Determination of Lead in Fish Tissues by Electrothermal Atomic Absorption Spectrometry Using Bismuth Nitrate as Chemical Modifier

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(Received January 12, 1993)

The electrothermal atomic absorption spectrometry has been used to develop a routine and interference-free method for the determination of lead in fish tissues. Bismuth(III) nitrate was used as a chemical modifier. The analytical curves of lead were studied by using pyrolytically coated graphite tubes. The instrumental conditions, electrothermal atomizer programme, concentration of the modifier, the calibration and addition curves were studied. A comparative study was also made between $Pd(NO_3)_2$ and $Bi(NO_3)_3$ as chemical modifiers. Not only a better limit of detection value has been obtained in comparison to $Pd(NO_3)_2$ modifier but also a lower atomization temperature, hence an extension of the life period of the graphite tube compared to magnesium nitrate+ammonium dihydrogen phosphate mixed modifier could be achieved in the present method.

Lead is a wide spread non-essential metal that is highly toxic to humans and animals.[1,2] There are several investigations on the toxic effects and bioaccumulation of lead in fishes.[3,4] Flame atomic absorption spectrometry (AAS) method is not suitable to determine lead directly at ppb levels[5] and electrothermal AAS is increasingly the method of choice.[6] Chemical modifiers have been shown to be important for the successful determination of lead in many matrices.[7] Most workers have used $NH_4H_2PO_4$ or $(NH_4)_2HPO_4$ for the said purpose.[8—13] It has been found to be preferable to use a mixture of $Mg(NO_3)_2$ and $NH_4H_2PO_4$ as a mixed matrix modifier for lead in many cases.[10] In several matrices lanthanum salts have also been used.[14—16] Regan and Warren[17] found 1% ascorbic acid to be useful in removing the effect of the alkaline earth metals on lead. Tominaga and Umezaki[18,19] found that ascorbic acid reduced chloride interferences on lead. The same modifier was also effective for determination of lead when sulphuric acid was used for wet oxidation of the sample.[20,21] Ammonium nitrate has been used as a matrix modifier by Halliday et al.[22] Xiao-quan and Zhe-ming[23] used Pd or Pt in HNO_3 as a matrix modifier for the determination of lead. A mixed modifier consisting of vanadium+palladium showed an excellent stabilization for various elements including lead.[24]

Until recently, the choice of an appropriate modifier has been primarily guided by the intuition and knowledge of the empirical part of inorganic chemistry. An attempt has been made by Tsalev et al.[25] to find out some guidelines for selecting and predicting an appropriate modifier. An analyte being isomorphous with the matrix modifier would be retained stronger within the crystal lattice of the modifier. According to Vlasov[26] criteria there exists a perfect of good isomorphism between lead and bismuth. With this end in view, in the present work lead has been determined in fish tissues using bismuth nitrate as a chemical modifier.

Experimental

Apparatus. A Shimadzu Model AA-646 atomic absorption spectrometer equipped with a GFA-4A electrothermal atomizer was used. A Shimadzu lead hollow cathode lamp worked as the resonance line source. Sample aliquots were injected into the furnace with a Shimadzu AIU-1 autosampler. Shimadzu pyrolytic coated graphite tubes were used throughout the work. The instrumental conditions and furnace programme have been shown in Tables 1 and 2 respectively. Argon was used as a purge gas. In the atomization step argon-flow was programmed to zero.

Reagents. A stock solution of Pb(II) (5490 μg ml) was prepared by dissolving lead(II) nitrate (E. Merck, Germany) in 0.1 M HNO_3 in a 250 ml volumetric flask and filling upto the mark with 0.1 M HNO_3, from which working solutions were made by serial dilution (1 M=1 mol dm^{-3}). A 0.7% bismuth(III) nitrate (E. Merck, Germany) solution was used as a chemical modifier. All other reagents used were of analytical reagent grade. Seronorm Trace Elements Urine (Batch No. 009024), Nycomed Pharma AS, Oslo was used as a reference material.

General Procedure. The stock solutions were diluted to the appropriate concentration with doublly distilled water in a 10 ml volumetric flask containing 0.2 ml of 0.7% $Bi(NO_3)_3$ solution. 10 μl of the resulting solution was injected into the furnace. The calibration curve was constructed using aqueous standard solution mixed with 0.7% of $Bi(NO_3)_3$

Table 1. Instrumental Parameters for Lead

Wave length	283.3 nm
Lamp current	10 mA
Slit width	0.19 nm
Mode	Background correction (D_2 lamp)
Sample volume	10 μl

Table 2. Temperature Programme for Electrothermal Atomizer

Stage	Temp(°C)	Time(s)	Gas(L min^{-1})	Mode
Drying	120	20	1.5	Ramp
Ashing	300	20	1.5	Step
Atomization	1300	4	0.0	Step
Clean	2000	5	1.5	Step
Cool	30	10	1.5	Step

solution.

Analysis of Biological Samples. Collection, storage, and solution preparation of fish tissues (*Anabas testudineus*) were done according to Pastor et al.[27] Definite quantity of each tissue was processed to colourless solution by wet digestion method. For this the different tissue samples were treated separately with concd HNO_3 (5 ml). If the colour of the solution still persisted, it was further treated with 5—7 drops of H_2O_2. Finally the stock solution was made from the resulting mixture. The reference material i.e., Seronorm Trace Elements Urine (5 ml) was taken into solution. Before running in ETAAS all the solutions were taken in 10 ml volumetric flasks and the volumes were made upto the mark after adding 0.2 ml of 0.7% $Bi(NO_3)_3$ solution. 10 μl sample was used in each case.

Results and Discussion

Optimization of Furnace Programme. Temperatures and times of various stages of drying, charring, and atomization were investigated and optimized to achieve the best working condition (Figs. 1 and 2). The pyrolytic graphite tubes were cleaned off at 2000 °C for 5 s twice after each run. Nevertheless, in the present method a lower atomization temperature (1300 °C) could be used in comparison to a widely used method (1800 °C).[6]

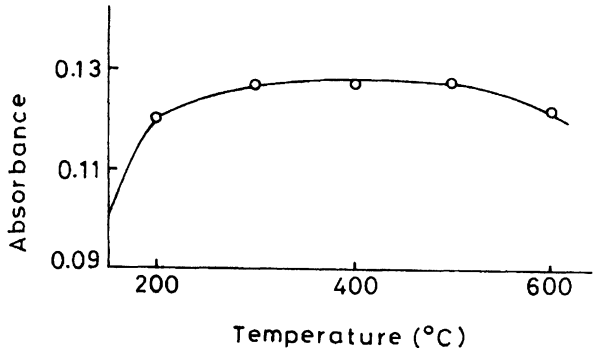

Fig. 1. Peak absorbance values of lead as a function of ashing temperature when atomizing at 1300 °C.

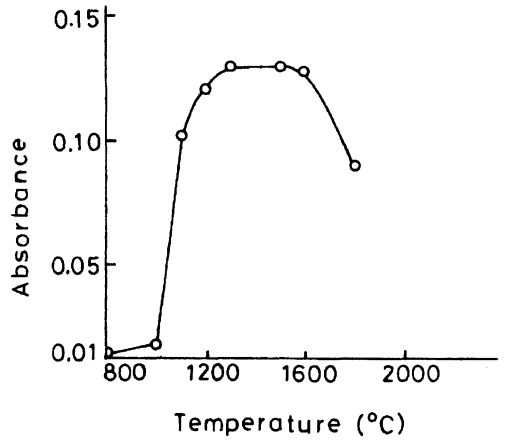

Fig. 2. Peak absorbance values of lead as a function of atomization temperature after ashing at 300 °C.

Choice of Chemical Modifier. The effect of the few compounds of $Ba(NO_3)_2$, $Bi(NO_3)_3$, $Cd(NO_3)_2$, $IrCl_3$, $Pd(NO_3)_2$, $(NH_4)_2MoO_4$, Na_2WO_4, $Hg(NO_3)_2$, and $Ni(NO_3)_2$ was investigated as chemical modifier. According to Vlasov[26] there are possibilities of perfect isomorphism between pairs of the analyte (Pb) and matrix elements like Ba, Bi, Cd, Ir, Pd, Mo, or W and moderate isomorphism between pairs of the lead and matrix elements like Hg or Ni. So the effects of the various metals as modifiers were investigated. The results are presented in Fig. 3. $Bi(NO_3)_3$ and $Pd(NO_3)_2$ show comparable and better results than the others. So $Bi(NO_3)_3$ was selected in the present work. Concentration of $Bi(NO_3)_3$ was varied and optimized to the 0.7%; 0.2 ml of which was sufficient.

Spectral Interferences. Early publications on the determination of lead in various samples reported numerous interferences both spectral and non-spectral, which in part were dependent on the volatility of lead.[7,28] So different foreign ions were tested which might be present in biological samples. It was found that iron, magnesium and phosphate caused severe background interferences when present at high concentrations in the sample solution, resulting in an absorption signal depression. Such background absorption could be eliminated by employing matrix modification (Fig. 4). Therefore for the determination of lead in biological samples bismuth was employed as the matrix modifier.

Analytical Figures of Merit. To obtain the calibration graph a series of standard solutions with the optimum amount of the chemical modifier was subjected to the electrothermal atomizer programme. The calibration graph was linear upto 80 ng ml^{-1}. The slope value of the calibration graph (sensitivity)[29] is 0.5 ml ng^{-1}. The limit of detection (LOD)[29] defined as three times the standard deviation (σ_b) for consecutive measurements of the signal from a reagent blank (3 σ_b) was 0.9 ng ml^{-1}. This LOD value is better than that (1

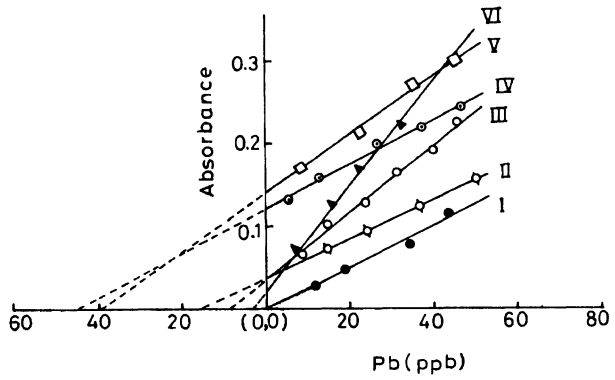

Fig. 3. Effect of various matrix modifiers upon lead absorption signal. Curve I: Standard lead only; Curve II: lead+tungstate; Curve III: lead+barium; Curve IV: lead+bismuth; Curve V: lead+palladium; Curve VI: lead+cadmium.

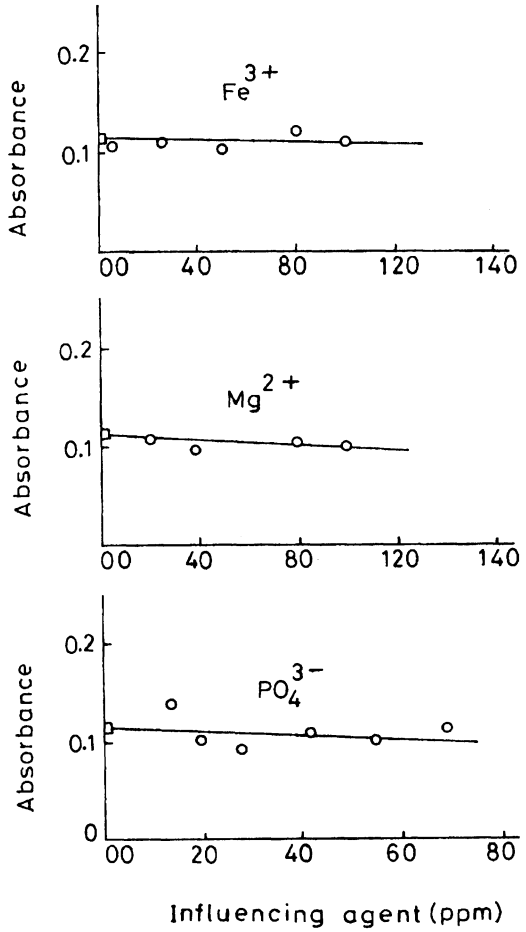

Fig. 4. The influence of iron, magnesium, and phosphate on the determination of lead (□ on the absorbance axis indicates 35 ppb Pb only; -o-o- indicates 35 ppb Pb+various amounts of an influencing agent+0.2 ml 0.7% bismuth nitrate).

Table 3. Analytical Results of Pb in Fish Tissue and Reference Material

Description of samples	Lead found (ppm) Without modifier	with modifier
Fish tissue		
Kidney	2.81	3.40[a)]
Liver	3.67	4.26[a)]
Brain	5.93	7.26[a)]
Stomach	7.85	9.70[a)]
Seronorm Trace Elements : Urine[b)]	0.09	0.18[c)]

a) in $\mu g\,g^{-1}$, b) Certified value-0.19 $\mu g\,ml^{-1}$, c) in $\mu g\,ml^{-1}$.

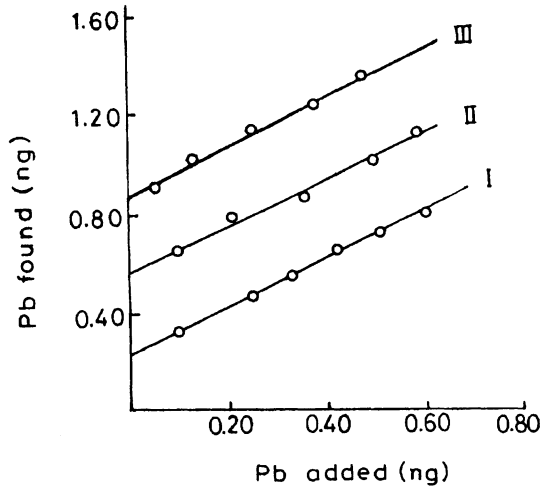

Fig. 5. Recovery of spiked lead. Curve I (Fish kidney): Y=1.00X+0.24; Curve II (Fish brain): Y=0.96X+0.56; Curve III (Seronorm reference): Y=0.96X+0.86.

$ng\,ml^{-1}$) reported by using $Pd(NO_3)_2$ modifier. Nevertheless, limit of quantitation (LOQ)[29] defined as 10 times the standard deviation ($10\sigma_b$) was 2.8 $ng\,ml^{-1}$. The relative standard deviation or coefficient of variation of the method including instrumental and matrix factors of one of the tissue samples (kidney) was determined for 10 replicate analyses and the result was 4.8%. In order to verify the accuracy of the method a reference material, Seronorm Trace Elements Urine was used; the results for lead obtained by using the present method (183.6±3.9 $ng\,ml^{-1}$) were in good agreement with certified value (190 $ng\,ml^{-1}$).

Application. The proposed method was applied for determination of lead in fish tissue samples viz. kidney, liver, brain, and stomach of *Anabas testudineus*. A reference biological material viz. Seronorm Trace Elements Urine was also analysed by the proposed method. The results are presented in Table 3. The feasibility of the method was examined by recovery studies of lead added to different biological samples. A good correlation was obtained between lead added and found: the line of best fit for the different matrices had a slope value close to 1.0 (Fig. 5).

Conclusion

The concept of isomorphism between pairs of chemical modifier (Bi) and analyte (Pb) has been involved in electrothermal AAS in view of the behaviour of isomorphous pairs[26] during thermal behaviour. Thus the present method using $Bi(NO_3)_3$ as a chemical modifier is effective and can be successfully applied for determination of lead in biological samples. There are at least two novelties of the present method viz. a better limit of detection value in comparison to $Pd(NO_3)_2$ modifier[23] and a lower atomization temperature, hence an extension of the life period of the graphite tube compared to $Mg(NO_3)_2+NH_4H_2PO_4$ mixed modifier[10] or lanthanum modifier.[14—16]

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