



Determination Of Heavy Metals In The Liver

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ABSTRACT

The article discusses data on the solubilization of heavy metals in the liver of cattle in the form of aqueous extracts and ensuring the complete separation of heavy metals in the liver, the determination of the amount of mercury, cadmium, lead and iron in the liver of cattle by the method of potentiometric oximetric titration on the device "Ionomer U- 130" and spectrophotometric determinations were carried out on the "Spectrophotometer KFK-3".

Keywords:

aqueous liver extract, mercury, cadmium, lead and iron ions, potentiometric titration and spectrophotometry, redox couple, equivalent point, ionomer potential, optical densities, UV spectra, calibration diagram.

Introduction

The problem of environmental security in our republic has become a common problem not only for the people of our republic but also for all mankind. Nature and man relate to each other based on certain laws. Violation of these laws will lead to irreparable environmental disasters. Violation of the ecological balance of the environment and its constituent objects; has a negative impact on soil, water, air, plants, animals and people. Based on the existing problems, we conducted scientific research work on the detection of heavy metals in the liver of cattle.

The studied literature also shows that world scientists are paying great attention to this field. Scientists and researchers have developed various criteria for determining the contamination of the environment with heavy metal ions as a result of systematic

examination of soil, air, water, one or another nutrient, plants, etc. For example, the degree of damage to the environment can be determined by the amount of heavy metals in the leaves of trees.

Literature revive

Japanese researchers [1] used atomic absorption spectrometry to determine the content of Cd, Pb, and Cu in roasted beef liver. For this, the samples were prepared by dry ashing and then extracted with sodium diethyldithiocarbamate of methyl isobutyl ketone. As a result of the tests, the detection limits of heavy metals in the samples were 0.02-0.43 mg/kg Pb, with 0.1 mg/kg Cd, 1.0 mg/kg Pb and 0.5 mg/kg Cu; <0 Amounts of .01-0.03 mg/kg Cd and 0.01-2.4 mg/kg Cu were found.

The atomic emission method was used to determine heavy metals in human blood serum, its various organs, and the organism of monkeys, soil and plants.

The authors [2] used the induction plasma atomic emission spectroscopy method to examine the blood serum of people who consumed food products sterilized by irradiation. After burning, the sample was melted and 12 trace elements (Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sn, Se, Zn) were determined from the solution. The lower limit of their detection is 10-2-10-3mg/l. When the results of the analysis of 78 samples were analyzed statistically, it was found that there were no significant deviations from the values of the control groups. Troshkova and Yudelovich [3] developed a chemical atomic emission method for the determination of Bi, Ti and V in serum and blood with a relative standard deviation of 0.15-0.23 and a lower detection limit of 0.8-5.0 µg/l came out

Induction plasma mass spectrometry (MS) was used to determine the isotope ratio of Fe in human blood serum [4]. The sample was directly introduced in an unprepared form by electrothermal evaporation. This method allows for the express determination of the amount of Fe in the ratio of $^{54}\text{Fe} / ^{56}\text{Fe}$ and $^{57}\text{Fe} / ^{58}\text{Fe}$ in the blood serum of non-pregnant and pregnant women [5], in the work Li, Fe, Co, Cu, Zn, Br, Rb from human blood serum. , Mo, Sn, I, Cs, Ba and Bi were used to determine very small amounts of Inp mass spectrometry. In this case, In is added as an internal standard (up to 100 µg/l) to account for the interfering effects of significant amounts of Na, K, and Ca. As a result of the analysis, the results of the determination of Fe, Co, Cu, Zn, Rb, Mo and Cs using the methods of X-ray spectrometry with neutron activation and nuclear particles were obtained. The authors [6] developed methods for determining Fe, Co, Cu, Zn, Rb, Mo and Cs in blood serum. Sample preparation requires minimal work, for which the serum sample is diluted with 0.14 M HNO₃ and spiked with an internal standard of In. Standard solutions of NaCl, NaNO₃, Ca(NO₃)₂, cysteine, and internal standard destroying elements in acid solution with similar composition and amount are

checked to adjust the overlap of signals of polyatomic ions. Relative error: (%) 16 – Fe, 21 – Co, 5 – Cu, 2.2 – Zn, 0.16 – Rb, 12 – Mo and 5.4 – Cs were determined. The same authors [7] developed a methodology for the determination of Li, Fe, Co, Cu, Zn, Rb, Mo, Sn, Cs, Ba and Bi from human blood serum by Inp MS method. The serum sample was diluted 5-10 times with 0.14 M HNO₃ solution to which salt (up to 100 µg/l) was added as an internal standard. To evaluate the error of the method, the biological materials related to the second generation were examined. Certified estimates of elements in these materials were established using INAA, Inp AEmS, and electrothermal AAS methods.

2 schemes of neutron-activation detection were developed to determine trace amounts of 24 chemical elements from a human liver sample [8]. In this case, the sample is irradiated for a short time using a nuclear reactor, and elements such as Al, V, Mg, Cl, Cu, Na, K, Mn and others are destructively determined. Other elements are radiochemically isolated from the irradiated sample by liquid chromatography and extraction. In order to increase the sensitivity of γ-spectrometric detection, a background reduction system due to Compton scattering is used. The results of the determination of Cr, As, Se, Mo, Ag, Sn and Sb from 30 liver samples are presented. 12 chemical elements (Cl, Br, K, Al, Na, Hg, Mn, S, Cu, Zn, Mg and Ca) in the hair of residents of different regions of Japan [9] were determined by the neutron-activation method. Statistical values of these results are presented in the work.

Experimental part

The liver of cattle reared in the Buloq-boshi district of Andijan region was taken from the liver market of Buloq-boshi district of Andijan region as an object of investigation for determination of heavy metals in food products. A 300 g sample of the black beef liver was taken as a control object. The resulting object sample is finely ground. This sample was then placed in a one-litre beaker and 300 mL of chemically pure concentrated acetic acid (CH₃COOH) was added. The container was

sealed and the solution formed from the bovine liver sample in acetic acid was separated by filtration in a Buechner funnel. The extracted liver sample was washed 5-6 times with distilled water. As a result, 1.5 litres of the solution was extracted from the sample of cattle liver. Separated solutions are concentrated using the distillation method at a temperature of 70-80 °C. The separated liver filtrate was concentrated by evaporation to a volume of 800 ml. The concentrates were used to determine heavy metals in the liver [10].

When the filtrates were found to contain heavy metals, the remains of the liver samples were washed twice with distilled water. In the filtrates from the sixth washing, qualitative reactions were carried out to determine the ions of heavy metals. There were no changes as above. In this way, the complete transition of heavy metals in the sample to the solution was achieved. After collecting all the filtrates in one container, they were heated to 70-80 temperature and concentrated using the distillation method. As a result, the volume of the liver sample filtrate was brought up to 800 ml. This solution was used to determine heavy metals in the liver [10].

For potentiometric analysis of heavy metals in the liver sample solution prepared for analysis, 400 ml of the liver sample solution was separated. This solution was divided into 3 parts for potentiometric determination of Cd^{2+} , Pb^{2+} , and Hg^{2+} ions in the obtained solution. Then, the amount of Cd^{2+} , Pb^{2+} and Hg^{2+} ions was determined from this solution. Before starting the potentiometric determination of Cd^{2+} , the U-130 ionomer was run. Then, 5 ml of the filtrate obtained from the isolated liver sample for Cd^{2+} was taken using a 10 ml graduated pipette and placed in a 50 ml titration beaker. 1 drop of 0,03 M $\text{K}_3[\text{Fe}(\text{CN})_6]$ solution was added to the solution to form a redox couple. Then 10 ml of distilled water was added to the solution in the beaker.

The ionomer was adjusted to measure the potential, and the titration was performed by adding titrant portions every 40-60 seconds from a standard solution of $\text{K}_3[\text{Fe}(\text{CN})_6]$ in a 25 mL micro burette.

After 40-60 seconds after adding each portion (drop) of titrant, the display of ions becomes constant, and the potential values during this period are recorded in the titration report. The volume of titrant to the drop corresponding to the largest potential jump was taken as the volume corresponding to the equivalence point. The amount of Cd^{2+} was calculated based on the results of 6-7 repeated experiments conducted in parallel. In addition to Cd^{2+} , the amount of Pb^{2+} and Hg^{2+} ions in liver solutions was determined in the same way as in the potentiometric titration of Cd^{2+} [11].

In this case, a 0,005 M $\text{K}_2\text{Cr}_2\text{O}_7$ solution was used as a titrant to determine Pb^{2+} and a drop of a 0,05 M CrCl_3 solution was used to form an oxidation-reduction pair, and the amount of Pb^{2+} was found.

In the potentiometric determination of Hg^{2+} , a 0.1 M solution of $\text{NaJ}\cdot 2\text{H}_2\text{O}$ was used as a titrant, and 1 drop of a 0.005 N solution of J_2 in alcohol was added to the solution to form a redox couple. Thus, the amounts of Cd^{2+} , Pb^{2+} and Hg^{2+} ions in the solution taken from the liver sample were determined by potentiometric titration and the obtained results were discussed.

A dilute solution of NaOH was added to the second portion of the filtrate of the liver sample to be tested. As a result, heavy metal hydroxides precipitated. The resulting precipitate was separated and washed 3-4 times in distilled water. The washed precipitate was dissolved in a solution of concentrated HNO_3 (1:10). The resulting solutions were used for spectrophotometric determinations. To determine the amount of Cd^{2+} , Hg^{2+} va Fe^{3+} ions in the filtrates, the solutions converted to nitrates were divided into three parts, and spectrophotometric determinations were made from the separated solution for each metal ion. 30 minutes before starting the spectrophotometric determinations, the KFK-3 photometer was started with the cover of the cuvette compartment open. We used the gradation plot method for spectrophotometric determinations. For this, standard solutions of $\text{Cd}(\text{NO}_3)_2\cdot 4\text{H}_2\text{O}$, $\text{Hg}(\text{NO}_3)_2\cdot 2\text{H}_2\text{O}$, and $\text{Fe}_2(\text{SO}_4)_3\cdot 9\text{H}_2\text{O}$ were used, and the optimal conditions for detection were selected using

these solutions. First, the extract of the complex formed by the effect of dithizone on a 0.1 M chloroform standard solution of the tested metal ion in a cuvette with a thickness of 10 mm, chloroform was added to the second, and the optical density of the solution was measured [12].

The determination of mercury is carried out in the same way as the determination of Cd^{2+} by the method of formation of dithizone complexes. A 0.001% solution of dithizone in chloroform was used to extract Hg^{2+} from the standard solution. The extracts obtained in several steps were combined, and a dilute solution of ammonia was added to remove free dithizone from the extract and the mixture was shaken. The extract was then mixed with dilute acetic acid by shaking. As a result, a reddish-yellow solution was formed. The resulting reddish-yellow solution of mercuric dithizone was transferred from a separatory funnel to a 50 ml volumetric flask, and chloroform was added up to the volumetric level of the flask. In the above order, a ranking chart was created [13].

The Hg^{2+} ion in filtrates from the bovine liver was extracted at $\text{pH}=0$ (in the above procedure). The extract is reddish-yellow in colour and has a maximum optical density at a wavelength of 540 nm. The resulting extract was tested at this length.

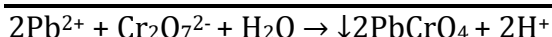
The optical density of the extract was measured at 630 nm (maximum absorption area of dithizone) to eliminate the effect of copper(II)-ion present in the extract. After that, KJ solution is added to the extract, and when shaken, the mercury dithionate in the extract breaks down, and Hg^{2+} turns into HgJ_4^{2-} -complex at $\text{pH}=4$. The amount of mercury was determined based on the difference in absorption by measuring the optical density of dithizone formed in an equivalent amount during the decomposition of mercuric dithizone at 620 nm.

Spectrophotometric determination of Fe^{3+} was carried out in the presence of sulfosalicylic acid, by the method of a graduation diagram. For this, a 0.1 M standard solution of $\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ was first prepared. Fe^{3+} ions were adjusted to the pH value of the 0,1 M

$\text{Fe}_2(\text{SO}_4)_3 \cdot 9\text{H}_2\text{O}$ standard solution prepared in accordance with the pH values of the filtrates of the samples separated for determination. Then, 10 ml was taken from the standard solution using a graduated pipette and placed in a 50 ml volumetric flask. It was mixed by adding 10 ml of 10% sulfosalicylic acid solution. After 1 minute, 10 ml of 10% NH_3 solution in water was also added. Then distilled water was poured up to the mark of the flask. After five minutes, the yellow solution formed in the first cuvette, the second cuvette was filled with a solution containing ammonia and sulfosalicylic acid (without iron) and the optical density of the solution was measured. From the rest of this solution, 0,05, 0,025; 0,0125; 0,00625; 0,003125; 0,0015625 M concentration solutions were prepared using distilled water. The optical densities of these solutions were measured at a selected wavelength (680 nm). A gradation plot was constructed based on the measured values. Then, for the spectrophotometric determination of the amount of Fe^{3+} in the filtrates of the samples separated for the Fe^{3+} ion, 10 ml was measured in a pipette and placed in a 50 ml measuring flask. 10 ml of 10% sulfosalicylic acid solution was added to the solution and mixed. After 1 min, 10 ml of 10% NH_3 solution in water was also added and distilled water was added up to the volumetric level of the flask. The optical density of the resulting solution at a wavelength of 680 nm was determined and the obtained value was placed on the calibration chart. On this basis, the amount of Fe^{3+} in the liver was determined [14].

Results and discussion

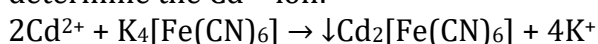
For potentiometric determination of heavy metal ions (Pb^{2+} , Cd^{2+} , Hg^{2+}) their oxradiometric capabilities were used. Since the Pb^{2+} ion is electroactive in the aqueous solution under the given conditions, to determine the endpoint, the oxidation-reduction pair is formed at the expense of the titrant, that is, 1 drop of 0.02884 M CrCl_3 solution is added to the solution to form the $\text{Cr}_2\text{O}_7^{2-}/\text{Cr}^{3+}$ pair. During the titration, PbCrO_4 precipitates. The resulting precipitate has low solubility, ($K^0_s=1,8 \cdot 10^{-14}$), is enough.



The last drop of K₂Cr₂O₇ added when the titratable Pb²⁺ in the solution was exhausted caused an increase in the electrode potential. The rise of the potential after the equivalence point can be explained using the formula

$$E = 1,33 + \frac{0,059}{6} \lg \frac{[\text{Cr}_2\text{O}_7^{2-}] * [\text{H}^+]^{14}}{[\text{Cr}^{3+}]^2}$$

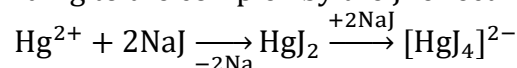
According to the results of titration, the amount of Pb²⁺ ion in the liver was on average 9.02 mg/kg. The number of parallel detections was equal to 7 in the first case and 9 in the second case. The following reaction was used to determine the Cd²⁺ ion:



Due to the low content of Pb²⁺ in cattle liver, titration was performed by dropwise addition of titrant (0,004903 M K₂Cr₂O₇). At the endpoint of the titration, the potential jump was around 15 mv. The solubility of the resulting precipitate is low and is K_s=3,2*10⁻¹⁷. A drop of a 0.0291 M solution of K₃[Fe(CN)₆] was added to the test solution to generate the redox potential.

amount of Cd²⁺ in the liver (Lahm) was 7.49 mg/kg.

The determination of the Hg²⁺ ion was based on its binding to the complex by the J- effect:



A drop of 0.005 N solution of iodine was added to the test solution to form a redox couple. In this, the change in potential during the titration

$$E = 0,536 + \frac{0,059}{2} \lg \frac{[\text{I}_2]}{[\text{I}^-]^2}$$

is explained using the formula.

Determination of the amount of Hg²⁺ in the liver was also performed on the basis of dropwise titration. According to the obtained results, the amount of Hg²⁺ was 7.22 mg/kg. 5 and 7 parallel experiments were performed to evaluate the reproducibility of mercury determination.

The results of potentiometric determination of the amount of heavy metals in cattle liver are presented in Table 1.

Table 1. Results of potentiometric determination of heavy metal ions in the liver

Object	R, mg			S, mg			Error, %		
	Pb ²⁺	Cd ²⁺	Hg ²⁺	Pb ²⁺	Cd ²⁺	Hg ²⁺	Pb ²⁺	Cd ²⁺	Hg ²⁺
Liver	13,07	8,09	9,15	0,05	0,04	0,04	0,88	1,21	0,99

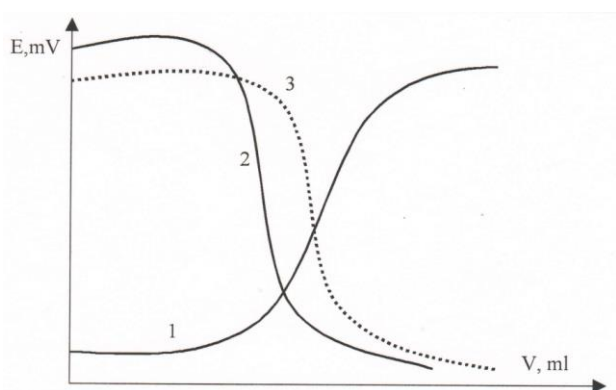


Figure 1. Potentiometric titration curves of heavy metal ions

1 — Pb²⁺, 2 — Hg²⁺, 3 — Cd²⁺

Reduction of potential after the equivalence point:

$$E = 0,356 + 0,059 \lg \frac{[\text{Fe}(\text{CN})_6^{3-}]}{[\text{Fe}(\text{CN})_6^{4-}]}$$

is represented by the formula.

The titrant (K₄[Fe(CN)₆] 0,004229 M) was also added dropwise in the determination of Cd²⁺ in the liver. The potential jump at the equivalence point of the titrant was around 50 mv. The number of conducted parallel experiments according to (4 and 7). The

The accuracy of the obtained results was evaluated using the spectrophotometric method.

For the spectrophotometric determination of heavy metal ions (Fe³⁺ Cd²⁺ Hg²⁺), we used their formation of coloured complexes with various organic compounds. Iron (III) ion forms a yellow complex compound with sulfosalicylic acid in an ammonia medium. The hg²⁺ ion forms an orange complex in chloroform solution, and the Cd²⁺ ion forms a pink complex.

The optimal conditions for detection were selected using a 0.05 M solution of the resulting colour solution. For this purpose, a corresponding solution was placed in a cuvette

with a thickness of 10 mm, and its optical density was measured in the wavelength range from 310 nm to 998 nm in relation to the non-ionic solvent under investigation. The resulting spectra are presented in Figure 2. The experiments showed that the Hg^{2+} ion had the maximum optical density at a wavelength of 540 nm (Fig. 2, curve 1).

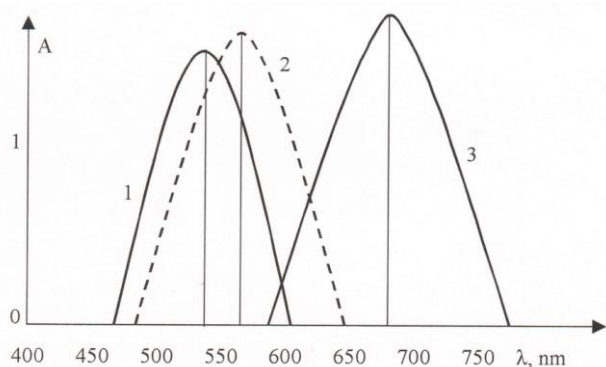


Figure 2. UV spectra of heavy metal solutions:
1 - Hg^{2+} ; 2 - Cd^{2+} ; 3 - Fe^{3+}

Optical densities showed a linear relationship (Fig. 3, curve 2) in the concentration range of 0.00625 - 0.05 M. And the Cd^{2+} ion has the maximum optical density at a wavelength of 560 nm (Fig. 2, curve 2), and the optical densities show a straight line in the concentration range of 0.0125 - 0.1 M got the appearance (Fig. 3, line 1). The maximum optical density of Fe^{3+} ion (Fig. 2, curve 3) corresponded to the wavelength of 680 nm. Iron (III) ion had a straight line (Fig. 3, line 3) in the concentration range of 0.0015625 - 0.05 M. Parallel experiments were performed to ensure the reproducibility of the analysis. Ranking plots were made based on the average of the results of 3-4 parallel experiments. 4 parallel experiments were conducted to determine the amount of iron in cattle liver. The obtained results (Table 2) show that the average amount of iron in the liver was 0.48 mg/kg. This is very close to the established norm. 5 and 9 parallel experiments were conducted to determine the amount of mercury (in bovine liver extracts), respectively. Based on the obtained results (Table 2), shows that the amount of mercury in cattle liver is 7.24 mg/kg, which is much higher than the specified

values. The results of determining the amount of metals in liver products using the spectrophotometric method are presented in Table 2. 5 and 9 parallel experiments were conducted to determine the amount of mercury (in bovine liver extracts), respectively. Based on the obtained results (Table 2), shows that the amount of mercury in cattle liver is 7.24 mg/kg, which is much higher than the specified values. The results of determining the amount of metals in liver products using the spectrophotometric method are presented in Table 2. 5 and 9 parallel experiments were conducted to determine the amount of mercury (in bovine liver extracts), respectively. Based on the obtained results (Table 2), shows that the amount of mercury in cattle liver is 7.24 mg/kg, which is much higher than the specified values. The results of determining the amount of metals in liver products using the spectrophotometric method are presented in Table 2.

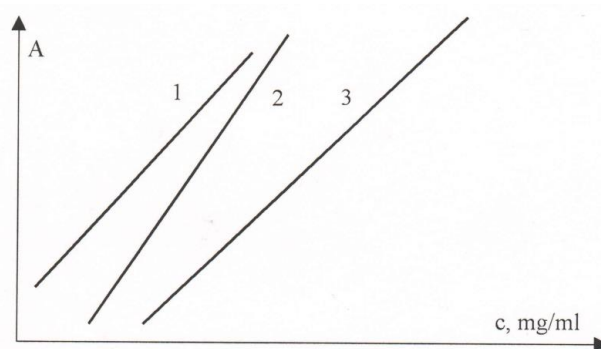


Figure 3. Grading charts of heavy metal ions:
1 — Cd^{2+} ; 2 — Hg^{2+} ; 3 — Fe^{3+}

Liver cadmium content was 7.48 mg/kg, and 5 and 7 parallel experiments were performed to assess the reproducibility of the results, respectively. All obtained results were evaluated using mathematical-statistical methods. The standard deviation did not exceed 0.05 mg. This indicates that they are sufficiently accurate.

Table 2. Results of spectrophotometric determination of heavy metal ions in the liver

Object	X, m	S, mg	Error,
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	g			%					
	Fe 3+	C d ² +	H g ² +	Fe 3+	C d ² +	H g ² +	Fe ³ +	Cd ² +	H g ² +
Liver	0,70	9,47	9,12	0,04	0,04	0,05	15,90	1,06	1,24

The results of spectrophotometric determinations were compared with the results of potentiometric titration and their accuracy was evaluated. The closeness of the results obtained using both methods and in many cases, the compatibility, indicates the correctness of the results.

Conclusion

1. The method of transferring heavy metal ions from cattle liver into solution was developed. Taking advantage of the fact that the nitrates of the examined heavy metal ions are well soluble in water, it was possible to transfer them to the solution in the form of nitrates.
2. Potentiometric oximeter titration and spectrophotometric methods were used to determine the amount of heavy metal ions in cattle liver.
3. Potentiometric oxradiometric titration of mercury ion in cattle liver with NaI (with I₂), titration of a lead ion with K₂Cr₂O₇ (with Cr³⁺) and titration of Cd²⁺ ion with K₄[Fe(CN)₆] (K₃[Fe(CN)₆]) was conducted using
4. For spectrophotometric determination of the number of heavy metal ions in cattle liver, coloured complexes of Fe³⁺ formed by sulfosalicylic acid, Hg²⁺ and cadmium in chloroform solution with ditozone were used.
5. The values obtained by potentiometric and spectrophotometric methods were used to assess the accuracy and correctness of the results. The mutual compatibility of the obtained results indicates their correctness.

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