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Direct Photometric Determination of Lead by Manual and Flow Injection Methods with Gallocyanine

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A direct photometric method was developed for the determination of sub-nanogram levels of lead. The method is based on complexation between lead and gallocyanin, which form a coloured dye (λ_{max} 550 nm) at pH 8.0. The determination was based on the use of kinetic approach and in the lead concentration range of 1.0×10^{-3} µg/mL to 1.0×10^{1} µg/mL, a smooth calibration curve was obtained. The reaction system can also be successfully adapted to flow injection analysis (FIA). The dynamic range of the proposed flow injection method was 1.0×10^{-3} µg/mL to 1.0×10^{2} µg/mL and detection limit was 1.6 ng/mL at a sampling rate of 30 injections per hour. At 1:1 mole ratio of lead to the interfering ion, mercury, iron, aluminium, citrate and fluoride were found to interfere most during the determination.

Key Words: Flow injection analysis, Lead determination, Gallocyanin, Photometry.

INTRODUCTION

Heavy metals in the water treatment field refers to heavy and dense. The metallic elements occur even in trace levels in water are very toxic and tend to accumulate in atmosphere. Most heavy metals are too rarely found in water and lead is the most significant of all the heavy metals because it is both toxic and common. It gets into water from corrosion of plumbing materials. In addition lead can be found in the solder used to join copper pipes and in fittings and faucets made from brass. Drinking water is the major source of lead exposure for the general population. Lead seldom occurs naturally at a level higher than 5 µg/L in surface water supplies such as rivers and lakes[1].

Lead is a general protoplasmic poison that is cumulative, slow acting and subtle and produces a variety of symptoms. Like other heavy metals, it has an affinity for sulfur. Though it exerts much of its activity through sulfhydryl inhibition, lead also interacts with carboxyl and phosphoryl groups. The element interferes in heme synthesis[3].

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Recently, lead determinations at ppb-ppm levels[4-6] are performed using preconcentration techniques followed by atomic absorption spectrophotometry (AAS) with reported detection limit from 0.08 to 1.00 µg/L. These methods are problematic when a large number of samples have to be determine since the preconcentration step is usually laborious and time consuming.

Other techniques used for lead determination include isotope dilution gas chromatography-mass spectrometry[7], flow injection Donnan dialysis-inductively coupled plasma-AAS[8,9], anodic stripping voltammetry[10,11], potentiometry[12], laser atomic fluorescence spectrophotometry[13,14], the flow injection analysis (FIA) based on crown ether dicyclohexyl-18-crown-6 extraction method[15], the flow injection analysis based on 4-(2-pyridilazo) resorcinol complex method[16] and the use of 5,10,15,20-tetra(4-*n*-sulfoethylpyridinium)porphyrin[17]. Kinetic methods include catalytic persulfate oxidation of hemotoxylin[18] and reduction of resazurin by sodium sulfate[19] have also been reported.

The objective of this research is to develop a simple, reliable flow injection analysis method for lead ion determination in water without preconcentration with a detection limit at pbb level.

EXPERIMENTAL

All chemicals used were of analytical grade and deionized water was used throughout for solution preparation. A 1.5×10^{-3} g/mL of gallocyanine (BDH) solution is prepared by dissolving 0.15 g of gallocyanine in 100 mL of buffer pH 8. A stock lead solution (5.0×10^{3} µg/mL) was prepared by dissolving 0.5 g of $PbNO_3$ (AnalaR) in 100 mL of water. Working standards solutions were prepared by appropriate dilution before use. Buffer solutions used were prepared according to methods from Handbook of Basis Tables for Chemical Analysis[20].

A Shimadzu 160A spectrophotometer was used for the absorbance measurements with 10 mm quartz cell. The flow injection system consisted of peristaltic pump (Gilson), rotary injection valve (Rheodyne 7725) and a Shimadzu 160A spectrophotometer equipped with built in flow through cell. The configuration of flow injection manifold used is shown in Fig. 1 with optimum conditions.

Manual procedure: The pH at which the absorbance of the lead-gallocyanine complex is at maximum and the position of the maximum, were first determined. Suitable proportions of the solutions were mixed, buffered to the requisite pH and the absorbance of the buffered lead-gallocyanin complex were obtained over a range of wavelength from 200 to 700 nm.

The effect of gallocyanine concentration on the lead complex formation were studied by varying the volumes of the 1.5×10^{-3} g/mL gallocyanin (1-10 mL) whilst maintaining the volume of the buffer solution (2.5 mL) and the concentration of the analyte (1 mL of 1.0×10^{-2} µg/mL). All these solutions were mixed into a 100 mL volumetric flask and was diluted to the mark with deionized water.

The sensitivity of gallocyanine with lead was determined as follows. Different concentration (600 µL of 1.0×10^{-8} µg/mL - 1.0×10^{3} µg/mL) of lead solution were introduced into a cuvette containing of 1 mL of 1.5×10^{-3} g/mL gallocyaninee and 2.5 mL of buffer solution. In all these cases the amount of gallocyanin is in excess for complete complex formation.

Repeatability of the method was determined by measuring the absorbance of 10 different batch of similar proportions of mixed solutions containing 1 mL of 1.5×10^{-3} g/mL gallocyanine, 2.5 mL of buffer solution and 600 µL of 1.0×10^{-2} µg/mL of lead solution.

The effect of diverse ions on the determination of lead were studied by introducing different volumes of interfering ions (so that the ratio of lead:diverse ions varies *i.e.*, 1:1, 1:3 and 1:5) into a cuvette containing 50 µL of 1×10^{-2} µg/mL lead solution, 1 mL of 1.5×10^{-3} g/mL gallocyaninee and 2.5 mL of buffer solution.

Flow injection procedure: The same method used formerly in manual procedure was adapted for flowing system. Optimization of the flow system was carried out upon parameters such as flow rate, pH, reagent's concentration, repeatability, dynamic range of Pb(II) concentration and interference.

As shown in Fig. 1 the carrier solution which is the reagent itself was pumped at a flow rate of 1.2 mL/min. A 25 µL of lead solution is injected into the carrier stream. The colour development proceeds in the reaction coil and then passed through the flow through cell. The absorbance of the complex produced is monitored continuously at λ = 550 nm.

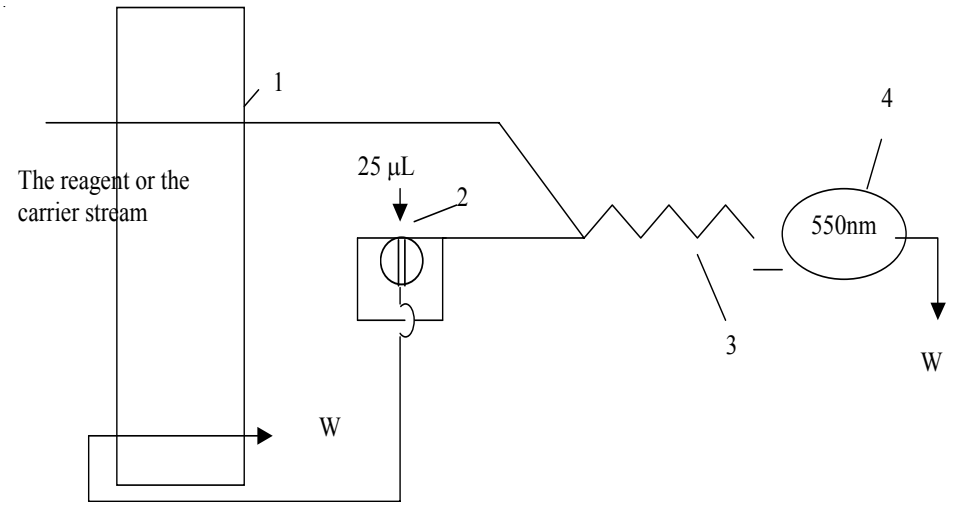

Fig. 1. Manifold for the spectrophotometric determination of lead which consist of peristaltic pump (1), injection valve (2), reaction coil (3), detector (4) and waste (W)

The optimization of the parameters mention before (pH, flow rate *etc.*) was studied by using the same procedures as mentioned earlier. In the case of flow rate, the flow is varied whereas for the pH, a series of buffer solution with different pH was introduced into the analyte solution before it was injected.

Reproducibility of the method was done by injecting the same concentration of Pb(II) (1×10^{-2} µg/mL) repeatedly. The dynamic range of Pb(II) concentration was studied by injecting a series of analyte with different concentration ranging from (1.0×10^{-8} µg/mL - 1.0×10^{3} µg/mL). The study on diverse ion effect was carried out by mixing the diverse ion with the analyte in fixed proportion (so that the ratio of lead:diverse ions varies *i.e.*, 1:1, 1:3 and 1:5) before injecting the mixed solution into the injection valve.

RESULTS AND DISCUSSION

Optimization of the manual mode: As shown in Fig. 2, gallocyanine showed a maximum absorbance at λ = 620 nm, whereas the complex showed a maximum absorbance at λ = 550 nm. This difference in the maximum values for gallocyanine and its complex is due to a sharp colour change of the gallocyanine and Pb(II)-gallocyanine complex from navy blue to violet, respectively. Gallocyanine is a green crystalline solid, practically insoluble in cold water, slightly soluble in hot water and is soluble in alcohol and glacial acetic acid. It is soluble in alkali carbonate to give a red solution and is soluble in concentrated hydrochloric acid to give a blue solution which becomes red when diluted with water. Gallocyanine reacts with Pb(II) ion to give a compound which structure is shown in Fig. 3.

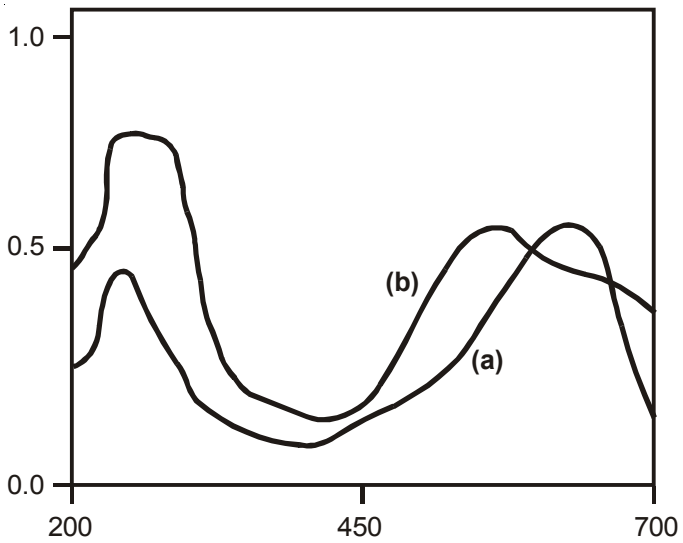

Fig. 2. Absorbance spectrum for gallocynin (a) and lead-gallocynin complex (b)

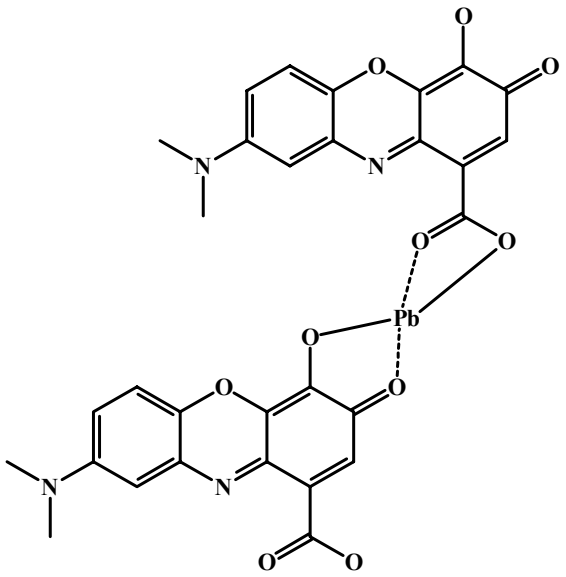

Fig. 3. Proposed structure of the complex formed between Pb(II) and gallocynine

The maximal colour development was obtained at pH 8, as shown in Fig. 4. For pH above 8 (9 to 13), studies have not been carried out because precipitation occurred immediately after addition of the buffer into a solution containing of gallocyanine and Pb(II). In acidic medium (pH < 4), gallocyanine exists as diprotonated ligand and appear in red-violet colour whereas in basic medium (pH 5.5 to 8), gallocyanine exist as monoprotonated ligand and appear in blue colour. At pH > 8, gallocyanine exists as non-protonated ligand and appear in red-violet colour. The most suitable condition for detection of Pb(II) with gallocyanine is within pH range of 5.5 to 8 since the reagent appear in blue colour instead of red-violet colour in pH < 4 and pH > 8.

The effect of gallocyanine concentration on the complex formation was examined by varying their concentration. The absorbance increases with the increasing of gallocyanine. A maximum absorbance was obtained at a gallocyanine concentration of 0.15 % (g/mL) (Fig. 5). Thus this concentration was recommended for further analytical use. The reproducibility of the results were satisfactory with relative standard deviation (RSD) of 5.69 %.

The linear range under the optimum condition was obtained over the range of 1.0×10^{-3} µg/mL to 1.0×10^{1} µg/mL of lead. The limit of detection (LOD) for the proposed method is 2.9 ng/mL. Khan *et al.*[21] reported a detection limit of 1 mg/L level of lead by using the 1,5-diphenylthiocarbazone in micellar media. Takeshi and Yuzuru[22] reported a detection limit of 4.8 ng/mL based on complex formation with 2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfo-propylamino)phenol. The method proposed here offer a superior detection limit than others.

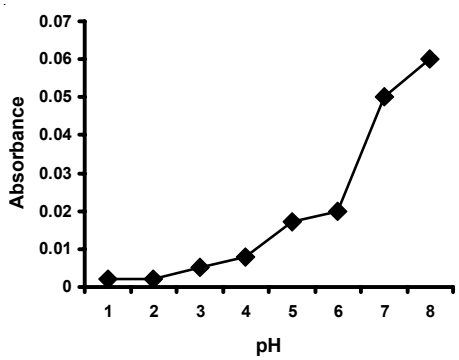

Fig. 4. Effect of pH on the absorption of Pb(II)-gallocynin complex

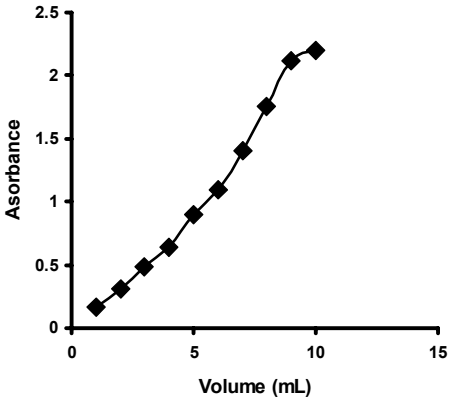

Fig. 5. Effect of gallocyanin concentration on Pb(II)-gallocynin complex formation

Table-2 shows the degree of the interference in presence of variety of diverse ion. Generally, negative interference was observed for anions whereas cations showed positive interference. Negative interference can result from the reaction of the interference with the analyte being determined, leading to an incomplete reaction[23]. The most common type of such interference is the complexing of the analyte by the interfering ion. Many cations form complexes in solution with a variety of substances that have a pair of unshared electrons (*e.g.*, on N, O, S atoms in the molecule) capable of satisfying the coordination number of the metal.

The degree of anion interference is varied in the order $F^- > NH_4^+ >$ citrate > EDTA. This is due to bulky structures of the ligands. Fluoride is the least bulky while EDTA is the most bulky ligand. Bulky structure reduces the interaction between the ligand and the metal.

Positive interference normally occur when the interfering ion react with the reagent along with the analyte and produce more intense coloured species resulting a higher reading in absorbance. Aluminium, iron(III) and mercury interfere most during the determination. However problem of these interfering ions can be eliminated by the use of conventional methods, such as application of a masking agent or more practical method, such as synchronous derivative spectrometry.

Optimization of the flow system: The sensitivity of the proposed method is largely dependent upon parameters such as flow rate and coil length. The effect of varying the flow rate was studied over the range 0.4 mL/min to 1.8 mL/min while other variable constant. By observing the plot in Fig. 6 it is reasonable to conclude that by decreasing the flow rate, the detector response increase due to a longer sample residence time in the mixing coil and a reduce in sample dispersion. The same observation has also been made by Nabi and Worsfold[24]. A flow rate of 1.2 mL/min was chosen in this study in order to achieve a reasonable timing in getting a peak. The pH of the waste solution which was passed through the coil was measured. A maximum pH for the colour development remains the same as in manual mode.

Reproducibility in this flow injection system is much more satisfactory compared to the manual mode (RSD of 1.94 %).

By changing the sensitivity of the detector and increasing the volume of the sample, a lower detection limit is achieved compared to the manual method. The linear range was obtained over the range of 1.0×10^{-3} µg/mL to 1.0×10^{2} µg/mL (Fig. 7). The LOD for this method is 1.6 ng/mL.

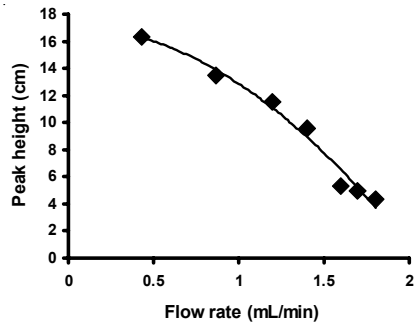

Fig. 6. Effect of pump flow rate on the peak height

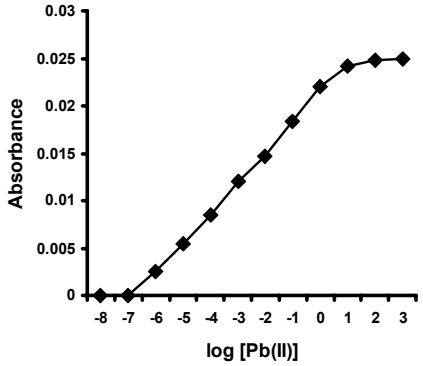

Fig. 7. Response curve the gallocynin reagent towards different concentration of lead

Studies on diverse ions do not significantly differ from the manual mode (referring to Table-1). This is expected since the interaction between the ions is the same whether in manual or dynamic mode. This method has been validated against inductive coupled plasma optical emission spectroscopy (ICPOES) method. A graph of the reading obtained from ICPOES *versus* the reading obtained from the sensor was plotted (Fig. 8) ($R^2 = 0.976$, n = 5). Statistical analysis (t test) was carried out upon the data gathered by the proposed method and inductive coupled plasma optical emission spectroscopy (ICPOES).The mean from both methods were not significantly different indicating that the results from both methods are comparable.

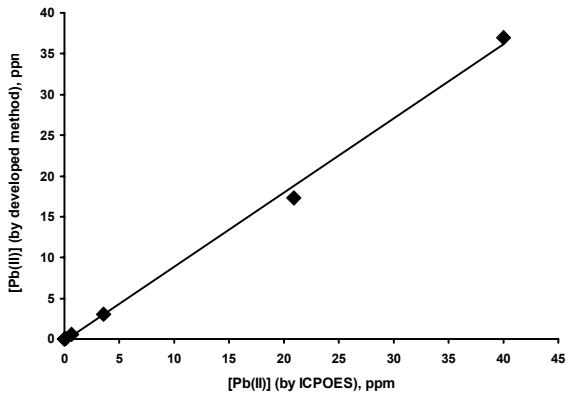

Fig. 8. Validation of the developed method with ICPOES

TABLE-1
DEGREE OF INTERFERENCE IN LEAD(II) DETERMINATION BY MANUAL AND FLOW INJECTION ANALYSIS METHOD

Diverse ion	Ratio	Manual	FIA
Ag	1:1	+7.8	+6.7
	1:3	+9.4	+8.3
	1:5	+6.1	+8.4
Al	1:1	+7.5	+6.9
	1:3	+14.2	+13.2
	1:5	+7.2	+14.1
Fe	1:1	+4.1	+3.2
	1:3	+13.4	+12.5
	1:5	+11.4	+12.7
Hg	1:1	+12.0	+11.3
	1:3	+13.9	+12.5
	1:5	+12.0	+12.5
Mg	1:1	+8.3	+7.2
	1:3	+10.3	+9.4
	1:5	+2.5	+9.3
Na	1:1	+1.9	+1.5
	1:3	+13.9	+11.5
	1:5	+6.7	+11.0
Citrate	1:1	-20.6	-16.0
	1:3	-15.0	-16.0
	1:5	-15.3	-15.0
EDTA	1:1	-5.3	-3.7
	1:3	-10.3	-9.8
	1:5	-7.2	-10.0
Fluoride	1:1	-32.4	-30.0
	1:3	-9.7	-30.0
	1:5	-9.2	-31.0
Ammonium	1:1	-26.5	-24.0
	1:3	-7.8	-20.0
	1:5	-6.7	-25.0

Conclusion

The experimental procedures developed in this work have enabled the determination of lead ions in solution over a wide concentration range. The sensitivity of the manual and flow injection photometric methods proposed here is superior to that of others[21,22]. The repeatability of the methods was satisfactory. The advantage of the method reported here is the simplicity of the determination (without preconcentration, extraction, *etc.*) and the low detection limit which is suitable in the analysis of most environmental samples including drinking water (for which the recommended upper safe limit is 50 μg/L)[25].

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