

## Spectrophotometric determination of Uranium through complex formation with roxarsone

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**Abstract:** A new sensitive, accurate, and non-extractive spectrophotometric method was developed for the rapid determination of Uranium in pure form and Uranium ore using roxarsone. The procedure was based on the complex formation between Uranium (VI) and roxarsone. This showed maximum absorption at  $\lambda_{\max}$  395 nm with a linear relationship in the concentration range from (20-100  $\mu\text{g mL}^{-1}$ ) with a molar absorptivity  $9.57 \times 10^3 (1\text{mol}^{-1} \text{cm}^{-1})$ . Inductively Coupled Plasma mass spectroscopy (ICP-MS) was used in the study of the interferences caused by some metallic ions, which were effectively masked by tartaric acid and diethylenetriaminepenta-acetic acid (DTPA). The method holds its accuracy and precision well when applied to the determination of the studied Uranium in its pure form and Uranium ore.

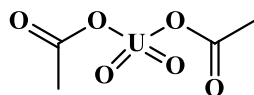
**Keywords:** Roxarsone; Uranium; Spectrophotometry; Uranium Ore.

### 1. Introduction

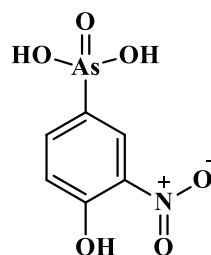
Uranium is a dangerous radioactive material that is found naturally in soil, water, or in the dust. The estimation of Uranium is essential to decide on the feasibility of extracting it from a specific area and avoiding direct exposure to high doses of radiation or high doses of dust containing Uranium, which causes serious health hazards in the case of swallowing. Therefore, the determination of Uranium in a new, quick and cheap method is important, roxarsone was used in the present study as a reagent of detecting the concentration of uranium ion in a sample of water and rocks.

The reaction mechanism is expected to be via complex formation between U (VI) with roxarsone across the O atom from the arsenic group ( $\text{H}_2\text{AsO}_3$ ), the hydroxyl group atom, and the Nitro group ( $\text{NO}_2$ ) atom. Roxarsone has not been used to determinate Uranium or any other metal spectrophotometrically before.

Spectrophotometry applies to the estimation of much lower concentrations than titration methods and has the advantage of being easy to carry out <sup>1,2</sup>. It refers to a polymer formed by electrostatic interaction between polyelectrolytes, which are oppositely charged <sup>3,4</sup>.



Uranyl acetate



Roxarsone

Uranium ore always contains other metal impurities <sup>5</sup>, so the leaching, adsorption, or biosorption of Uranium vary according to the type of the rock <sup>6,7</sup>. Many techniques have been previously developed for the determination of Uranium; these methods include inductively coupled plasma spectrometry (ICP-AES) <sup>8</sup>, inductively

coupled plasma-mass spectrometry (ICP-MS) <sup>9,10</sup>, ion chromatography (I.C.) <sup>11</sup>, capillary zone electrophoresis (C.Z.E.) <sup>12</sup>, flow injection analysis (F.I.A.) <sup>13</sup> and gamma spectrometry <sup>14</sup>. However, these analyses offer restricted accessibility due to the need for a rather valuable apparatus and higher costs. Spectrophotometry is a comparatively easy substitute

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method, which has been applied in the determination of Uranium concentrations<sup>15,16</sup>.

Roxarsone (4-hydroxy-3-nitrophenylarsonic acid) is an aromatic arsenical compound used in the poultry industry, and for the treatment of coccidial intestinal parasites<sup>17</sup>. Biological activities result in the incorporation of arsenic into organic molecules such as arsenobetaine, arsenosugars, and arsenolipids, which are found in many marine organisms<sup>18</sup>.

Uranium was determined spectrophotometrically in other methods using synthesized sulfacetamide azo dye derivative<sup>19</sup>, meloxicam<sup>20</sup>, 2-(2-thiazolylazo)-p-cresol (tac)<sup>21</sup>, arsenazo iii<sup>22</sup>, 2-ethanolimino-2-pentylidino-4-one<sup>23</sup>, a mixture of - xylene and benzene<sup>24</sup>, azide ions<sup>25</sup>, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol<sup>26</sup>, 2-Hydroxy-1-naphthaldehyde-P-hydroxybenzoic hydrazone<sup>27</sup>, thiocyanate<sup>28</sup> and 7-iodo-8-hydroxyquinoline-5-sulfonic acid (ferron)<sup>29</sup>. Uranium was used in the spectrophotometric determination of piroxicam and tenoxicam<sup>30</sup>, sulfur-containing compounds<sup>31</sup>, phosphorylated proteins<sup>32</sup>, tetracyclines<sup>33</sup>, acylthiosemicarbazide<sup>34</sup>, diiodoquin, clioquinol<sup>35</sup> and serum cholesterol<sup>36</sup>. The spectrophotometric reaction was used in the present study as a method for detecting the concentration of uranium ion in an unknown sample.

## 2. Experimental

### 2.1. Instruments

Metertech Inc. SP-8001 UV-VIS spectrophotometer (Taiwan, R.O.C.) with 1 cm quartz cells connected to an I.B.M. computer loaded with software application, Icp mass spectroscopy (ICP-MS) Hewlett-Packard 4500 (HP 4500) inductively coupled plasma mass spectrometer (ICP-MS), the system can measure trace elements as low as one part per trillion (ppt) and quickly scan more than 70 elements.

### 2.2. Materials and Reagents of the working solution

Chemicals used for the method were of the highest purity as available from their sources in the form of pure analytical grade:

Uranyl acetate obtained from Fluka/Sigma-Aldrich (St. Louis, MO): 0.5 mgmL<sup>-1</sup> solution in distilled water.

Roxarsone purchased from Sigma with purity greater than 98%: prepared as 0.15% w/v by dissolving 0.15 g of roxarsone in 100mL distilled water.

Diethylenetriaminepenta-acetic acid (DTPA) purchased from Aldrich Chemical Company (USA.): prepared as 0.02 % w/v by dissolving 0.02 g of DTPA in 100ml deionized water.

Tartaric acid: prepared as 1 %. W/v by dissolving 1 g of tartaric acid (Merck) in 100 ml deionized water.

Phosphate rock from Nile valley Elsebaeiya, Egypt.

### 2.3. The procedure of standard Uranium in pure form

With high accuracy (0.5-2.5 mg) of pure standard uranyl acetate was transferred to a 25 mL volumetric flask, 3mL of 0.15% solution of roxarsone was added at room temperature then diluted with 20 mL distilled water at room temperature. The volumes were completed with distilled water, and the absorbance was measured at  $\lambda_{max}$  395 nm against blank similarly prepared to neglect uranyl acetate, and the results obtained were compared with the reference method<sup>37</sup> (Table 1).

#### 2.3.1. Uranium ore method

This part was done to confirm the validity of the method and the possibility of the application on Uranium ore. The rock used was phosphate rock from Nile valley Elsebaeiya which contain 100 ppm uranium, 650 ppm Mn (II), 1700ppm Ca (II), 1850ppm Fe (III), 21.3 ppm Ni (II), 13.2 ppm Cu (II), 1250ppm Al (III), 132 ppm Zr(IV) (analyzed by ICP MS).

The rock was crushed with a rock crusher, then the resulting powder was divided into 20 parts, and a portion was taken from each part until it reaches the required weight. The contents of 15 g finely powdered rock were weighed and mixed well, transfer from powdered rock an accurately weighed quantity equivalent to 1 mg uranyl acetate, 50 mL of 4 N HNO<sub>3</sub> was added. The mixture was evaporated to near dryness on a hot plate. The addition of 15 mL followed this concentrated H<sub>2</sub>SO<sub>4</sub> 98%. It evaporated to dryness on a hot plate, dissolved in 2×8 ml portions of deionized water. The mixtures were homogenized by shaking for 5 minutes, then filtered into a 25 ml volumetric flask, 2 mL of DTPA, 1 mL of tartaric acid, and 3 mL of roxarsone reagent solution were added. The solution was filled up to the mark with deionized water. The absorbance of the complex was measured at 395 nm and determined against a reagent blank as the reference prepared simultaneously. The results obtained were compared with the official method<sup>37</sup>, as shown in (Table 2).

### 2.4. Interference Study

#### 2.4.1. Metals interference

The effect of different metals on the determination of Uranium with roxarsone was carefully studied, a solution containing other metals was treated as the procedure step by step. The data demonstrate that Uranium could be determined in the presence of other minerals that interfere with Uranium in other spectrophotometric methods. Metal ions 60 mgmL<sup>-1</sup> concentration like Mn (II), Ca (II), Fe (III), Ni (II), Co (II), Cu (II) and Al (III) were found not to interfere significantly up to concentration 150 mgmL<sup>-1</sup> with Average of three experiments (Recovery%) 99.6, 99, 98.7, 100.3, 99.4, 99.8, 99 and 98.8 respectively. The presence of Zr (IV), Mo (VI) and Pb (II) up to 120 mgmL<sup>-1</sup> was successfully masked by DTPA and tartaric acid with recovery%

99.1, 98.7 and 98.6, respectively. This step was detected by Inductively coupled mass spectroscopy (ICP-MS) to confirm the validity of the study. The data obtained gave the same results as those of roxarsone spectrophotometry with a good recovery percentage.

#### 2.4.2. Clay, silt and sand interference

Each of these rock contents examined alone after will grinding and powdering then take 10 µg mixed with 60 µg mL<sup>-1</sup> uranium complexed with roxarsone and repeated three times to inshore the statistical recovery %.

### 3. Results and Discussion

The previous procedures were applied to

**a-** Different concentrations of pure Uranium, the results obtained, and compared with the reference method <sup>37</sup> and shown in (Table 1).

**b-** Uranium ore and the results compared with the reference method <sup>37</sup> are shown in (Table 2).

**c-** 60 µg mL<sup>-1</sup> of Uranium in the presence of clay, slit, and finely powdered sand, the results obtained are shown in (Table 3).

Statistical analysis of the results indicates that the proposed method was precise and accurate (Table 4).

**Table 1.** Determination of pure Uranium using roxarsone at λ<sub>max</sub> 395 nm.

Uranium		
Taken (µg mL <sup>-1</sup> )	Found (µg mL <sup>-1</sup> )	Recovery <sup>b</sup> %
20	20.09	100.45
40	39.36	98.40
60	59.67	99.45
80	79.12	98.90
100	100.2	100.2
Mean recovery	99.48±0.860	
N	5	
Variance	0.740	
S.E	0.384	
Molar absorptivity	9.57×10 <sup>3</sup> (1mol <sup>-1</sup> cm <sup>-1</sup> )	
Sandell's sensitivity	2.4×10 <sup>-2</sup> (µg cm <sup>-2</sup> )	
t-test	0.3	
F-test	1.09	

(<sup>a</sup>)Mean ± S.D.

(<sup>b</sup>)Average of three experiments

**Table 2.** Determination of Uranium in 100 ppm uranium ore by complexation with roxarsone using standard addition technique.

uranium ore 100 ppm			
Taken (µg mL <sup>-1</sup> ) Uranium from ore	Added (µg mL <sup>-1</sup> ) standard Uranium		Recovery %
5	15		101
5	35		102
5	55		99.6
5	75		98.1
5	95		98.2
Mean recovery	99.78±1.72		
N	5		
Variance	2.94		
S.E	0.768		
t-test	0.51		
F-test	4.32		

(<sup>a</sup>)Mean ± S.D.

(<sup>b</sup>)Average of three experiments.

**Table 3.** Effect of some common ingredients on the determination of Uranium using roxarsone.

Other Ingredients 10 µg. added	Uranium	
	Taken ( µg mL <sup>-1</sup> )	Recovery %
—	60	99.6
clay	60	99
slit	60	98.7
sand	60	100.3
carbonate	60	99.8

(a) Average of three experiments

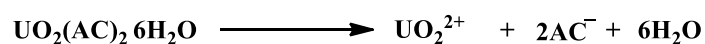
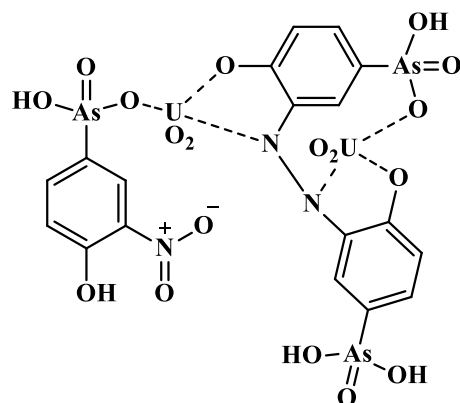
**Table 4.** Evaluation of the accuracy and precision of the proposed methods.

Compared method Statistical parameter	Uranyl acetate			
	Taken (µg mL <sup>-1</sup> )	20	60	100
Found ± SD <sup>a</sup>		19.8±0.01	60.3±0.02	99.7±0.02
RSD(%)		0.071	0.035	0.047
SAE <sup>b</sup>		0.063	0.009	0.010
Confidence limit <sup>c</sup>		0.012	0.018	0.020

<sup>a</sup> Mean ± standard deviation for five determinations.<sup>b</sup> Standard analytical error.<sup>c</sup> Confidence limits at P = 0.95 and 4 degrees of freedom.

These results and precise statistical data were due to a new complex formation with a dark yellow color; it was obtained due to the interaction of uranyl ion with the oxygen in –O.H. and NO<sub>2</sub> functional groups. The

complex achieved may be due to the interaction of uranyl ion with roxarsone as explained following mechanism (Scheme 1).

**Step 1****Step 2****Scheme 1.** A suggested complex obtained may be due to the interaction between uranyl ion with roxarsone

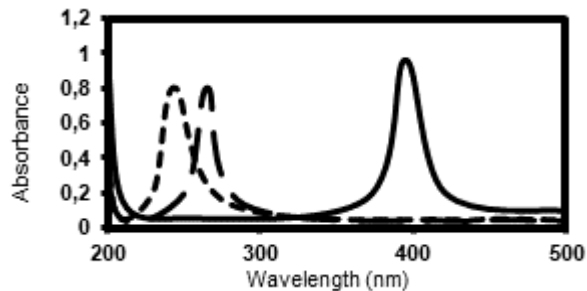
The reaction product is suitable for spectrophotometric measurement in this study, the

studied uranium exhibit colored condensation product with roxarsone that was measured at

$\lambda_{\max}$  395 nm but uranyl acetate alone was measured at 265 nm while roxarsone  $\lambda_{\max}$  was at 244 nm, this indicates the formation of the complex (Fig. 1).

### 3.1. Effect of solvent

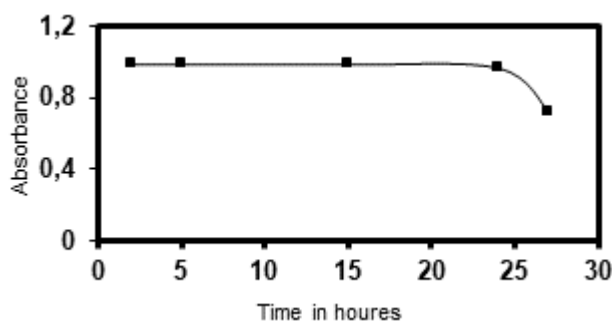
In this work, distilled water, ethanol, chloroform, toluene, methanol, benzene, and methylene chloride were studied. Distilled water represents the optimum diluting solvent with maximum absorbance.



**Figure 1.** Absorption spectra of  $100 \mu\text{g ml}^{-1}$  of Roxarsone 0.15% at -uranyl acetate at  $\lambda_{\max}$  265 nm, Reaction product of  $100 \mu\text{g ml}^{-1}$  - $\lambda_{\max}$  244nm and of uranyl acetate in distilled water with 0.15% roxarsone at  $\lambda_{\max}$ .

### 3.3. Reaction kinetics

The reaction was carried out at  $25 \pm 1^\circ\text{C}$ , and the complex formed with maximum absorbance quickly in 1 to 1.5 minutes and was stable for more than 20 hours (Fig. 3).



**Figure 3.** Effect of time on the color intensity of the reaction product between 3ml 0.15% roxarsone and  $100 \mu\text{g ml}^{-1}$  of uranyl acetate.

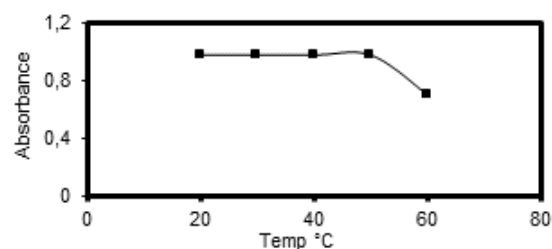
### 3.5. Effect of pH

pH was carefully examined by preparing buffered solutions of pH from 1 to 10<sup>38</sup>, Influence of pH on uranyl acetate solutions at different pH values (1- 10) were assayed to determine the variation of concentrations with pH on this protocol. The concentrations determined using this modified method was not affected significantly with the variation in the pH from 2 to 8. (Fig. 5) shows the

### 3.2. Effect of heating temperatures

Different temperatures varying from ambient to  $70^\circ\text{C}$  were studied. It was found that heating did not enhance the complexation reaction; at  $50^\circ\text{C}$  the absorbance starts decreasing, and the complex begins to disintegrate (Fig. 2).

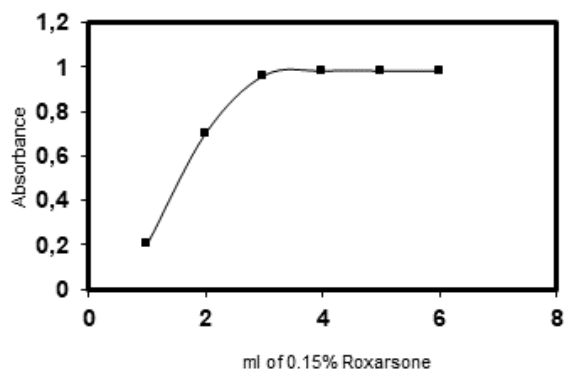
The optimal reaction conditions were carefully studied, as mentioned in the following paragraphs:



**Figure 2.** Effect of temperature on the color intensity of the reaction product between 3ml 0.15% roxarsone and  $100 \mu\text{g ml}^{-1}$  of Uranyl acetate.

### 3.4. Effect of reagent volumes

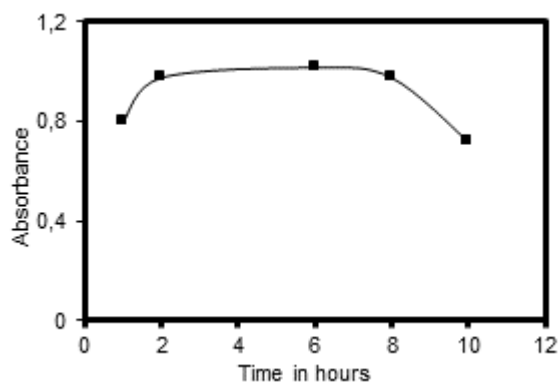
It was found that the sufficient volume was three ml of 0.15% roxarsone to achieve maximum color intensity (Fig. 4).



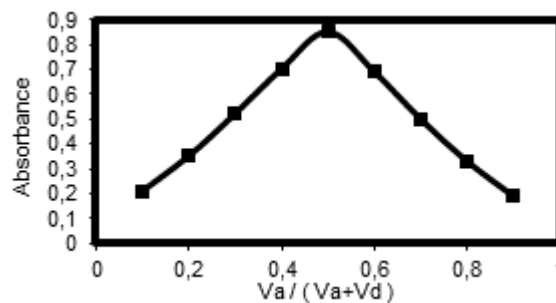
**Figure 4.** Effect of volume of 0.15% roxarsone on the colour intensity of the reaction product with  $100 \mu\text{g ml}^{-1}$  uranyl acetate. change of the color intensity of the reaction as a function of pH values.

### 3.6. Stoichiometry

Effect of the order of addition on studying the molar ratio of the studied uranyl acetate by the continuous variation method (Job's method)<sup>39</sup> of equimolar solutions in the presence of an excess amount of roxarsone; it was found to be 1: 1 of uranyl ion and roxarsone (Fig. 6).



**Figure 5.** Effect of pH on the color intensity of the reaction product between 3ml 0.15% roxarsone and  $100\mu\text{g mL}^{-1}$  of uranyl acetate.

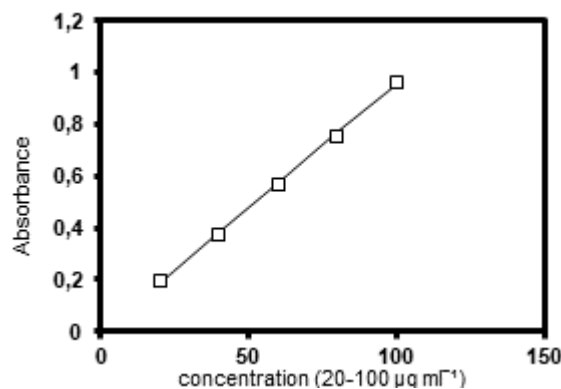


**Figure 6.** Continuous variation plot for ( $1.5 \times 10^{-3}$  M) uranyl acetate and ( $1.5 \times 10^{-3}$  M) of roxarsone Va=volum of uranyl acetate and Vd=volum of roxarsone.

### 3.7. Linearity and quantification

A linear relationship was obtained for the absorbance

of uranyl acetate with roxarsone in the concentration ranges of  $20\text{-}100\mu\text{g mL}^{-1}$  (Fig. 7).



**Figure 7.** Calibration curve of the reaction product of Uranyl acetate with roxarsone at  $\lambda_{\text{max}}$  395 nm.

The following equations describe the calibration graphs :

$$\text{at } \lambda_{\text{max}} 395 \text{ nm} \quad A = 0.00957 C$$

Where A is the absorbance and C is the concentration of uranyl acetate in the final solution in  $\mu\text{g mL}^{-1}$ .

### 4. Conclusion

The proposed method was successfully applied for the determination of Uranium, and the validity of the process was assessed by comparing the calculated t and F values with the reference method, the results showed no significant difference between them. DTPA and tartaric acid successfully masked the presence of Zr (IV), Mo (VI) and Pb (II) up to  $120\text{ mg mL}^{-1}$ . Clay, silt, sand, and carbonate do not interfere with the new complex formation. No extraction step is required and therefore the employment of organic solvents, which are commonly toxic pollutants, is avoided. Uranium ore was analyzed by the proposed method applying the standard addition technique and compared with the reference method. Statistical analysis of the results revealed that the proposed method was highly precise and accurate as of the reference one.

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