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Extraction of Arsenic with Copper from Biological Materials.

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Many authors have reported studies on the detection of metallic poisons. Some difficulties, however, have been encountered in the field of legal medicine to detect those poisons in biological materials. For instance, a troublesome digestion of the materials is required before quantitative analysis of the poisons can be carried out.

Gettler and Kave[1)] described a simple and rapid analytical method for mercury, bismuth, antimony, and arsenic in biological material without digestion. They attempted to extract the metals from materials with copper spiral and reported that 50% of the arsenic was detected by following determination by the Gutzeit method.

Extraction of metallic poisons with copper has long been known as Reinsch test, but the mechanism of this test for arsenic has not yet been clearly elucidated. According to Blyth,[2)] the arsenic stain on copper is a true alloy, consisting of one arsenic to five copper atoms. However, according to Gettler and Kave,[1)] one-half the amount of arsenic is combined chemically with copper while another half is deposited on the copper, and the arsenic deposited on copper is detectable by the Gutzeit method. In the presence of hydrochloric acid, arsenic will combine chemically with copper as follows[3)]:

$$As_2O_3 + 8Cu + 6HCl = Cu_5As_2 + 3CuCl_2 + 3H_2O$$

Attempt was made to extract micro-amounts of arsenic from various biological materials and to determine its amount by colorimetry.

Experimental

Copper Spiral—A spiral is prepared by winding a length of electric copper wire tightly and closely over a piece of glass rod ten times. The copper wire used is 1 mm. in diameter and the glass rod is 8 mm. in diameter. The copper spiral is washed before use with EtOH and then with Et_2O.

Procedure—To 20 cc. of arsenious acid solutions and 10～20 g. of finely macerated tissue, dried milk, or urine, in a 50-cc. Erlenmeyer flask, some HCl is added. To the tissue and dried milk, 10 cc. of H_2O is added. The copper spiral is immersed in the material in the flask and the content is gently boiled for approximately 30～60 min. in a water bath, the original volume in the flask being maintained by appropriate addition of 10% (by volume) of HCl. After extraction of As is over, the spiral is removed and washed with H_2O.

The washed spiral, which is stained with As, is placed in a bottle used for producing AsH_3 in the following procedure. The amount of As stained on the copper spiral was determined by the Gutzeit method or by colorimetry.[4)]

Results

Arsenious Acid Solution—After extraction of As with copper spiral, the amount of As stained on Cu was determined by both Gutzeit method and colorimetry.

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(1) Examination of the quantitative analytical methods for As: For the extraction of As with Cu, 20 cc. of As_2O_3 solution was added to 5 cc. of HCl and heated in a boiling water bath for 30 min. As shown in Tables I and II, under those conditions, more accurate results were obtained by colorimetry than by the Gutzeit method. About 50% of 10~20 γ of As was recovered as described by Gettler and Kave.1) Therefore, in all of the following experiments, As was determined by colorimetry.

TABLE I. Determination by the Gutzeit Method

Arsenic	Found (γ)						Mean square
(γ)	1	2	3	4	5	Average (%)	
5.0	2.0	1.6	2.7	2.0	2.0	43.2	21.415
10.0	5.0	6.2	5.0	4.2		50.8	22.651
20.0	12.5	12.5	10.0	10.0		56.3	17.473

TABLE II. Determination by Colorimetry

Arsenic	Found (γ)						Mean square
(γ)	1	2	3	4	5	Average (%)	
5.0	2.2	2.3	2.6	2.5	2.5	48.4	3.564
10.0	4.8	5.3	5.1	4.8	5.3	50.6	2.389
20.0	10.3	10.0	10.3	10.3	9.9	50.8	0.061
30.0	16.5	16.5	16.8	16.2	16.0	54.3	0.457

(2) Effect of the concentration of HCl on extraction of As with copper spiral: Extraction with Cu was attempted with various concentrations of HCl added on heating for 30 min. and the results obtained are shown in Table III.

TABLE III. Effect of the Concentration of Hydrochloric Acid

Arsenic	HCl added	Found (%)					
(γ)	(cc.)	1	2	3	4	5	Average
23.3	1.0	5.3	7.6	2.1	3.0	6.4	4.9
23.3	2.5	46.8	54.6	52.0	49.8	51.3	50.9
23.3	5.0	47.0	57.0	54.0	55.7	46.4	54.0
23.3	10.0	53.6	57.8	55.7	46.1	45.0	51.6

(3) Effect of period of heating: After addition of 5.0 cc. of HCl, the extraction of As with Cu was carried out at various intervals of heating and the highest recovery was obtained in 30 min. of heating, as shown in Table IV.

TABLE IV. Effect of Period of Heating

Arsenic	Period of heating	Found (%)					
(γ)	(min.)	1	2	3	4	5	Average
23.3	15	36.5	31.1	35.4	27.0	31.0	32.2
23.3	30	47.0	57.0	54.0	55.7	46.4	54.0
23.3	60	53.0	48.6	50.0			50.5
23.3	90	46.4	48.0	51.1			48.5

TABLE V. Recovery of Arsenic in using 1~3 Spirals

Number of spirals	Arsenic (γ)			
	10	20	30	40
1	5.03	8.5	16.3	15.1
	3.96	9.37		14.5
	3.83	9.63		15.4
	3.66	9.72		17.7
2	3.80	13.19	15.6	15.08
	4.56	13.68	12.9	16.04
	4.25	14.68	14.24	22.80
	6.31	14.10		23.70
3	4.25	10.80		22.00
	7.07	10.90		25.10
		12.16		19.80
		11.62		25.00

(4) Effects of increase in number of copper spirals on extraction of As : Experiments described above showed that about 50% of As was recovered when 5.0 cc. of HCl was added and the heating was continued for 30 min. Under the same conditions, the effect of increase in number of the spiral was tested by using 1~3 spirals. The results are shown in Table V.

The amount of As remaining in the residue after extraction was examined. After extraction with Cu, the solution which originally contained 23.3 γ of As was estimated by colorimetry and the results are shown in Table VI.

Table VI. Extraction with One Copper Spiral from Arsenious Acid Solution containing 23.3 γ of Arsenic

Found from Cu (%)	Found residue (%)	Total found (%)	Found from Cu (%)	Found residue (%)	Total found (%)
47.0	48.0	95.0	55.7	41.0	96.7
57.0	40.0	97.0	46.4	47.2	93.6
54.0	50.4	104.4			

Arsenic in Dried Milk—Samples were prepared by adding As_2O_3 to some dried milk. Ten g. of the sample was added to 10 cc. of H_2O and 5 cc. of HCl. Longer heating was required to obtain a good recovery rate and the mixture was heated for 90 min. Estimation of As stained on Cu was carried out by both the Gutzeit method and by colorimetry. The results are shown in Table VII.

Table VII. Arsenic in Dried Milk

Aresnic content (γ)	Found (%)						Mean square
	1	2	3	4	5	Average	
	(By Gutzeit's)						
5	40.0	36.0	40.0	32.0	40.0	37.6	4.556
10	38.0	50.0	47.0	50.0	44.0	45.8	8.424
20	55.0	47.5	42.5	42.5	57.0	48.8	15.949
	(By Colorimetry)						
5	42.0	46.0	40.0	46.0	40.0	42.8	3.093
10	48.0	47.0	45.0	47.0	48.0	47.0	0.493
20	51.0	49.0	50.0	47.5	51.5	49.8	2.575
30	51.0	51.0	50.7	50.7	50.0	50.7	0.221

Arsenic in Bovine Liver—Finely macerated bovine liver was mixed with As_2O_3 and 10 g. of the mixture containing 23.3 γ of As was used as the sample. Such a sample as animal tissue contains a large amount of protein and other biological substances, and the amount of HCl and the period of heating were tested under various conditions. The results are shown in Table VIII.

Table VIII. Effects of Period of Heating and Addition of Various Amounts of Hydrochloric Acid

HCl added (cc.)	Period of heating (min.)	Found (%)				HCl added (cc.)	Period of heating (min.)	Found (%)			
		1	2	2	Average			1	2	3	Average
2.5	30	0	0	0	0	10.0	30	57.9	54.7	53.6	52.2
	60	0	0	0	0		60	54.7	48.2	47.3	50.0
	90	0	0	0	0		90	45.0	54.0	45.4	48.1
5.0	30	57.9	56.9	52.9	55.7						
	60	64.3	76.1	76.1	72.1						
	90	60.8	67.2	69.8	65.9						

Arsenic in Urine—As_2O_3 was dissolved in human urine free of As. The urine sample containing 23.3 γ of As was used. Effect of the amount of HCl and the period of heating were tested and the results obtained are shown in Table IX.

Effect of Reducing Agents—Comparatively high recovery rate was obtained in detection of As in urine as described above. Various reducing agents naturally present in the urine were supposed to affect the detection of As, and the effect of various reducing agents on extraction of As with Cu was tested by using aqueous solution of As_2O_3 as a sample. To 25 cc. of the solution containing 23.3 γ of As, 4 cc. of HCl and 1 cc. of 1% solution of each reducing agent were added, and the mixture was heated for 30 min. The results are shown in Table X.

Table IX. Arsenic in Urine

Time of warming (min.)	HCl added (cc.)	Found (%)				
		1	2	3	4	Average
30	0.5	0	0			0
	1.0	32.1	43.9			38.0
	2.5	87.9	85.8	75.1	79.3	82.0
	5.0	81.5	81.5	81.5	70.3	78.7
	10.0	70.8	69.7	58.4	64.3	65.8
60	2.5	66.5	78.3	75.1	79.1	74.8
	5.0	68.6	69.2	78.3	81.5	74.4
	10.0	46.1	50.4	51.5	49.3	49.3

Table X. Effect of Reducing Agent

Reducing agent	Found (av.) (%)
L-Ascorbic acid	62.6
Hydroxylamine hydrochloride	46.4
Hydrazine sulfate	77.1
Hydroquinone	77.9

Hydrazine sulfate and hydroquinone had greater effect than L-ascorbic acid, while hydroxylamine hydrochloride did not affect the increase of recovery rate of As. Recovery rate of As was estimated for various amounts of hydrazine sulfate (Table XI) or hydroquinone (Table XII) added.

Table XI. Effect of 1% Hydrazine Sulfate

HCl added (cc.)	1% Hydrazine sulfate (cc.)	Found (%)					
		1	2	3	4	5	Average
2.5	0.5	72.9	86.9	83.4	79.8	85.3	81.7
	1.0	78.3	87.9	78.1	81.5	83.6	81.9
	2.0	87.9	78.3	86.9	84.9	90.1	85.6
5.0	0.5	69.7	80.4	75.3	74.6	72.1	74.4
	1.0	79.4	66.4	85.4	80.3	82.3	78.7
	2.0	78.3	75.1	80.4	86.9	81.7	80.5

Table XII. Effect of 1% Hydroquinone

HCl added (cc.)	1% Hydroquinone (cc.)	Found (%)					
		1	2	3	4	5	Average
2.5	0.5	82.4	81.5	90.1	84.0	79.8	83.6
	1.0	93.3	79.4	80.4	87.3	93.3	86.7
	2.0	94.4	87.9	82.4	87.0	80.4	86.4
5.0	0.5	66.5	71.8	74.0	77.2	70.5	72.0
	1.0	73.0	84.7	76.1	76.1	70.8	76.1
	2.0	75.1	83.7	83.6	80.7	77.3	86.1

Discussion

About 50% of 10~20 γ of arsenic in water was able to be detected by the present method as described by Gettler and Kave, and the recovery rate was obtained with accuracy, especially on addition of 5 cc. of hydrochloric acid and heating for 30 minutes. The recovery rate of 50%, however, varied according to the change in the amount of hydrochloric acid added, amount of arsenic in the sample, and in the period of heating.

These findings suggested that about one-half the amount of arsenic in aqueous solution was able to combine with copper, and the combined arsenic was completely changed to arsine in the analytical method that followed. However, increase in the number of copper spirals did not greatly improve the recovery of arsenic and the sum of the amount of arsenic estimated by this method and that remaining in the solution after extraction with copper were almost equal to the total amount of arsenic preliminarily present in the solution. These results showed that one-half the amount of total arsenic was all that was able to combine with the copper spiral, and the amount of arsenic combined with copper was indifferent to the width or surface area of copper spiral.

The arsenic was supposed to combine with copper in three ways as follows :

(1) Formation of copper arsenide (Cu_5As_2) by chemical reaction.
(2) Formation of an alloy of arsenic and copper.
(3) Deposition of arsenic on the surface of copper according to ionization tendencies of arsenic and copper.

The formation of copper arsenide will only proceed stoichiometrically and alloy formation will be affected by the physical condition of surface of copper, while polarization potential of copper will control the amount of arsenic deposited on copper.

A concentration of hydrochloric acid appears to have an effect on the formation of copper ion from metallic copper, on the physical change of surface of copper by corrosive action of the acid, and on the concentration of hydrogen ion. The period of heating apparently has an effect on the velocity of reactions described above. Consequently, the recovery rate of arsenic obtained by this method was influenced by the amount of hydrochloric acid added as well as by the period of heating. Under certain conditions, however, especially in certain concentration of each ion contained in the reaction system, the chemical reaction will reach the equilibrium, i.e., saturation of arsenic on the copper, and the amount of arsenic on the copper was 50% of the total amount for some reason and the recovery rate was not stoichiometric.

These considerations were confirmed by the following experiments. The recovery rate of arsenic contained in dried milk and animal liver, which are rich in proteins having buffer action, was less than 50%.

Marked increase in the recovery rate of arsenic was observed when urine was used as a sample. Some reducing agents naturally present in the urine were assumed to affect the recovery rate of arsenic and the assumption was confirmed by later experiment in which various reducing agents were added to the sample solution. The effect of these reducing agents on the recovery rate of arsenic seems to be due to acceleration of ionization of arsenic, $As_2O_3 \rightarrow As^{3+}$. Thus, greater amount of arsenic was found to combine with copper in the presence than in absence of a reducing agent. About 80% of arsenic was recovered when a suitable amount of hydrazine sulfate or hydroquinone was added. This finding gives two advantages in the practical analysis of arsenic in the field of forensic chemistry.

(1) In the pre-test of arsenic, known as Reinsch test, the limit of identification is increased four times or more by adding a small amount of hydroquinone or hydrazine sulfate to the sample.

(2) In practice, the minimum amount of arsenic contained in the biological material is concluded by multiplying a correction factor 100/80 to the value obtained by the present method.

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Summary

A rapid and simple method for the detection of arsenic, initiated by Gettler and Kave, was examined. Under a certain condition recovery of arsenic was 50%, but it was not a constant value as had been reported. The value of 50% was changeable by varying the amount of arsenic present in the sample, amount of hydrochloric acid added, and the period of heating. The mechanism of formation of arsenic stain on copper spiral was discussed. Increase in the recovery of arsenic above 80% was obtained by adding some kinds of a reducing agent.

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