DETERMINATION OF TRIETHYLLEAD, DIETHYLLEAD AND INORGANIC LEAD IONS IN URINE BY HYDRIDE GENERATION-FLAMELESS ATOMIC ABSORPTION SPECTROMETRY

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Abstract: A technique utilizing hydride generation-atomic absorption spectrophotometry was established for the fractional determination of triethyllead (Et_3Pb^+), diethyllead ($\text{Et}_2\text{Pb}^{2+}$), inorganic lead (Pb^{2+}) and total lead (total Pb) in the urine.

The extraction of the target lead compounds from the urine required the pretreatment of urine samples.

Sample solutions were prepared by the use of 0.5 M DL-Malic acid for the generation of Et_3Pb^+ , by the use of 0.75 M H_2O_2 -0.004 M HClO_4 for the generation of $\text{Et}_2\text{Pb}^{2+}$, and by the use of 1.6 M DL-Malic acid-0.05 M $\text{K}_2\text{Cr}_2\text{O}_7$ for the generation of Pb^{2+} ; and sodium borohydride was then added to the so treated sample solutions. The lead hydrides were once fixed in a U-trap cooled with liquid nitrogen beforehand, and the respective hydrides were then fractionated by volatilization; and the fractions were atomized in a quartz cell heated to 1000°C for atomic absorption spectrophotometry.

The recovery rates by this technique from 5 replications proved to be 99.7% for Et_3Pb^+ , 97.3% for $\text{Et}_2\text{Pb}^{2+}$, 91.4% for Pb^{2+} , and 95.9% for total Pb, with the coefficients of variation being 5.6% for Et_3Pb^+ , 7.5% for $\text{Et}_2\text{Pb}^{2+}$, 2.1% for Pb^{2+} and 2.9% for total Pb. The detection limits of this technique also proved to be 0.005 μgPb for Et_3Pb^+ and $\text{Et}_2\text{Pb}^{2+}$ and 0.1 μgPb for Pb^{2+} .

Keywords: Atomic absorption spectrophotometry—Hydride generation—Human urine—Triethyllead—Diethyllead—Inorganic lead

INTRODUCTION

Tetraethyllead is known to be degraded into diethyllead via triethyllead and further into inorganic lead through physical and chemical actions in Nature;¹⁾ and similar degradation of this compound can presumably take place *in vivo*.²⁾ This fact means that the determination of triethyllead (Et_3Pb^+), diethyllead ($\text{Et}_2\text{Pb}^{2+}$) and inorganic lead (Pb^{2+}) in the urine and blood is indispensable for assessment of the severity of tetraethyllead poisoning or biological monitoring of workers at lead processing plants.

Colorimetric methods for the determination of triethyllead have been reported by Henderson and Snyder³⁾ (1961), Bolanowska⁴⁾ (1968) and Imura *et al.*,⁵⁾ (1971) and there are also established microanalytical methods utilizing gas chromatography.⁶⁾ Besides, there are a few reports of colorimetric analysis for diethyllead.³⁾⁷⁾ However, it has been found considerably difficult to apply these colorimetric methods for the analysis of not a few biological samples because of their poor sensitivities.

In the last few years, analytical methods for arsenic⁸⁾ and tin⁹⁾ by means of hydride generation have been presented, making it possible to fractionate different chemical species of metal compounds and determine them with high sensitivities. The present authors¹⁰⁾¹¹⁾ have determined different chemical species of arsenic compounds in biological samples. Furthermore, Jin *et al.*¹²⁾ (1978) have reported an analytical method for inorganic lead utilizing hydride generation.

The present paper offers an improvement on the analytical method for arsenic previously developed by the authors¹⁰⁾ so as to be suitable for the determination of lead, which, as the result of a study of its conditions for the determination of Et_3Pb^+ , $\text{Et}_2\text{Pb}^{2+}$ and Pb^{2+} , has proved capable of determining these lead compounds in the urine with high sensitivities by the use of the same system.

APPARATUS AND REAGENTS

1. Apparatus

The Shimadzu model AA-610S atomic absorption spectrophotometer was used for atomic absorption spectrophotometry. The Hamamatsu TV Co. hollow cathode lamp was used as the lead light source (2833Å). The manifolds for the generation, fixation and atomization of lead hydrides are illustrated in Fig. 1. The U-trap for the fixation

Fig. 1. Sample reaction chamber, U-trap and detector arrangement.

A: gas flow meter, B: generator, C: magnet mixer,
D: water trap, E: ice, F: ice+NaCl, G: liquid N_2 ,
H: U-trap, I: quartz wool, J: quartz cell, K: nichrome
wire

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and volatilization of lead hydrides was a quartz tube 5 mm in caliber, 7 mm in external diameter, and 200 mm in height, packed homogeneously with quartz wool. The quartz cell for the atomization of lead hydrides was 10 mm in caliber, 14 mm in external diameter, and 140 mm in length. A nichrome wire was used for heating the quartz cell to 1,000°C.

2. Reagents

Working standard solution of Et_3Pb^+ : Triethyllead chloride (Alfa Division, Ventron Corp., U.S.A.) was purified with water-alcohol. The lead content of the purified triethyllead chloride was 98% of the theoretical value. This product contained not more than 0.1% inorganic lead,¹³⁾¹⁴⁾ and no diethyllead ions⁷⁾ (not more than 0.1%); and 3.30 mg of this compound was dissolved in 100 ml of water.

Working standard solution of $\text{Et}_2\text{Pb}^{2+}$: Diethyllead dichloride was synthesized from tetraethyllead (Strem Chemicals Inc., U.S.A.) via diethyllead dibenzoate by Heap's method,¹³⁾ and purified with water-alcohol. The lead content of the purified diethyllead dichloride was 99% of the theoretical content. This purified product contained 0.3% inorganic lead and no triethyllead ions (not more than 0.1%⁵⁾); and 3.35 mg of this purified compound was dissolved in 100 ml of water.

Working standard solution of Pb^{2+} : This solution was prepared by dissolving 100 mg of metal lead (purity: 99.99% ; Soekawa Chemical Co., Ltd., Tokyo) in 100 ml of 1N HNO_3 .

These 3 working standard solutions were diluted properly before use.

10% w/v solution of sodium borohydride (NaBH_4): After 1N NaOH solution (E. Merck AG, West Germany) had been adjusted to 0.2N, NaBH_4 was dissolved in this solution. This NaBH_4 solution was prepared before use.

Glyoxal-bis(2-hydroxy-anil) solution⁷⁾ (GHA): This solution was prepared by dissolving 0.24 g of dotite GHA (Dojindo Co., Ltd., Kumamoto) in 100 ml of methanol; and the solution was allowed to stand at room temperature, kept from light, for 24 hours before use.

The acids used were all reagents of super special grade, and all the other reagents were of special grade. Water was subjected to ion exchange after distillation.

PROCEDURE

The working standard solutions of lead compounds (Et_3Pb^+ , $\text{Et}_2\text{Pb}^{2+}$ and Pb^{2+}) or a sample (containing not more than 1 μg as Pb) was transferred into the sample reaction chamber, and one of 3 different buffers (Figs. 3, 4 and 5) was added at a time. The reaction solution was kept to a constant volume. Helium gas was allowed to flow through the chamber at the flow rate of 5 liters/minute to replace the air in it completely. Then, 2 ml of 10% NaBH_4 was added into the sample reaction chamber while cooling the U-trap with liquid nitrogen, and allowed to react for 2 to 5 minutes. The lead hydrides generated in this reaction were fixed to the quartz wool in the U-trap. After the end of the reaction, the U-trap was warmed to room temperature; the lead hydrides

were then volatilized; the lead hydrides so fractionated were atomized in the quartz cell heated to 1,000°C, respectively, and determined with the atomic absorption spectrophotometer. This procedure gave the peaks of lead hydrides illustrated in Fig. 2.

RESULTS AND DISCUSSION

1. *Conditions of buffers for Et_3Pb^+ , $\text{Et}_2\text{Pb}^{2+}$ and Pb^{2+}*

Sodium borohydride, acids and oxidizing agents are indispensable for the conversion of leads into their hydrides. Therefore, the acids and oxidizing agents suitable for

Fig. 2. Corrected retention time of leads.

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analysis of the respective leads were investigated.

1-1. Et3Pb^+

DL-Malic acid buffers were used for the determination of Et3Pb^+ . As illustrated in Fig. 3, DL-malic acid buffers at not less than 0.4 M proved to give a constant high sensitivity; and 0.5 M DL-malic acid buffer was used in the subsequent experiments. The reaction solution required stirring with a magnetic stirrer for not less than 2 minutes.

1-2. Et2Pb^{2+}

The HClO_4 and H_2O_2 system was used for the determination of Et2Pb^{2+} . As depicted in Fig. 4, absorption spectrophotometry was performed by varying the concentration of H_2O_2 while maintaining the HClO_4 solution at 0.004 M. As a result, it was shown that maximum sensitivity was attained with H_2O_2 at 0.75 M. Then, the concentration of HClO_4 was raised while H_2O_2 was maintained at 0.75 M: this experiment proved that the sensitivity was greatly improved, but it was associated with the degradation of

Fig. 3. Effect of D, L-Malic acid concentration on sensitivity to triethyllead.

- 1) Et3Pb^+ ; 0.1 $\mu\text{g Pb}$
- 2) 10%w/v NaBH_4 2 ml

Fig. 4. Effect of hydrogen peroxide concentration on sensitivity to diethyllead.

- 1) Et₂Pb²⁺; 0.1 μ g Pb
- 2) 0.004 M HClO₄
- 3) 10%w/v NaBH₄ 2 ml

Et₂Pb²⁺. For this reason, the concentration of HClO₄ was decided to be 0.004 M. The reaction by the use of this system required 5 minutes.

1-3. Pb²⁺

Pb²⁺ was determined by the use of the DL-malic acid and K₂Cr₂O₇ system. As illustrated in Fig. 5, the concentration of K₂Cr₂O₇ was varied while DL-malic acid was maintained at 1.6 M. This experiment showed that maximum sensitivity was attained with the concentration of K₂Cr₂O₇ between 0.05 and 0.06 M. The reaction by the use of this system required 2 minutes.

2. Influences of coexistent metal ions

Table 1 shows the influences of coexistent metal ions on the determination of Et₃Pb⁺, Et₂Pb²⁺ and Pb²⁺.

It was found that Ag⁺ and Cu²⁺ were the potent interfering ions for the determination of Et₃Pb⁺; Ag⁺, Co²⁺, Cr⁶⁺ and Cu²⁺, for the determination of Et₂Pb²⁺, and Co²⁺

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Fig. 5. Effect of potassium dichromate concentration on sensitivity to inorganic lead.

- 1) Pb^{2+} ; 0.1 μg Pb
- 2) 1.6 M D, L-Malic acid
- 3) 10%w/v $NaBH_4$ 2 ml

and Mn^{2+} , for the determination of Pb^{2+} .

3. Methods for extraction of Et_3Pb^+ , Et_2Pb^{2+} and Pb^{2+} from the urine

The direct determination of total lead in the urine is possible. However, the direct determination of Et_3Pb^+ , Et_2Pb^{2+} and Pb^{2+} required the pretreatment by the following extraction procedures for the interference by the organic substances and inorganic ions present in the urine, while the direct determination of Et_3Pb^+ in the fresh urine is possible.

3-1. Et_3Pb^+ in the urine

After 20 ml of a urine sample was measured into a glass-stoppered centrifuge tube, 4 ml of 60% $HClO_4$, 8 g of $NaCl$ and 10 ml of benzene were added into the tube, and the contents were shaken vigorously for 20 minutes. The tube was then centrifuged at 3,000 r.p.m. for 20 minutes. The benzene layer separated in the top layer was transferred into a separating funnel, and the Et_3Pb^+ in the layer was back-extracted into 1%

Table 1. Effect of diverse metal ions.

Ion	Et3Pb ⁺	Et2Pb ²⁺	Pb ²⁺
Ag ⁺	0.2	<0.1	5
As ³⁺	1000	1000	1000
Be ²⁺	1000	1000	1000
Ca ²⁺	1000	1000	1000
Cd ²⁺	50	10	100
Co ²⁺	1000	<0.1	0.5
Cr ⁶⁺	3	0.1	
Cu ²⁺	0.5	<0.1	5
Fe ³⁺	10	2	5
Hg ²⁺	1000	10	1000
Mg ²⁺	1000	1000	10
Mn ²⁺	1000	5	0.5
Na ²⁺	100	1000	100
Ni ²⁺	1000	10	5
Zn ²⁺	3	5	10

1) Et3Pb⁺, Et2Pb²⁺ and Pb²⁺; 0.5 μ g Pb

2) The values in the above table denote the number of times for each metal ion against the level of each lead when the level of latter was determined to be within 10% of the addition level.

HNO₃. This solution was adjusted to pH 8 with NaOH for use as the sample. In this instance, Et2Pb²⁺ is not extracted with benzene.

3-2. Et2Pb²⁺ in the urine

After measuring 20 ml of a urine sample into a separating funnel, 4 ml of 60% HClO₄, 8 g of NaCl and 10 ml of MIBK were added into the funnel; and the contents were then shaken vigorously for 10 minutes. After the mixture had been allowed to stand for 10 minutes, the MIBK layer containing Et3Pb⁺ was discarded, and the water layer was adjusted to pH 7 with ammonia water. This solution was transferred into another separating funnel, and after the addition of 2 ml of 3% sodium diethyldithiocarbamate (DDTC) and 10 ml of MIBK, the mixture was shaken. The Et2Pb²⁺ in the MIBK layer was then back-extracted into 1% HNO₃. This solution was adjusted to pH 8 with NaOH for use as the sample. In this instance, Pb²⁺ is also extracted. However, because of different reaction conditions for Et2Pb²⁺ and Pb²⁺, only Et2Pb²⁺ is detected.

3-3. Pb²⁺ in the urine

After measuring 20 ml of a urine sample into a separating funnel, the same procedure as described in 3-2 was accomplished; and the MIBK layer containing Et3Pb⁺ was discarded. The water layer was adjusted to pH 9.8 with NaOH, and then transferred

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into another separating funnel. After the addition of 3 ml of the GHA solution and 10 ml of MIBK, the mixture was shaken, and after it had been allowed to stand for 10 minutes, the MIBK layer containing $\text{Et}_2\text{Pb}^{2+}$ was discarded. The water layer was adjusted to pH 7 with NaOH; and 2 ml of 3% DDTC and 10 ml of MIBK were added to extract Pb^{2+} into the MIBK layer. The Pb^{2+} in the MIBK layer was back-extracted into 1% HNO_3 , and this extract was adjusted to pH 8 with NaOH for use as the sample.

4. Recovery rates, accuracies and sensitivities

To 20 ml of a urine sample were added 20 μg Pb of Et_3Pb^+ , 20 μg Pb of $\text{Et}_2\text{Pb}^{2+}$ and 20 μg Pb of Pb^{2+} ; assay samples were then prepared from the mixture by the extraction methods described in 3 above, and assayed by the standard procedure. Table 2 shows the recovery rates and coefficients of variation of the leads from 5 replications. Et_3Pb^+ in the urine was directly determined without the extract procedure. Total lead in the urine was likewise determined directly. $\text{Et}_2\text{Pb}^{2+}$ and Pb^{2+} in the urine were determined by the extraction methods. The recovery rate for Pb^{2+} was 91%, but those for the other leads were as high as not less than 95%. The coefficients of variation were 2.9% for total Pb, 5.6% for Et_3Pb^+ , 7.5% for $\text{Et}_2\text{Pb}^{2+}$, and 2.1% for Pb^{2+} : this technique thus seems to have high reproducibility.

The detection limits of this technique proved to be 0.005 μg Pb for both Et_3Pb^+ and $\text{Et}_2\text{Pb}^{2+}$, and 0.1 μg Pb for Pb^{2+} . The calibration curves for the leads gave a satisfactory linear relation for the levels of not more than 1 μg Pb, respectively.

Even if Et_3Pb^+ , $\text{Et}_2\text{Pb}^{2+}$ and Pb^{2+} are coexistent in a urine sample, the extraction methods described in 3 above permit only the target lead or leads to be determined.

Table 2. Recovery and coefficient of variation of leads from urine.

	Total lead	Et_3Pb^+	$\text{Et}_2\text{Pb}^{2+}$	Pb^{2+}
Average recovery (%)	95.9	99.7	97.3	91.4
Coefficient of variation (%)	2.9	5.6	7.5	2.1

n=5

CONCLUSION

A method utilizing hydride generation and atomic absorption spectrophotometry permitted rapid fractionation and determination of Et_3Pb^+ , $\text{Et}_2\text{Pb}^{2+}$ and Pb^{2+} in the urine with high sensitivities and accuracies by the use of the same system. The determination by this technique of leads in the urine of persons exposed to tetraethyllead permits the chemical species of the leads to be got hold of accurately.

Besides the assay of the urine for Et_3Pb^+ , $\text{Et}_2\text{Pb}^{2+}$, Pb^{2+} and total lead, the assay of many other biological samples seems to be possible by the pretreatment of the samples similar to that of urine samples.

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REFERENCES

- 1) Calingaert, G., Shapiro, H., Dykstra, F.J. and Hess, L. (1948). *J. Am. Chem. Soc.*, **70**, 3902.
- 2) Arai, F., Yamamura, Y. and Yoshida, M. (1981). *Jpn. J. Ind. Health*, to be published. (In Japanese)
- 3) Henderson, S.R. and Snyder, L.J. (1961). *Anal. Chem.*, **33**, 1172.
- 4) Bolanowska, W. (1968). *Br. J. Ind. Med.*, **25**, 203.
- 5) Imura, S., Fukutaka, K., Aoki, H. and Sakai, T. (1971). *Bunseki Kagaku*, **20**, 704. (In Japanese)
- 6) Hayakawa, K. (1971) *Jpn. J. Hyg.*, **26**, 377.
- 7) Imura, S., Fukutaka, K. and Kawaguchi, T. (1969). *Bunseki Kagaku*, **18**, 1008. (In Japanese)
- 8) Braman, R.S., Johnson, D.L., Foreback, C.D., Ammons, J.M. and Bricker, J.L. (1977). *Anal. Chem.*, **49**, 621.
- 9) Braman, R.S. and Tompkins, M.A. (1978). *Anal. Chem.*, **51**, 12.
- 10) Yamauchi, H. and Yamamura, Y. (1979). *Jpn. J. Ind. Health*, **21**, 47. (In Japanese)
- 11) Yamauchi, H., Iwata, M. and Yamamura, Y. (1980). *Jpn. J. Ind. Health*, **22**, 111. (In Japanese)
- 12) Jin, K., Taga, M., Yoshida, H. and Hikime, S. (1978). *Bunseki Kagaku*, **27**, 759. (In Japanese)
- 13) Heap, R., Saunders, B.C. and Stacey, G.J. (1951). *J. Chem. Soc.*, 658.
- 14) Imura, S. and Fukutaka, K. (1965). *Bunseki Kagaku*, **14**, 1167. (In Japanese)