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Spectrophotometric Assay of Lead in Human Hair Samples by using alizarin red (S) in Samarra area

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ABSTRACT

A new spectrophotometric method for determination of lead in human hair samples was developed. The method is based on the reaction of lead(II) with alizarin red(S) in basic medium to form a pink red water soluble complex which gave a maximum absorbance at 518 nm. The optimum conditions (effect of Nitric acid and hydrogen peroxide pH of medium, time effect, heating effect and reagent concentration) were investigated. The method obeyed to beer's law over the concentrations range 1.50 ppm -9.00 ppm with molar absorptivity = 32522.96 l.mol-1.cm-1 and Sandell index 0.0064µg.cm-2. It was applied successfully to determination of lead in pure and human hair samples with high sensitivity and good validity.

Introduction:-

Heavy metals are an important class of pollutants and derive from both point sources (e.g., sludge dumping, industrial effluents, mine tailings) and diffuse sources (e.g., highway runoff)(1), Their content present in the environment is a result of its release by natural processes and a long history of anthropogenic use of lead. Its increasing use in the various industries such as storage batteries, gasoline, cable manufacture, paint industry motorization and increasing use of communal and industrial waste water for liming and fertilizing soil as well as long-lasting use of some pesticides may lead to excessive concentrations of lead, cadmium, zinc, copper or mercury in the soil, and ammunition have resulted in recurring environmental contamination in developing and industrialized areas of the world(2). It has been discovered that there is a correlation between content of heavy metals in soils and in vegetables grown on it. Lead is accumulated throughout the food chain and ultimately reaches the human organism (the maximum daily dose of lead which does not lead to accumulation is 0.50 mg which consider a great importance to human health(3-6)

The normal amounts of lead in female and male hairs in normal Pakistan peoples were 6.41-7.37 and 7.37-10.17 respectively while these amounts were 15.51-18.73 and 18.76-25.78 respectively in skin disease patients(7,8) .Due to it's toxicity, it's determination is important and several techniques such as voltametry by using banana tissue as modifier of carbon paste electrode for voltametric method(9), by using differential pulse anodic stripping voltametry with hanging mercury drop electrode(10), by continuous flow square-wave anodic stripping voltametry at a gold electrode flow cell(11), mass spectrometry- inductively coupled plasma (12) to determination of organic Pb in oil, milk powder, and estuarine sediment, inductively coupled plasma atomic emission (13), by using poly[benzo-18-crown-6] and column chromatography(14), atomic absorption spectrometry (AAS) in biological, soil and vegetable samples(15-18), and spectrophotometry by simultaneous determination of Pb (II) in complexes with 1,2-Diaminocyclohexane-N,N,N,N-tetra-acetic

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acid(19), by inhibition of the arsenazo I peroxide(20), with 2-(5-bromo-2-piridylazo)-5-diethylaminophenol (21), with dibromo-p-methyl-chlorosulphonazo (DBMCSA) to give blue coloured complex of maximum absorbance at 663nm(22), with1,5-diphenylthiocarbazone and sodium dodecyl sulphate resulting an intense red-blue complex with a suitable absorbance at 500nm(23), with 2-(5-bromo-2-pyridylazo)-5-(diethylamino) phenol immobilized on an anion exchange resin(24) or By flow injection spectrophotometric using 1,5-diphenylthiocarbazone in aqueous micellar(25) , ion selective sensors(26), have been used for its determination at low concentration level .

Because there is no any study indicated the levels of lead around Samarra area to know if there is any pollution with this poising metal although it is a crowded town with cars and the workers treated with alloys containing a lead in its active industrial area, so this work was designed to investigate these levels.

Experimental method :

Sampling and treatment:-

This study was conducted on 36 Iraqi persons (males and females at different ages) from Samarra City, Salah-addine province , Iraq.

The hair was cutted close in suboccibital area of the head (about 1-2 cm) after ascertaining that no coloring agent had been used, the hair samples were washed twice , first with acetone and then with DDW and let to dry completely.

Procedure assay of Human Hair:-

Before analysis, each individual hair sample was cut into approximately 0.5 cm long pieces and mixed to allow representative sub–sampling of the hair specimen. Then, each 0.05 gm of sample was assayed with 2 ml of concentrated nitric acid and heated in covered beaker for 5-10 minutes till boiling and the sample had been left over night to complete the assaying of the hair samples, then 1 ml of 30% of H2O2 was added and heating until the color of the solution became clear .

Preliminary investigations :-

When the Pb (II) solution was added to alizarin red (S) in basic medium a red pink water soluble complex was obtained.

Developing method :-

The solutions to be studied were prepared in series of 10 ml volumetric flasks, exactly measured 1ml of Pb (II) (1x10-3M) of pb(II) and 2 ml of alizarin red (S) (1x10-2M) and the final pH modified to 12± by using 5M NaOH, Then the volume was completed to the mark with DDW. Absorbance of the solutions was measured against corresponding blank similarly prepared at 518 nm.

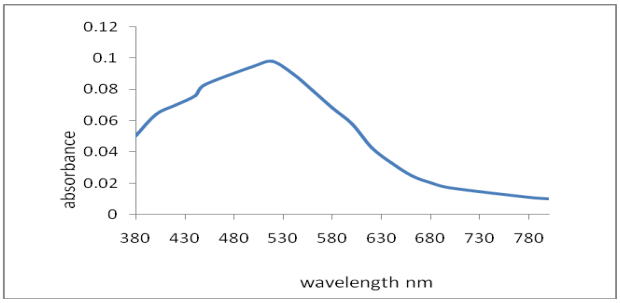
Results and discussion :-

Optimum variables:

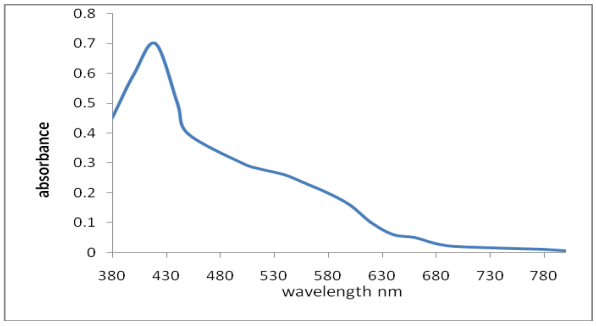
Some optimum variables were studied in order to establish the optimal working conditions for the quantitative determination of Pb(II) and prediction the stoichiometry of the complex.

Influence of the wavelength:-

The spectrum plotted for solution containing Pb(II) complex is presented in figure (1) from 380-780 nm. The maximum absorbance appeared at 518 nm so all further measurements have been performed versus a corresponding blank , otherwise the alizarin red(S) solution gave maximum absorbance at 420nm versus DDW as in figure(2).



Figure(1) the absorbance spectra for the complex vs. blank



Figure(2) the absorbance spectra for the alizarin red (S) vs. DDW

Effect of Nitric acid volume:

The effect of Nitric acid volume on the color intensity of complex with no effect on the nature of reagent and complex was studied in order to fix the needed amount to digest hair samples by using of adding different volumes(0.5-4) ml of concentrated acid to form the complex as in table (1) where 2ml showed maximum absorbance.

Table(1) Nitric acid effect

Nitric acid Vml	absorbance
0.5	0.567
1	0.651
1.5	0.692
2	0.766
2.5	0.732
3	0.711
3.5	0.690
4	0.657

Hydrogen peroxide volume effect:

In order to fix the volume of hydrogen peroxide needed to clear the color of the hair solutions with no effect on the nature of reagent and complex, variant volumes of 30% H2O2 were used. The results showed 1ml was the suitable volume though maximum absorbance of the complex as in table (2).

Table(2) Hydrogen Peroxide volume effect

H2O2 Vml	Absorbance
0.25	0.44
0.5	0.56
1	0.69
1.5	0.65
2	0.58
2.5	0.56
3	0.53
3.5	0.49
4	0.46

Alizarin red (S) volume effect:-

To 10 ml volumetric flasks contain (0.5 ml - 4 ml) of 1 ×10-2 M of alizarin red (S), 1 ml of 1×10-3 of Pb (II) was added, and measured the absorbance of the red complex at 518 nm. The results shown that 2 ml of Alizarin red (S) was the suitable volume for reaction formation as in table (3).

Table (3) Alizarin red (S) volume effect:-

Alizarin red (S) volume ml	absorbance
0.5	0.51
1	0.66
1.5	0.93
2	0.95
2.5	0.82
3	0.77
3.5	0.60
4	0.55

Temperature effect:-

Several experiments were done to establish the effect of heat over the temperature range 20oC-85oC. The maximum absorbance of the colored complex showed between 35 to 40 oC as in figure (3).

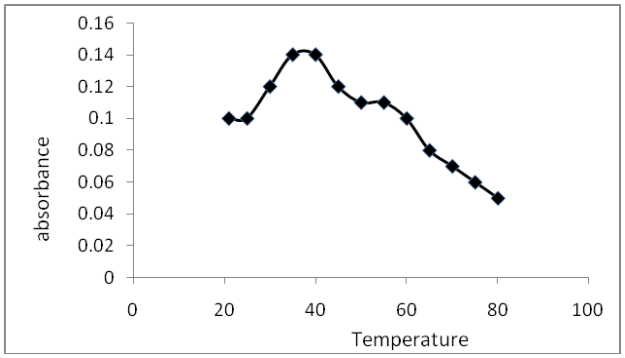


Figure (3) the effect of temperature on the complex's stability

At low temperatures the reaction didn't complete, and at high temperatures the complex began degradation, So 35oC to 40 oC temperature was the best range in this method .

Reaction time and stability of complex:-

After mixing the reactants , The complex appears directly and its color intensity increase with time till 30 minutes and be constant to above 90 minutes as in figure (4). This stability gives good chance to do all the requiring measurements. After that the complex might be began digridation according to the dicrese in absorbance with time.

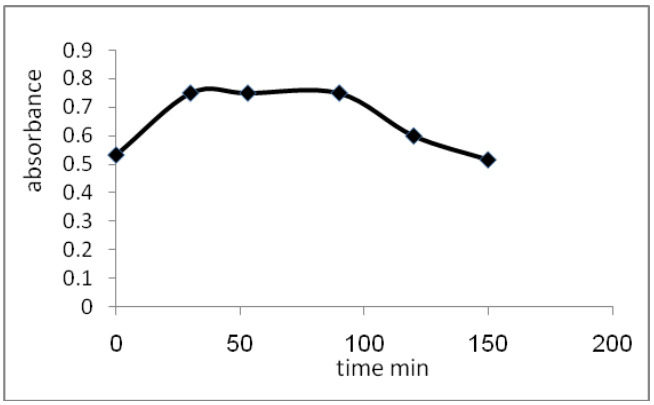


Figure (4) shows the time effect on the stability of complex

pH effect:-

The effect of pH on the stability of the complex was studied over the range1.5-13.5 pH unit. The results were shown pH= 12± is the best pH requiring to complete the reaction, otherwise the complex decomposes in strong acid and basic media. These results are shown in figure (5).

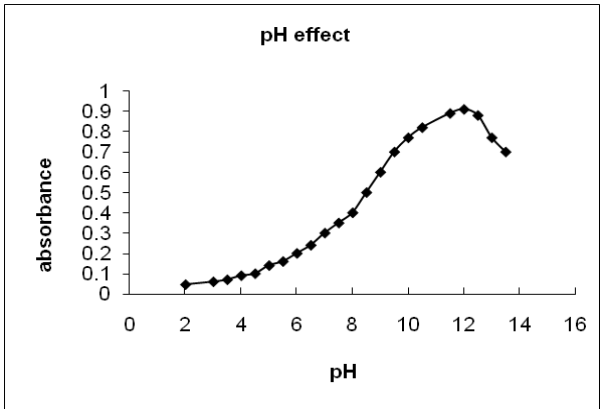


Figure (5) pH effect on complex formation

Stoichiometry of the complex:-

In order to establish the stoichiometry of the complex, the ratio between ligand and Pb(II) was examined by measuring the absorbance of the solutions containing fixed concentration of Pb(II) and different concentrations of reagent (mole ratio method), and by Job's method which include mixing versus volumes of ligand and reagent (they have same concentration), the final volumes of two methods were 10ml.

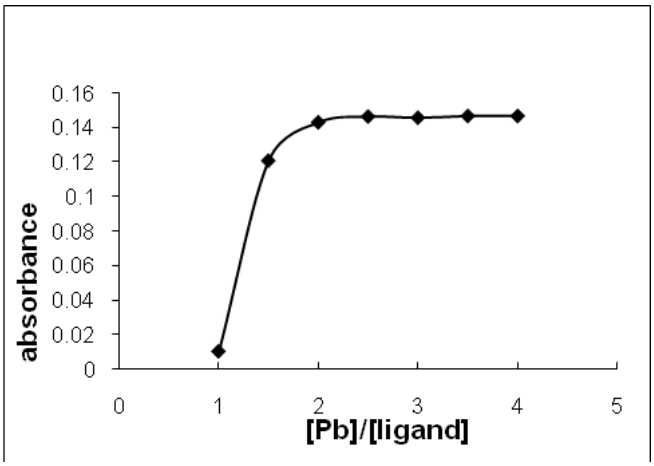
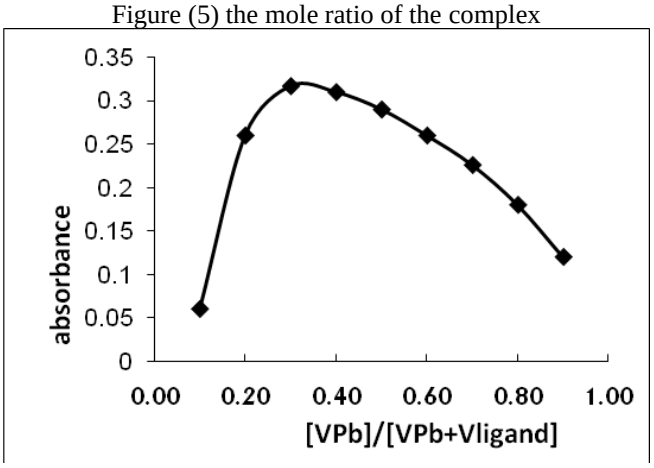
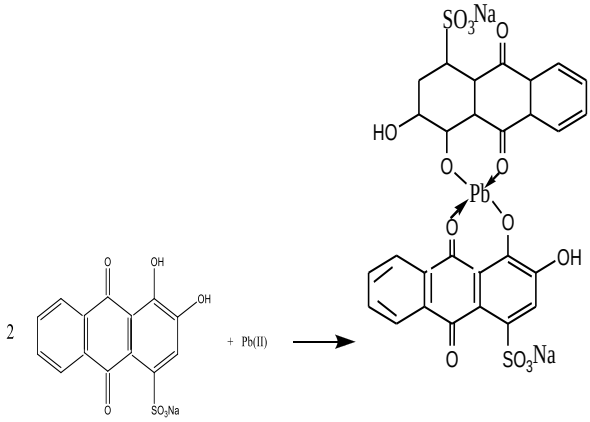


Figure (5) the mole ratio of the complex



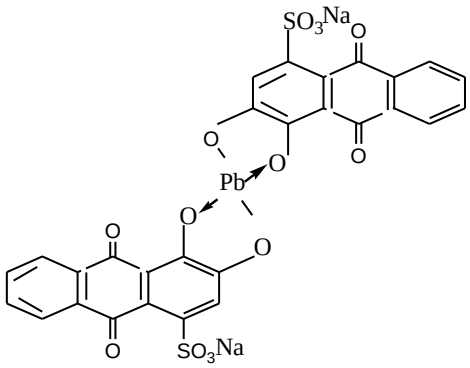
Figure(6) Job curve of the complex

The results indicated the form of complex is 1Pb (II): 2 reagent as in figures (5 and 6). The colored complex probably occurs as forms 1 or 2.



Alizarin Pb complex (1)

or



Pb complex (2)

Calibration Curve:-

To a series of 100ml volumetric flasks containing aliquot volumes (0.72 ml – 4.35 ml) of 1×10^{-3}M of Pb (II), 2ml of 1×10^{-2}M of alizarin red (S) was added. The volumes were completed to the final volume with DDW water and employing other optimized conditions, a linear calibration graph for Pb(II) is obtained in (Figure 7).

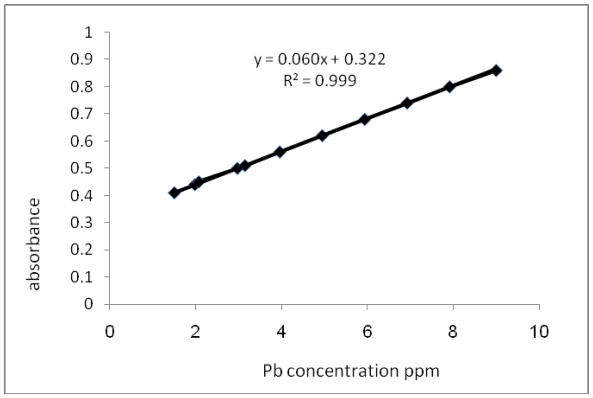


Figure (7) Calibration Curve of the complex

The results showed that Beer's law is obeyed over the concentration range of (1.5ppm – 9ppm), with correlation coefficient (R) of 0.998. The molar absorptivity of the red product formed was found to be 32522.96 $L.mol^{-1}.cm^{-1}$, Sandell index was 0.0064, the limit of detection (LOD) was 0.223 mg/liter and limit of quantification (LOQ) was 0.67 mg/liter. These results indicated high sensitivity of the method.

Method Validation:-

Accuracy and precision:-

Accuracy of the method was assessed as the percentage relative error (Err%) and percentage recovery (REC%) while the precision was assessed as a Percentage relative standard deviation (R.S.D%) of the predicted concentrations from the regression equation, So different concentrations of Pb (II) were prepared and analyzed five times (n = 5).

The accuracy and precision of 4.5, 7, 13, 15.4, 17, 20, 24, and 27 µg/ml were ranged in (table 4). The percentage recovery values, from 96.67% to 102 .86%, with relative error percentage of not more than +2.86%, established the high accuracy of the proposed method, while the RSD% (less than 2.1%) show high precision.

Table (4) The accuracy and precision of the method

Pb present ppm	Pb found ppm	±RSD%	Err%	Rec%
1.5	1.56	1.3	-0.23	99.77
2.07	4.76	2.1	+3.06	103.06
3.15	7.16	1.7	-0.42	99.58
4.95	11.96	0.8	+0.49	100.49
6.93	16.76	0.98	+0.58	100.58
9	19.16	1.34	-0.39	99.61

Application of method :-

1 ml of each hair sample solution was transferred in to 100 ml volumetric flask, added 2 ml of 1×10-2alizarin red (S) And completed the volume to the mark with DDW and employing other optimized conditions. Table (5) shows the results of the analysis of the Pb(II) in the samples.

Table (5) The Pb concentration of Pb in human hair samples

No	Sample ppm	No	Sample ppm	no	Sample ppm	No	Sample ppm
1	1.94	10	2.08	19	2.40	28	5.92
2	N.D	11	1.79	20	3.31	29	2.46
3	1.82	12	2.42	21	2.34	30	4.21
4	N.D	13	2.42	22	4.12	31	5.80
5	2.50	14	3.29	23	3.21	32	N.D
6	1.50	15	2.33	24	6.11	33	3.94
7	3.20	16	4.12	25	6.11	34	6.41
8	1.50	17	3.22	26	5.40	35	8.99
9	1.94	18	6.10	27	8.22	36	3.71

The Pb concentration in samples was not more than 8.97 ppm indicating not high pollution with Pb in Samarra area (7, 8).

To provide an additional support to the accuracy and precision of the developed assay method, a standard addition method was employed, which involved individual each solution of hair sample to two portions, one of them used to assay Pb(II) in human hair and another portion were used in addition standard method by

Addition of fixed concentration of Pb (II) (2 µg/ml) to it, the found concentrations were determined using the proposed methods (n=5). The percentage recovery, relative error percentage, and relative standard deviation of the added pure drug were calculated as in table (6).

The results showed the method gave good precision and accuracy. The RSD percentage was between (0.82% – 1.3%), the Error percentage and recovery percentage between were between (-4.5%-+5.5%) and (96%-105.5%) respectively. This method indicated there is no effect of the interfering on the determination of Pb(II) in the samples.

Table (6) shows the analysis results of the method

Sample No	Pb ppm indicated in hair	Pb ppm added	Pb ppm total	Pb ppm found	RSD%	Err%	Rec%
1	1.94	2	3.92	3.9	1.03	-1	99.00
7	3.2	2	5.03	5.07	0.82	+2	102.0
12	2.42	2	4.38	4.3	1.02	-4	96.00
15	4.09	2	6.09	6	0.95	-4.5	95.50
17	6.07	2	8.07	8	1.10	-3.5	96.50
18	8.76	2	10.76	10.68	0.82	-4	96.00
20	7.49	2	9.49	9.6	1.33	+5.5	105.5
31	5.78	2	7.78	7.88	0.83	+5	105.0

Comparing the method:-

To determination the advantages of this method, it is compared with reference methods as in table(5).

The results of comparing with another spectrophotometric methods showed the developing method have five advantages (good limit of detection and quantification, the linearity range is more wide, correlation coefficient and Rec%) .

A sensitive spectrophotometric method had been performed for determination of Lead in human hair. It‘s accuracy, and precision were good. The method has high sensitivity (through molar absorptivity 32522.96 and Sandell index 0.0025 µg.cm-2) and can be used without interfering effect with mean linearity of lead over 1.5µg/ml -66.6 µg/ml.

Table(5) Comparing the developing method with another references methods

Parameter	Developing method	Reference method(28)	Reference method(27)
Reagent	Alizarin red (S)	Dichlorohydroxylporphyrin-triton x-100	4-(2-pyridylazo) resorcinol
RSD% average	0.99	0.44	2.4
LOD µg/ml	0.2	1.45	11
Linearity µg/ml	1.5-9	Up to 0.46	0.05-.9
Color	Pink red	Yellow	Red
Wavelength nm	518	482	523
L.O.Q µg /ml	0.67	4.4	------
Regression equation	Y=0.06×+0.322	Y=0.093×+0.0012	Y=1.522×-0.031
Correlation coefficient (R)	0.998	0.9992	0.9963
Molar absorptivity L.mol-1.Cm-1	3.2523×104	2.32×105	------
Sandell sensitivity mg/cm2	0.0064	-----	------
Rec% average	100.15	-----	102
pH	12±	9	9

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التقدير الطيفي للرصاص في نماذج شعر بشري باستخدام كاشف الاليزارين الأحمر(S) في منطقة سامراء

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الخلاصة

تم تطوير طريقة طيفية جديدة لتقدير الرصاص في نماذج شعر بشري . تستند الطريقة تفاعل الرصاص الثنائي مع الاليزارين الاحمر (S) في وسط قاعدي لتكوين معقد احمر وردي ذائب في الماء اعطى اعظم امتصاص عند 518 نانومتر. وتم تثبيت الظروف المثلى للتفاعل مثلتاثير حامض المنتريك وبيروكسيد الهيدروجين الدالة الحامضية وتاثير الزمن وتاثير التسخين وتركيز الكاشف. وقد بينت الطريقة المطاوعة لقانون بير عند التراكيز 1.5− 9جزء من المليون وكانت قيمة معامل الامتصاص المولاري 32522.96 لتر.مول−1.سم−1وحساسية ساندل 0.0064 مايكروغرام.سم−2. طبقت الطريقة بنجاح في تقدير الرصاص في النماذج النقية وفي نماذج الشعر البشري بحساسية جيدة.