

## NEW METHOD FOR ESTIMATION OF MICROAMOUNTS OF URANIUM IN BLOOD AND URINE

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### ABSTRACT

*A rapid and sensitive method for the microdetermination of uranium in blood and urine is described. The method is based on the oxidation of the organic materials found in both blood and urine and the induced fluorescence of the uranyl complex when subjected to a laser beam in the ultraviolet region.*

*Newly proposed phosphoric acid, phosphate and pyrophosphate mixtures were prepared and used instead of "Fluran" the proprietary reagent (patent). The new proposed solution mixtures proved to have the same uniform and high luminescence or even better than Fluran.*

*The effect of associating cations in major and trace amounts with uranium in blood and urine have been investigated.*

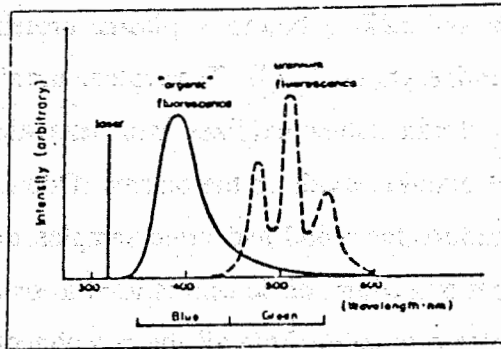
*The new method have been applied for the estimation of uranium in blood and urine of 20 normal persons and the normal levels of uranium are thus given.*

*This new method can be used for routine checking of exposure conditions to uranium miners.*

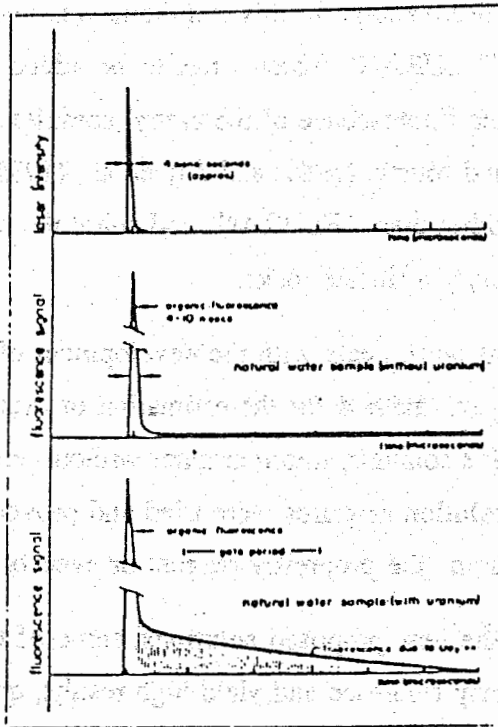
## INTRODUCTION

Among the analytical techniques for uranium estimation that are characterized by their high sensitivity are the neutron activation, fission track and plasma emission spectrometry. All these methods need equipped laboratory or reactor facility. A new instrumental method for the direct measurement of laser induced fluorescence of microamounts of uranium was developed. The detection limit was 0.05 ppb (Robbins, 1978). In this technique the ultraviolet laser beam excites fluorescence of the uranyl ion and the natural dissolved organics, Fig. 1. The fluorescence of the former persists longer than that of the organics when the laser is switched off. The uranyl ion fluorescence can be isolated and measured by modulating the laser beam and tuning the detector.

The measurements of uranium fluorescence in organic rich samples like blood and urine may give lower results. The organic rich solution show slower uranium response, presumably the reaction of the enhancing materials with metallo organic species is slower than the equivalent inorganic reaction. A similar effect has been noted in the laboratory samples spiked with high humic acid concentrations. In these cases, about 80% of the final uranium values is reached after about 70 seconds or so. Samples throughput in these cases would be correspondingly less. Conclusively, it is recommended to treat the blood and urine samples for oxidation of the organics before the measuring procedures.



Fluorescence of uranium and organics



Persistence of fluorescence

Fig (1) : Characteristics of Uranium and Organics fluorescence .

#### Estimation of uranium in blood and urine

Uranium is found in blood complexed with the bicarbonate in plasma to 47% and 32% is bound to plasma proteins and 20% to erythrocyte (Hodge et. al. 1973). Tetravalent uranium introduced into the blood stream either oxidizes into hexavalent uranium or forms colloidal uranium oxide in the plasma (Dounce, 1949). In the suggested procedure for blood and urine samples treatment before the measurement procedure, nitric acid is used to oxidize the organic materials, release uranium from all the components in blood and to oxidize the tetravalent uranium to the hexavalent state.

The only disadvantage in this technique was that a propriety reagent named "FLURAN" (patent) has to be added to each sample to enhance the fluorescence of the uranyl complex. The research work of Tikoo and Murty, (1980) and Aly et, al., (1985) proposed a buffer solution other than "FLURAN" and adapt the method for estimation of uranium in silicate rocks.

The present work deals with the development of a new, rapid and highly sensitive method for the estimation of uranium in blood and urine which is found in micro amount without extraction steps. New proposed solution mixtures were tried and proved to be equivalent to the "Fluran" the propriety reagent or even better.

By using the new proposed solutions, some of the associated trace elements may fluoresce and yield high results, quench the uranyl fluorescence or act as an inner filter. These effects have been

examined in order to stabilize the method and obtain reproducible results with minimum interference level suitable for blood and urine analysis.

## EXPERIMENTAL

All the chemicals used were of analytical grade reagents namely;  $\text{HNO}_3$  (sp.gr. 1.42), Uranyl Nitrate ( $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), Zinc metal (Zn), Copper metal (Cu), Cobalt Chloride ( $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ), Sodium Chloride, (NaCl), Potassium Chloride (KCl), Calcium Carbonate ( $\text{CaCO}_3$ ), Magnesium Nitrate ( $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), Ferric Iron Oxide ( $\text{Fe}_2\text{O}_3$ ), Potassium permanganate ( $\text{KMnO}_4$ ), phosphorus Pentaoxide ( $\text{P}_2\text{O}_5$ ) and Ammonium Meta-vanadate ( $\text{NH}_4\text{VO}_3$ ).

The stock solutions (100 ppm) were prepared by dissolving the metal or its salt in the proper acid solutions. The solutions are evaporated with nitric acid. The final acidity is adjusted to 10 ml 4N  $\text{HNO}_3$  per liter. Different concentrations are prepared in 50 ml measuring flasks from 0 to 50 ppm in 1 ppm U solutions.

### Collection of blood samples:

Twenty healthy persons (male) were chosen clinically free from illness. They were examined for cardiovascular system, chest, abdomen, nervous system, blood and urine analysis and proved to be healthy. Venous blood samples were collected with disposable

#### Estimation of uranium in blood and urine

sterile syringes. The specimens were then transferred into sterile plastic tubes and kept at  $-20^{\circ}\text{C}$  until used.

#### Sample treatment:

Five ml blood samples are heated with 3 ml  $\text{HClO}_4$  and 5 ml  $\text{HNO}_3$  in 150 ml beakers till the evolution of gases are terminated. Make up to 45 ml with deionized water and 0.5 ml 4N  $\text{HNO}_3$ . Allow the beaker to stand for a few minutes in order to redissolve any uranium and dilute up to volume in 50 ml measuring flasks with deionized water, Uranium fluorescence is measured in each solution following proposed procedure listed below.

#### The standard addition method for estimation of uranium:

One ml of the prepared sample solution is transferred to the measuring cell of the uranium analyzer and the volume is adjusted to 8 ml with 0.8 ml of the enhancement solution and deionized water and the induced fluorescence of the uranium complex is then measured. To account for any interference that might be imparted by of the concomitant ions with uranium in the measured solution, the standard addition is proposed to be used in the present work. The volume of the standard added is kept small so that the volume is not affected and calculation is simplified. The following equation is used for calculating the concentration of uranium:

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Uranium concentration of the sample (ppm) =  $S_1 / S_2 - S_1 \cdot V_1 / V_2 \cdot CD$  where,

$S_1$  = deflection of the meter due to sample,  $S_2 - S_1$  = increased of deflection due to the standard added,  $V_1$  = volume of standard solution added,  $V_2$  = volume of sample,  $C$  = uranium concentration (ppm) of standard solution added and  $D$  = sample dilution factor.

**Perparation of the new proposed enhancement solution:**

**Solution I:** 250 ml of conc. phosphoric acid is diluted to one liter with deionized water.

**Solution II:** 125 gm of sodium dihydrogen orthophosphate is dissolved in one liter of deionized water.

**Solution III:** 133 gm sodium pyrophosphate is dissolved in one liter of deionized water.

**Instrument:**

A Scintrex UA-3 laser Fluorimeter is used in the present work. The unit is equipped by Nitrogen Laser as a pulsed ultraviolet source (337 nm), Fig. (2).

## Estimation of uranium in blood and urine

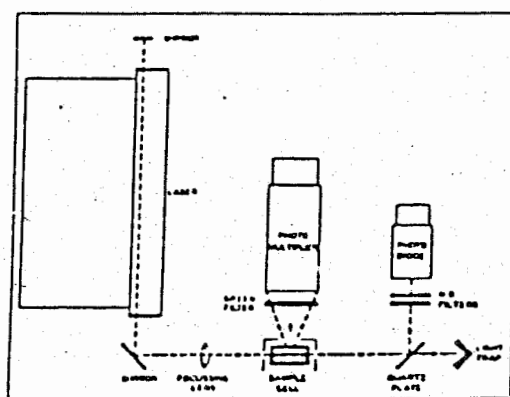


Fig (2) : Optical layout of the UA-3 instrument.

## RESULTS AND DISCUSSIONS

Results using the laser fluorescence technique on uranium solutions containing the different proposed enhancement solutions are correlated to that solution containing Fluran, the propriety reagent. The results showed that the new proposed solutions proved to have the same uniform and high luminescence or even better than Fluran, Table (1).

The effect of added elements expected to be present in blood or urine in major or trace amounts on the induced fluorescence of the uranyl complex ion have been examined. Different concentrations of each element have been prepared using the stock solutions ranging from 2 ppm to 10 ppm in 1 ppm uranium solution. The obtained results are presented in Fig. (3). The proposed range of the trace ele-



ments in the measured solution is chosen after literature survey for the normal abundance of trace elements in blood and urine, Table(2).

The present study showed that the dilution procedures recommended by many authors to account for some cations interference is not sufficient in this method as some of the interfering cations still affect the uranium signal even when they are present in ppb concentration level. Table (3) shows the cation effect on the aparent fluorescence of uranium.

Table (1): Fluorescence intensities of uranyl solution in the presence of the proposed enhancement solutions.

Conc. of uranium solution in ppb	Fluorescence intensity of uranyl solution				
	Without enhancement solution	with Fluran "Patent" Solution	with Solution (I)*	with Solution (II)*	with Solution (III)*
1	1.5	120	122	125	133
2	3.5	241	241	252	256

\* Enhancement solution proposed in the present work.

Estimation of uranium in blood and urine

Table (2) : Normal abundance of trace elements in blood and urine.

Element	Blood	Urine
Al	0.39 mg /L	800-10000 ug / 24 hrs
Fe	400-500 mg /L	130-1200 ug/ 24 hrs
Ca	2.2-2.6 m mol /L	2.5-10 m mol /L
Mg	40.00mg /L	114 mg / 24 hrs
Na	153-155 m mol /L	43-217 m mol /L
K	3.5-5.5m mol /L	26-132 m mol /L
Mn	0.012 mg /L	3- 300 ug /L
P	2.5-4.8 mg / dL	0.34-1.0 g / 24 hrs
V	1.5 ug /kg	
Cu	1.01 mg /L	48 ug /24 hrs
Zn	6.46 mg /L	200-2000 ug /L
Co	0.9 - 3.9 ug /L	0.5-2.2 ug /L

Fig. 3- Cation interference in uranium fluorescence.

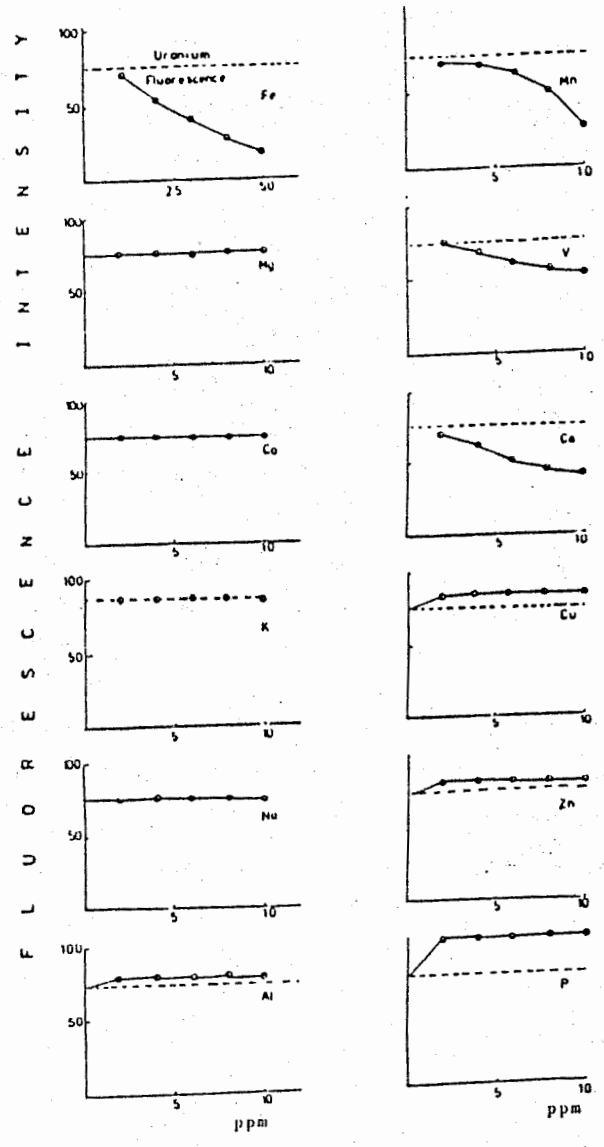


Fig. (3) : Cation interference in uranium fluorescence.

Estimation of uranium in blood and urine

Table (3) : Cation effect on the apparent fluorescence of uranium.

Cation	Max. Conc. of cation in the measured soln.	Type of Interference	% Error
Al	10	Enhancement	+ 13.3
Fe	50	Inner Filter	- 80.8
Ca	91	No Interference	
Mg	10	No Interference	
Na	10	No Interference	
K	10	No Interference	
Mn	10	Inner Filter	- 66.7
P	10	Enhancement	+33.3
V	10	Inner Filter	- 32.2
Cu	10	Enhancement	+ 7.6
Zn	10	Enhancement	+ 3.7
Co	10	Life Time Quencher	- 48.7

In the present work the types of effects imparted by different cation species which interfere with the measurement of uranium using the uranium analyser are identified and listed in Table (3). Those effects are summarised in the following observations:

**1- Inner filters**

Materials which absorb the excitation light at wave length (337 nm) and represented in the present study by Fe-Mn-V. When the concentration of iron in the measured solution reaches 50 ppm, it suppresses the apparent fluorescence of uranium by about 80%. Manganese, titanium and vanadium give the same effect and the

percentages of the suppression are listed in Table (3).

## **2- Lifetime quenchers**

Materials which shorten the lifetime of the light emission from the uranyl ion and represented in the present study by cobalt which if present in a concentration of 10 ppm in the measured solution will suppress the apparent fluorescence of uranyl ion by 48.7% . Besides, organic matter if present, acts also as lifetime quencher but as mentioned before , the samples are treated in a way which gets rid of the organic matter found in both blood and urine.

## **3- Self enhancement**

Materials which give rise to significant apparent fluorescence before the addition of the enhancement solution. Consequently the background signal will be high and the balance control becomes relatively ineffective. Those are represented mainly by phosphorus which gives an apparent results magnified by about 33.3% when present with a concentration of 10 ppm in the measured solution. Aluminium, copper, and zinc give also the same enhancement effect with variable values as given in Table (3).

According to the above mentioned observations it is recommended to use the standard addition method to compensate for the variability of constituents that might be present in blood and urine. An experiment is designed to check for the validity of the standard addition method to minimize the error due to the interfering elements using six solutions with variable concentrations of uranium and a variable amount of iron. The six solutions are measured by

#### Estimation of uranium in blood and urine

the normal method (external standard method) and the standard addition method suggested in the present work and the results are given in Table (4).

Table (4): Estimation of uranium by the external standard method and the standard addition method in presence of 20 ppm iron as interfering cation.

Added uranium concentration	Uranium in ppb by external standard method	Uranium in ppb by standard addition method
2.0 ppb U + 20 ppm Fe	1.21	1.96
2.2 ppb U + 20 ppm Fe	1.33	2.17
2.4 ppb U + 20 ppm Fe	1.44	2.37
2.6 ppb U + 20 ppm Fe	1.57	2.52
2.8 ppb U + 20 ppm Fe	1.66	2.70
3.0 ppb U + 20 ppm Fe	1.81	2.95

This experiment showed that uranium can be estimated in the presence of any interfering element or elements as long as the proper method for treating the sample and the proper method of measuring the fluorescence (the standard addition method) are used.

A trial was made to estimate uranium content in blood and urine of normal persons taking in consideration all the above mentioned observation and results beside the recommended procedures for sample treatment and fluorescence measurement . This work is

very important to research works dealing with the occupational hazards that might arise from inhalation or ingestion of uranium bearing materials and tracing its pathway inside that human body.

Uranium compounds introduced into the blood stream are rapidly distributed throughout the human organism where tetravalent uranium if present is oxidized to the hexavalent state is likely to occur. The major part would be rapidly excreted by the kidney while the rest would be deposited in the kidney and in bone and detectable amounts could be found in the liver. Thus urinary excretion of uranium may reflect recent exposure levels and can be used for routine checking of exposure conditions (Boback, 1975).

In the present work, blood and urine samples were taken from twenty normal persons to estimate the normal uranium levels using the laser induced fluorescence technique. The results obtained are listed in Table 5.

Estimation of uranium in blood and urine

Table (5) : Uranium content in blood and urine of normal persons

Sample No.	Uranium in blood (ppb)	Uranium in Urine (ppb)
1	0.25	0.11
2	0.24	0.18
3	0.13	0.09
4	0.15	0.09
5	0.37	0.20
6	0.19	0.09
7	0.11	0.08
8	0.20	0.16
9	0.18	0.08
10	0.29	0.20
11	0.18	0.09
12	0.26	0.17
13	0.25	0.09
14	0.13	0.10
15	0.20	0.09
16	0.18	0.10
17	0.17	0.09
18	0.10	0.08
19	0.18	0.10
20	0.30	0.12
Range	0.10 - 0.37	0.08 - 0.21
$\bar{X} \pm SE$	$0.203 \pm 0.015$	$0.120 \pm 0.010$

X = mean

SE = Standard error

The uranium content found in the blood of normal persons was found to range from 0.10 to 0.37 microgram / liter with an average of  $0.203 \pm 0.015$  while in urine the normal content ranges from 0.08 to 0.21 microgram / liter with an average of  $0.120 \pm 0.010$ .



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## طريقة جديدة لتقدير كميات شحيحة

### من اليورانيوم فى دم وبول الإنسان

أ.د. منير محمد على د . سهير محمد أنور السيد

هيئة المواد النووية هيئة الطاقة الذرية

يتناول هذا البحث شرح طريقة حديثة لتقدير عنصر اليورانيوم فى الدم والبول تمتاز بالحساسية العالية والسرعة . وتعتمد هذه الطريقة على معالجة العينات للتخلص من المواد العضوية باكسدتها ثم تعرض المحاليل الناتجة لأشعة الليزر حيث ينبعث منها وميض مستحدث يتناسب طردياً مع كمية اليورانيوم الموجودة .

هذا وقد تم اقتراح مجموعة من المحاليل المحفزة المكونة من مخلوط حامض الفوسفوريك ومركبات الفوسفات والبيروفوسفات التى أمكن استخدامها بدلاً عن محلول الفلوران الذى يورد مع جهاز الليزر وهو غير معروف التركيب ( براءة الاختراع ) وبهذا فقد أمكن توفير العديد من العملة الصعبة التى تستنفذ فى شراء هذا المحلول بالإضافة إلى أن هذه المحاليل المقترحة قد فاقت فى تأثيرها عن الفلوران مما أدى إلى زيادة حساسية الطريقة لكميات اليورانيوم الضئيلة جداً .

وقد أمكن بهذه الطريقة تقدير مستوى اليورانيوم فى دم وبول الإنسان لعدد عشرون رجلاً من الأصحاء .

ويساعد هذا البحث فى متابعة تواجد عنصر اليورانيوم فى محاليله بنسب قد تصل إلى جزء فى البليون مما يمكن المسئولين على رعاية عمال مناجم اليورانيوم وتتبع حالات التلوث الإشعاعى لهم .