. Since Ti(IV), Mo(VI), Cr(III), and Fe(III) had no interference with Se(IV) they could be determined at appropriate electrolyte conditions. Selenite, on the other hand, was determined using its hydrogen catalytic peak. The results obtained under 2 conditions, where interference was eliminated and not eliminated, were compared. It was found that the quantities for Cu, Cd, Pb, and Zn were larger in medium where interference does not take place.

Key Words: Liver; trace elements; determination; polarographic.

Introduction

Trace elements play a vital role in the body (and is very rich and varied). While some of them are essential to life others are toxic even at very low concentrations. Since these elements are obtained mostly from the human diet, the determination of their concentration is very important. The liver is known to accumulate many trace elements; thus, cow liver is a potent source of heavy elements.

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The consumption of liver may partially supply human nutritional requirements for essential elements such as Fe, Cu, Co, Se, and Zn. However, several metals, such as Pb, As, and Cd are known to be potentially toxic. Lead acts as a metabolic poison and accumulates in bones, replacing calcium. Cadmium on the other hand accumulates in the liver and kidney. Since many elements important in the biological life of the body accumulate in the liver, their concentrations have to be determined very accurately.

Some elements such as Co, Se, Mo, and Cu in the liver have been analyzed by flame and electrothermal AAS after extraction of their complexes with APDC into chloroform.[1] In another study, it was found that the quantitative distribution of trace elements (P, Fe, Zn, Cu, Mn, Mo, and Co) in the liver lobes of cattle and pigs was not uniform, as a result of different metabolisms in different parts of the organ.[2] The trace elements in human liver biopsy samples were determined using ICP-MS and TXRF techniques.[3] Porcine liver, on the other hand, was analyzed for its trace element content using online HPLC-ESI-MS after extraction and chromatographic separation.[4] For the trace rare earth elements in pig liver ICP-AES has been used after on-line separation and pre-concentration.[5] Using DPP the quantities of trace elements in cow liver were determined.[6]

Determination of trace elements in biological materials is usually difficult because of long and tedious pre-concentration techniques such as ion exchange, solvent extraction, or hydride generation to lower the risks of interference problems. These are all time-consuming procedures and losses of elements are also possible.

With electrochemical methods, the interference problems can be solved by changing only either the supporting electrolyte or the pH.[7,8] These methods also have the advantage that they require relatively inexpensive instruments, are capable of determining elements accurately at trace and ultra-trace levels,[9] and have demonstrated an ability for multi-element determination.

Using cathodic stripping voltammetry the selenium content in garlic,[10] by differential pulse stripping voltammetry the selenium and lead contents in milk,[7] and by differential pulse polarography (DPP)[11] the selenium and lead present in blood were determined.

In our selenium determination studies in the presence of some ions using polarographic or voltammetric techniques, we found strong interference. During anodic stripping voltammetric (ASV) studies,[12] in the presence of both copper and selenite ions, a new peak appeared, which was due to the reduction of a CuSe intermetallic compound. This intermetallic compound formation was confirmed in cyclic voltammetric (CV) studies.[13] Similar behavior was reported to occur[14] between selenium and copper during the anodic and cathodic stripping voltammetric determination of selenium. In our differential pulse polarographic (DPP) studies we observed that selenite and some ions, such as cadmium, lead, copper, and zinc diminished the peaks of each other, and new peak formations at more positive potentials for the corresponding ions appeared. This observation was attributed to the formation of an intermetallic compound (Somer mechanism),[15] between selenium and the ions present. It was shown that corrections had to be made during quantitative determination of these ions by taking into account these interferences. However, our further studies have shown that this interference can be used for the trace determination of tin.[8] We investigated also the pH dependence of the interference between selenite and some ions and the optimum conditions for the elimination of interference was proposed.[16] Under these conditions it was possible to determine copper, cadmium, lead, zinc, and selenite (each 10^{-5} M) in the presence of 50 to 100 times of selenite with great accuracy.

The purpose of this investigation was to establish a simple polarographic method for the determination of as many trace elements as possible in cow liver in which the elimination of interference can be accomplished.

According to our previous work,[15,16] while some of the ions present in liver will form intermetallic compound some will not. In this work the ions present in cow liver were determined under 2 different conditions. In the first one the interference with Se was eliminated and in the second one the interference was not eliminated. Thus, the results obtained under 2 conditions were compared and discussed. In this proposed method there is no need for sophisticated instruments or tedious separation procedures.

Experimental

Apparatus

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

Reagents

All chemicals used were of analytical reagent grade (Merck, Darmstadt, Germany) and triply distilled water was used in the preparation of their solutions and at all stages of analysis. The reagents used were Na_2SeO_3, $(NH_4)_2Mo_7O_{24}$. $4H_2O$, $CuSO_4.2H_2O$, $Zn(NO_3)_2$, $Na_2H_2Y.2H_2O$, $Ti(Cl)_4$, $Cr(NO_3)_3$, CH_3COONa, and various acids. Solutions of 10^{-3} M and more dilute ones were prepared before every use in order to avoid the aging process of solution.

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 (3.0 M) and water columns in the form of fine droplets by using a platinum sieve. The collected mercury was dried between sheets of filter paper. Before use, a DPP polarogram of this mercury was recorded in order to confirm the absence of impurities.

Procedure

Digestion of samples

Cow liver was first cut into fine pieces and then dried for 48 h in an oven at 80 °C to remove the water content and obtain a constant weight. Two different samples of about 3 g (S_1 = 3.03 g, S_2 = 2.75 g) from dry liver were transferred into a 50 mL long-necked glass flask. For each sample, 10.5 mL of acid mixture (5 mL of HNO_3; 5 mL of $HClO_4$, and 0.5 mL of H_2SO_4) were added. First 10.5 mL of this acid mixture was added and left overnight with a glass funnel covering the mouth of the flask. The next day the flask was heated over a flame by turning the flask until nitrogen oxide fumes were completely given off. When the digestive sample turned yellowish to deep dark brown there was a danger of explosion, and so about 5 mL of HNO_3 and 5 mL of $HClO_4$ had to be added, followed by cooling of the flask for about 2 min before addition. The sample became clear

and it was evaporated until about 1 mL of solution remained. After cooling 2 mL of HCl was added and it was heated to convert all selenium to selenium(IV) and then evaporated to near dryness. The digested sample was cooled to room temperature, the funnel was rinsed with water into the flask, and the contents were transferred into a 10.0 mL Teflon flask, which was made up to the mark with triply distilled water. This solution was later diluted before its addition to the polarographic cell.

Polarographic determination

In a general procedure a total of 10.0 mL of acetic acid-acetate buffer (1.0 M) in the polarographic cell was de-aerated by stream of nitrogen gas (99.999%) for about 5 min. Polarograms were taken by scanning the potential from 0.0 to –2.0 V at a scan rate of 5 mV s^{-1}. If addition of EDTA was needed then 8.0 mL of buffer and 2.0 mL of 0.1 M EDTA were used as the electrolyte. The peak potentials of Se(IV), Fe(III), Cu(II), Ti(IV), Cr(III), Pb(II), Zn(II), Cd(II), and Mo(VI), which are commonly found in cow liver, were determined at different pH values, i.e. 2, 4, 5, and 6, in acetate buffer and in the presence and absence of EDTA. The pH of the digested sample was adjusted to the desired value before addition to the polarographic cell.

Two kinds of determination were carried out. In the first one, trace elements were determined in a medium where the interference effect was not eliminated. In the second one determinations were made in a medium (pH 8.5 with or without EDTA) where interference was eliminated. The medium is given for each element in the Results and Discussion section.

The polarogram of the digested sample was taken under various conditions and the trace elements in the sample were determined by standard additions.

Results and discussion

Preliminary experiments

The peak potentials of several elements that may be found in cow liver such as iron, copper, lead, cadmium, selenite, chromium, and zinc were determined in various supporting electrolytes. For this purpose HCl and acetate buffer over a wide range of pH values in the presence or absence of EDTA were studied. 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. The determination of trace elements was performed under 2 different conditions. In the first determinations were carried out where the interference of ions was not considered. In the second the pH was held at pH 8.5 in order to eliminate the interference.

Determination of trace elements in liver

Polarograms of digested cow liver sample were taken in acetate electrolyte at various pH values and in the presence and absence of EDTA. Thus, the presence of elements could be validated by determining their quantities under different conditions.

As an example the polarogram of digested cow liver sample taken at pH 2 with acetate electrolyte and EDTA is given in Figure 1. The DP polarograms had peaks at –0.1 V, –0.36 V, –0.71 V, –0.85 V, –1.1 V, and

–1.2 V. According to our preliminary studies the peak at about –0.1 V may belong to Fe(III) or Cu(II) or both, since their peak potentials overlap in this medium, the peak at –0.36 V may belong to Mo-EDTA, the peak at –0.71 V to Pb-EDTA, the peak at –0.85 V to Cd-EDTA, the peak at –1.2 V to Cr-EDTA, and the peak at –1.1 V to As-EDTA. Their presence was confirmed by standard additions and by the polarograms taken under different conditions.

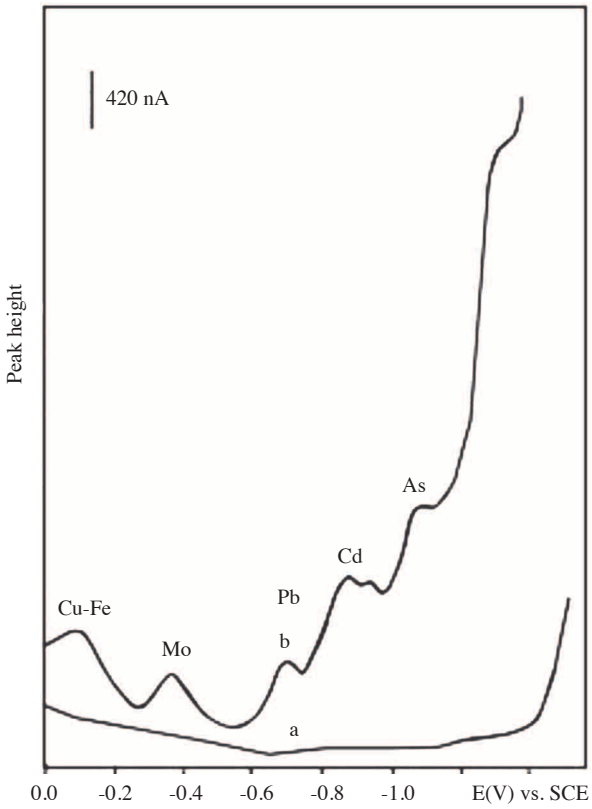

Figure 1. DPP polarogram of digested cow liver sample, (a) 10 mL 0.1 M acetate + 1.0 mL 0,1 M EDTA, (pH 2), (b) curve a + 0.1 mL digested liver sample.

The polarogram of the liver sample taken at pH 4 in acetate buffer and EDTA had peaks at –0.3 V, –0.65 V, –0.75 V, and –0.9 V. According to our previous work, the peak at –0.3 V may belong to Ti(IV), the peak at –0.75 V to Pb, and the peak at –0.9 V to Cd.

The polarogram of the liver sample taken at pH 4 in acetate buffer had peaks at –0.4 V, –0.6 V, –0.78 V, –1.0 V, and –1.25 V; the peak at –0.4 V belongs to Pb, the peak at –0.6 V to Cd, and the peak at –1.0 V to Zn. The quantities of Cd, Pb, and Zn were determined by standard additions in this medium.

Each ion in the liver was determined from its peak where it had a sharp peak and where it was best separated from the next peak. The quantities found in different electrolytes for each ion were compared so that the results for each ion could be confirmed.

According to our previous work intermetallic compound formation takes place between selenite and Cu, Cd, Pb, and Zn.[15] Thus, in the presence of selenite the results obtained may be plausible. It was found

that this interference could be eliminated by using pH 8.5 acetate electrolyte.[16] Since it is known that liver contains high quantities of selenite, the above mentioned elements have to be determined by taking account this interference.[6,19] The elements such as Cr(III), Fe, Mo(VI), and Ti(IV) that do not form intermetallic compounds with selenite can be determined in appropriate electrolyte conditions.

In this work the elements present in cow liver were determined under various conditions where the interference was eliminated and not eliminated, so that a comparison could be made.

Determination of lead and molybdenum

For the determination of lead the digested liver sample was first warmed up so that the precipitated $PbCl_2$ could be dissolved. An aliquot from the cooled sample was taken and added to pH 2 acetate electrolyte in the presence of EDTA. From its peak at –0.71 V lead content could be determined. The result found was 290 ± 10 μg g^{-1} (Table 1). Molybdenum found in the same medium from its peak at –0.36 V was 130 ± 10 μg g^{-1} (Table 2). The same cow liver was analyzed at pH 8.5 for lead, so that interference between selenite and lead would not take place. Since in the presence of EDTA the peak could be better separated **a** polarogram of the liver sample was taken with EDTA. The result found was 520 ± 20 μg g^{-1} for lead (Figure 2) (Table 3).

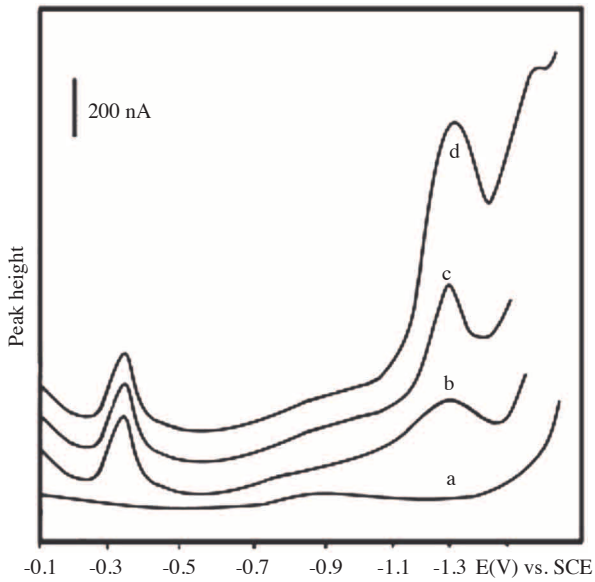

Figure 2. Determination of lead in cow liver, pH 8.5 acetate (inhibited interference of Se), (a) 9.0 mL 0.1 M NaAc + 0.1 mL liver sample, (pH 8.5), (b) curve a + 0.2 mL 0.1 M EDTA, (c) curve b + 0.1 mL 1 × 10^{-3} M Pb(II), (d) curve c + 0.1 mL 1 × 10^{-3} M Pb(II).

Determination of cadmium and titanium

Cadmium and titanium were separated best in EDTA at pH 4 acetate buffer from the elements present in liver. Cadmium was determined from its peak at –0.9 V and titanium from the peak at –0.3 V (Figure 3). Cd content in the presence of interference with selenite was 175 ± 15 μg g^{-1} (Table 1). On the other hand, Ti

was determined in the same medium without any interference and the result was 160 ± 10 μg g^{-1} (Table 2). The same digested liver sample was analyzed in acetate electrolyte at pH 8.5 where no interference for Cd was taking place. Cadmium content was found as 290 ± 10 μg g^{-1} from its peak at –0.6 V (Figure 4), as expected it was larger in this medium where interference of selenite was eliminated (Table 3).

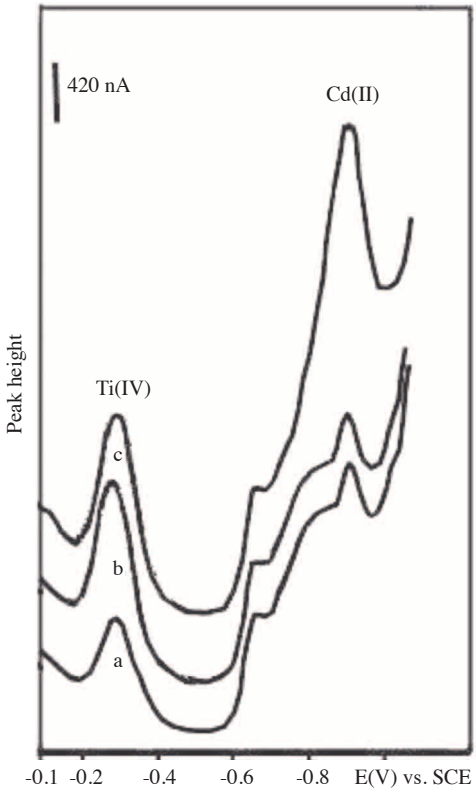

Figure 3. Determination of Cd(II) and Ti(IV) in cow liver, (a) 10 mL 0.1 M HAc-NaAc + 1.0 mL 0.1 M EDTA + 0.1 mL liver sample, (pH 4), (b) curve a + 0.1 mL 1 × 10^{-3} M Ti(IV), (c) curve b + 0.1 mL 1 × 10^{-3} M Cd(II), (b) curve a + 0.1 mL 1 × 10^{-3} M Cd(II).

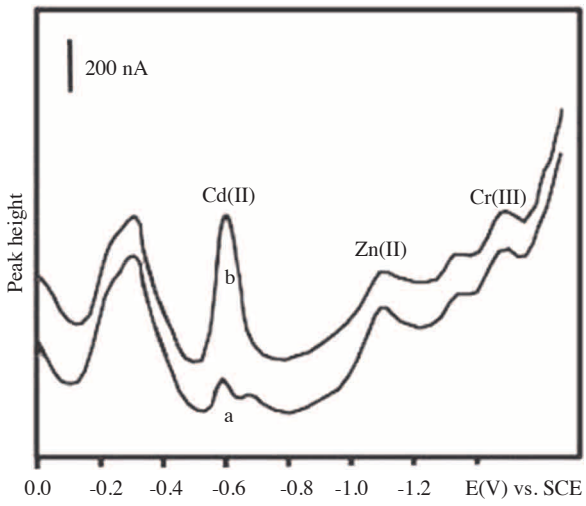

Figure 4. Determination of Cd in cow liver pH 8.5 acetate (inhibited interference of Se), (a) 7.0 mL 0.1 M NaAc + 0.1 mL liver sample, (pH 8.5), (b) curve a + 0.1 mL 1 × 10^{-3} M Cd(II).

Determination of chromium and copper

Both of these ions were determined in pH 6 acetate buffer and EDTA. Chromium(III) had a peak at –1.25 V and its quantity found was 170 μg g^{-1} (Table 2); the copper peak (Figure 5) was at –0.37 V and its quantity was 160 ± 15 μg g^{-1} (Table 1). As known, Cu will form an intermetallic compound with selenite in this medium although it can be well separated from iron. Thus, a polarogram of the same liver sample was taken in acetate electrolyte at pH 8.5 in the presence of EDTA (Figure 6). Copper quantity was determined from its peak at –0.37 V as 170 ± 15 μg g^{-1} (Table 3). As can be seen there is not much difference between the quantities obtained in the 2 media. The reason is that the intermetallic compound of copper is not as strong as it is for Cd, Zn, and Pb.[16]

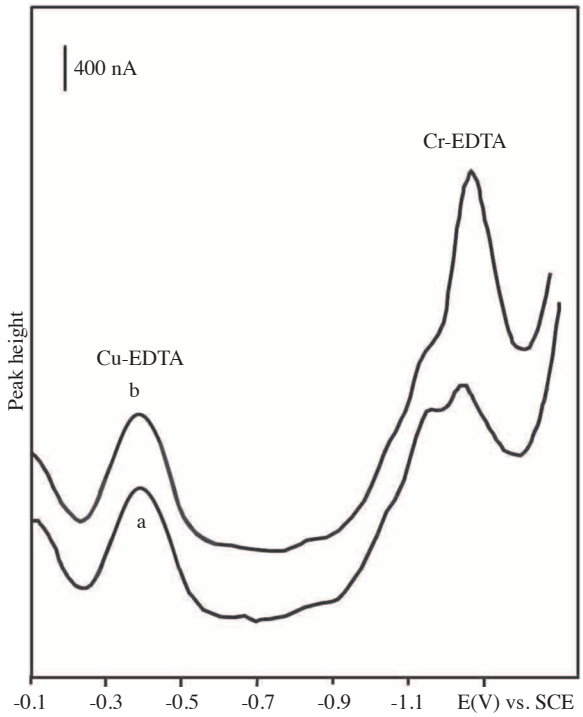

Figure 5. Determination of Cr(III) in cow liver sample, (a) 10 mL 0.1 M NaAc + 1 mL 0.1 M EDTA + 0.1 mL liver sample, (pH 6.0), (b) curve a + 0.1 mL 1 × 10^{-3} M Cr(III).

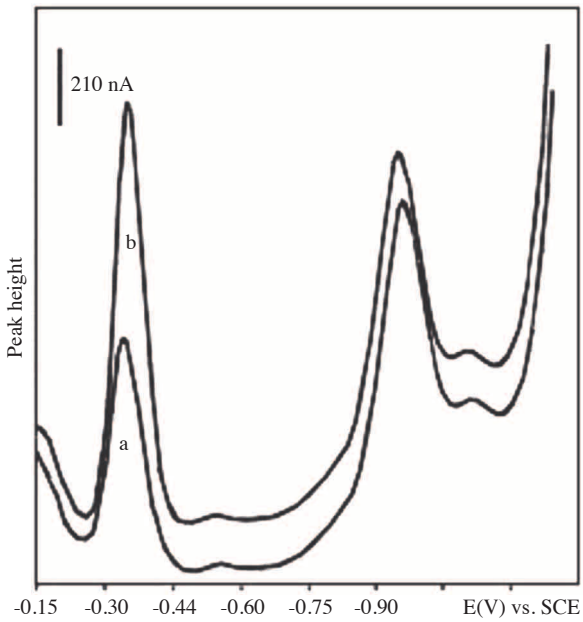

Figure 6. Determination of Cu in cow liver pH 8.5 (inhibited interference of Se), (a) 10.0 mL 0.1 M NaAc (pH 8.5) + 1.0 mL liver sample + 1.0 mL 0.1 M EDTA, (b) curve a + 0.1 mL 1 × 10^{-3} M Cu(II).

Determination of iron

According to the preliminary experiments, iron and copper peaks may overlap at lower pH values at about –0.1 V. Addition of EDTA at pH 5 enabled their separation: the copper peak was at about –0.32 and iron peak was at about –0.1 V (Figure 7). Iron content found from this peak was 210 ± 10 μg g^{-1} (Table 2).

Determination of zinc

Zinc was determined in acetate buffer at pH 4 from its peak at about –1.0 V (Figure 8). The quantity found was 200 ± 10 μg g^{-1} (Table 1). Because of interference between selenite and zinc in this medium the same cow liver was analyzed in acetate electrolyte at pH 8.5 where the interference was eliminated. The quantity found using the peak at about –1.0 V was 320 ± 10 μg g^{-1} (Table 3). As can be seen, because of the interference at pH 4, the quantity found was smaller.

Determination of selenium

In acidic medium a hydrogen catalytic peak appeared at about –1.1 V when Se(IV) and Mo(VI) were present together in a solution.[9] We used this peak for the determination of very low concentrations of both ions in blood.[17] The detection limit for these ions was about 1.5 × 10^{-9} M. For the determination of one of these

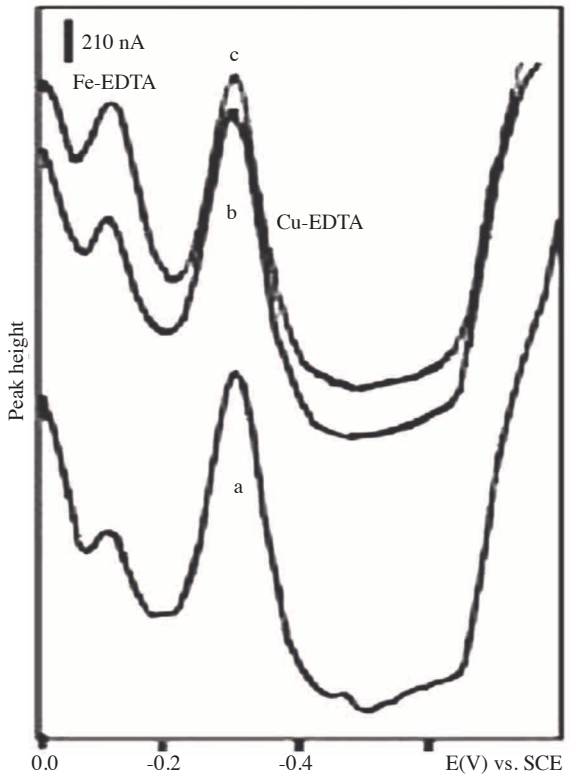

Figure 7. Determination of Fe(III) in cow liver pH 5 HAc-NaAc buffer, (a) 8.0 mL HAc-NaAc (pH 5) + 0.1 mL liver sample + 1.5 mL 0.1 M EDTA, (b) curve a + 0.1 mL 1×10^{-3} M Fe(III), (c) curve b + 0.1 mL 1×10^{-3} M Fe(III).

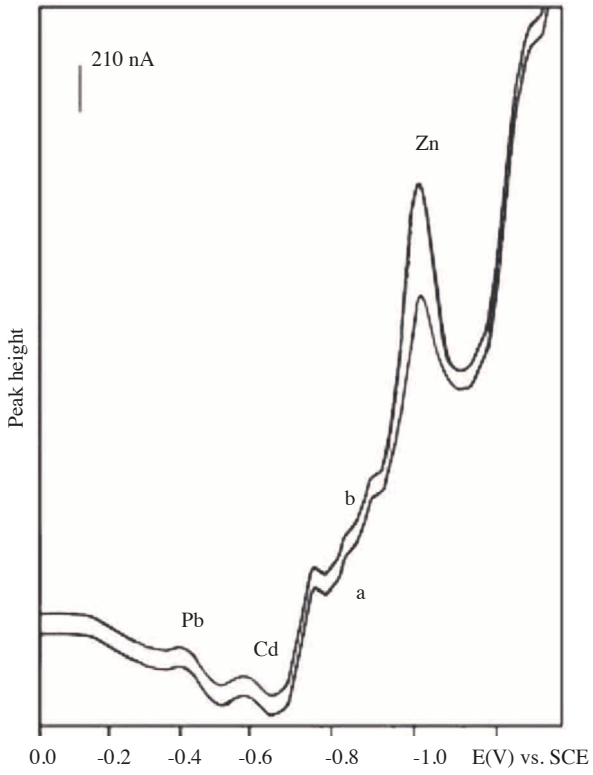

Figure 8. Determination of Zn in cow liver using DPP, (a)10 mL HAc-NaAc + 0.5 mL cow liver sample, (pH 4), (b) curve a + 0.1 mL 1×10^{-3} M Zn(II).

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 observe 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 addition of selenite the quantity of it can be determined. In this work a 0.1 mL sample solution from the digested and 100 times diluted liver sample was taken and added to 10 mL acetate buffer at pH 3.3 and a DP polarogram was taken (Figure 9). The sample had to be diluted 100 times since at lower dilution the catalytic peak was off the scale. As can be seen there was no peak for selenite at about –0.57 V, but by the addition of 2×10^{-5} M Mo(VI) a hydrogen catalytic peak appeared at about –1.1 V. This peak increased by standard additions of 3×10^{-6} M selenite, as long as Mo(VI) was present in the solution. The content of selenite found was 1800 ± 40 μg g^{-1} (Table 2). As seen, selenite content was quite high, the reason may be that Se is being used in food for prevention of white muscle disease.[18] A similar quantity of Se was also observed in cow[6] and chicken liver samples,[19] and it was attributed to food composition.

As can be seen, the selenite content in liver is high and therefore the interference between copper, lead, cadmium, and zinc has to be considered.

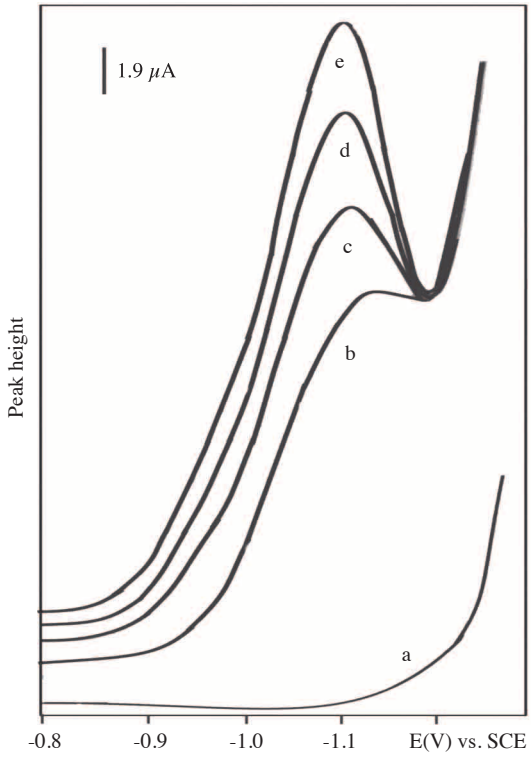

Figure 9. Determination of selenium in cow liver by using catalytic peak, (a) 10.0 mL acetate buffer (pH 3.3) + 0.1 mL 100 times diluted cow liver sample, (b) curve a + 0.2 mL 1×10^{-3} M Mo(VI), (c) curve b + 0.3 mL 1×10^{-4} M Se(IV), (d) curve c + 0.3 mL 1×10^{-4} M Se(IV), (e) curve d + 0.3 mL 1×10^{-4} M Se(IV).

Table 1. Determination of Cu, Cd, Pb, and Zn in cow liver sample (interference with selenite).

Ions	Cd(II)μg g^{-1}	Pb(II) μg g^{-1}	Zn(II) μg g^{-1}	Cu(II) μg g^{-1}
Media	pH 4 HAc-NaAc, EDTA	pH 2 HAc-NaAc, EDTA	pH 4 HAc-NaAc	pH 6 HAc-NaAc, EDTA
Quantity	175 ± 15	290 ± 10	200 ± 10	160 ± 15

Note: N = 4, 90% confidence interval.

Table 2. Determination of Cr, Fe, Se, Mo, and Ti in appropriate electrolyte conditions with no interference from Se.

Ions	Cr(III) μg g^{-1}	Fe(III) μg g^{-1}	Se(IV) μg g^{-1}	Mo(VI) μg g^{-1}	Ti(IV) μg g^{-1}
Media	pH 6 HAc-NaAc, EDTA	pH 5 HAc-NaAc, EDTA	pH 3.3 HAc-NaAc	pH 2 HAc-NaAc, EDTA	pH 4 HAc-NaAc, EDTA
Quantity	170	210 ± 10	1800 ± 40	130 ± 10	160 ± 10

Note: N = 3, 90% confidence interval.

Table 3. Determination of Cd, Zn, Pb, and Cu under conditions with and without interference from selenium.

Ions	Cd(II) μg g^{-1}	Pb(II) μg g^{-1}	Zn(II) μg g^{-1}	Cu(II) μg g^{-1}
Different conditions (with interference)	pH 4 175 ± 15 pH 4	pH 2 290 ± 10 pH 2	pH 4 200 ± 10 pH 4	pH 6 160 ± 15 pH 6
pH 8.5 0.1 M NaAc (without interference)	290 ± 10	520 ± 20	320 ± 30	170 ± 10

Note: N = 4, 90% confidence interval.

Conclusions

Trace elements found in cow liver such as Cu, Cd, Pb, Zn, Se, Ti(IV), Mo(VI), Cr(III), and Fe(III) can be determined with a simple and cheap instrumental technique, DPP, from one digested sample solution without any extraction or pre-concentration. The accuracy of the results was confirmed by measuring the element quantities under different conditions and with a synthetic sample containing similar quantities of ions present in cow liver.

The quantities of trace elements found in cow liver with no interference from Se, such as Cr, Fe, Mo, and Ti, were determined in appropriate electrolyte conditions.

Trace elements found in cow liver such as Cu, Cd, Pb, Zn, and Se exhibited interference with selenite during their DPP determination. This interference could be eliminated by using pH 8.5 medium. These trace elements found in cow liver were determined using DPP in 2 media where interference with Se will occur and will not occur. It was found that the quantities for Cu, Cd, Pb, and Zn were larger in medium where interference does not take place. This method is suitable for the routine analysis of large number of liver samples and it can be applied to other biological samples containing complex mixtures of elements. There is no need for time-consuming extraction and separation procedures with danger of contamination.

References

1. Parsley, D. H. *J.Anal. Atom. Spectrom.* **1991**, *6*, 289-293.
2. Taucins, E.; Svilane, A. *Akad. Nauk Latv. SSR, Inst. Biol.* **1962**, *3*, 159-164.
3. Varga, I.; Szebeni, A.; Szoboszlai, N.; Kovacs, B. *Anal. and Bioanal. Chem.* **2005**, *383*, 476-482.
4. Nischwitz, V.; Michalke, B.; Kettrup, A. *J. Anal. Atom. Spectrum.* **2003**, *18,* 444-451.
5. Zhang, N.; Huang, C.; Hu, B. *Anal. Sci.* **2007**, *23*, 997-1002.
6. Somer, G.; Guliyeva, G.; Ekmekçi, G.; Şendil, O. *Can. J. Chem.* **2003**, *81*, 31-36.
7. Inam, R.; Somer, G. *Food Chem.* **2000**, *69*, 345-350.
8. Ekmekci, G.; Inam, R.; Somer, G. *Anal. Sci.* **2000**, *16*, 1151-55.
9. Inam, R.; Somer, G. *Talanta* **1999**, *50*, 609-616.

10. Inam, R.; Somer, G. *Food Chem.* **1999,** *66*, 381-385.
11. Inam, R.; Somer, G. *Talanta* **1998**, *46,* 1347-1355.
12. Aydın, H.; Somer, G. *Anal. Sci.* **1989**, *5*, 89-93
13. Somer, G.; Karacan, M.S. *Electroanal.* **1994**, *6*, 527-530.
14. Adeloju, S. B.; Bond, A. M.; Briggs, M. H.; Hughes, C.H. *Anal. Chem.* **1983**, *55*, 2076-2082.
15. Inam, R.; Somer, G. *Anal. Sci.* **1998**, 14, 399-404.
16. Somer, G.; Yılmaz, U. T. *Talanta* **2005,** *65,* 598-602.
17. Inam, R.; Ekmekci, G.; Somer, G. *Talanta* **2000**, *51*, 825-830.
18. Somer, G.; Caliskan, A.C. *Turk. J. Chem.* **2007**, *31*, 411-422.
19. Somer, G.; Ekmekçi, G.; Sendil, O. *Turk. J. Chem.* **2003**, *27,* 347-355.