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Simultaneous Determination of Copper, Zinc and Selenium in Chicken Liver by Differential Pulse Polarography

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A differential pulse polarographic method for the determination of copper, zinc and selenium in chicken liver is described. It was possible to determine these elements in a single sample solution after acid digestion. Dried liver samples weighing about 3 g were digested using HNO_3 and $HClO_4$ acids. Britton-Robinson or acetate buffer has been found suitable as the supporting electrolyte. For the determination of selenium, the hydrogen catalytic peak was used after the addition of molybdenum(VI) to the buffer solution at about pH 3. Then the pH of the same solution was increased to 4 and zinc was determined. For the determination of copper, EDTA was added and it was determined from its peak at –0.24 V. The trace element quantities for four different dry liver samples were 15-30 mg/g for copper, 2.2-3.6 mg/g for selenium and 0.4-0.9 mg/g for zinc. The validity of the method has been demonstrated using a synthetic sample resembling the liver in composition; consistency was shown for the quantity of zinc using AAS.

Key Words: Chicken liver; determination; copper; selenium; zinc; differential pulse polarography

Introduction

The roles of some trace and ultra trace elements in the body are so rich and varied that in many instances they are essential to life, while in others they are toxic even at very low concentrations [1–3]. Since these elements are taken mostly from the diet, their determination in food is very important. In terms of nutrition chicken liver is quite important for humans. The liver is known to deposit many trace elements and it is expected that the quantity of the elements in the liver will depend on the chicken's nutrition. Some elements are added to chicken feed to protect the animals from disease. Of these, selenium is of special interest since selenium-deficient feed causes white muscle disease, which results in paralysis.

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The chemistry and concentrations of trace elements in biological material are such that different techniques and methods of analysis[4−6] are usually required for their determination. The determination of trace levels of elements in biological material is usually difficult. Analysis by atomic absorption spectroscopy (AAS) has been shown to be quick and simple. Although there are several references to this method, there are also a number of factors generally overlooked, such as interference, matrix effects and losses through volatilization at high temperatures. The limit of detection is also poor compared to voltammetric methods and thus pre-concentration is usually needed. Parsley[7] proposed a method for the determination of cobalt, copper, selenium and molybdenum in liver using flame and electrothermal AAS. This method has been applied to the elements in the NIST SRM 1577 standard, after complex formation and extraction. A stripping voltammetric technique was used by Bond et al.[8] for the determination of selenium in bovine liver standard reference material (U.S. NBS). However, ion-exchange separation was needed for the interfering ions because of their suppression effect on the selenium peak. A method was described[9] for the determination of cadmium, selenium, copper and lead in bovine liver using differential pulse voltammetry. Selenium IV had to be oxidized by permanganate to avoid its interfering effect on the peaks of the present ions. The excess of permanganate was removed by adding hydrazine sulfate.

Electrochemical methods have the advantage that they require relatively inexpensive instrumentation, are capable of determining elements accurately at trace and ultra-trace levels[10,11] and have demonstrated an ability for multi-element determination[12,13]. Using cathodic stripping voltammetry the selenium content in garlic[10] and by differential pulse stripping voltammetry the selenium and lead contents in milk[11] have been determined. The cadmium, lead and selenium present in *Medicago sativa*[12], and the selenium and lead present in blood were determined by differential pulse polarography[13] (DPP).

The present work describes a simple differential pulse polarographic determination of copper zinc and selenium in chicken liver. Although the detection limit is higher than that of voltammetric stripping methods, DPP has the advantage of having better reproducibility and being more straightforward. Its detection limit is sufficient for the determination of trace elements in liver so that there is no need for extraction pre-concentration procedures. It is possible to determine these elements in a single sample solution after acid digestion.

Materials and Methods

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 three-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) and triply distilled water was used in the preparation of their solutions and at all stages of analysis. The reagents used were SeO_2,

$(NH_4)_2Mo_7O_{24}$. $4H_2O$, $CuSO_4.2H_2O$, $Zn(NO_3)_2$, $Na_2H_2Y.2H_2O$, CH_3COONa and various acids. Dilute solutions were prepared before every use from their stock solutions to prevent the solution from ageing. Britton-Robinson (BR) buffer solution was prepared in such a way that 2.3 mL of glacial acetic acid, 2.7 mL of phosphoric acid (85%) and 2.47 g of boric acid dissolved by dilution with water to 1.0 l. Fifty milliter portions of this solution were taken and the pH was adjusted by the addition of an appropriate amount of 2.0 M NaOH.

Digestion of Chicken Liver

Certain amounts of chicken liver (four different liver samples of similar weight) were dried for 48 hours in an oven at 120 °C to remove the water content and obtain a constant weight. The water content was 72 ± 2%. Four different samples of about 3 g (S_1 = 2.86 g, S_2 = 2.99 g, S_3 = 3.12 g, S_4 = 3.18 g) from dry liver were transferred into a 50-mL long–necked glass flask. For each sample, 4.5 mL of acid mixture (2 mL of HNO_3, 2 mL of $HClO_4$ and 0.5 mL of H_2SO_4) were added. A pre-cleaned glass funnel was inserted into the flask to prevent rapid evaporation. The solution was kept in an oil bath at 50 °C until the foaming was over. Then the temperature was increased to 150 °C and heating continued until the evolution of brown fumes of nitrogen oxides ceased. When the sample turned to a dark color after the flask cooled, 4 mL of HNO_3 was added to avoid danger of explosion. 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 into the polarographic cell. 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 during digestion were between 88 and 97%.

Procedure

A total of 10.0 mL (1.0 M to 0.2 M) of acetate or BR buffer (pH 3) in the polarographic cell was deoxygenated by a stream of high purity nitrogen gas for 3-4 min. The polarogram was taken by scanning the potential from 0.0 V to –1.5 V at a scan rate of 5 mV/s. To this solution was added 0.1 mL of sample solution, diluted according to the concentration of elements present, and once more the polarogram was taken. Then, as necessary, 0.1 mL of 10^{-2}-10^{-4} M MoVI solution was added and selenite was determined from the hydrogen catalytic peak formed at about –1.1 V. After that the pH was increased to 4 with concentrated NaOH, and zinc was determined from its peak at about –0.97 V. For copper, 2 mL of 0.1 M EDTA was added to the same solution, and copper was determined from its peak at about –0.23 V.

Results and Discussion

Preliminary experiments

For the optimum working conditions, supporting electrolytes including HCl, KCl, acetate and BR buffer solutions at various pH values were used. The peak potentials of several elements that may be found in liver such as iron, copper, lead, cadmium, selenium and zinc were determined. Both BR and acetate buffer were

found to be suitable for the digested liver sample solution. BR buffer has the advantage of a wide pH range, but in some cases the compounds in it may interfere with the ions in the sample. Since copper and iron are possibly present in liver, for their separation the addition of EDTA was needed. In this medium at about pH 4, iron had a peak at about 0 V and copper at –0.23 V, which allowed good separation for both ions.

Trace Elements in Chicken Liver

To find out which elements are present in chicken liver above the detection limit of DPP, a polarogram of the liver sample has to be taken. The digested liver sample was stored in 10.0 mL of acidic water (about 3 g of dry liver in 10 mL). From this solution 0.1 mL was added to 10.0 mL of buffer solution in the polarographic cell. When a polarogram of a liver sample in buffer at pH 4.0 was taken, only a peak for zinc at about –0.95 V could be observed at a current range of 0.2 mA. The copper peak was off the scale under these working conditions, indicating a large amount of it. Copper could be determined at a higher current range or by using dilute solutions of liver sample. The peak of selenium at about –0.57 V can usually not be observed at low concentrations, but according to our previous work[14] it can be determined very accurately using the hydrogen catalytic peak. Since no peaks for lead and cadmium were observed, it was decided that they may be present in amounts below their detection limits.

Determination of Copper

A 10 mL solution containing 0.2 M acetate buffer and 0.02 M EDTA in a polarographic cell was deoxygenated by passing nitrogen through it, and the polarogram was taken by scanning the potential from 0.0 to –0.5 V. Then 0.1 mL of digested liver sample S_4 (3.18 g of dry liver in 250 mL) was added and once more the polarogram was taken. Here, since the copper concentration was high the sample solution was diluted into 250 mL. In this way, a current range of 0.2 mA was used, without dilution higher current ranges had to be used. As can be seen in Figure 1, the copper peak appeared at -0.23 V. Copper content was determined by standard additions and the determinations were repeated five times for each sample. Copper content for four different samples (at different weights) varied from 30 mg/g to 15 mg/g (dry weight). The results with their standard deviations are summarized in Table 1. Although iron was expected to be in liver, no peak for iron was observed since it was hidden under the mercury oxidation peak.

Table 1. Determination of copper in chicken liver.

sample	average (mg/g)	standard deviation	RSD (%)	CI = X ± ts / $\sqrt{}$ n (mg/g)
S_1	29.7	3.2	10.6	30 ± 4
S_2	15.3	2.1	13.6	15± 3
S_3	20.7	1.2	6.0	21 ± 2
S_4	21.4	0.8	3.9	21 ± 1

RSD, relative standard deviation. CI: confidence interval: 95% (n = 5)

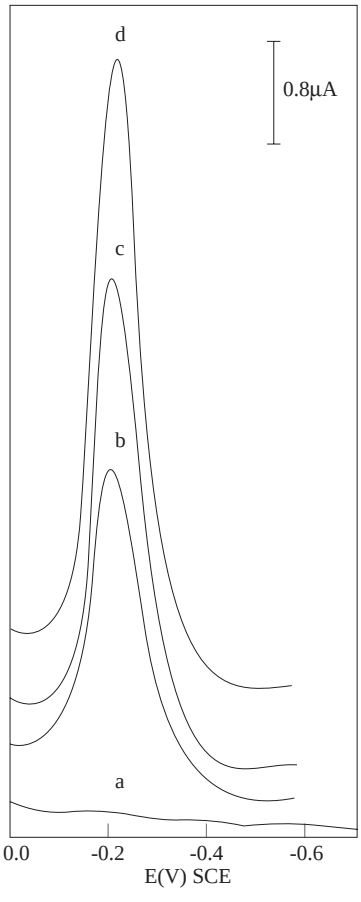

Figure 1. Determination of copper in chicken liver by DPP
10 mL HAc/Ac buffer, EDTA (pH 4.0)
a + 0.1 mL S_4 (3.18 g dry liver in 250 mL)
b + 200 μl 1×10^{-3} M Cu^{2+}
c + 200 μl 1×10^{-3} M Cu^{2+}

Determination of Selenium

The selenium peak could not be observed because of its low concentration in the polarogram taken for the determination of copper. In a previous study[14] we observed that at very low concentrations of selenium a peak appeared at about –1.1 V when Mo(VI) was added. This peak was attributed to a hydrogen catalytic peak and it could be used for the determination of very low concentrations of both of these ions. A linear relationship was obtained for both selenium and molybdenum. 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 that of the other ion[14]. 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. Lead, cadmium, copper, zinc and thallium exert almost no interference in 20-fold higher amounts. The selenium in blood has been determined [15] with this method without any separation or extraction procedure.

For the determination of selenium in chicken liver, 0.1 mL of digested sample solution (2.86 g of dry

liver in 50 mL) was added to 10.0 mL of BR buffer at pH 3 and the DP polarogram was taken by scanning the potential from –0.5 V to –1.4 V. As can be observed from Figure 2, although there was no peak for selenium at –0.57 V after the addition of Mo(VI) a hydrogen catalytic peak appeared at about –1.1 V. This peak increased by standard additions of selenite as long as Mo(VI) was present in the solution. The selenium content of four different liver samples was determined using this method. Each sample was analyzed five times. The results are given in Table 2 along with their standard deviations. The selenium contents for four different samples varied between 2.2 and 3.6 mg/g. Because of the very large catalytic peak, the current range had to be taken as 2 mA so that it would not be off the scale. At this current range the zinc peak (about –1.0 V) was not observable and thus did not interfere with the catalytic peak. Zinc was determined at a 0.1 mA current range since its concentration was low in liver. In the presence of a higher concentration, zinc interference on the catalytic peak would be expected, but in this case either EDTA addition would be needed[14] to complex the zinc ion or the sample solution had to be diluted. The selenium content of the S_2 sample was also determined with the cathodic stripping voltammetric method[10] and 3.2 ± 0.2 mg/g Se(IV) was found, which was consistent with the result (3.3 ± 0.2 mg/g) obtained with this method.

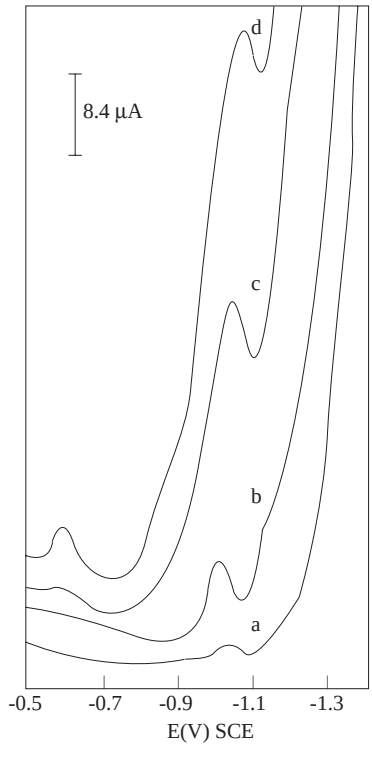

Figure 2. Determination of selenium in chicken liver, using catalytic hydrogen wave.
10.0 mL BR buffer (pH 3.0) + 0.1 mL S_1 (2.86 g dry liver in 50 mL)
a + 150 μl 1×10^{-2} M Mo(VI)
b + 25 μl 1×10^{-2} M Se(IV)
c + 25 μl 1×10^{-2} M Se(IV)

Determination of Zinc

The DP polarogram taken with 10 mL BR of buffer, pH 4.0, and 0.1 mL of sample solution (2.99 g of dry liver in 50 mL of water) is shown in Figure 3. The peak at about –0.97 V increased with standard additions

of zinc. Four different digested chicken liver samples were analyzed, and for each sample five determinations were performed. The zinc levels for four different samples varied from 0.4 to 0.9 mg/g. The results are summarized in Table 3 along with their standard deviations.

Table 2. Determination of selenium in chicken liver.

sample	average (mg/g)	standard deviation	RSD (%)	CI = X ± ts / $\sqrt{}$ n (mg/g)
S_1	2.2	0.2	7.8	2.2 ± 0.2
S_2	3.3	0.1	4.4	3.3 ± 0.2
S_3	3.0	0.9	2.9	3.0 ± 0.1
S_4	3.6	0.1	3.3	3.6 ± 0.2

RSD, relative standard deviation. CI: confidence interval: 95% (n = 5)

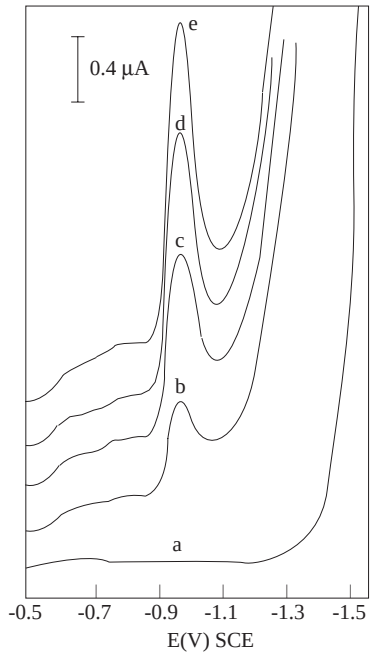

Figure 3. Determination of zinc in chicken liver by DPP 10.0 mL BR buffer (pH 4.0).
a + 0.1 mL S_2 (2.99 g dry liver in 50 mL)
b + 50 μl 1×10^{-3} M Zn^{2+}
c + 50 μl 1×10^{-3} M Zn^{2+}
d + 50 μl 1×10^{-3} M Zn^{2+}

Table 3. Determination of Zn in chicken liver

sample	average (mg/g)	standard deviation	RSD (%)	CI = X ± ts / $\sqrt{}$ n (mg/g)
S_1	0.54	0.03	6.3	0.54 ± 0.04
S_2	0.90	0.07	7.8	0.90 ± 0.09
S_3	0.61	0.04	7.2	0.61 ± 0.05
S_4	0.40	0.01	3.6	0.41 ± 0.02

RSD, relative standard deviation. CI: confidence interval: 95% (n = 5)

For the validity of the method, zinc levels in three different samples were determined using flame AAS. The quantities found were for the S_1 sample 0.62 mg/g, for S_3 0.66 mg/g and for S_4 0.51 mg/g. The relative error was -12% for S_1, -8% for S_3, and about -19% for S_4.

Synthetic Sample

To check the validity of the suggested method, a synthetic sample was prepared containing similar quantities of the metal ions present in liver. The elements were determined with the proposed method. For this purpose, to 10.0 mL of buffer (pH 3) solution was added 0.1 mL of synthetic sample containing zinc, copper and selenium. The final concentrations were in the sample solution. 1.0×10^{-4} M, 1.0×10^{-5} M and 2.0×10^{-5} M for copper, zinc and selenium, respectively. First Se(IV) was determined from the catalytic peak after the addition of Mo(VI); the current range had to be high because of the large catalytic peak. Then pH was increased to 4 by the addition of NaOH and zinc was determined and finally, after the addition of EDTA, copper was determined. The results are given in Table 4. This method clearly enabled the determination of trace elements with high accuracy and precision.

Table 4. Determination of copper, zinc and selenium in four synthetic samples.

	Concentrations in synthetic sample (M)	Found (M) $x \pm$ t s $/\sqrt{n}$	Relative error (%)	RSD (%)
Cu	1.00×10^{-4}	$(1.0 \pm 0.1) \times 10^{-4}$	+4	4.0
Zn	1.00×10^{-5}	$(1.2 \pm 0.1) \times 10^{-5}$	+7	4.6
Se	2.00×10^{-5}	$(2.0 \pm 0.2) \times 10^{-5}$	-1	6.4

RSD, relative standard deviation. t:confidence interval: 95% (n = 4).

Conclusion

The determination of trace elements in chicken liver is important from the nutritional point of view. Chicken is consumed frequently in many countries.

Chickens are fed food rich in elements, and thus it is important to determine their levels in the liver. With the proposed method it was possible to determine these elements simultaneously from one single sample solution after digestion. It was found that copper, selenium and zinc were present in chicken liver, but lead and cadmium concentrations were below the detection limit. In conclusion, with the present method, which was used for the first time for the simultaneous determination of trace elements in chicken liver, it was possible to determine zinc, selenium and copper, without any separation or pre-concentration techniques. 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 metals.

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