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The Quantitative Spectrographic Determination of Lead in Urine

BY JACOB CHOLAK

Chemical methods for the estimation of lead in biological materials are laborious, time-consuming, and depend on the use of large samples for their accuracy. Moreover, the methods currently employed differ in sensitivity to such an extent that the results are not strictly comparable. The use of the spectrographic method has effected improved results and, consequently, the detailed procedure is presented here together with a discussion of its sensitivity and accuracy.

Equipment and Method

The spectrograph used was a Bausch and Lomb Littrow-type quartz spectrograph, capable of taking the region from λ 2100 to λ 8000 Å. on three 25-cm. plates. Its dispersion was such that elements like iron, with many spectral lines, did not mask the lines of other elements.

An arc between graphite electrodes (7 mm. Acheson regraphitized spectrographic electrodes whose spectra did not give a lead line) was adopted as the means of excitation, because its high temperature adapted it well to the detection of very small quantities of metal;[1] detectability was further enhanced by placing the sample in a deep crater of the negative electrode.[2] With 10 amperes and 60 volts, four-minute exposures were found to be adequate.

The quantity of lead excited by the arc was determined by inserting a revolving logarithmic sector in the light beam between the arc and the spectrograph slit.[3] This procedure made it possible to take the altitude of the lead line on the photographic plate instead of its intensity, as the measure of the amount of lead present in the arc. The measurement of the line altitude was facilitated by the use of a wide slit (0.05 mm.); but it then became imperative to cut down the light intensity, especially of the background, by inserting an ordinary rotating sector between the arc and the logarithmic sector; the latter was cut according to the formula $\log \alpha = b \times 1$ to expose a slit 15 mm. long.[3,4]

The most persistent lead line (λ 4057.8 Å.) could not be used because it lay in a region covered with cyanogen bands, consequently the line λ 2833.2 Å. was employed. Other lines such as λ 2614.3 Å. do not appear with lead concentrations smaller than 0.10 mg. per 100 cc.

The amount of lead present in the samples was determined as follows. Known amounts of lead and a constant amount of bismuth were added to solutions having approximately the same salt composition as normal urine. These standard solutions were introduced into the arc, their spectra were photographed, the altitudes of the lead line λ 2833.2 Å. and of the bismuth line λ 2898.1 Å. were measured and their ratios were plotted against their lead content. (Bismuth was adopted as the "internal standard"[4,5,6,7] because it is rarely present in urine in significant amount, and because it vaporizes in the arc under the same conditions as lead. Chromium, tin, zinc and cadmium were abandoned after unsatisfactory trials.) The urine sample containing an unknown quantity of lead was provided with the same internal standard, introduced into the arc, and photographed on the same plate; the altitudes of the lead and bismuth lines were measured, and by means of their ratio the amount of lead present in the sample was read from the previously constructed curve. On each plate three known lead solutions were used, containing 0.01, 0.10 and 0.20 mg. of lead per 100 cc., respectively, 5 mg. of bismuth, and ten times the concentration of the various inorganic salts usually present in the urine of an average person on an average diet;[8] 0.2 cc. of solution (corresponding to 2 cc. of normal urine) was arcked. Similarly, the solution of the urine ash was made up in such a way that 0.2 cc. would correspond to 2 cc. of urine. The concentration of the inorganic salts can vary 50% above or below the normal without producing significant variations in the intensities of the lead and bismuth lines.

One may see from Fig. 1 why it is essential to photograph both the known and the unknown solutions on the same plate. The heavy straight line represents the mean slope of the curve obtained when the statistical means of the intensity ratios of the standard solutions from 66 plates were plotted against the logarithms of the concentration of lead per liter of urine. Of the plates thus analyzed, only 25% had curves the slopes of which fell on the mean, and only 70% fell in the area bounded by the lighter solid lines representing the plus and minus deviations from the mean; the remaining 30% fell either above or below the deviations from the mean. The slopes of curves on two such plates are indicated by the broken lines marked No. 466 and No. 489.

Experimental Technique

To 100 cc. of urine in a 200-cc. silica dish (cleaned by repeated washing with hot nitric acid and repeated rinsing with hot doubly distilled water) 7.0 cc. of redistilled nitric acid[9] is added and the contents of the dish are evaporated to dryness on a hot-plate. The dish is covered with a quartz-plate and placed in a muffle furnace where ashing

(1) Nitchie, *Ind. Eng. Chem., Anal. Ed.*, **1**, 1 (1929).

(2) Mannkopff and Peters, *Z. Physik*, **70**, 444 (1931).

(3) Scheibe and Neuhäusser, *Z. angew. Chem.*, **41**, 1218 (1928).

(4) Gerlach and Schweitzer, "Foundations and Methods of Chemical Analysis by the Emission Spectrum," Adam Hilger, Ltd., London, 1929.

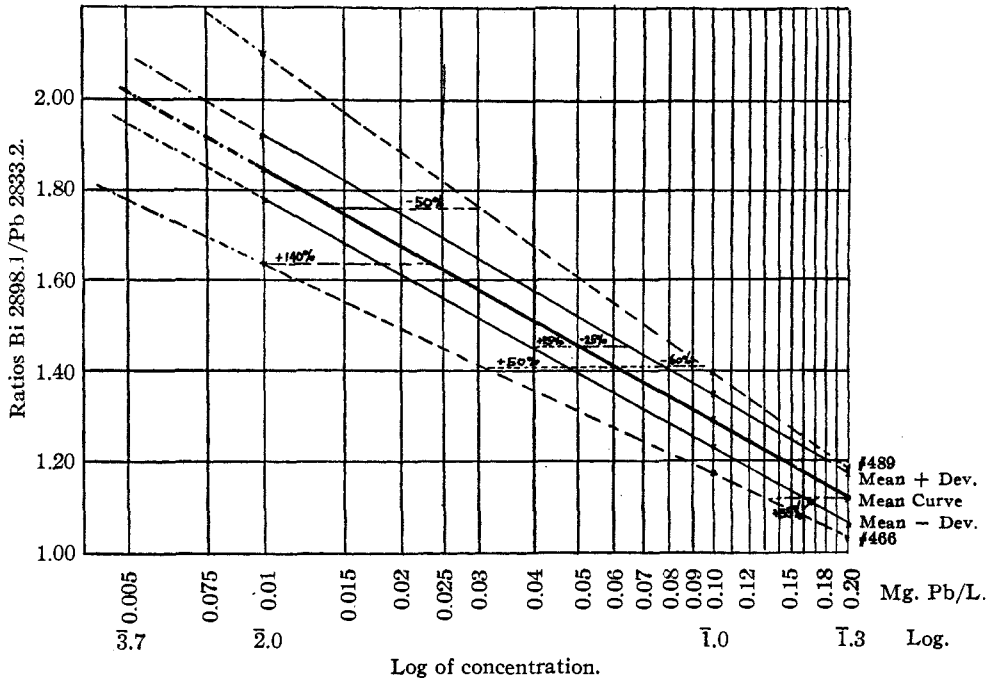
(5) Lundegårdh, "Die quantitative Spektralanalyse der Elemente," Gustave Fischer, Jena, 1929.

(6) Nitchie and Standen, *Ind. Eng. Chem., Anal. Ed.*, **4**, 182 (1932).

(7) Twyman and Hitchen, *Proc. Roy. Soc.* (London), **A133**, 72 (1931).

(8) Mathews, "Physiological Chemistry," 5th ed., 1930, p. 730.

(9) Only the middle fraction of the acid obtained by distilling the reagent C. P. nitric acid from a silica still is used. Freshly distilled acid is preferable since acid kept in Pyrex bottles for several months takes up as much as 0.01 mg. of lead per liter from the glass. (The glass contains about 0.08 mg. per gram.)



Constants for mean curves (66 plates). 0.01 mg. = 1.84 ± 0.07 deviation. 0.10 mg. = 1.29 ± 0.06 deviation. 0.20 mg. = 1.11 ± 0.06 deviation.

Fig. 1.—Showing the mean slope and the standard deviation of curves obtained from a series of observations made on the standard solutions (sixty-six plates), together with the slopes of two individual curves which fall outside the limits of the standard deviation.

is accomplished at a temperature controlled by pyrometer so as not to exceed 500°. After cooling, the ash is taken up in 2 cc. of redistilled nitric acid and 3 or 4 cc. of doubly distilled water, warmed if necessary, and washed into a clean graduated 15-cc. Pyrex centrifuge tube, the final volume being kept below 9.5 cc. The solution of ash is tested spectrographically for the presence of bismuth by arcking 0.1 cc. on a graphite electrode. In the absence of bismuth in concentrations in excess of 0.10 mg. per liter, as determined by the absence of its line at λ 2898.1 Å., 0.5 cc. of a bismuth solution (1 cc. = 1 mg. Bi) is added and the volume is made up to 10 cc. with doubly distilled water. (If the preliminary test reveals the presence of bismuth in the sample, one of two courses may be followed. The bismuth may be removed as the oxychloride before adding the desired amount of bismuth as the "internal standard." This procedure involves some loss of lead and is therefore somewhat unsatisfactory. More reliable results are obtained by ignoring the bismuth line produced by the sample, thereby dispensing with the "internal standard" for the specific sample, and using the average of the bismuth lines in the standards as the standard of intensity. Such steps are rarely required, however, on account of the infrequent occurrence of bismuth in urine in amounts sufficient to produce the line at λ 2898.1 Å.)

The solution to be tested and each of the standard solutions are introduced into the 3 × 10 mm. craters of their respective graphite electrodes in 0.2-cc. amounts, by the repeated addition from capillary pipets and the subsequent evaporation of 0.1-cc. portions. The electrodes are dried in an oven for several hours (preferably overnight) after which they are used as the negative poles of the arc and the spectra of sample and standards are photographed on a 10 × 25 cm. Eastman No. 33 plate. The spectral region taken is that between λ 2500 and λ 3400 Å. After developing, fixing and drying the plate, the lengths of the lead line at λ 2833.2 Å. and of the bismuth line at λ 2898.1 Å. are measured to 0.1 mm. with a magnifying eyepiece carrying a 20-mm. scale divided into tenths. A curve is plotted from the data obtained on each plate.

Accuracy and Limitations of the Method

To test the accuracy of the method, several series of samples were prepared by adding known quantities of lead to solutions of urine salts equivalent to one-liter samples of urine. The quantities of lead added were such that they fell below the quantity contained in the highest urine standard, 0.20 mg. per liter. The results are recorded in Table I, from which it can be seen that the error in dealing with amounts of lead from 0.001 to 0.010 mg. per 100 cc. (equivalent to 0.01–0.10 mg. per liter) is about 0.001 mg., which corresponds to 0.01 mg. of lead per liter. For amounts greater than 0.01 mg. of lead (corresponding to more than 0.10 mg. per liter), the error is somewhat greater. Reduction in the size of the sample does not increase the relative error—samples as small as 10 cc. can be employed

provided they contain at least 0.0001 mg. of lead (equivalent to 0.01 mg. per liter). This offers a distinct advantage over present chemical methods, in which the sensitivity of detection is limited by the absolute amount of lead present. Slight losses of lead result, unavoidably, from the use of chemical methods which require the separation of lead, by means of its salts, from interfering metals such as copper, iron, tin and aluminum, which are present in biological materials.[10] (For example, the maximal loss entailed in the use of one such method, which we have described,[11] amounts to 0.07 mg. per sample.) By eliminating the steps necessary for the separation of lead from other metals, the spectrographic method avoids these losses.

TABLE I

Pb added, mg.	0.01	0.01	0.01	0.01
Pb found, mg.	.02	.01	.01	.01
Pb added, mg.	.05	.05	.05	.05
Pb found, mg.	.06	.06	.07	.07
Pb added, mg.	.10	.10	.10	.10
Pb found, mg.	.09	.10	.09	.10
Pb added, mg.	.15	.15	.15	.15
Pb found, mg.	.11	.12	.15	.12

The limits of lead concentrations accurately measurable by this technique lie between 0.01 and 0.20 mg. per 100 cc. These limits are demonstrated by Fig. 2, in which the change in ratios of the bismuth and lead lines with the change in lead concentration is expressed in a curve. Below 0.01 mg. the ratios become indistinguishable because the threshold of sensitivity of the plate has been reached. Above 0.20 mg. of lead per 100 cc., the curve becomes asymptotic to its axis, because the intensity of the lead line at λ 2833.2 Å. reaches a point beyond which increasing concentrations of lead fail to produce correspondingly increasing degrees of blackness of the photographic plate. The difficulty due to high concentrations of lead may be overcome by diluting the sample. When this is done it is advisable to add such amounts of salts as will keep the concentration of the various salts equivalent to that in the standards. Most urine samples, however, will not require dilution. (If groups of samples should be encountered which regularly contain more than this limit, another line such as the one at λ 2614.3 Å. might be chosen and its range of applicability similarly determined.)

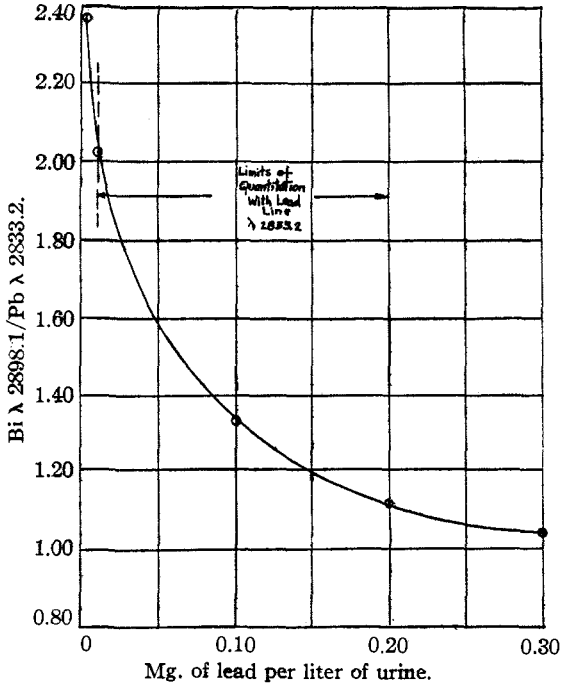


Fig. 2.—Variation in the ratio of intensities of bismuth and lead lines with variations in lead concentration.

Another factor in the establishment of the lower limit of quantitation is the fact that lead-free standards cannot be prepared. The salts used in the preparation of the standards, though of the highest purity, always showed appreciable quantities of lead. Such lead can be removed partially by precipitation with hydrogen sulfide, but despite this measure we have found it impossible to prepare standards which contain less than 0.001 mg. of lead per 100 cc. of solution.

With the above limitations the method has been found to be applicable to the measurement of lead in all kinds of biological material, provided standards approximating the composition of the samples are prepared.

The Limits of Qualitative Detection of Lead.—The sensitivity of the spectrographic detection of lead varies with the composition of the materials introduced into the arc. When using 0.2-cc. portions of pure lead salt solutions, we have found that almost 0.10 mg. of lead must be present in 100 cc. of the solution in order to produce the lead line at λ 2833.2 Å. On the other hand, solutions containing urinary salts and minute amounts of lead (prepared by adding known amounts of lead to solutions equivalent in salt content to average samples of urine), produce this line when 0.2-cc. portion of dilutions repre-

(10) Taylor, *Proc. Roy. Soc. New South Wales*, **61**, 315 (1928).
(11) Kehoe, Thamann and Cholak, *J. Ind. Hyg.*, **15**, 257 (1933).

senting as low as 0.0002–0.0003 mg. of lead per liter are arcked (equivalent to 1 part per 3 to 5 billion of urine). Moreover, 0.1-cc. portions of fresh urine always give a lead line, and since some of these in our experience contain as little as 0.01 mg. of lead per liter, the indicated sensitivity without resort to other than the routine procedures of the method as described, is of the order of 1 part per 100 million.

Summary

1. A method is described for the quantitative spectrographic determination of minute quantities of lead in urine.

2. This method is suitable for the determination of lead in samples so small that a chemical method cannot be used.

3. Concentrations of lead in urine as low as 1 part per 100 million can easily be detected. Concentrations which can be measured accurately by the technique described lie between 0.01 and 0.20 mg. per liter.

4. For amounts of lead up to 0.10 mg. per liter the accuracy of the method is ±0.01 mg. per liter. For amounts above 0.10 and up to 0.20 mg. per liter the error is somewhat greater.

5. The method may be made applicable to the measurement of lead in all kinds of biological material.

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The Reaction of Alkyl Sulfates and *p*-Toluenesulfonates with the Grignard Reagent and the Preparation of Dialkylmagnesium Compounds

BY C. M. SUTER AND HOWARD L. GERHART

This investigation was a result of the observation that in the reaction of equimolecular quantities of *n*-butyl sulfate and *n*-butylmagnesium bromide there was no appreciable decrease in the amount of basic magnesium. This reaction was first[1] formulated as

$$n\text{-}C_4H_9MgBr + (n\text{-}C_4H_9)_2SO_4 \longrightarrow n\text{-}C_4H_9MgOSO_3C_4H_9 + n\text{-}C_4H_9Br$$

but further investigation has shown that only a small percentage of the basic magnesium is present in the precipitate formed in the reaction. If any large amount of *n*-butylmagnesium *n*-butyl sulfate is produced it undergoes disproportionation.[2]

$$2n\text{-}C_4H_9MgOSO_3C_4H_9 \longrightarrow (n\text{-}C_4H_9)_2Mg + Mg(n\text{-}C_4H_9SO_4)_2$$

The results of some preliminary experiments comparing the behavior of *n*-butyl sulfate, methyl sulfate and methyl *p*-toluenesulfonate with typical Grignard reagents are listed in Table I. In these experiments only the amount of basic magnesium remaining in solution was determined.[3]

Benzene, *n*-butylbenzene and *n*-butyl bromide were isolated from the phenylmagnesium bromide–*n*-butyl sulfate reaction mixture by fractionation of the ether solution after hydrolysis. Fractionation before hydrolysis gave *n*-butyl bromide and *n*-butylbenzene.

TABLE I

Magnesium compound	Ester	Decrease in basic magnesium, % 1 mole ester 15 min. reflux	2 moles ester 2 hrs. reflux
n-C_4H_9MgBr	$(n\text{-}C_4H_9)_2SO_4$	<5	30
n-C_4H_9MgCl	$(n\text{-}C_4H_9)_2SO_4$	<5	43 (3 hrs.)
C_6H_5MgBr	$(n\text{-}C_4H_9)_2SO_4$	36	58
n-C_4H_9MgBr	$(CH_3)_2SO_4$	18	66
C_6H_5MgBr	$(CH_3)_2SO_4$	52	100
C_6H_5MgBr	$CH_3OSO_2C_7H_7$	42	100

In Table II are given more complete data for the reaction of a number of esters with two Grignard reagents. Since the chief reaction involves the formation of an alkyl bromide, one mole of ester was used per equivalent of halogen present in the Grignard solution. The phenylmagnesium bromide was 0.63 *N* in basic magnesium[3] and 0.68 *N* in bromide ion; the *n*-butylmagnesium bromide, 0.59 *N* and 0.62 *N* in these components. A volume of solution containing 0.03 equivalent of halogen was used for each reaction. The reaction time was one hour except where otherwise specified.

The figures given in the fourth column represent a purely arbitrary method of expressing the variation in reactivity of the alkyl sulfates and

(1) Suter and Gerhart, THIS JOURNAL, **55**, 3496 (1933).

(2) This paper as originally submitted for publication contained a discussion of the structure of the Grignard reagent in the light of its reaction with alkyl sulfates. The subsequent appearance of the work of Cope, *ibid.*, **56**, 1578 (1934), which contains an excellent discussion of this topic makes its treatment here unnecessary.

(3) Gilman, Zoellner and Dickey, *ibid.*, **51**, 1576 (1929).