

## **Determination of Chromium in blood serum using Atomic Absorption Spectrophotometric (AAS)**

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### **Abstract:-**

A new approach for the direct determination of chromium by Electrothermal Atomic Absorption spectrometry (ETAAS) is proposed, the method employs a 1.0 cm X 0.3 cm Tantalum metal coil placed in graphite tube. The experimental conditions for the determination of chromium via this method have been optimized. Pyrolysis and atomization curves are constructed using uncoated graphite tube without and with coil. The calibration curves were linear over the rang 10- 50 ng/ml of chromium concentration, with relative standard deviation values, at 0.013, 0.018 and 0.024 $\mu$ g/ml. the accuracy and precision of the method was evaluated by determining recoveries percentage between (99.7-101) and standard deviation and using different concentrations of standard solutions of this method was applied successfully for the determination of chromium in human blood serum.

**Key words :** Blood serum , Chromium ,Electrothermal and Tantalum.

### **الخلاصة**

تضمنت الدراسة تطوير طريقة جديدة لتقدير عنصر الكروم مباشرة باستخدام مطيافية الامتصاص الذري الكهروحراري بوجود منصبة معدنية من عنصر التنتاليوم بابعاد 1سم طول وعرض 0.3 سم يتم وضعها داخل الفرن الكرافيتي بشكل ملف.

تم دراسة الظروف الالية لتقدير عنصر الكروم باستخدام الطريقة الجديدة ورسم منحني الترميد والتذرية باستخدام الفرن الكرافيتي بوجود وبدعم وجود الملف ووجد تطور كبير في المعطيات التحليلية بوجود الملف وكان منحني المعايرة خطيا لعنصر الكروم مع انحراف قياسي نسبي مؤوي قليل لمحاليل قياسية معلومة وطبقت الطريقة بنجاح لتقدير عنصر الكروم في نماذج من مصل الدم.

## **Introduction:-**

Chromium recognized to be essential for human since the 1960. May have function in the control of glucose and lipid metabolism. Chromium deficiency leads to impaired lipid and glucose metabolism and results in high cholesterol levels. Although, the determination of chromium species Cr(III) and Cr(VI) is important, the total concentration of the element is very useful, especially if one form is determined and other form is calculated by differences<sup>(1-6)</sup>.

Chromium exists in different oxidation states in groundwater, industrial waste water and soil. In the form  $\text{HCrO}_4^- / \text{Cr}_2\text{O}_7^{2-}$  (pH 1-6) or  $\text{CrO}_4^{2-}$  (pH > 6). Is highly irritating and toxic to humans and animals<sup>(7-10)</sup>

Recently a number of methods using FAAS<sup>(1,7)</sup>, ICP-OES<sup>(3)</sup>, ETAAS<sup>(2,8,12)</sup>, OE and absorption spectroscopy<sup>(5)</sup>, CPE combination FAAS,<sup>(7,17)</sup> AAS using chemically modified chloromethylated polystyrene – PAN Ion Exchange extraction solid- phase<sup>(8,9)</sup>, ICP-MS and ICP-AES<sup>(10,13,15)</sup>, ICP<sup>(11)</sup>, ICP-AAS<sup>(14)</sup> forked ETAAS<sup>(16)</sup>, Coil tungsten<sup>(16,18-21)</sup>, X- Ray Fluorescence<sup>(22)</sup> and tantalum second surface<sup>(23)</sup>

In this study a new atomization medium for Chromium determination via ETAAS was developed. It is simple constructed, available and gave accurate and precise results under mild heating cycle conditions in comparison with commercial uncoated graphite tube and no need for chemical modifiers to be used.

Materials and Methods:-

## **Apparatus:-**

Shimadzu model AA-6200 flame atomic absorption spectrophotometer and Graphite Furnace Flameless Atomic Absorption GFAAS-6200, Furnace Uncoated Shimadzu, Coil –tantalum length 50 centimeter, Centrifuge, micropipette.

### **Chemicals:-**

A commercially available Stock solutions of each element studied were prepared by dissolving the appropriate mass of pure metals (99.99%) in a slight excess of nitric acid and the solution then made up (Aldrich/ 1000 $\mu$ g/ml in 1% HNO<sub>3</sub>) Cr (as K<sub>2</sub>CrO<sub>4</sub> FLXANAL) for each element and subsequent dilution is then carried out

to obtain calibration curve. Working solutions were freshly prepared by diluting aliquots of this solution with deionized water before use. Nitric acid, sulfuric acid and hydrochloric acid (Analytical grade, FLUKA) was used.

### **Procedure:**

A series of chromium are standard solutions (10-50) ng/ml prepared in 1% HNO<sub>3</sub> were injected in a uncoated graphite furnace in which a piece of tantalum metal (1.0cmX0.3cm) coil was inserted. The atomic absorption signal for each standard was measured after subjecting it to the heating cycle under optimum conditions.

In this study we use tantalum filament after we put coil on graphite furnace , a wire which has a length suitable (1.5 centimeter) and radius (1.2 mm) , and then put the furnace with the coil-tantalum in instrument (AAS) , regular the furnace whereas the resonance radiation for element is pass without intercept with coil wire and complete regularly position to fall down droplet, it must be above wire coil completely and not on furnace wall or down the surface furnace show to fall down droplet by mirror constant on the basic of instrument to embrace using for this purpose.

This is done in three ``stages for both elements:-

The first stage:- the temperature of atomization and ashing for both elements is found out by making atomization and ashing curves each individually.

The second stage:- finding the temperature of atomization and ashing elements after introducing single spiral in the graphite furnace and making atomization and ashing curves each individually.

The third stage:- finding the temperature of atomization and ashing for both elements after introducing couple spiral in the graphite furnace and making atomization and ashing curves each individually. The concentration (0.03 µg/ml) was used to find out the optimum temperature of ashing and atomization for both elements chromium within three stages. The ashing curve of manganese has been made when the furnace empty from the single spiral then the furnace contain double spiral, the atomization curve has been made by using the same method. The atomization and ashing curves of chromium element have been made by applied the previous methods.

These methods were applied on a number of human bloods in serum samples, and the levels of detection limit were good.

**Sample collection and Serum preparation:**

Blood samples were taken into plastic disposable from the controls subjects and placed in acid washed tubes. Serum were separated within two hours of collection and kept in refrigerator at freezing point until the day of analysis. All specimens were then thawed at ambient temperature, mixed thoroughly, centrifuged briefly to precipitate fibrin and then processed.

## **Results and discussion**

Under optimum conditions graphite furnace atomic absorption spectroscopy method which was use above to study the effect of variable on the absorbance intensity that gave the optimum condition shown in tables for manganese and cobalt.

**Table(1). Instrumental conditions for a Shimadzu for four states of Element Chromium**

<b>Element Chromium</b>	<b>Drying Temp.(C°)</b>	<b>Ashing Temp.(C°)</b>	<b>Atomize Temp.(C°)</b>	<b>Cleaning Temp.(C°)</b>
Uncoated Without Coil	100	1100	2400	2600
Coated furnace	100	1000	2300	2500
Uncoated & single coil Tantalum	100	1000	2300	2500
uncoated&platform Ta	100	900	2200	1800

Figures a calibration graph for chromium has been obtained for 10 $\mu$ l volume injection for several concentrations. Figures show the absorbance measurements of a series of standard solutions prepared in different concentrations and the triplicate

mode for each set of standard, in this figure the absorbance measurements refer very clearly to the good reproducibility which has been obtained indicating that the method of preparing the standard solution are done in good methodology. It can be seen from the results obtained that improved sensitivity is achieved with coil Ta compared to another atomization.

The statistical treatments for calibration graphs are summarized in table (2)

**Table(2): Analytical values of statistical treatments for the chromium**

<b>Parameters (Cr)</b>	<b>Without coil-Ta</b>	<b>Coated furnace</b>	<b>With single Coil - Ta</b>	<b>With platform-Ta</b>
Correlation coefficient	0.9994	0.9997	0.9999	0.9999
Regression equation	63.766X-0.0111	76.484X+0.0008	85.697X+0.001	93.135X-0.0016
Slope (ml/ $\mu\text{g}^{-1}$ ),b	63.766	76.484	85.697	93.135
Intercept a	0.0111	0.0008	0.001	0.0016
Standard deviation for residual Sy/x	0.00263296	0.00760698	0.001376	0.0037228
Limit of Detection ( $\mu\text{g/ml}$ )	0.0000507	0.00002104	$1.2797 \times 10^{-5}$	0.0000117869
Limit quantification ( $\mu\text{g/ml}$ )	0.002633	0.000994	$1.606 \times 10^{-3}$	0.00039973
Sensitivity	0.00000664	0.0000046684	$4.112 \times 10^{-6}$	0.000003447

The accuracy and precision of the determination (Chromium) was studied depending upon the value of the relative error (E %), Recovery (Rec %) and Relative standard deviation (RSD %) respectively.

For five replicate measurements of each of concentration were done and the average value were used, the results were listed in table (3) which

show good accuracy and precision in aqueous solutions and in blood sera samples.

**Table(3): Accuracy & precision of Selenium**

Chromium	Conc. µg/ml		E%	Rec%	RSD%
	Present	Found			
Without coil	0.006	0.0059	- 1.667	98.333	0.173
Coated furnace	0.006	0.00602	+0.333	100.333	0.165
single coil Ta	0.006	0.00609	+1.5	101.5	0.107
Platform- Ta	0.006	0.00612	+2	102	0.098

**Table(4). Accuracy & precision of Chromium in serum samples**

Samples with coated furnace	Serum Chromium Conc. µg/ml			Error %	Rec. %	RSD%
	Present	Added	Found			
1.	0.026	0.001	0.0262	0.769	100.769	0.121
2.	0.032	0.002	0.0323	0.938	100.938	0.1
3.	0.048	0.003	0.0485	0.833	100.833	0.06711

**Table(5). Accuracy & Precision of Chromium in serum samples**

Samples & single coil - Tantalum	Serum Chromium Conc. $\mu\text{g/ml}$			Error %	Rec. %	RSD%
	Present	Added	Found			
1.	0.021	0.001	0.0213	1.428	101.428	0.165
2.	0.032	0.002	0.0324	1.25	101.25	0.11
3.	0.048	0.003	0.0485	1.46	101.46	0.06711

**Table(6). Accuracy & precision of Chromium in Serum samples**

Samples & platform Tantalum	Serum Chromium Conc. $\mu\text{g/ml}$			Error %	Rec. %	RSD%
	Present	Added	Found			
1.	0.021	0.001	0.0214	+1.9	101.9	0.175
2.	0.032	0.002	0.0327	2.2	102.2	0.168
3.	0.048	0.003	0.0489	+1.875	101.875	0.112



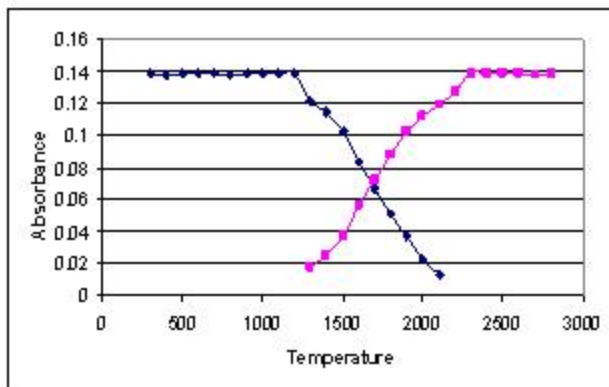


Figure (1) Ashing Curves and Atomization Curves for uncoated GFAAS chromium

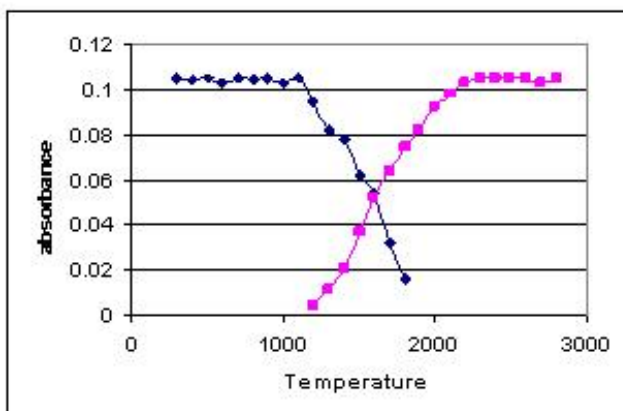


Figure (2) Ashing Curves and Atomization Curves for coated GFAAS chromium

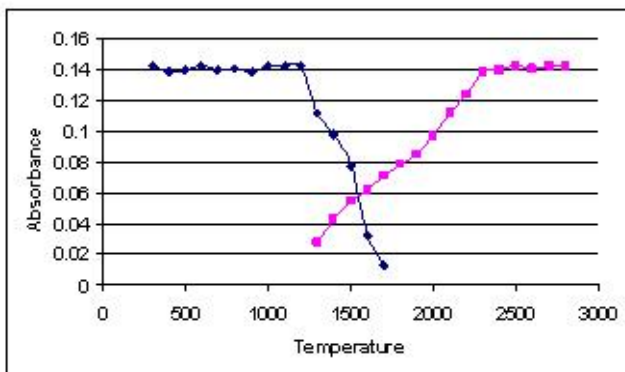


Figure (3) Ashing Curve and Atomization Curve for Uncoated GFAAS with single coil of Chromium

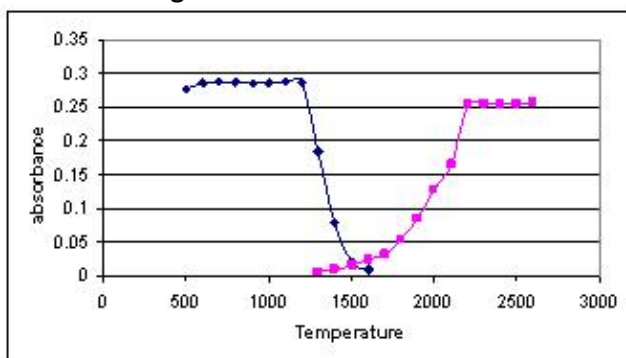
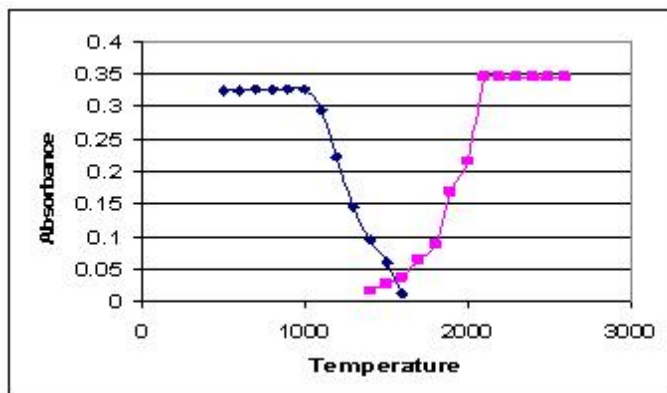


Figure (4) Ashing Curve and Atomization Curve for uncoated GFAAS with single coil tantalum of Chromium



**Figure (5) Ashing Curve Atomization of uncoated with platform Tantalum of Chromium**

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