Sensitive and Selective Spectrophotometric Assay of Lead in Aqueous Solution

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ABSTRACT

A sensitive and selective spectrophotometric method for the determination of lead (II) in aqueous solution using zincon reagent has been developed. The pinkish red coloured complex was formed at pH 9.5 which shows maximum absorption at 543 nm. Beer's law is obeyed in the range 0.04 –1.2 ppm with a molar absorbtivity of 9.84 x 10^4 l.mol $^{-1}$cm^{-1}. The established stoichiometry is l:l ligand: metal. The method seems to be selective through the study of cationic and anionic interferences, easy, rapid, accurate, reproducible, and the complex is stable for 24 hours. The optimum conditions and the applications of the method have been studied for the assay of lead in different water samples.

Keywords: Lead determination, Zincon reagent, Aqueous solution, Masking agents, Spectrophotometry, Aanalytical applications.

(II)

(9.5)   .(   )

(1.2-0.04)   (543)

(   ) $^{1-}$.$^{1-}$.   (10^4x 9.84)

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(24)

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INTRODUCTION

The determination of lead is very important from both the health and pollution points of view. Lead is the most important element which affects the physical properties of steel, alloys and high purity metals (Taher, 2003). A regular absorption of small quantities of lead may cause serious injuries to health such as encephalopathy and kidney, (Dos Santos *et al.,* 2004). Lead poisoning is related to anemia, because the activity of heme synthetic enzymes can be inhibited by lead ions (Rubio, 2005). Lead is also a risk factor for hypertension in women (Houston and Johnson, 1999). Pb(II) determination in natural waters is of a considerable interest in the environmental sciences. (Nollet, 2000). In natural water, its typical concentration lies between 2-10 μg L^{-1}, whereas the upper limit recommended by WHO is less than 10 μg L^{-1} (Tewari and Singh, 2002). Concentrations of 5-10 μg lead in drinking water may increase urinary coproporphyrins (Wilson, 1966). The increased emphasis on trace lead determination developed a need for more rapid and accurate methods of analysis for this metal. Various methods are introduced to determine lead. Flame atomic absorption spectrometry (FAAS) is a more frequently used technique due to its simplicity and lower cost, although this method has a limited sensitivity for lead so a preconcentration step is often required to improve the detection limit (Vinas *et al.,* 1997). Other detection techniques are also used such as inductively coupled plasma mass spectrometry (ICP-MS) (Hung *et al.,* 1999). Spectrophotometric methods based on using the first order and second derivative for the determination of lead in synthetic alloy samples is developed (Nagalakshmi *et al.,* 2011). 3,5-Dimethoxy-4-hydroxy benzaldehyde isonicotinyol hydrazone (DMHBIH) is used as an analytical reagent for lead determination. This reagent gives a bright yellow coloured water soluble complex in alkaline buffer solution at λ =430 nm and ε =1.82x10 ^{4}. An on-line flow injection analysis system for the determination of lead in water samples with a preconcentration step and spectrophotometric detection was investigated (Klamtet *et al.,* 2008). The system is based on the preconcentration of lead on a column packed with Amberlite XAD-4 resin. A new and selective method for the determination of trace amounts of lead in aqueous environmental samples was reported (Tan and Ngocle, 2010). The method is based on the interaction between Pb(II) and benzoic acid azophenyl calix (4) arene reagent. A simple and accurate flow injection analysis system coupled with spectrophotometric detection was developed (Klamtet *et al.,* 2007) to the preconcentration and determination of lead in aqueous samples. This system is based on the reaction of Pb(II) with PAR at pH 9.0 with absorption maximum at λ= 523 nm. The determination of lead content in medicinal plants is developed by (Campos *et al.,* 2009), using pre-concentration flow injection analysis flame A.A.S. This method exhibited a good performance with high precision and repeatability and an excellent linearity. A simple, economical, reliable and versatile method for lead was developed (Lang *et al.,* 2009). The procedure has been validated by standard addition method and an ICP-MS method, it is based on the complex formation of lead with dithizone. A new flow injection on-line preconcentration system adapted to flame atomic absorption for lead and cadmium determination at the μg.l^{-1} was developed (Dadfarnia *et al.,* 2007). The procedure was applied to tap water, river water, pine leaf and blood samples. A procedure for the determination of lead in water samples by solid phase spectrophotometry was developed (Pellerano *et al.,* 2006). It is based on the reaction of Pb(II) ion with (5-Br-PADAP) reagent immobilized on an anion exchange resin that gives rise to a coloured complex, whose absorbance is measured directly in the solid phase. However, most of the used and applied

methods to lead determination are based on spectrophotometry because of their simplicity, sensitivity, ease of handling and comparatively low cost as well as the high speed, precision and accuracy. During an investigation of a number of oraganic reagents selected as possible analytical reagents because of their structure, the reagent 2-carboxy -2- hydroxy -5 sulphoformazyl benzene (zincon), gives a highly coloured product with lead.
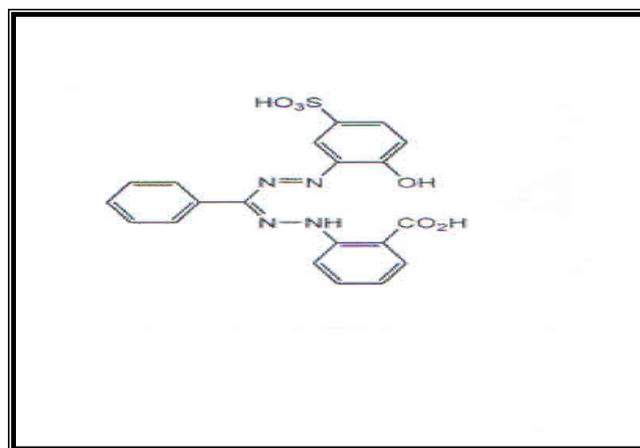
Zincon reagent structure

Zincon has been known as an excellent reagent for copper and zinc in aqueous solution (Sabel *et al.,* 2010). Based on these findings, from the above literature survey of lead; this paper reports a spectrophotometric method using zincon reagent for a fast, sensitive and selective assay of lead traces in aqueous solution.

EXPERIMENT

Reagents

Unless otherwise stated, all chemicals used were of analytical reagent grade, and double distilled water was used all over the work.

Stock lead (II) (100ppm) ≡ 4.826 × 10^{-4} M solution.

This solution is prepared by dissolving 0.1598 g of lead nitrate [Pb $(NO_3)_2$] (Fluka AG) in double distilled water containing a few drops of HNO_3. The volume is completed in 1000 ml volumetric flask, this solution is standardized against EDTA-Na_2 solution using xylenol – orange as an indicator. (Vogel, 1961)

Working lead (II) (10 ppm) solution.

This solution is prepared by diluting 10 ml of the above solution to 100 ml with double distilled water ,in a volumetric flask.

Composite complexing solution; complexing solution No(l).

This solution is prepared by dissolving 0.076 g of thiourea, 0.2542 g of 5-sulphosalicylic acid, and 0.0419 gm of sodium fluoride, in 80 ml water, the pH of the solution is brought to 9.5 with 0.1 NaOH and the volume is completed to 100 ml with double distilled water in a volumetric flask.

Composite complexing solution No (II).

This solution was prepared daily by dissolving 0.1761 g of ascorbic acid in 80 ml of water and the volume is completed to 100 ml with double distilled water.

Zincon reagent 5×10^{-4} M solution.

It is prepared by dissolving 0.0023 g of zincon powder in 2 ml of 1 M sodium hydroxide and diluting to 100 ml. This solution was stable for about one month, and it was stored in amber flask in the dark. **Note** that zincon is obtained from BDH(England) $C_{20} H_{15} N_4 O_6$ S.Na, Mwt =462.42, Product no. 13138.

Buffer solution (pH 9.5).

This solution is prepared by mixing 50 ml of (0.1 M boric acid and KCl) and 34.6 ml of NaOH, the volume is completed to 100 ml with double distilled water. (Dempsey and Perrin, 1974).

Apparatus

Absorption spectra are recorded using shimadzu UV-160 UV-Visible computerized recording double –beam spectrophotometer. Absorbance reading is carried out on a Pye-Unicam SP-30 UV digital double–beam spectrophotometer, using 1-cm matched glass cells. pH readings (at 25 C°) are made on PW-9421 pH meter (Philip's),reading to ± (0.001)pH unit.

A.A.S Instrumental conditions

Atomic absorption measurements are carried out using Shimadzu A.A 670 (Graphic printer).

Principle line 217.0 nm

Flame: air – acetylene

Lamp current: Maximum 6 mA. Sensitivity: 0.035 mg / l. Band pass:0.2 -0.5nm.

RESULTS AND DISCUSSION

Preliminary investigation

The visible spectra of the pinkish – red colour formed on mixing dilute Pb (II) solution and zincon reagent show an absorption band at (535 – 545) nm whereas the reagent blank shows maximum absorption at 476 nm. This colour contrast ($\Delta \lambda \cong 69$) nm, indicates a definite reaction in basic medium between Pb (II) and zincon reagent.

Study of the optimum reaction conditions

The effects of various parameters on the absorption intensity of the coloured complex are studied, and the reaction conditions have been optimized.

Effect of pH

The effect of pH on color intensity is first examined. Different volumes of 0.01 M of HNO_3 and 0.01 M NaOH solutions are added separately to an aliquot of solution containing 25 μg of Pb (II) and 5 ml of 5×10^{-4}M zincon reagent. The absorbances are read at λ max of each solution and the pH of the final coloured solutions is measured. The results are shown in Table (1 and 2). From the results it can be observed that the pH 9.5 is considered the optimum because of high sensitivity, low blank value. Higher pH values lead to higher blank and resulting in a weak colour contrast, while the absorbance decreases largely at low pH values. Therefore a pH 9.5 is selected for the subsequent investigations. Different buffers of pH 9.5 are prepared and their effects on the absorbance and the pH of the final reaction mixtures are studied. Five buffer solutions of pH 9.5 have been tested. These are:boric acid– NaOH (B_1), sodium borate-NaOH (B_2), glycine-NaOH (B_3), ammonia-

ammonium chloride (B$_4$) and Na$_2$CO$_3$ – NaHCO$_3$ (B$_5$). The results are summarized in Table (3).

Table 1: Effect of HNO$_3$ on absorbance

Ml of 0.01 M HNO$_3$	λ_{max} (nm)	Absorbance	Final pH
0.0	401	0.288	6.25
0.1	405	0.221	5.87
0.3	405	0.076	5.10
0.5	406	0.007	4.22
0.7	405	0.002	3.86
1.0	401	0.005	3.65

Table 2: Effect of NaOH on absorbance

Ml of 0.01 M NaOH	λ_{max} (nm)	Absorbance	Final pH
0.1	401	0.204	6.45
0.3	468	0.242	7.15
0.5	505	0.247	9.22
0.6	520	0.310	9.29
0.7	528	0.322	9.49
0.9	530	0.337	9.53
1.0	533	0.339	9.87
1.5	537	0.411	10.23
2.0	541	0.445	10.58
2.5	542	0.476	10.67
3.0	543	0.492	10.80

Table 3: Effect of buffers on absorbance

Ml of buffer solution	Absorbance/ml of buffer added				
	B$_1$	B$_2$	B$_3$	B$_4$	B$_5$
0.5	0.46	0.37	0.33	0.31	0.29
1.0	0.47	0.38	0.35	0.33	0.30
2.0	0.48	0.40	0.36	0.34	0.32
3.0	0.48	0.42	0.39	0.34	0.33
5.0	0.49	0.45	0.41	0.36	0.36
7.0	0.51	0.59	0.43	0.37	0.35
λ_{max} (nm)	540-543	540-543	540-543	540-543	540-543
Final pH of the reaction mixture	9.48-9.52	9.10-9.34	9.15-9.56	9.15-9.37	9.25-9.53

Although buffer solutions B1, B$_2$, B$_3$, B$_4$ give high sensitivity, 5 ml of boric acid – NaOH (B$_1$) has been used for the subsequent experiments because of its relatively good capacity, lower blank value (0.076) and stronger colour contrast.

Effect of masking agents:

In order to enhance the selectivity of zincon reagent as the case of some organic reagents which are reactive with a wide variety of metal ions, various masking agents were examined (Wilkinson, 1987). These are: sodium fluoride, oxalic acid, 5- sulphosalicylic acid, 1-10, phenanthroline, thiourea, ascorbic acid, citric acid, tartaric acid and hydroxyl amine hydrochloride. The results indicate that sodium fluoride, 5-(S.S.A), thiourea, and ascorbic acid have little effects on the absorbance of lead. Therefore, two solutions are prepared in which the first one contains 0.01 M of each of sodium fluoride, 5-S.S.A and thiourea (ClS), and the second contains 0.01 M of ascorbic acid (CIIS). The pH of the two solutions is adjusted to 9.5, and effect of different amounts of CIS in the presence of the same amounts of CIIS is tested. The results are shown in Table (4).

Table 4: Effect of composite complexing solution

MI OF CIS	Absorbance	Ml OF CIIS	Absorbance
0.5	0.385	0.5	0.388
1.0	0.392	1.0	0.389
2.0	0.480	2.0	0.485
3.0	0.483	3.0	0.487
4.0	0.484	4.0	0.487
5.0	0.483	5.0	0.487

3 ml of CIS and 4 ml of the above complexing solutions were chosen in order to increase the selectivity.

Effect of reagent amount:

The effect of zincon reagent concentration on the maximum formation of the coloured complex was studied by using the described procedure and adding to a series of lead concentration (1.0 - 10.0) ml of zincon solution. The experimental data showed that 7 ml of the used reagent seems to be optimum, because of a more stable absorbance reading of the complex is obtained here. The results are given in Table (5).

Table 5: Effect of reagent amount on complex formation

Ml of reagent 5×10^{-4} solution	Absorbance
1	0.399
2	0.445
3	0.482
4	0.484
5	0.485
7	0.487
9	0.487
10	0.486

Effect of surfactants:

The effect of the presence of surfactants (cationic: a = cetylpyridinium chloride, b = cetyltrimethyl ammonium bromide, anionic: c = sodium dodecyl sulphate and d =gelatin as neutral) on the colour intensity of the complex are examined. The experiment reveals the presence of such compounds gives no useful effect.

Effect of Time

To define the suitable time for absorbance measurement, the effect of time on the development and stability of coloured complex is investigated under the optimum conditions. Complete colour formation occurs after 5 min., and the absorbance of the complex remains constant for at least 6 hours.

Order of the addition of reagents

To test the effect of order of the addition of the reagents on the absorbance, solutions of order (I - IV) are tested as shown in Table (6). The experiment has shown that the order of addition NO.1: (M+CIS+CIIS+B+R) gives the best absorbance in each of the all order of additions tested. M= Pb^{+2} ion solution; CIS,CIIS (first and second complexing solutions); B= buffer solution; R= zincon reagent solution. Therefore, this order is selected for the subsequent experiments.

Table 6: Order of the addition of reagents

Reaction components	Order number	Absorbance
M + CIS + CIIS + B + R	I	0.488
M + CIS + CIIS + R + B	II	0.478
M + B + CIS +CIIS + R	III	0.473
M + B + R + CIS + CIIS	IV	0.470

Final absorption spectra

When a buffered aqueous solution of lead (II) ions are mixed with zincon reagent at pH 9.5, a characteristic pinkish – red colour of binary complex is immediately formed (Fig. 1) shows the absorption spectra of the coloured complex with maximum intensity at 543 nm treated according to the recommended procedure, but no absorption obtained with the reagent at this wavelength (the reagent absorbs light at λ= 476 nm). Therefore, all measurements were made at 543 nm.
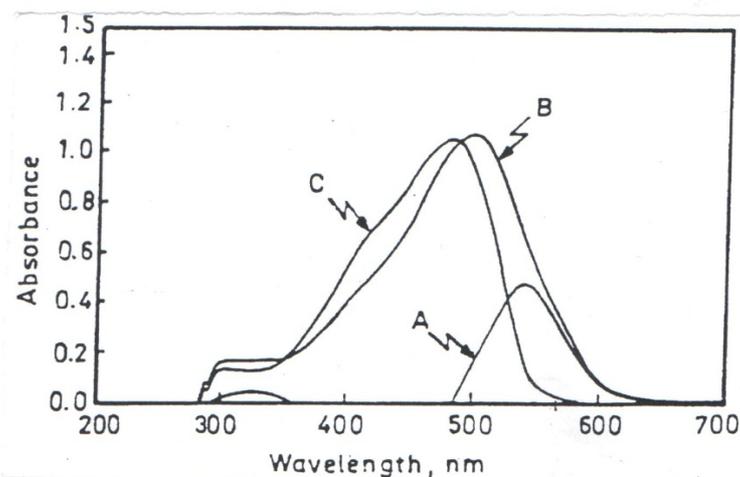
Fig. 1: Absorption spectra of 20 μg Pb(II)/ 25ml measured, (A) against blank, (B) against distilled water and (C) blank against distilled water.

Recommended Procedure and Calibration Graph

To a series of 25 ml volumetric flasks, increasing volumes of working Pb(II) solution are transferred to cover the range of 1.0-50 μg Pb (II). 3 ml of CIS and 4 ml of CIIS solutions are introduced 5 ml of buffer pH 9.5 and 7.0 ml of 5×10^{-4} M zincon reagent solution, are then added. The volumes are made to the mark with double distilled water. The reaction mixtures are mixed and absorbances are measured at 543 nm, against a reagent blank prepared in the same manner but without lead. The absorbance remains constant for more than 24 hrs. A plot of absorbance versus determinand concentrations (Fig. 2) shows that Beer's law is obeyed over the range of (1.0-30.0) μg lead in a final volume of 25 ml, i.e, (0.04-1.2) ppm. The molar absorptivity being 9.84×10^4 l.mol^{-1} .cm^{-1}, and a Sandell sensitivity index of 0.0021 μg.cm^{-2}.
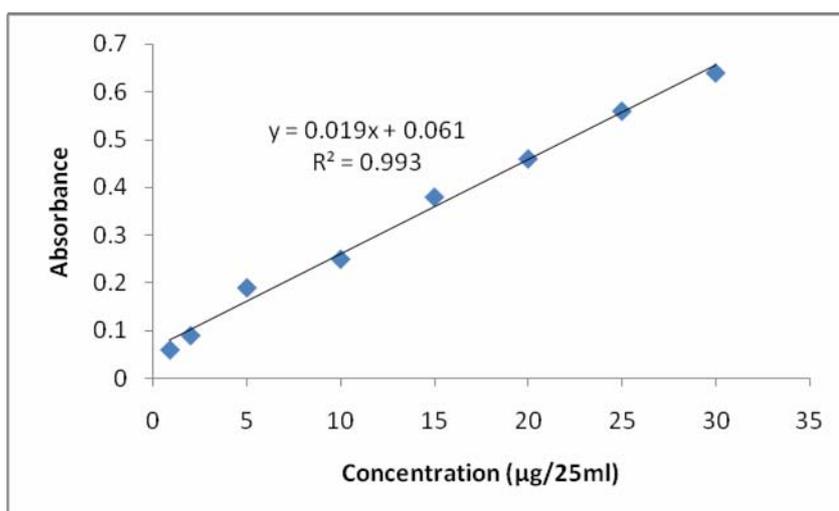
Fig. 2: Calibration graph for lead(II) determination with zincon reagent in aqueous solution

Accuracy and precision of the calibration graph

Under the experimental conditions which included in the recommended procedure, the recovery and precision have been checked by performing five replicate determinations for 1.0,10, 30 µg of Pb (II), the recovery and relative standard deviation are 100.01,100.08, 99.75 and $\pm$ 4.44, $\pm$ 0.76, $\pm$ 0.47 respectively, indicating a good accuracy and precision.

Nature of the complex

The nature of the complex was determined by Job's method of continuous variations and mole ratio method. (Hargis, 1988). The results reveal that the combination ratio of Pb (II): reagent is 1:1. Thus, the empirical formula of the coloured complex may be written as below.
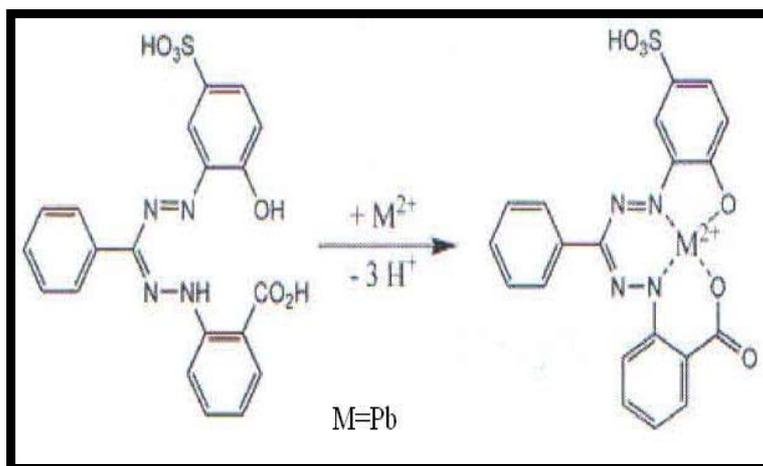
Fig. 3: Suggested structure of the complex

Stability constant of the complex

The conditional stability constant of the 1:1 Pb(II): reagent can be estimated using the following formula, (Hargis, 1988): $K = \frac{1-\alpha}{\alpha^2 c}$. The mean value of K is found to be 2.27 x 10^{+5} l. mol^{-1}.

Effect of Organic Solvents

The effect of different organic solvents on the absorbance of the coloured complex has been examined. The results obtained show that there are no colour contrast, also shift in wavelength of maximum absorption and the decrease in the intensity of the coloured complex are observed when solvents (methanol, acetic acid, formic acid, tetrahydrofuran, acetone and pyridine) are used. The effect of these organic solvent can be attributed to the decrease in the dielectric constant of the solution, and may be to the pH effect. (Ivanov, 1976). Therefore, water has been recommended for dilution because of high sensitivity, low cost, availability and simple handling.

Study of interferences

In order to assess the analytical applications of the proposed method, the interfering effects of foreign ions are examined by determining 20 µg of Pb (II) in the presence of each of the interfering ions that often accompany lead in real samples to be examined latter using the recommended procedure. The results obtained are summarized in Table (7).

Table 7: Effect of diverse ions on the determination of 20 μg of Pb (II) ion

Foreign ion	Form added	Amount added (μg)	Interference* %
Na (I)	$NaNO_3$	2000	1.5
Ca(ll)	$CaCl_2$	500	2.4
Mg(ll)	$MgSO_4. 7H_2O$	500	1.9
Fe(ll)	$FeSO_4.7H_2O$	250	3.6
Cd(ll)	$Cd(CH_3COO)_2.2H_2O$	100	1.4
Ag(l)	$AgNO_3$	1000	3.10
Al(lll)	$Al(NO_3)_3.9H_2O$	1500	4.15
Ba(ll)	$BaCl_2.2H_2O$	500	2.74
Co(ll)	$CoCl_2.6H_2O$	250	4.9
Cu(ll)	$CuSO_4.5H_2O$	500	3.3
Mn(ll)	$Mn(NO_3)_2.4H_2O$	500	2.94
Mn(Vll)	$KMnO_4$	500	2.18
As(lll)	$NaAsO_2$	1000	1.89
NH_4^{+1}	NH_4NO_3	2000	0.8
Th(lV)	$Th(NO_3)_4.6H_2O$	500	2.7
U(Vl)	$UO_2(NO_3)_2.6H_2O$	1000	3.2
$NO_3^{(-1)}$	$NaNO_3$	2000	1.54
$Cl^{(-1)}$	NaCl	500	3.7
$SO_4^{(-2)}$	Na_2SO_4	500	4.2
$PO_4^{(-3)}$	NaH_2PO_4	500	1.9
$CO_3^{(-2)}$	Na_2CO_3	150	1.1
$SO_3^{(-2)}$	Na_2SO_3	200	-1.4
$I^{(-1)}$	NaI	200	-1.8
$F^{(-1)}$	NaF	500	0.7
$Br^{(-1)}$	KBr	250	0.6

*This work seems to be selective with respect to the species cited above in the Table.

Comparison of Methods

The present work proves to be simple, selective, sensitive and needs no extraction. Table (8) shows some characteristics of the spectrophotometric methods used for lead determination. These methods in general lack applications, except the dithizone method which needs extraction into organic solvent.

Table 8: Comparison of methods for Pb (II) determination.

Reagent	pH	λmax (nm)	Molar absorptivity l.mol⁻¹.cm⁻¹	Ref.
Zincon	9.5	545	9.84×10^4	Present work
Dithizone	7-10	520	6.8×10^4	(Sandell, 1959)
Arsenazo(lll)	4-6	600	2.8×10^4	(Michaylovo and Kuleva, 1980)
4-(2-pyridy1 azorecorcinol	10.0	520	3.7×10^4	(Khopkar, 1971)
Pyrocatechol Violet	7.6	645	5×10^4	(Jarosz, 1988)
DMHBIH	9.0	430	1.82×10^4	(Nagalakshim et al., 2011)
BAPC	12.5	440	1.82×10^4	(Tan et al., 2010)
(5-Br-PADAP)	9.2	550	2.32×10^4	(Pelleramo et al., 2006)
Dithizone	7.0-9.5	520	------------	(Lang et al., 2009)
(5-Br-PADAP)	5.5	(597&629)	3.5×10^4	(Taher, 2003)

Applications of the method
Lead in water samples

The present method has been applied to the determination of lead in various water samples. Samples of water were collected and to each was added known amount of lead followed by extra pure HNO_3 (5 ml), the solutions were evaporated to dryness and the volume made up to 25 ml with distilled water (Joshi, 1985). An aliquot of each sample was then treated according to the developed procedure (this work).The results complied in Table (9) show that the method is suitable for determining lead in waters with satisfactory recovery.

Table 9: Determination of lead in water samples

Sample	Ml of sample	Pb(II) added (µg)	Pb(II) found (µg) spectr.	Recovery* %	Pb(II) found (µg) A.A.S
Tap water	1	5	5.15	103.0	5.21
		10	10.21	102.1	9.87
		25	25.15	100.6	24.96
	3	5	4.95	99.0	5.05
		10	10.14	101.4	10.21
		25	24.87	99.48	25.06
	5	5	4.97	99.4	4.56
		10	9.48	94.8	10.31
		25	25.54	102.16	25.87
Tigris river water (Mosul City)	1	5	5.02	100.4	5.34
		10	9.96	99.6	9.24
		25	24.89	99.56	25.15
	3	5	5.03	100.6	4.88
		10	10.12	101.2	9.87
		25	24.79	99.16	25.12
	5	5	4.83	96.6	4.69
		10	10.46	104.6	10.85
		25	24.59	98.36	25.14
Ground well water (Mosul city) (Bartela city)	1	10	10.15	101.5	9.65
	3	15	14.95	99.6	15.05
	5	25	25.12	100.48	24.85

(*) Average of five determinations

CONCLUSION

The development of simple, sensitive, selective and inexpensive spectrophotometric method for the determination of lead in aqueous solution has been carried out. The method is based on the reaction of lead with zincon reagent at pH 9.5 and λmax of 543 nm to form a binary 1:1 chelate which is water soluble and stable. Beer's low is obeyed over the range 0.04-1.2 ppm and $\varepsilon = 9.84 \times 10^4$ l.mol^{-1}.cm^{-1}. The method is applied successfully to assay lead in various water samples.

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