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RESEARCH ARTICLE

Development of method for the determination of lead in teeth samples by flow injection hydride generation atomic absorption spectrometry in the presence of $K_3Fe(CN)_6$, HNO_3 and $NaBH_4$

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Development of method for the determination of lead at teeth samples by flow injection hydride generation atomic absorption spectrometry in the presence of $K_3Fe(CN)_6$, HNO_3 and $NaBH_4$

Summary

Flow-injection hydride generation (FI-HG), coupled with atomic absorpsion spectrometry (AAS) was used for the determination of lead (Pb) at teeth samples. Plumbane (PbH_4) from acid solution was generated by the reaction of potassium ferricyanide ($K_3Fe(CN)_6$) and sodium tetrahydroborate ($NaBH_4$). Forty teeth samples were analyzed and Pb levels were found between 0.66 and 15.60 μg g^{-1}. Characteristic sensitivity and limit of detection value were found to be 0.6 μg L^{-1} and 0.4 μg L^{-1} respectively. The precision of the method in terms of relative standard deviation, R.S.D%, for 11 replicate measurements of blank signal was found as 3.8%. External calibration graph was linear between 10- 60 μg L^{-1} Pb at 283.3 nm wavelength. The accuracy of the method was tested using standard reference material (SRM), NIST-SRM 1468 Bone Meal. The results were in good agreement at 95% confidence level statistically.

Key Words: *Lead hydride, plumbane, tooth, atomic absorption spectrometry*

$K_3Fe(CN)_6$, HNO_3 ve $NaBH_4$ varlığında akışa enjeksiyonlu hidrür oluşturmalı atomik absorpsiyon spektrometri ile diş numunelerinde kurşun tayini için yöntem geliştirilmesi

Özet

Akışa enjeksiyonlu-hidrür oluşturma (FI-HG) sistemi ile birleştirilmiş atomik absorpsiyon spektrometri (AAS) diş numunelerinde kurşun (Pb) tayini için kullanılmıştır. Plumbane (PbH_4), asitli ortamda potasyum ferrisiyanür ($K_3Fe(CN)_6$) ve sodyum tetrahidroborat ($NaBH_4$) tepkimesi sonucu oluşturulmuştur. 40 diş numunesi analiz edimiş ve Pb düzeyleri 0.66 ile 15.60 μg g^{-1} aralığında bulunmuştur. Karakteristik duyarlık ve tayin sınırı sırasıyla 0.6 μg L^{-1} ve 0.4 μg L^{-1} olarak bulunmuştur. Yöntemin kesinliği, bağıl standart sapma cinsinden, R.S.D%, kör sinyalinin ard arda 11 ölçümü sonucu %3.8 olarak bulunmuştur. Dış kalibrasyon grafiği, 283.3 nm dalga boyunda 10- 60 μg L^{-1} Pb derişim aralığında doğrusaldır. Yöntemin kesinliği standart referans madde (SRM), NIST-SRM 1468 Bone Meal kullanılarak test edilmiştir. Sonuçlar %95 güven aralığında istatistiksel olarak uyumludur.

Anahtar Kelimeler: *Kurşun hidrür, plumbane, diş, atomik absorpsyon spektrometri*

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INTRODUCTION

Some elements like iron, copper, zinc, manganese, cobalt, nickel, selenium, and fluorine are considered essential elements for life. Few elements, such as arsenic, cadmium, mercury and lead are present in human body due to the environmental pollution (1,2). Some of these elements are natural component of soil, water, vegetation, and air (3,4). Increasing environmental pollution has given rise to concern about the accumulation of elements in human body. Lead is the one of these elements known to be poison (4,5), which has severe effects upon the central nervous system, particularly upon children (3,6). Lead is absorbed into the bloodstream, from where, especially in the case of inorganic lead, it is distributed to soft tissue or calcified tissues like bones and teeth (95% in bones and teeth) for a long period of time (4,7). So trace or ultra trace determination of lead in various biological samples continues to be a focus of interest.

Tooth is a biological material which is classified as "hard tissue" like bone (1,2). The pretreatment procedures for hard tissues are more complicated than soft tissues (e.g organs) or biological fluids (e.g blood, urine, sweat). Pretreatment procedures (washing, drying, homogenization, filtration, centrifugation, digestion etc.) vary according to the nature of the samples, analysis method, the elements to be determined and their concentration levels prior to other processing and detection (8). For example, analytes are extracted from the solid biological matrix in the form of solution using ultrasonic leaching in dilute acidic solutions (9,10) microwave-assisted in wet digestion (11,12) or heat (13). Efficient decomposition of the biological material is required in order to attain suitable recovery. This is carried out by digestion with HNO_3/HF (14), HNO_3 (11) or HNO_3/H_2O_2 (12,15) to release the total element from the biological sample. Analysis method has to be chosen according to the elements to be determined and concentration of elements.

Hydride generation (HG) is one of the most popular sample introduction technique with excellent matrix separation capability (16,17). It can be applied for the determination of elements (e.g As, Sb, Bi, Pb, Sn, Se, Ge, and Te) that form relatively stable and volatile hydride (8,16).

The limited sample amount of many biological materials is an important problem for researchers to develop analytical procedures (8). Flow injection (FI) system reduced the sample and reagent consumption. Moreover all manipulations are done automatically in an enclosed environment, thus the risk of sample contamination are minimized (8).

Atomic spectrometry is the most commonly used method to determine trace elements and generally requires the analyte to be in solution. Coupling flow injection with HG-AAS for the determination of hydride forming elements in a variety of samples offers number of advantages (16). The direct transfer of the volatile species to any atomizer (quartz, metallic or graphite tube) improves the sensitivity (8,16). The other advantages of generation of volatile species are low cost and detection limit, efficient separation of the analyte from the matrix, good precision and high sample throughput (16). Seperation of analyte from the matrix results in reducing the matrix related interferences and higher efficiency in sample introduction as compared with conventional nebulization (18).

Atomic absorption spectrometry can be coupled with HG which is one of the most sensitive technique for the determination of lead at trace levels (19). Lead is one of the hydride forming element but hydride generation yield and thermal stability are low when compared with other hydride forming elements (20,21). Generation of lead hydride depends largely on the experimental conditions, and on the nature of the acids and oxidizing agents used. However, it has been demonstrated that the use of acidic oxidizing media increases the reaction rate and sensitivity (22). Most common oxidizing agents used in presence of various organic (lactic (22), tartaric (23), malic (24) and oxalic (25), citric (26) and acetic (26) and mineral acids (HCl (27), HNO_3 (26), $HClO_4$ (28), and H_2SO_4 (28)) are hydrogen peroxide (29), ammonium or sodium peroxodisulphate (24), potassium dichromate (22) and potassium ferricyanide (14, 27, 30). Highest sensitivity has been reported with a relatively mild oxidant such as $K_3Fe(CN)_6$ which presumably oxidize the lead to the +4 state (27). Generation of plumbane with a mixture of potassium ferricyanide, sodium tetrahydroborate

and hydrochloric or nitric acid (BH_4^- + $3H_2O$ + Pb (IV) → H_3BO_3 + $3H^+$ + PbH_4) has been successfully applied to the determination of Pb in various samples, including calcium matrices (20,27), urine (31), dialysis concentrates (30), geochemical deposits and paint (32).

The aim of our work is to develop and verify a method for the determination of lead at teeth samples using flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) in the presence of $K_3Fe(CN)_6$, HNO_3 and $NaBH_4$ reagents. Since the analyte is efficiently separated from biological matrix by hydride generation, matrix related interferences are eliminated.

MATERIALS AND METHODS

Reagents and equipment

All chemicals used were of analytical-reagent grade. Deionized water (18 MΩcm) from a Milli-Q water purification system (Millipore, Milford) was used to prepare all solutions. Laboratory glassware was kept overnight in a 10% (v v^{-1}) nitric acid (HNO_3) solution. Afterwards it was rinsed thoroughly with deionised water. Each tooth sample was digested with HNO_3 (Merck) in Teflon beaker by open wet digestion separately. All working standard solutions of lead were prepared freshly by appropriate dilutions of 1000 mg L^{-1} stock lead solution (Fisher Scientific, UK). $NaBH_4$ (Aldrich) was used as reducing agent at the concentration of 1.5% (m v^{-1}) including 0.5% (m v^{-1}) sodium hydroxide (NaOH) (Merck). Standards and sample solutions were prepared in 2.5% (m v^{-1}) $K_3Fe(CN)_6$ (Merck) including 0.2 mol L^{-1} HNO_3 (Merck).

Instrumentation

ATI-UNICAM 939 atomic absorption spectrometer coupled with a hydride generation system and a flame heated quartz T-tube, as atomizer, was used throughout the experiments. Unicam data coded lead hollow cathode lamp was employed at 283.3 nm, with 7.5 mA and 0.5 nm spectral band pass. Deuterium background correction was used.

Three channels ALITEA VS-3 midi peristaltic pump, Tygon peristaltic pump tubing (1.8 mm i.d), Rheodyne Model 5020 injection valve with 750 µL injection loop were used in flow injection system to pump the carrier, reducing and sample solutions. A standard U-Type gas-liquid separator of UNICAM VP 90 vapour system was used for the gas liquid separation. Nitrogen was used as carrier gas to sweep out the lead hydride to the atomiser. The quartz atomizer was 13 cm in length with a 10 mm i.d.; the T-connection was 8 cm in length with 4 mm i.d. The flow injection manifold is shown in Figure 1.

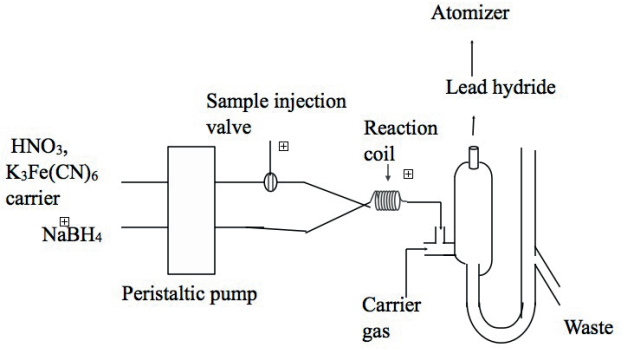

Figure 1. Flow injection manifold for the determination of lead using FI-HG-AAS

OHAUS analytical balance, Nüve MK418 electrical heating, Eppendorf research micropipettes were used for this study.

Preparation of tooth sample

During the sample preparation of teeth following procedure was used. First of all; each tooth sample was cleaned individually by the method proposed by Delves *et al.* (33) then was soaked overnight in a 5.0 mL 10% (v v^{-1}) solution of hydrogen peroxide (H_2O_2) in polyetilene tubes and then rinsed six times with deionised water. Teeth were kept in acetone for 30 min for defatting and then rinsed six times with deionised water then dried in an oven at 45 °C for 24 hours (33). When the samples cool down to room temperature, each teeth was weighted into Teflon beaker and dissolved in 2-7 mL concentrated HNO_3 on a hot plate (34). Amounts of HNO_3 used to digest tooth with respect to their weights were given in Table 1. The digested samples were heated up to dryness and the residues dissolved in 5 mL of 0.7- 2.9 mol L^{-1} HNO_3 according to their weights and they were diluted to 10 mL. Then, HNO_3 and $K_3Fe(CN)_6$ concentrations of each samples were adjusted to 0.2

Table 1. Weight of tooth and the volume of HNO_3 for digestion

Dried tooth weight (g)	Volume of concentrated HNO_3 (mL)
<0.2	2
0.2 – 0.4	3
0.4 – 0.6	4
0.6 – 0.9	5
0.9 – 1.1	6
>1.1	7

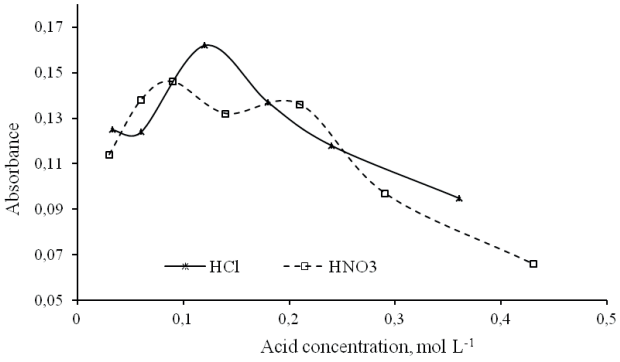

Figure 2. Effects of HCl and HNO_3 concentrations on signal intensity

mol L^{-1} and 2.5% (m v^{-1}) respectively with proper dilutions. Blanks were also prepared according to the amounts of solvents used in each digestion process. SRM was prepared by using the same digestion procedure.

Recommended analytical procedure

Lead standard solutions were prepared including 2.5% (m v^{-1}) $K_3Fe(CN)_6$ and 0.2 mol L^{-1} HNO_3. The carrier solution was 2.5% (m v^{-1}) $K_3Fe(CN)_6$ in 0.2 mol L^{-1} HNO_3. 1.5% (m v^{-1}) $NaBH_4$ as the reducing agent was prepared including 0.5% (m v^{-1}) NaOH. An aliquot of 750 mL standard or sample solutions were injected to the flowing stream. Lead hydride was generated in the mixing coil with the reaction of $NaBH_4$ and then carried to the quartz tube atomizer by nitrogen gas (flow rate 271 mL min^{-1}). All measurements were recorded as peak height absorbance. External calibration method was used for the FI-HG-AAS measurements to determine the amount of lead at teeth and SRM.

RESULTS AND DISCUSSION

Optimisation studies

All optimisation studies were made with the same teeth solution. For this purpose, ten teeth were digested in the same medium according to the procedure described above and the residue was dissolved with HNO_3 then diluted.

The parameters that directly affect the analytical signal in FI-HG-AAS measurement (e.g. sample volume, flow rates and concentrations of various reagents, nitrogen gas flow rate, and reaction coil length) were optimized.

Sample acid concentration was an important factor that influences the generation of lead hydride. HNO_3 and HCl are the most common acids used for carrier solution with $K_3Fe(CN)_6$, so the effects of two acids with $K_3Fe(CN)_6$ in FI-HG-AAS system were investigated (Fig.2). HNO_3 is widely used to digest the biological samples, to dissolve the digested residue. Thus, using HNO_3 is more advantageous than using HCl. The highest signals were obtained between 0.1 and 0.2 mol L^{-1} HNO_3 and 0.1 mol L^{-1} HCl concentrations; beyond this range the sensitivity decreased drastically as shown in Fig.2. This narrow range of HCl concentration is a general shortcoming of HG-AAS for lead determination.

Other parameters that affect the hydride generation efficiency are $K_3Fe(CN)_6$ and $NaBH_4$ concentrations. The optimisation study of oxidizing agent ($K_3Fe(CN)_6$) and reductant ($NaBH_4$) were shown in Figure 3. Lead signal was stable between 2.0 and 3.0% (m v^{-1}) $K_3Fe(CN)_6$ and 1.0 and 2.0% (m v^{-1}) $NaBH_4$. Thus 2.5% (m v^{-1}) $K_3Fe(CN)_6$ and 1.5% (m v^{-1}) $NaBH_4$ were used for further experiments.

The absorbance signal was strongly dependent on the flow rate of $K_3Fe(CN)_6$ and $NaBH_4$. Effects of flow rate of the solutions and the length of reaction coil were investigated in Figures 4. The signal intensity is directly proportional to the flow rate of the solutions. Optimum flow rate value was choosed

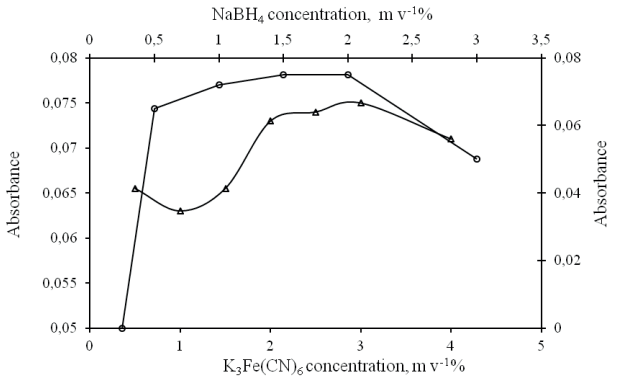

Figure 3. Effects of $K_3Fe(CN)_6$ (Δ) and $NaBH_4$ (o) concentrations on signal intensity.

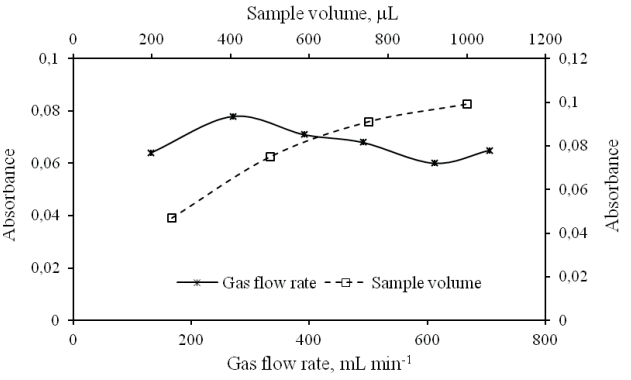

Figure 5. Effects of nitrogen gas flow rate and sample volume on signal intensity

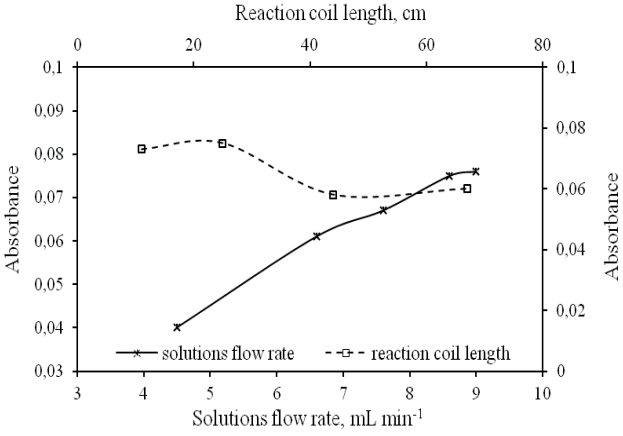

Figure 4. Effects of flow rate of solutions and reaction coil length on signal intensity

as 8.6 mL min^{-1} according to the peak shape and highest signal intensity. The length of reaction coil has varied depending on hydride generation kinetics of the elements, concentrations and flow rate of the reactives. The length of the reaction coil was selected considering the sensitivity and peak shape of the signal. The highest sensitivity was obtained at 25 cm reaction coil length.

The gas–liquid separation can be controlled by the flow rate of carrier gas, which also influences the transfer to the atomizer and dilution in the gas phase. Effects of nitrogen gas flow rate and sample volume on lead signal intensity were shown in Figure 5. Optimum flow rate and sample volume were chosen 270 mL min^{-1} and 750 µL, respectively for further studies.

Analytical figures of merit

The slopes of external calibration and the standard addition method were not different statistically in FI-HG-AAS. So the external calibration method was used to determine the lead at teeth with FI-HG-AAS. Forty teeth samples were analyzed and Pb levels were found between 0.66 and 15.60 µg g^{-1} (Table 2). The detection limit ($3s_b$ m^{-1}, where s_b is the standard deviation of the blank signals, m is the slope of the calibration graph) and characteristic sensitivity (concentration which gives the 0.0044 absorbance) for 750 µL sample volume were found to be 0.4 µg L^{-1} and 0.6 µg L^{-1}, respectively in FI-HG-AAS. The calibration graph was linear between 10 and 60 µg L^{-1} for standard solutions. The equation of the calibration graph obtained by linear regression was y = 0.0078C + 0.0015 (R^2 = 0.9992), where y is the peak height absorbance of lead signal and C is the concentration of lead in µg L^{-1} (Fig. 6). The precision of the system in terms of R.S.D% for 11 replicate measurements of blank was found as 3.8%. The sampling frequency was 70 measurements per hour. The life of quartz tube was 300 measurements for teeth samples.

Accuracy of the FI-HG-AAS method was tested using SRM. Lead concentration of SRM obtained with the FI-HG-AAS (1.35 ±0.02 µg g^{-1} Pb) method was in agreement with the certified value (1.335 ±0.014 µg g^{-1} Pb) using Student's t-test (p = 0.05).

Typical signal obtained with teeth solution in FI-HG-AAS was shown in Fig.7. It should be noted

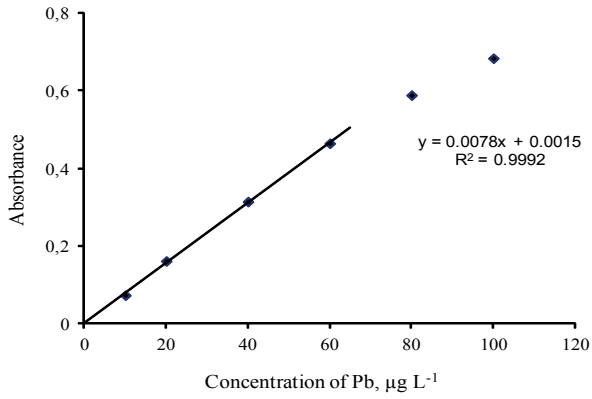

Figure 6. Calibration graph of Pb with FI-HG-AAS

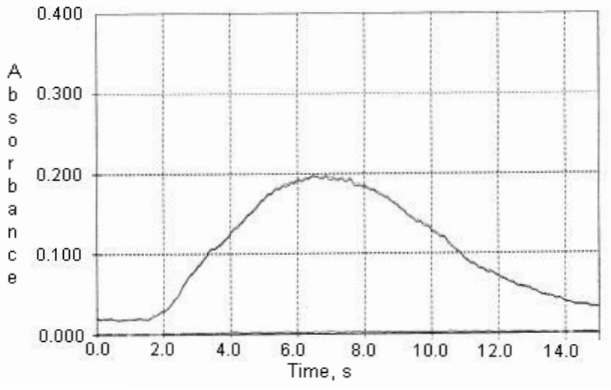

Figure 7. Flow injection profile of lead in diluted tooth using FI-HG-AAS

that almost no background signal was observed in the signal; this is the distinct advantage of the hydride technique.

CONCLUSION

Chemical vapour generation technique has an advantage to decrease the interferences due to the selectivity of the analyte to the vapour phase. Since no significant background signal was observed with sample solution. Using external calibration method in FI-HG-AAS has a big advantage in routine analysis in terms of time.

Quartz t-tube lifetime was found approximately 300 measurements due to the fluoride in teeth. This is comparable with graphite tube in ETAAS. No significant difference was observed statistically in lead concentration in SRM with FI-HG-AAS. Therefore FI-HG-AAS is sensitive, fast, and economical method to analyse lead in tooth sample.

Table 2. Concentrations of lead in teeth samples with FI-HG-AAS

Sample no.	Concentration of Pb mg g^{-1} (n = 3)
1	2.78 ±0.10
2	7.09 ±0.66
3	3.20 ±0.08
4	1.46 ±0.06
5	2.94 ±0.23
6	4.68 ±0.06
7	4.40 ±0.21
8	1.37 ±0.07
9	1.07 ±0.10
10	0.97 ±0.01
11	2.20 ±0.30
12	2.35 ±0.08
13	1.72 ±0.08
14	3.25 ±0.40
15	5.16 ±0.24
16	2.53 ±0.13
17	5.42 ±0.51
18	7.83 ±0.36
19	15.60 ±1.24
20	1.56 ±0.19
21	6.90 ±0.60
22	1.65 ±0.24
23	3.56 ±0.17
24	2.06 ±0.25
25	4.29 ±0.26
26	5.71 ±0.15
27	1.88 ±0.08
28	3.23 ±0.24
29	1.20 ±0.06
30	4.55 ±1.29
31	5.13 ±0.10
32	3.56 ±0.06
33	4.82 ±0.10
34	2.20 ±0.08
35	5.57 ±0.51
36	1.11 ±0.06
37	0.66 ±0.03
38	2.74 ±0.75
39	1.81 ±0.09
40	2.60 ±0.04

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