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Spectrophotometric Determination of Uranium(VI) in Bacterial Leach Liquors Using Arsenazo-III

Tariq M. Bhatti, Abdul Mateen, M. Amin, Kauser A. Malik & Ahmad M. Khalid*

National Institute for Biotechnology and Genetic Engineering, PO Box 577, Jhang Road, Faisalabad, Pakistan

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ABSTRACT

A highly sensitive and precise spectrophotometric method for the direct determination of uranium(VI) in bacterial leach liquors, obtained by the action of Thiobacillus ferrooxidans *and* T. thiooxidans, *from low-grade sandstone uranium ores, has been developed. Arsenazo-III formed an intense pink–violet complex at pH 2·0 ± 0·1, which showed maximum absorption at 655 nm. This method was found to obey Beer's law up to 6 μg* cm^{-3}, *giving the values of molar absorptivity* (ε) *and Sandell sensitivity* (S) *as* $4{\cdot}6 \times 10^4$ mol^{-1} cm^{-1} *and 0·0052 μg* cm^{-2}, *respectively. Interference due to different metal ions, such as Al, Ca, Co, Cr, Fe, Mn, Mo, Zn and Zr, was successfully masked by diethylenetriaminepenta-acetic acid without inhibiting the formation of the uranium(VI)–arsenazo-III complex. This method was also found suitable for detecting low levels of uranium(VI) in mine waters, acid leach liquors and tailings liquids. The results obtained were found to be in close agreement with the values determined by fluorometric and indirect spectrophotometric methods.*

Key words: spectrophotometric determination of uranium(VI), uranium(VI)–arsenazo-III complex, bacterial leach liquors, *Thiobacillus ferrooxidans*, *T. thiooxidans*, sandstone uranium ore.

INTRODUCTION

Among the practical applications of biotechnology is mineral biotechnology, which deals with the bioprocessing of ores of many kinds. Bioprocessing of low-grade

*To whom correspondence should be addressed.

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ores of uranium, copper and zinc is being carried out commercially.[1,2] *Thiobacillus ferrooxidans* and *T. thiooxidans*, which are acid-producing, chemolithoautotrophic bacteria, oxidize ferrous and/or reduced inorganic compounds of sulphur, thereby fulfilling their energy requirements. As a result of metabolic activities of these *Thiobacilli*, strong oxidants like ferric sulphate and sulphuric acid are produced, which indiscriminately solubilize metallic ions present in the ores. Therefore, the bacterial leach liquor from uranium bearing sandstone ore is laced with other ions (Al, Ca, Cu, Co, Fe, Mn, etc.) in addition to uranium.

Spectrophotometric determination of uranium in the presence of various metals has been of wide interest, and numerous methods for measuring uranium in low levels do exist.[3] The bisazo derivatives of chromotrophic acid are among the most sensitive reagents for the spectrophotometric determinations of uranium and among these arsenazo-III, which contains arsenic acid, has been shown to be the most sensitive.[4,5]

Arsenazo-III (1,8-dihydroxynaphthalene-3,6-disulphonic acid–bis(azophenyl arsenic acid)), which is a bisazo dye based on chromotrophic acid and *o*-aminophenylarsonic acid, is commonly used for the determination of thorium and uranium(IV), but it also gives a very sensitive colour reaction with uranium(VI).[6] The method proposed by Savvin[4] for the spectrophotometric determination of uranium involves either a preliminary reduction of uranium(VI) to uranium(IV) or an extraction using EDTA and diphenylguinidine. The extraction procedures usually involve solvents which are hazardous, as well as expensive, rendering these methods inappropriate for adaptation in industrial process control operations.

A direct spectrophotometric method for measuring uranium contents in solids, as well as in solutions of various kinds, has been developed. This method eliminates the cumbersome extraction procedures and is found to be rapid, precise and reasonably accurate.

EXPERIMENTAL

Reagents

Standard uranium solution (1 mg cm^{-3})

Analytical grade uranyl nitrate hexahydrate, $UO_2(NO_3)_2.6H_2O$ (0·2110 g), was weighed accurately on a watch glass. Complete removal of nitrate ions was carried out by heating the salt, which was moistened with 2 or 3 drops of deionized water and 2 to 3 cm^3 concentrated H_2SO_4, to dryness. This nitrate-free residue was dissolved in 100 cm^3 deionized water to give a stock solution containing 1 mg cm^{-3} uranium. Further dilution of this stock solution (1:20) was carried out to yield the working solution containing 50 μg cm^{-3} uranium.

Arsenazo-III

The solution was prepared by dissolving 0·5 g of the reagent in dilute NaOH. The final volume (200 cm^3) was made up with deionized water (pH 2·0).

DTPA solution

The solution (2·5%) was prepared by dissolving 25 g diethylenetriaminepentaacetic acid (DTPA) in 500 cm^3 deionized water, with dropwise addition of 1 M NaOH and constant stirring and heating. The solution was diluted to 1 dm^3 with deionized water.

Protocol

An aliquot of the sample solution, containing 0·5 to 100 μg of uranium, was pipetted into a 50 cm^3 volumetric flask, and 2 cm^3 of complexing solution (2.5% DTPA) and 1 cm^3 of arsenazo-III reagent solution were added. The volume was made up with dilute H_2SO_4 (pH 2.0) and the solution was allowed to stand for 2 to 3 min. The pink–violet coloration that developed, due to formation of the uranium–arsenazo-III complex, was measured at 655 nm against a corresponding reagent blank, using a 10 mm path cell in a Shimadzu UV-120-02 spectrophotometer.

Other analytical procedures employed

For a comparative study, the uranium contents of different samples were also determined by employing an indirect spectrophotometric method[7] in which methyl isobutyl ketone (MIBK) was used as a solvent for extracting uranium. Dibenzoylmethane (DBM) was used as a complexing agent, and the absorbance of golden-yellow complex formed was measured at 410 nm in a 10 mm cell.

Fluorimetric determination of the uranium present in leach liquors was carried out by the method described by Centanni *et al.*,[8] which was modified by Mateen *et al.*[9]

Bacteria used and leaching conditions

Chemolithotrophic iron and sulphur oxidizing bacteria were isolated from the different ore samples, which were collected from the mine sites. Enrichment techniques were employed for isolating these microorganisms from these samples. Basal salts solution (9K-medium) as described by Silverman and Lundgren[10] containing (g dm^{-3}): $(NH_4)_2SO_4$ (3·0), KCl (0·1), K_2HPO_4 (0·5), $MgSO_4 \cdot 7H_2O$ (0·5), and $Ca(NO_3)_2$ (0·01), was used. Ferrous sulphate (5%) or elemental sulphur (1%) was added as energy source. Two strains, TMB–TFe and TMB–TTh, were isolated. These bacteria were Gram negative, aerobic, 0·5–0·7 × 1–1.5 μm rods. They were motile, mesophilic (growth temperature, 25°–35°C), acidophilic (growth pH 1–3·5), obligately autotrophic bacteria. Strain TMB–TFe was found to oxidize elemental sulphur in addition to ferrous ions, while strain TMB–TTh could not oxidize ferrous ions and grew on elemental sulphur or sulphides. Taxonomic studies of these isolates indicated that they closely resembled *T. ferrooxidans* and *T. thiooxidans*, respectively.

PVC columns (25 cm diameter and 2 m height) were filled with 100 kg of sandstone uranium ore ($U_3O_8 = 0{\cdot}027\%$). These columns were irrigated with mine water containing indigenous microflora of these microorganisms. Effluents from these columns were collected and were monitored for uranium content.

RESULTS AND DISCUSSION

Absorption spectra of uranium–arsenazo-III complex

Absorption spectra of the arsenazo-III reagent formed with UO_2^{2+} (Fig. 1) were obtained with the addition of 1 cm^3 of 0·25% dye and 2 cm^3 of 2·5% DTPA solution in order to complex interfering metals. As shown, the maximum absorption of the pink–violet uranium dye complex occurred at 655 nm. At this wavelength, the dye (arsenazo-III) did not show any appreciable absorption. The large differences between the wavelengths of the absorption maxima of complexes (λ_{max} 655–675 nm) and the free dye reagent (λ_{max} 520–530 nm) are of paramount importance. The absorbance of the UO_2^{2+}–arsenazo-III complex was found to be linearly related to the concentration of uranium ions.

Figure 2 presents the relationship between concentration of uranium ions and absorbance at λ_{max} 655 nm, as determined previously. The value of molar absorptivity (ε) of the uranium(VI)–arsenazo-III complex was calculated from the standard curve and was found to be $4{\cdot}6 \times 10^4$ mol^{-1} cm^{-1}, which corresponded to a Sandell sensitivity (S) value of 0·0052 $\mu g\ cm^{-2}$. Kadam *et al.*[5] found that the molar absorptivity (ε) of this complex was $2{\cdot}0 \times 10^4$ mol^{-1} cm^{-1} ($S = 0{\cdot}012$ $\mu g\ cm^{-2}$) at pH 5·5, adjusted with 10% hexamine at 640 nm.

In another report,[11] the uranium(IV)–arsenazo-III complex was found to have an ε value of $4{\cdot}5 \times 10^4$ mol^{-1} cm^{-1} in the presence of 4 to 6 M HCl, $HClO_4$ or HNO_3 at 646 nm. The Sandell sensitivity of this method was 0·0053 $\mu g\ cm^{-2}$. As indicated, this newly developed direct method is *c.* 40% more sensitive than the earlier procedure described by Kadam *et al.*[5]

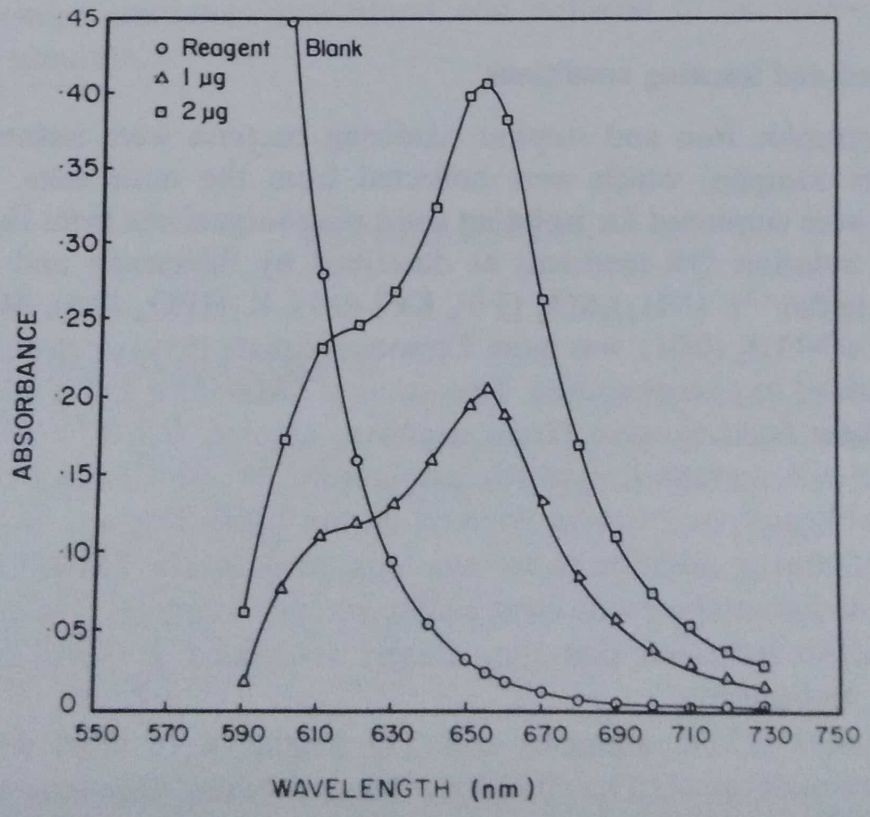

Fig. 1. Absorption spectra of arsenazo-III complex formed with uranyl ions. ○, Reagent blank; △, 1 µg of U; □, 2 µg of U.

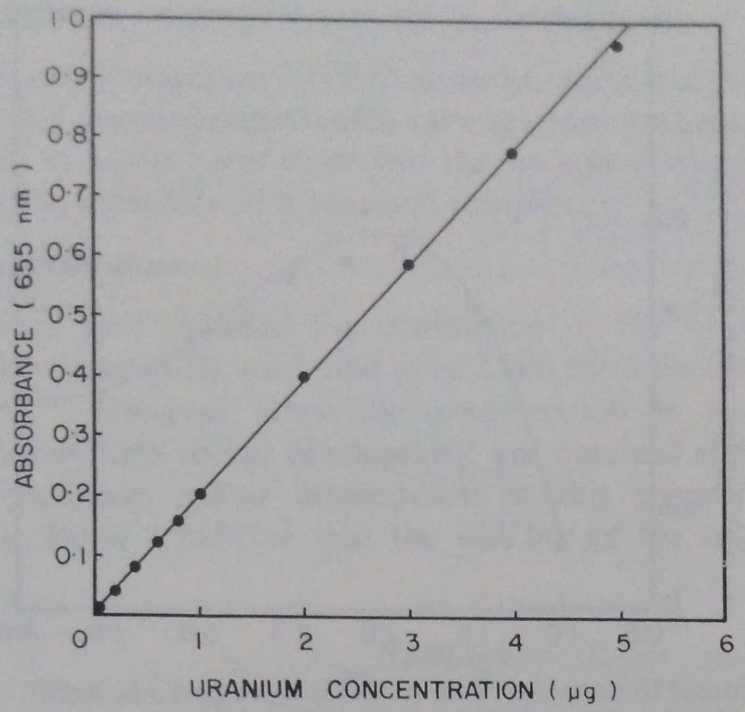

Fig. 2. Standard curve for spectrophotometric determination of uranium. The points represent average of six replicates. Colour intensity was measured at 655 nm.

Effect of pH on colour development

A series of solutions containing 1 cm^3 of 50 μg uranyl ions, 2 cm^3 of 2·5% DTPA solution and 1 cm^3 of 0·25% arsenazo-III solution of different pH values were prepared. The absorbance of these solutions was measured at 655 nm against a series of reagent blanks of similar pH values, but containing no uranium. The blanks were also measured against deionized water (pH 2·0). After the absorbance measurement, the final pH values of the solutions were again determined. These results are plotted in Fig. 3 and indicate that the optimum pH range was rather narrow. The maximum colour development was obtained at pH 2·0 and 2·1. No significant complex between UO_2^{2+} and arsenazo-III was formed below pH 1·5 or above 2·5.

The colour reaction between uranium(VI) and arsenazo-III at a pH of 5·5 provided a sensitive and selective method for uranium determination, using DPTA as masking agent.[5] Zirconium, hafnium, thorium, scandium, titanium(IV), tin(IV), bismuth, iron(III) and the rare earth elements were found to interfere above certain concentrations and required separation or masking with certain complexans prior to analyses.[11] Strelow and VanderWalt[12] have shown that, in the colorimetric determination of uranium(VI)with arsenazo-III at pH $1{\cdot}8 \pm 0{\cdot}2$, the complexans EDTA and 1,2-diaminocyclohexanetetra-acetic acid were found to be less suitable, because at low pH (1–2) they tend to precipitate, while DTPA proved to be especially attractive for masking other elements in the determination of uranium(VI) at low pH.

Effect of dye concentration on colour development

The effect of different concentrations of arsenazo-III reagent solution on the

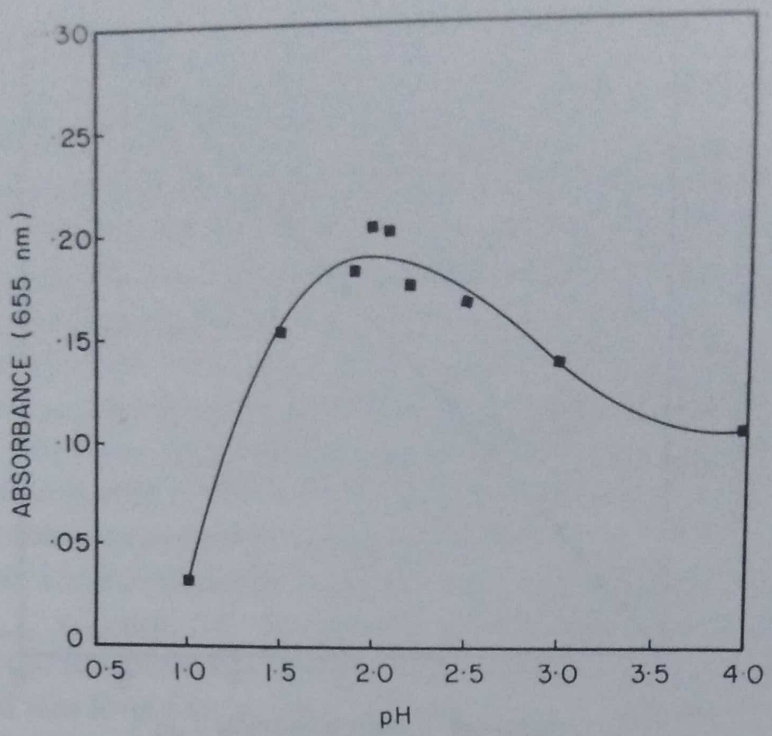

Fig. 3. Effect of pH on development of colour by the interaction of uranyl ions and DTPA.

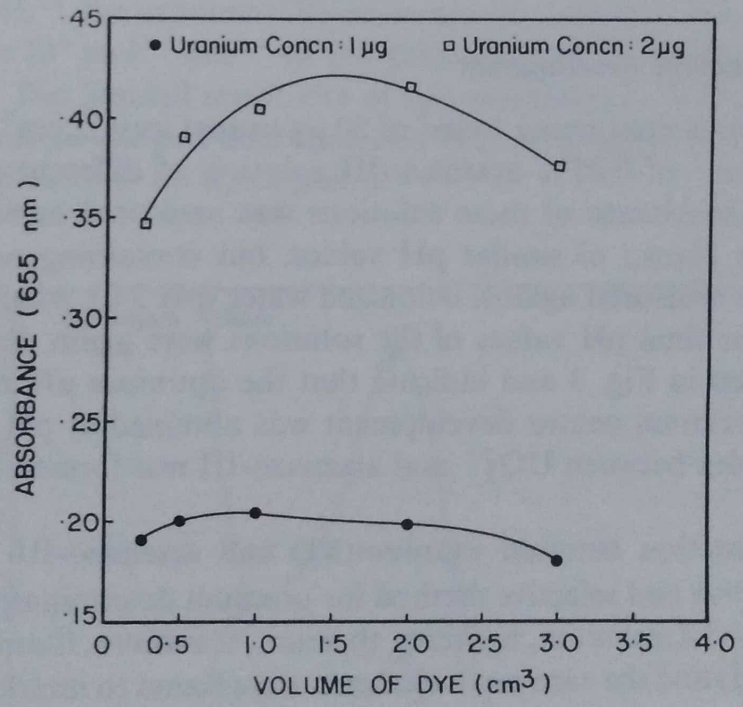

Fig. 4. Effect of concentration of dye (arsenazo-III) on colour production with 1 and 2 $\mu g\ cm^{-3}$ of uranyl ions. ●, 1 μg of U; □, 2 μg of U.

formation of the uranium–arsenazo-III complex, in the presence of 1 and 2 $\mu g\ cm^{-3}$ uranium, was also studied. Results in Fig. 4 indicate that 1 cm^3 of arsenazo-III solution (0·25%) was sufficient to complete the formation of the colour complex in a 1:1 and 1:2 ratio. This observation is in agreement with the results reported in the literature.[5] When arsenazo-III was added in excess ($>3\ cm^3$) a dark red colour developed.

Effect of complexan concentration on colour development

The effect of complexing agent (DTPA) on the production of the uranium–arsenazo-III complex was also investigated with varying concentrations of DTPA. The data are presented in Table 1 and show that the amount of complexan did not affect significantly the formation of a coloured complex.

Optimum reaction time

The minimum time required for completion of the colour development of uranium(VI)–arsenazo-III was found to be 2 min when the uranium concentration was 1 $\mu g\,cm^{-3}$. However, when the concentration of uranium was doubled (2 $\mu g\,cm^{-3}$) only 75% colour development was obtained after 2 min, and it took 5 min for maximum colour development at this concentration. The results presented in Table 2 indicate that the stability of the colour complex, when

TABLE 1
Effect of Complexan (DTPA) on Production of Uranium(VI)–Arsenazo-III Complex

Volume of DTPA (2·5%) (cm^3)	*Absorbance (655 nm; U, 1 $\mu g\,cm^{-3}$)*
None	0.204
0.5	0.212
1.0	0.207
2.0	0.203
3.0	0.202
4·0	0.190
5.0	0.180
10·0	0.180

TABLE 2
Effect of Reaction Time on Formation of Coloured Complex Uranium(VI)–Arsenazo-III

Time	*Absorbance (655 nm)*	
	1 $\mu g\,cm^{-3}$ uranium	*2 $\mu g\,cm^{-3}$ uranium*
2 min	0·206	0·306
5 min	0·207	0·407
10 min	0·207	0·407
30 min	0·209	0·408
1 h	0·209	0·405
2 h	0·203	0·402
6 h	0·202	0·401
24 h	0·201	0·401
48 h	0·201	0·400
96 h	0·200	0·400

measured after different time periods (varying from 2 min to 96 h), no appreciable effect on the absorbance of the complex was found.

Effect of interfering metallic ions on complex formation

To study the interference of various metals on the absorbance of the uranium(VI)–arsenazo-III complex, a solution containing both uranium and metal ion was treated according to the prescribed protocol. The results obtained are given in Table 3. These data show that uranium could be determined in the presence of many heavy metals which frequently interfere with other spectrophotometric methods for uranium estimation. Metal ions like Cd(II), Mn(II), Cu(II), Hg(II), Ni(II), Fe(III) and Mo(VI) were found not to interfere significantly up to 100 $\mu g\ cm^{-3}$ concentration. The presence of Al(III), V(IV) and Ca(II) up to 20 $\mu g\ cm^{-3}$ and Cr(III) up to 5 $\mu g\ cm^{-3}$ was successfully masked with DTPA solution. Zirconium(iv) was found to interfere seriously in the determination of uranium and could only be masked at levels up to 2 $\mu g\ cm^{-3}$ in the sample solution.

Comparison of different methods for uranium estimation

A comparative study of different methods commonly available for uranium estimation was also undertaken. Analytical results of the two most frequently used methods for uranium estimation, i.e. MIBK/pyridine–DBM spectrophotometric[7] and fluorimetric[8,9] methods, both involving solvent extraction of uranium by MIBK, were compared with values obtained by the arsenazo-III direct method, and the results are presented in Table 4. As shown, the arsenazo-III direct method

TABLE 3
Masking Effects of DTPA on Uranium Recovery in the Presence of Interfering Metallic Ions

Metallic ions	*Amount of ions added ($\mu g\ cm^{-3}$)*	*Absorbance[a] (655 nm)*	*Recovery of uranium (%)*
None	—	0·204	100·0
Al(III)	20	0·204	100·0
Ca(II)	20	0·210	102·9
Cd(II)	100	0·200	98·0
Co(II)	20	0·200	98·0
Cr(III)	5	0·212	103·9
Cu(II)	100	0·206	101·0
Fe(III)	100	0·200	98·0
Hg(II)	100	0·202	99·0
Mn(II)	100	0·201	98·5
Mo(VI)	100	0·198	97·1
Ni(II)	100	0·206	101·0
Zr(IV)	2[b]	0·207	101·5

[a] Uranium concentration 1 $\mu g\ cm^{-3}$.
[b] Addition of 1 cm^3 of tartaric acid (10%) can enhance the masking effect up to 3 $mg\ cm^{-3}$.

TABLE 4

Comparison of Uranium Concentration Found in Various Samples by Different Methods

Sample	*Concentration ($\mu g\ cm^{-3}$)*		
	Arsenazo-III method	*DBM–Pyridine method*	*Fluorimetric method*
Bacterial leach liquors			
BLL-1	438·0	425·0	440·0
BLL-2	230·0	222·0	228·0
BLL-3	48·0	43·0	49·0
Mine water			
MW-GD3	3·0	<5·0	3·2
MW-GD4	1·8	ND[a]	1·7
MW-GD5	0·42	ND[a]	0.4
Acid leach liquors			
ALL#1	642·0	624·0	648·0
ALL#2	532·0	520·0	536·0
ALL#3	336·0	328·0	336·0
Tailings liquor			
TL-1	7·0	<5·0	5·5
TL-2	5·0	<5·0	5·0
Carbonate leach liquor[b]			
CLL-1	221·0	214·0	220·0
CLL-2	98·0	90·0	98·0
CLL-3	52·0	44·0	53·0
Eluants			
EL#1	1·8	ND[a]	2·0
EL#2	7·6	ND[a]	7·4
EL#3	2·5	ND[a]	2·5
Solids[c]			
Rock phosphate	80·0	76·0	84·0
Super phosphate	48·0	45·0	54·0
Phosphogypsum	5·0	<5·0	6·0

[a] Not detectable.
[b] Acidified with sulphuric acid.
[c] Leached by sulphuric acid prior to estimation.

and the fluorimetric method were reasonably comparable. As illustrated, the arsenazo-III direct method was also capable of determining the uranium present in solid samples.

Recovery of uranium added in bacterial leach liquors

The preciseness of the arsenazo-III direct method was determined by addition of known amounts of uranium to the bacterial leach liquors and then analysing these solutions for their uranium contents. Results are depicted in Table 5. The recovery of uranium from these samples was almost 100%.

TABLE 5
Recovery of Uranium from Bacterial Leach Liquors by Arsenazo-III

Sample	*Uranium concentration* ($\mu g\ cm^{-3}$)			
	Initial	*Added*[a]	*Total*	*Found*
A-1	10	None	10	10
A-2	10	20	30	29
A-3	108	None	108	108
A-4	108	40	148	148
A-5	200	None	200	200
A-6	200	50	250	250

[a]Added as uranyl sulphate solution.

CONCLUSIONS

The arsenazo-III direct spectrophotometric method is suitable for low-level detection of the uranium(VI) content in strongly acidic sulphate and phosphate solutions. DTPA was found to mask successfully the interference caused by Al, Ca, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, and Zr. This method for the determination of uranium(VI) in bacterial leach liquors and mine waters was rapid, precise and accurate. The protocol developed can also be used for monitoring environmental pollution levels for uranium(VI).

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REFERENCES

1. Torma, A. E., Leaching of metals. In *Biotechnology*, Vol. 6b, eds H.-J. Rehm & G. Reed. VCH Verlagsgesellschaft, Weinheim, 1988, pp. 367–99.
2. Tuövinen, O. H., Acid leaching of uranium ore materials with microbial catalysis. *Biotechnol. Bioeng. Symp.*, **16** (1986) 65–72.
3. Korkisch, J. & Hubner, H., Determination of uranium in minerals and rocks. *Talanta*, **23** (1976) 283–8.
4. Savvin, S. B., Analytical applications of arsenazo-III. Part II. Determination of thorium, uranium, protactinium, neptunium, hafnium and scandium. *Talanta*, **3** (1964) 1–6.
5. Kadam, B. V., Maiti, B. & Sathe, R. M., Selective spectrophotometric method for the determination of uranium(VI). *Analyst*, **106** (1981) 724–6.
6. Marczenko, Z., *Spectrophotometric Determination of Elements*. Ellis Horwood, Westergate, Sussex, UK, 1976, pp. 574–87.

7. Nagi, F. I., Anwar, J., Naeem, M., Umar, Z. & Ali, A., Spectrophotometric determination of uranium in rock samples by dibenzoyl methane. *J. Natural Sci. and Mathematics*, **14** (1974) 165–77.
8. Centanni, F. A., Ross, A. M. & DeSesa, M., Fluorimetric determination of uranium. *Anal. Chem.*, **28** (1956) 1651–7.
9. Mateen, A., Amin, M., Khan, A., Bhatti, T. M. & Shirazi, S., Determination of low levels of uranium by fluorimetric method. *Proc. Natl Chem. Conf.*, ed. M. Arfan & M. A. Khawaja. Department of Chemistry, University of Peshawar, Pakistan, 1989, pp. 238–62.
10. Silverman, M. P. & Lundgren, D. G., Studies on the autotrophic iron bacterium *Ferrobacillus ferrooxidans*. I. An improved medium and a harvesting procedure for securing high cell yields. *J. Bacteriol.*, **77** (1959) 642–7.
11. Florence, T. M., Johnson, D. A. & Farrar, Y. J., Analytical procedure for the spectrophotometric determination of uranium in ores and waters. In *Proc. Symp. on the Analytical Chemistry of Uranium and Thorium*, ed. T. M. Florence. Australian Atomic Energy Commission, Lucas Heights, Sydney, 7–8 May 1970, AAEC/TM552, pp. 5.1–5.12.
12. Strelow, F. W. E. & VanderWalt, T. N., Comparative study of the masking effect of various complexans in the spectrophotometric determination of uranium with arsenazo-III and chlorophosphonazo-III. *Talanta*, **26** (1979) 537–42.