Synthesis of Schiff Base Unsaturated Oligomeric Polyester for Determination of Trace Lead

Samy K. Badr * and Alaa S. Amin
Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

Abstract
A new very sensitive and selective chromogenic reagents prepared by the reaction of Schiff base with maleic anhydride (MA) and 1,4-butane (I), ethylene (II), 1,6-hexamethylene (III) and 1,2-propane diol (IV), respectively, were synthesized and studied for the determination of trace lead in detail. In 0.1molL−1 NaOH medium, lead reacts with polyester to form a yellow complex, which gives a maximum absorption at 462, 478, 482 and 495 nm using I, II, III and IV respectively. Beer’s law is obeyed over the concentration ranges 0.04-1.20μg/mL−1, whereas optimum concentration ranges as adopted from Ringbom method was 0.10-1.05μg/mL−1. The molar absorptivity, Sandell sensitivity, detection and quantification limits are also calculated Ca (3500-fold), Mg (2250-fold), Sr (1250-fold), Ba (1000-fold), Al (1500-fold), Bi (800-fold), Fe (2500-fold) Co (1100-fold), Ni (1000-fold), Cu (900-fold), Zn (1300-fold), Cd (2500-fold), and Ag (750-fold) do not interfere with the determination of lead. The chromogenic system is remarkably superior to other reagents, especially porphyrin compounds. The influence caused by oxygen in air or in solution can be easily eliminated by adding Na2SO3. The reaction is very stable, the stability constant of the complex being 1.44 x 1045. The chromogenic reaction is completed within 2.0 min at room temperature. The proposed method has been applied to the direct determination of trace lead in clinical samples. The accuracy and precision are both very satisfactory.

Keywords: Unsaturated polyester; Spectrophotometry; Lead determination; Clinical analysis

Introduction
It is well known that absorbed superfluous lead is vital to human, but people would also sicken serious diseases such as encephalopathy, kidney damage, and memory loss even if exposure a small quantity of it due to its cumulative characteristic in organism and bones [1,4]. Lead and its compounds are hazardous, lead poisoning was cumulative and its toxic effects are many and severe [5,6]. Because of environmental pollution (especially in developing countries), clinical lead analysis is becoming increasingly important. Analysis of clinical samples must be reliable, are simple and quick as possible. Flame atomic absorption spectrometry (FAAS), electro-thermal atomic absorption spectrometry ( ETAAS), flow injection (FI) [7-10], and electrochemical sensor [11-13] were used for the analysis of lead. However, FAAS was reliable down to about 0.2μg L−1 and so was not always suitable for the analysis of lead in clinical samples ETAAS and FI are generally used to determine lead at low concentrations, but they were complex to use; moreover, some type of preconcentration or extraction step must be used to meet the requirement of accuracy and precision. Spectrophotometric methods were versatile and economical.

Dithizone [14], diethylidythiocarbamate [15-17], arsenazo III [14], 4-(2-pyridylazo)-resorcinol (PAR) [18], malachite green [19], butylrhodamine-B [20], xylene orange [21], 2-(2-thiazolylazo)-p-cresol, dithiobenzic acid [22], dithiobenzic acid iodide– ethylene violet- polyvinyl chloride [23], and a copolymer consisting of protoporphyrin disodium salt and acrylamide [24] were suitable for the trace analysis of lead in clinical samples due to their low detection limit. Dibromo-p-methylsulfonazo [25] was sensitive, but Ca2+ and Mg2+, which exist in clinical samples were reasonable amounts, interfere with the determination of lead and require prior separation with potassium iodide-methylisobutylketone extraction.

Polymers having the amino acid moieties in the main chain or in the side chain find a variety of useful applications, such as chelating agents for metal ions, ion-exchange resins, polymer catalysts with enzyme like behavior, and promotes for preparing polymeric carrier’s drugs [26–31] polyesters with pendant amino groups and carbonyl oxygen of the ester group as donor sites per unit may yield metal-coordinated polymers with interesting properties. However, few investigations had been reported on the synthesis of polyesters having amino acid moieties in the main chain. Earlier, reported the synthesis and characterization of poly (ethylenespiaspirate)-metal complexes [32] which were found to be thermally more stable than the polyesters itself.

The present investigation aimed to synthesize polyester based on Schiff base derived from amino acid have attached much attention as analytical regents owing to their very high sensitivity, however, certain problems seriously effect their application, such as solubility and selectivity (especially transition metal ions interfere with the determination of lead).

All foreign ions studied in reasonable amounts do not interfere with the determination of trace lead. The proposed method has been applied to the direct determination of lead in clinical samples with good precision and accuracy. The method is one of the most sensitive and efficient techniques for the determination of trace lead at the present time.

Experimental
Materials
DL aspartic acid (Aldrich Comp), salicylaldehyde (WIN LAB, U.K.)

*Corresponding author: Samy K. Badr, Chemistry Department, Faculty of Science, Benha University, Benha, Egypt, E-mail: drsamysh@yahoo.com

Received August 30, 2010; Accepted October 04, 2010; Published October 06, 2010

Citation: Badr SK, Amin AS (2010) Synthesis of Schiff Base Unsaturated Oligomeric Polyester for Determination of Trace Lead. J Chem Eng Process Technol 1:102. doi:10.4172/2157-7048.1000102

Copyright: © 2010 Badr SK, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Preparation of azomethine schiff base

The Schiff base monomer was prepared by reacting 1.22g (0.01mol) of salisylaldehyde with 1.33g (0.01 mole) of aspartic acid as acidic catalysis in reaction flask which dipped in an oil bath (thermostatically controlled), provided, with mechanical stirrer and Dean-Stark trap to collect the water, stream of dry nitrogen insert in atmosphere [33], poured into ice-cold water and then allowed to stand for 1.0hr. The precipitated solids were filtered off, washed repeatedly with demineralized water and recrystalized from ethanol. The Schiff base was isolated as yellow solid. The yield was about 70%.

Preparation of unsaturated polyesters

Polycondensation were carried out by the reaction of maleic anhydride (0.5mol) and different Glycols (1.0mol) at temperature of 140-170°C about (4.0hr) using traces of p-toluene sulphonic acid as acidic catalysis in reaction flask which dipped in an oil bath (thermostatically controlled), provided, with mechanical stirrer and Dean-Stark trap to collect the water, stream of dry nitrogen insert in the flask to facilitate the removal of water [34], 0.5mol of Schiff base added in the second step and the temperature raised gradually 180-210°C (6-8hr). The unreacted monomer and other by-product were removed from the reaction mixture by evaporation for 0.5–1.0hr. at the end of the reaction. The copolymer was purified by dissolving in DMF and precipitating into large amount of rapidly stirred petroleum ether (40–60°C). The precipitated viscous copolymer dried in a vacuum oven at 40°C for 6.0hr.

Characterization of polyesters

Viscosity measurements were carried by Ostwald viscometer using 1.0% solution in dioxane at 30°C depending on the solubility of polyester. The inherent [37] viscosities of the polymer solutions in DMSO were determined at 30°C. The physical properties of the prepared unsaturated polyesters (I-1V) were listed in Table (1). The solubility characteristics of the polyesters (I–IV) are shown in Table 2. It can be seen from Table 2 that all polyester was insoluble in halogenated solvents such as chloroform and tetrahydrofurane (THF). Most of the polyester were soluble in dimethylformamide (DMF), dimethylsulphoxide (DMSO) and partially soluble in acetone. The solubility characteristics of the polyesters (I–IV) are shown in Table 2. It can be seen from Table 2 that all polyester was insoluble in halogenated solvents such as chloroform and tetrahydrofurane (THF). Most of the polyester were soluble in dimethylformamide (DMF), dimethylsulphoxide (DMSO) and partially soluble in acetone.

The relation between acid value and time of the polyester preparation by raise the temperature circuly after each one hr and calculate the progress of polymerization by acid value determination at the end of this temperature as shown in Figure (1). Finally, we operate the polymerization till the least degree of acid value which extend conjugation which help for the accuracy of this ligand in spectrophotometric determination of traces metal under application. The unsaturation of copolymer give high degree of stability and extend conjugation which help for the accuracy of this ligand in spectrophotometric determination of traces metal under application. The unsaturation of copolymer give high degree of stability and extend conjugation which help for the accuracy of this ligand in spectrophotometric determination of traces metal under application.
The 1H-NMR of the copolymers I-IV were illustrated in Table 3. The method of Baddar, [38] was used in this present work for the qualitative but quantitative analysis of these copolymers by the 1H-NMR spectroscopy. The results of both qualitative and quantitative were clear the molar ratios of the glycol to the acid from Table 3, also the study of 1H-NMR indicate the formation of the copolyester and also indicate the relation between the calculated amount of moles of acid and glycol were almost the same from quantitative analysis of the 1H-NMR spectra of the product copolymers.

**Chromogenic reaction of polyester with metal ions**

Polyesters were found to react mainly with Zn$^{2+}$ and Pb$^{2+}$. Zn$^{2+}$ reacts with reagent to form complex in pH 10 (borax buffer solution) with $\lambda_{\max}$ 462, 478, 482 and 495 nm [Figure 2] and molar absorptivity are 1.05 x 10$^5$, 1.12 x 10$^5$, 1.57 x 10$^5$ and 1.31 x 10$^5$Lmol$^{-1}$cm$^{-1}$. The highly sensitive reagent with Pb$^{2+}$ is examined now.

**Effect of medium**

The influence of the pH on the absorbance of the Pb$^{2+}$ reagent complex was studied. At pH > 9.0, the complex absorbance remained almost constant. The final pH was controlled by adding 2.0mL of 0.1molL$^{-1}$ NaOH to 10mL calibrated flask in order to keep the colour reaction reproducible, constant and sensitive.

**Effect of polyester concentration**

Under the conditions employed, the volume of 0.05% reagent solution required to obtain a maximum and constant absorbance for 1.0µg of lead was 0.4-0.7mL. Therefore, 0.5mL of 0.05% reagent solution was introduced (Figure 3).
Polymer Component Chemical shift δ(ppm) Assignment No. of proton Peak areas Relative Mole amount Mole %

I G 1.7, 3.7 and 4.2 (8H) \( CH_2 \) \( OH \) (1H) 11 70.2 5.5 44.42
A* 1.9, 3.9, 4.3, 5.2, 6.2, 7.1 and 7.5 (2H) \( CH_2 \) \( COO \) (1H) \( CH=NH \) (1H) \( OH \) (2H) = (4H) Ar 11 70.2 5.5 44.42

II G 4.2 (8H) COOCH
A 1.7, 3.6, 4.1, 5.2, 6.1, 7.3 and 7.8 (2H) \( CH=O \) (1H) \( CH=N \) (1H) \( CH=NH \) (1H) \( OH \) (2H) = (4H) Ar 11 81.4 7.4 46.54

III G 4.2 (8H) COOCH
A 1.7, 3.7, 4.0, 5.2, 6.1, 7.1 and 7.7 (2H) \( CH=O \) (1H) \( CH=N \) (1H) \( CH=NH \) (1H) \( OH \) (2H) = (4H) Ar 11 113.8 7.8 52.0

IV G 0.9, 4.1, and 4.3 (6H) 2CH3 (4H) COOCH2)2 and (2H) (COOCH)2 (2CHOCO-) 12 85.6 7.2 48.0
A 1.7, 3.7, 4.0, 5.2, 6.1, 7.1 and 7.7 (2H) \( CH=O \) (1H) \( CH=N \) (1H) \( CH=NH \) (1H) \( OH \) (2H) = (4H) Ar 11 113.8 7.8 52.0

Table 3: 1H-NMR Spectral data of unsaturated polyester I–IV.

| Parameter | Reagent |
|-----------|---------|
| \( \lambda_{max} \) | 462 | 478 | 482 | 495 |
| Beer’s law, \( \mu g \text{ mL}^{-1} \) | 0.05-1.20 | 0.05-1.00 | 0.5-1.10 | 0.05-1.15 |
| Ringbom conc, \( \mu g \text{ mL}^{-1} \) | 0.15-1.05 | 0.20-0.85 | 0.1-1.0 | 0.15-1.05 |
| Molar absorptivity, L mol\(^{-1}\)cm\(^{-1}\) | \( 1.05 \times 10^2 \) | \( 1.2 \times 10^2 \) | \( 1.57 \times 10^2 \) | \( 1.31 \times 10^2 \) |
| Sandell sensitivity ng cm\(^{-2}\) | 1.97 | 1.85 | 1.32 | 1.58 |
| Detection limit, ng mL\(^{-1}\) | 15 | 14 | 16 | 17 |
| Quantification limit, ng mL\(^{-1}\) | 48 | 45 | 50 | 54 |
| Stability up to bray | 7.0 | 7.0 | 9.0 | 10.6 |
| Regression equation * | | | | |
| (a) intercept | -0.013 | 0.009 | 0.012 | -0.008 |
| (b) slope | 0.51 | 0.54 | 0.76 | 0.63 |
| Correlation coefficient * | 0.9992 | 0.9990 | 0.9996 | 0.9988 |
| Standard deviation % | 0.38 | 0.61 | 0.72 | 0.54 |
| Relative standard deviation % | 0.87 | 1.13 | 1.27 | 0.96 |
| Error % | ±1.3 | 1.6 | ±1.7 | ±1.5 |

\*A = a +bC where C is the concentration of Pb\(^{2+}\) in ng mL\(^{-1}\)

Table 4: Physical and analytical parameter for Pb - complexed with polyester I-IV.

Effect of surfactants and organic solvent

The effects of various surfactants and organic solvents on the Pb\(^{2+}\)-reagent chromogenic system were studied. It was found that the absorbance increased remarkably in the presence of non-ionic surfactants. The order of the increase was Triton x 100 > Tween 80 > octylphenyl ether > polyethyleneglycol. Cationic surfactants such as cetyltrimethyl ammonium bromide and cetylpyridinium chloride were added; the absorbance of the complex obviously diminished.

The addition of appropriate amounts of various organic solvents did not affect the absorbance of the complex. Hence, Triton X-100 is the best sensitizing agent and 0.3-0.7mL of 2.0% Triton X-100 solution gives a constant and maximum absorbance. Therefore, 0.5mL of 2.0% Triton X-100 was adopted.

Stability of the system

Oxygen in air or in the solution can affect the absorbance of the polymeric reagent and the complex. We have found that this easily be eliminated by adding 0.5 mL of 2% Na\(_2\)SO\(_3\) solution. The absorbance of the Pb\(^{2+}\)-reagent complex then remains stable for at least one week at room temperature.

Calibration graph

According to the properties of the complex, Beer’s law was obeyed for 0.04-1.20\( \mu g \text{ mL}^{-1} \) of lead, whereas optimum concentration ranges as adopted from Ringbom method was 0.1-1.05\( \mu g \text{ mL}^{-1} \). The calculated molar absorptivity and Sandell sensitivity are recorded in Table 4. The IUPAC detection limit (K=3) and the quantification limits (k=10) [39] were calculated for each sample system (Table 4) Ten replicate analysis of a test solution containing 1.0\( \mu g \text{ mL}^{-1} \) of lead using general procedure gave a mean of 2.01, 2.007, 1.994, 1.996 with a relative standard deviation of 0.87, 1.13, 1.27 and 0.96% using polyester I, II, III and IV, respectively.

Effect of interfering ions

Under the optimum conditions, the effects of various foreign ions on the determination of 1.0\( \mu g \text{ mL}^{-1} \) of Pb\(^{2+}\) were examined separately, with a relative error of less than ± 5.0%. According to the data provided in Table 5, the tolerance for ions Zn\(^{2+}\), Mo\(^{6+}\), Cr\(^{6+}\), V\(^{3+}\), W\(^{6+}\), Ag\(^{+}\) and Hg\(^{+}\) should be low. All the foreign ions studied can be tolerated in considerable amounts.

Analytical applications

The proposed method was applied to the determination of Pb\(^{2+}\) in...
clinical samples. The results are in good agreement with those found for lead by ETAAS with a prior separation by potassium iodide-methyl isobutyl-ketone (KI-MIBK) (Table 6). The performance of the proposed method was assessed by comparison with the ETAAS method. Mean isobutyl-ketone (KI-MIBK) (Table 6). The performance of the proposed method was assessed by comparison with the ETAAS method. Mean

Table 6: Determination of Pb²⁺ in clinical samples.

| Sample | Found μg g⁻¹ | ETAAS | Confidence level. |
|--------|--------------|-------|-------------------|
| I      | 0.155 ± 0.003 | 0.157 ± 0.004 | 0.159 ± 0.005 | 0.159 ± 0.005 | 0.159 ± 0.005 | 0.159 ± 0.005 |
| II     | 0.212 ± 0.004 | 0.215 ± 0.004 | 0.218 ± 0.004 | 0.218 ± 0.004 | 0.218 ± 0.004 | 0.218 ± 0.004 |
| III    | 0.114 ± 0.002 | 0.117 ± 0.005 | 0.119 ± 0.005 | 0.119 ± 0.005 | 0.119 ± 0.005 | 0.119 ± 0.005 |
| IV     | 0.445 ± 0.003 | 0.450 ± 0.004 | 0.448 ± 0.003 | 0.448 ± 0.003 | 0.448 ± 0.003 | 0.448 ± 0.003 |

References
1. Chen CT, Huang WP (2002) A highly selective fluorescent chemosensor for lead ions. J Am Chem Soc 124: 6246-6247.
2. Yusof NA, Ahmad M (2002) A flow cell optosensor for lead based on immobilized gallicynin in chitosan membrane. Talanta 58: 459-466.
3. Demirbas A (2004) Adsorption of lead and cadmium ions in aqueous solutions onto modified lignin from alkali glycerol delignification. J Hazard Mater 109: 221-226.
4. Mohan S, Sreeakshami G (2008) Fixed bed column study for heavy metal removal using phosphate treated rice husk. J Hazard Mater 153: 75-82.
5. Renner R (1995) Newly deposited lead may be more bioavailable. Environ Sec Technol 29 : 256-260.
6. Schneider JA, Hornig JF (1993) Spectrophotometric determination of lead in tap water with 5,10,15,20-tetra(4-N-sulfoethylpyridinium)porphyrin using merging zones flow injection. Analyst 118: 933-936.
7. Almestrand L, Betli M, Hua C, Jager D, Rehman L (1988) Convenient determination of trace lead in whole blood and other fluids. Anal Chim Acta 209: 339-343.
8. Nygren O, Nilsson CA, Gustavsson A (1988) Determination of lead in blood using flow injection and a nebuliser interface for flame atomic absorption spectrometry. Analyst 113: 591-594.
9. Roux KCP, Maltez HF, Carletto JS, Martandal E, Carasek E (2008) Application of factorial design and Doehlert matrix for determination of trace lead in environmental samples by on-line column preconcentration FAAS using silica gel chemically modified with niobium(V) oxide. Anal Sci 24: 365-370.
Citation: Badr SK, Amin AS (2010) Synthesis of Schiff Base Unsaturated Oligomeric Polyester for Determination of Trace Lead. J Chem Eng Process Technol 1:102. doi:10.4172/2157-7048.1000102

10. Liang P, Liu R, Cao J (2008) Single drop microextraction combined with graphite furnace atomic absorption spectrometry for determination of lead in biological samples. Microchim Acta 160: 135-139.

11. Han CL, Ahmad M, Taib MN (2005) Electrochemical sensor for the lead determination: development and characterization. Sensors and the International Conference on new Techniques in Pharmaceutical and Biomedical Research 118-126.

12. Reeder GS, Heineman WR (1998) Electrochemical characterization of a microfabricated thick-film carbon sensor for trace determination of lead. Sensors & Actuators B: Chem. 52: 58-64.

13. Pachetti I, Mascini M, Minumni M, Bilia AR, Vincieli FF (2003) Disposable electrochemical sensor for rapid determination of heavy metals in herbal drugs. J Pharm Biomed Anal 32: 251-256.

14. Naseri MT, Hosseini MRM, Assadi Y, Kiani (2008) A Rapid determination of lead in water samples by dispersive liquid-liquid microextraction coupled with electro thermal atomic absorption spectrometry. Talanta 75: 56-62.

15. Pan J, Chen Y, Yan H (1981) Chromogenic reagents and their applications in spectrophotometric analysis, Shanghai Science and Technology, Shanghai, p. 283.

16. Terboven JF, Detmar DA, Buijze C (1959) Optical test strip for trace lead based on doped sol-gel film Z. Anal Chem 167: 401-406.

17. Willemsen LC (1986) Hand book of lead chemicals, Project LC-116, International lead zinc research Organisation, New York.

18. Dongil RM, West TS, Young P (1965) Determination of lead with 4-(2-pyridylazo)-resorcinol—I: Spectrophotometry and solvent extraction. Talanta 12: 583-585.

19. Bate MN, Dave DP, Sawant AD (1995) Extraction and spectrophotometric determination of lead(II) with pyridine-2-acetaldehyde salicyloylhydrazone. Talanta 42: 1291-1296.

20. Trinder N (1966) The use of diphenylcarbazone for the determination of microgram amounts of lead. Analyst 91: 587-590.

21. Pan J, Chen Y, Yan H (1981) Chromogenic reagents and their applications in spectrophotometric analysis, Shanghai Science and Technology, Shanghai, p.411.

22. Daia S, Zhang B, Yub L, Yang Y (2010) The determination of trace lead in drinking water by flow injection spectrophotometry. Spectrochim Acta (A) 75: 330-333.

23. Asanoa T, Yabusaki K, Wang PC, Iwashika A (2010) Determination of lead(II) in fish ash leachate using a newly developed simple spectrophotometric method. Spectrochim Acta (A) 75: 819-824.

24. Ferreira SLC, Andrade MGM, Lobo IP, Costa ACS (1991) 2-(2-Thiazolylazo)-p-cresol (TAC) as a reagent for the spectrophotometric determination of lead(II). Anal Lett 24: 1675-1678.

25. Ferruti P, Ranucci E, Sartore L, Bignotti F, Marchisio MA, et al. (1994) Recent results on functional polymers and macromonomers of interest as biomaterials or for biomaterial modification. Biomaterials 15: 1235-1241.

26. Jazsz K (2007) Synthesis and characterization of new functional polyester-anhydride based on succinic and sebacic acids. Macromol. Symp. 245: 109-116.

27. Chen CH, Lu HY, Tsai CJ, Yang CS (2009) Synthesis and characterization of polyethylene succinate) and its copoly(mesters containing minor amounts of butylene Succinate). J appl Polym Sci 111: 1433-1439.

28. Khuhaswar MY, Mughal MA, Channah AH (2003) Synthesis and characterization of some new Shift base polymers. Eur polym J 11: 805-809.

29. Glen SK, Forrest M, (2006) Amphiliphic block copolymer micelles for nanoscale drug delivery. Drug Develop Res 67: 15-22.

30. Congming X, Yueying H, Hongme J (2006) Synthesis and characterization of novel degradable aliphatic polyester that contains monomeric lactate sequences. Macromol Rapid commun 27: 637-640.

31. Gismera MJ, Sevillea MT, Procopio JR (2006) Potentiometric carbon paste sensors for lead (II) based on dithiodibenzonic and mercaptobenzoic acids. Anal Sci 22: 405-410.

32. BaJpal A, RAI S (1998) Synthesis and characterization of coordination polymers of polyesters with pendant amino groups. J appl Polym Sci 69: 751-759.

33. Kamal S, Acharya S, Dey RK, Ray AR (2002) Synthesis and metal ion uptake studies of chelating resins derived from formaldehyde-furfuraldehyde condensed phenolic Schiff bases of 4,4’-diaminodiphenylether and o-hydroxycacetophenone. Talanta 57: 1075-1083.

34. Tawfik SY, Asaad JN, Sabaa MW (2006) thermal and mechinal behaviour of flexible poly(vinyl chloride) mixed with some saturated polymers , poly Deg and Stability 91, 2385-2392.

35. Chen S, Wang Y, Fan Y (2008) Synthesis of amphiliphic poly(tetrathylen glycol succinate) and thermo sensitivity of its aggregation in water. J Biomedical Resarch Part (A) 17: 771-777.

36. Bllmeyer FW, Jr (1962) Textbook of polymer science, 2nd ed; Wiely; New York, Chapter, 3, p. 62.

37. Nery L, Leebrvre H, Frade L (2005) Polyamide-Polyester multiblock copolymers by chain-coupling reactions of carboxy-terminated polymers with phenyleine and pyriidylen Bioxazolines. J Polym Sci (A) 43: 1331-1341.

38. Baddar FG, Nosseir MH, Messitha NN, Ikadlosue NE (1976) Polymesters. part IV. Structural elucidation of γ-phenyltolaceton polymesters by joint application of I.R., U.V. and NMR spectroscopy. Eur Polym J 12: 631-634.

39. Li Z, Zhu Z, Chen Y, Hsu CG, Jiaomal Pan (1999) Spectrophotometric determination of lead in biological samples with dibromo-p-methyl-methylsulfonozao. Talanta 48: 511-516.

40. Hildebrand GP, Reilly CN (1957) Nomenclature, symbols, units and their usage in spectrochemical analysis—II. Data interpretation Analytical chemistry division, Anal Chem 29:258-261; IUPAC, (1978) Spectrochim Acta (B)33:241-245.