THE MEASUREMENT OF NATURAL URANIUM IN URINE BY FLUOROMETRY

Mesure de l'uranium naturel dans l'urine par fluorimétrie

by

G.H. KRAMER, J.R. JOHNSON and W. GREEN

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Résumé

La méthode fluorimétrique de mesure de l'uranium naturel dans l'urine, laquelle est couramment employée au laboratoire des essais biologiques de Chalk River, a été mise à l'épreuve, optimisée et documentée.

Cette méthode, qui permet de mesurer la fluorescence de l'uranium dans une pastille de fluorure de sodium fondu, s'est révélée indépendante par rapport à la trempe. On l'emploie pour mesurer les concentrations d'uranium dans l'intervalle allant de 1 µg/L à 90 µg/L. Le fluorimètre a un intervalle dynamique échelonné entre 0,2 µg/L et 200 µg/L.

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ABSTRACT

The fluorometric method of measuring natural uranium in urine that is currently used by the Bioassay Laboratory at Chalk River Nuclear Laboratories has been tested, optimized and documented.

The method, which measures the fluorescence of uranium in a fused sodium fluoride pellet, has been shown to be quench independent and is routinely used to measure uranium concentrations in the range of 1 μg/L to 90 μg/L. The fluorimeter has a dynamic range of 0.2 μg/L to 200 μg/L.

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1. INTRODUCTION

The Bioassay Laboratory of the Biomedical Research Branch at the Chalk River Nuclear Laboratories (CRNL) routinely monitors personnel for internal contamination by radioactive substances. Among the variety of tests performed are analyses for uranium in urine. These analyses are done radiochemically (1) for enriched uranium and fluorometrically for natural or depleted uranium. The paper describes this later method.

The natural uranium content of urine has been measured by a number of different methods by other workers. Chakarvarti et al. (2) have determined trace concentrations of uranium in urine in the range (0.9 to 6.5) x 10^{-3} \mu g/L using a fission track etch technique. Holzbecher and Ryan (3) have developed a neutron activation technique for measuring down to 5 \mu g/L using a SLOWPOKE reactor; however, a substantial pretreatment of the sample is required. Zsoldos and Csovari (4) have described a spectrophotometric method for the estimation of natural uranium in urine that can be used for a concentration range of 10 to 500 \mu g/L of uranium. Voloder (5) has discussed a polarographic method for determining uranium in biological samples and urine. Depending on the type of polarographic technique

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used, a variety of sensitivities are available. The detection limits of the various methods are: d.c. polarography, 50 μg/L urine; a.c. polarography, 20 μg/L urine; pulse polarography, 5 μg/L urine. Hafez and Goma (6) have reviewed a number of different estimation methods and recommend fluorometry for natural uranium in urine. Dupzyk and Dupzyk (7) have described a fluorometric technique for the estimation of natural uranium in urine. The range of uranium concentrations was 0.1 to 100 μg/L but due to a lengthy sample pretreatment, the recovery of uranium was only 75 ± 5%. Bushaw and Whitaker (8) have developed a kinetic analysis of Laser-induced phosphorescence of uranium phosphate that allows complete processing of a urine sample in about 15 minutes. The dynamic range of the method is currently 0.02 to 20 μg/L.

This document describes the routine fluorometric procedure that has been used at CRNL for over 20 years. Except in unusual circumstances, the samples require no pretreatment, thus ensuring no loss of uranium during any ashing or preliminary separation steps. The range of concentrations accessible by this method is 0.2 to 200 μg/L. This document also describes the modifications to the original fluorimeter (9, 10) and some of the results of the experiments that were carried out to optimize the equipment and experimental method. The results of the comparison of this method with the standard addition technique are also discussed.

2. Experimental

2.1 Sample Dishes

The platinum dishes (1.4 cm diameter) are maintained as two separate lots. One lot of approximately 60 dishes is kept for samples and the other lot of approximately 30 dishes is kept for spiked samples. Each lot is processed as described below.
The platinum dishes are prepared for use by boiling in distilled water for about 10 minutes. The hot water is decanted and the dishes are washed with fresh distilled water. This procedure is then repeated. After the hot water has been decanted, the dishes are boiled in 5% v/v nitric acid for about 10 minutes. The hot acid is decanted and the dishes thoroughly rinsed with fresh distilled water. NOTE: From this point the dishes must be handled only with the platinum tipped forceps which have been previously cleaned by heating to red heat and quenching in concentrated hydrochloric acid several times. The forceps are rinsed with distilled water prior to use.

2.2 Apparatus and Reagents

The sodium fluoride (99% pure) was supplied by the Allied Chemical Company, Canada Limited. Except for storage in a desiccator the sodium fluoride is used as received.

The fluorimeter, (Figure 1) which was built at CRNL, has been described previously (9, 10); however, the photomultiplier assembly has been modified by including a circuit that gives a linear response, see Figure 2. The new circuit replaces the circuit shown in Figure 6 of reference 9.

The present configuration of equipment differs from that shown in reference 9 in that the fluorimeter is no longer attached to the automatic pellet fusing equipment.

2.3 Preparation of Samples

The platinum dishes are placed on the sample trays which contain 8 dishes using the platinum tipped forceps. An aliquot (100 μL) of the urine sample to be analysed is added to each dish and the dishes are dried under a heat lamp. The pipette is then rinsed thoroughly with 5% v/v nitric acid, distilled water and the uranium standard solution. An aliquot of the standard solution is placed in dishes 6, 7 and 8. The dishes are dried under a heat lamp.
Approximately 0.25 g of anhydrous NaF are placed on each dish using the pellet dispenser. The NaF pellet is gently broken up to cover the dish. The samples are then fused and annealed for one minute each using the old automatic fluorimeter's (9, 10) mechanism. After fusing and annealing the samples are allowed to cool to room temperature before measurements are started.

2.4 Fluorescence Measurements

The fluorimeter should be switched on for about 30 minutes prior to making any measurements to stabilize the temperature. The sample holder is removed from the fluorimeter and a platinum dish is placed onto the brass block (see Fig. 1) using the platinum tipped forceps. The sample holder is placed back into the fluorimeter and the shutter opened. It is necessary to rotate the sample holder to estimate the mean response of the fused sodium fluoride sample because the response is angle dependent due to slight inhomogeneities of the fused sample.

The first and last sample should be the glass standard. The results from the standard ensure that the equipment is working satisfactorily and that no drift (± 5%) has occurred during the fluorescence measurements. If drift has occurred then the cause must be found and the fluorimeter repaired before any meaningful results can be obtained.

The results are tabulated and the uranium concentrations are evaluated as shown in Appendix A.

3. OPTIMIZATION TESTS

In addition to the known factors (9) that influence the performance of the equipment and the accuracy of the uranium estimation a variety of other parameters have been investigated. The factors that have already been examined (9) are: the relative fluorescence as a function of transfer time from the fusing to the annealing burner,
this has been set to one second; relative fluorescence as a function of cooling time of the fused sample, this must be at least three minutes. In addition to these tests the following variables have been examined and are discussed below: effect of temperature on the instrument gain; degradation of sample with time; effect of variable amounts of sodium fluoride on the fluorescent yield and the effect of long term exposure of the photomultiplier tubes to the fluorescence.

3.1 Temperature Effect

The temperature of the instrument is controlled by cooling with tap water at a flow rate of 0.4 L/min. Obviously, the amount of cooling is dependent on the temperature of the tap water which varies not only from day to day but can also vary from hour to hour during the summer. The results of this test are shown in Figure 3 and it can be clearly seen that no correlation exists between the temperature of the water exiting from the instrument and the reading obtained from the glass standard; however, if the cooling water is turned off then the reading given by the glass standard continues to rise until it reaches approximately 100 µA. At this point the instrument is at about 50°C. It is believed that the rise in gain is due to an increased light output from the UV light source at higher temperatures and/or an increased sensitivity of the photomultiplier tube at higher temperatures.

3.2 Degradation of Sample with Time

A series of samples, some with urine and some without (sodium fluoride spiked with uranium) were studied over a period of several days to investigate the effect of time since fusing on the fluorescence of the samples. The results (Fig. 4) show that the response from the spiked sodium fluoride samples decreases with time but that the response of the urine - sodium fluoride samples increases with time.
Refusing the samples has no effect on the spiked sodium fluoride but the urine-sodium fluoride samples are restored to their original fluorescence. This study indicates that samples should be processed on the same day as they are prepared; however, if there is a substantial delay between fusing and measuring the fluorescence, then it appears that the urine-sodium fluoride samples can be restored by refusing them prior to measuring the fluorescence, but samples not containing urine should be reprepared.

3.3 Effect of the Amount of Sodium Fluoride on the Fluorescent Yield

This effect was studied by preparing spiked sodium fluoride pellets in the normal way and preparing identical spiked samples using half the amount of sodium fluoride. The results of these experiments are shown in Table 1. It can be clearly seen that the results for full pellet or half pellet are statistically indistinguishable.

The fluorescence observed appears to be directly proportional to the amount of uranium present (and not on the concentration of uranium) and is independent of the amount of sodium fluoride present at the amounts of uranium and sodium fluoride normally used.

3.4 Effect of Long Term Exposure of the Photomultiplier Tube to the Fluorescence

It was found that for long exposures the response of the instrument, which was measured using the glass standard, decreased significantly. The results are shown in Fig. 5. The decrease is thought to be due to changes in the voltages along the voltage divider (Fig. 2) as the temperature of the resistors at the anode end increase with the length of time the equipment is operated at high current.
4. DISCUSSION

Although it has been shown (11) that urine samples of 50 µL or less do not give quenching problems, the method of adding a known amount of uranium to the sample prior to fusing eliminates any possible error from quenching for larger sample sizes (11). This is the technique used in this laboratory because we use sample sizes of 100 µL. Price et al. (11) have also shown that this technique does not suffer any interference by other elements that may also fluoresce under UV light.

The fluorimeter has been characterized by performing a number of experiments as described above using spiked urine samples. The precision of the results obtained in these experiments is, of course, quite variable and it is clear that there exists a limit to the experimental precision. This limit is described in Figure 6 which shows the best experimental precision that is obtainable as a function of the concentration of uranium in the sample. These experiments also showed that the practical detection limit for the method is about 1.0 µg/L uranium. This precision and detection limit are more than adequate for radiation protection purposes as urinary excretion of uranium can vary by a factor of two from day to day (12) and normal levels of uranium in urine for non-occupationally exposed individuals range up to 1 µg/L (13).

In addition to estimating the precision of this method, the experiment was repeated using the standard addition technique with the expectation of further improving the precision of the method. The standard addition technique as applied to this study consisted of placing an aliquot (100 µL) of the unknown sample in each of the eight platinum dishes and an increasing amount of uranium standard in each of seven of the dishes (42 to 300 µg/L). The results of the test are shown in Table 2.

It can be seen that no significant increase in precision has been gained while the work load has been increased.
In terms of accuracy of the result both methods are essentially indistinguishable. The normal method was 3 values within 1 σ, 1 value within 2 σ and 1 value within 3 σ. The S.A. method was 2 values within 1 σ, 1 value within 2 σ, 1 value within 3 σ and 1 outlier. The small sample size of this test excludes any firm conclusion about accuracy; however, the increased work load does not appear to bring any benefit either in precision or accuracy and the S.A. method is not recommended.

The optical system of the apparatus described above has not been optimized. Figure 6 shows the percent of transmitted light through the source and photomultiplier filters as derived from the manufacturer's specifications. It can be clearly seen that at least 65% of the transmitted intensity of the UV light that causes the fluorescence is lost; furthermore, at least 47% of the intensity of the fluorescent light is also lost.

If these reductions were partially removed in some way, then the sensitivity of the instrument ought to improve; however, this modification is not essential because the equipment is currently quite capable of measuring normal levels of uranium.
REFERENCES


**TABLE 1**

**FLUORESCENT YIELD AS A FUNCTION OF AMOUNT OF SODIUM FLUORIDE IN THE PELLET**

THE UNCERTAINTIES GIVEN ARE SAMPLE STANDARD DEVIATION

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>FULL PELLET (µA)</th>
<th>HALF PELLET</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28.4 ± 1.1</td>
<td>30.2 ± 0.8</td>
</tr>
<tr>
<td>2</td>
<td>27.7 ± 1.4</td>
<td>29.5 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>26.4 ± 1.4</td>
<td>27.8 ± 0.6</td>
</tr>
<tr>
<td>4</td>
<td>24.9 ± 1.7</td>
<td>26.4 ± 1.0</td>
</tr>
<tr>
<td>5</td>
<td>29.5 ± 0.6</td>
<td>26.8 ± 0.4</td>
</tr>
<tr>
<td>6</td>
<td>28.7 ± 1.2</td>
<td>28.5 ± 1.4</td>
</tr>
<tr>
<td>7</td>
<td>28.0 ± 0.5</td>
<td>28.0 ± 0.3</td>
</tr>
</tbody>
</table>

**TABLE 2**

**ANALYSIS OF FIVE SAMPLES BY THE NORMAL METHOD AND THE STANDARD ADDITION METHOD**

THE UNCERTAINTIES GIVEN ARE SAMPLE STANDARD DEVIATION

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>QUOTED VALUES µg/L</th>
<th>NORMAL METHOD µg/L</th>
<th>SA METHOD µg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81</td>
<td>89 ± 5</td>
<td>82 ± 5</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>16 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>3</td>
<td>5.1</td>
<td>6.4 ± 0.5</td>
<td>8.3 ± 0.4</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>58 ± 3</td>
<td>63 ± 6</td>
</tr>
<tr>
<td>5</td>
<td>34</td>
<td>35 ± 2</td>
<td>36 ± 2</td>
</tr>
</tbody>
</table>
FIGURE 1 The fluorimeter

FIGURE 2 Photomultiplier circuit to replace the circuit shown in Figure 6 of reference 9.
FIGURE 3  The relative fluorescence as a function of cooling water temperature at discharge.

FIGURE 4  The relative fluorescence and apparent uranium concentration as a function of time
Left hand ordinate: urine samples
Right hand ordinate: spiked samples
FIGURE 5  The effect of prolonged exposure of the photomultiplier tube to the fluorescent light as a function of time. Shutter open: left open between measurements Shutter closed: closed between measurements

FIGURE 6  The relative precision of measurement as a function of the amount of uranium present.
FIGURE 7  The relative transmission of the filters as a function of wavelength as specified by the manufacturer.
APPENDIX A

CALCULATION OF URANIUM CONCENTRATIONS

The calculations in this appendix are for a set of inter-laboratory comparison samples that were analysed in this laboratory. The samples were supplied by the Radiation Protection Bureau (Ottawa). The method of analysis was as described in Section 2 of this report.

The calculations are performed as follows: dishes 1 to 5 and 6 to 8 are averaged for each sample A to F and the blank. Each average value is corrected for the blank value. The spiked set (set "Y") is corrected for the uranium content of the urine by subtracting the urine values (set "X"). The uranium content of urine is then calculated from:

\[
\text{uranium content (µg/L)} = \frac{X' \times 300}{(Y' - X')}
\]

The errors are propagated throughout yielding a final standard deviation of the sample.
RESULTS OF INTERCOMPARISON SAMPLES

<table>
<thead>
<tr>
<th>SET</th>
<th>DISH # (μA)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>0.307</td>
<td>0.315</td>
<td>0.305</td>
<td>0.305</td>
<td>0.303</td>
<td>3.63</td>
<td>3.70</td>
<td>3.85</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>0.063</td>
<td>0.065</td>
<td>0.062</td>
<td>0.064</td>
<td>0.065</td>
<td>3.35</td>
<td>3.38</td>
<td>3.22</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>0.730</td>
<td>0.725</td>
<td>0.750</td>
<td>0.760</td>
<td>0.745</td>
<td>4.05</td>
<td>3.95</td>
<td>4.04</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>0.590</td>
<td>0.615</td>
<td>0.603</td>
<td>0.600</td>
<td>0.590</td>
<td>3.85</td>
<td>3.73</td>
<td>3.70</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>0.157</td>
<td>0.144</td>
<td>0.145</td>
<td>0.154</td>
<td>0.155</td>
<td>3.17</td>
<td>2.98</td>
<td>3.18</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>0.404</td>
<td>0.418</td>
<td>0.423</td>
<td>0.403</td>
<td>0.425</td>
<td>3.49</td>
<td>3.52</td>
<td>3.70</td>
</tr>
<tr>
<td>BLNK</td>
<td></td>
<td>0.025</td>
<td>0.028</td>
<td>0.025</td>
<td>0.032</td>
<td>0.028</td>
<td>0.031</td>
<td>0.029</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Dishes 1-5 contain sample. This is set X.

Dishes 6-8 contain sample + 300 μg/L U spike. This is set Y.
### CALCULATIONS

<table>
<thead>
<tr>
<th></th>
<th>$\bar{X}$</th>
<th>$\bar{Y}$</th>
<th>$\bar{Y}'$</th>
<th>$\bar{X}'$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.307(0.005)</td>
<td>3.727(0.112)</td>
<td>0.279(0.005)</td>
<td>3.699(0.112)</td>
</tr>
<tr>
<td>B</td>
<td>0.004(0.001)</td>
<td>3.317(0.085)</td>
<td>0.036(0.003)</td>
<td>3.289(0.085)</td>
</tr>
<tr>
<td>C</td>
<td>0.742(0.014)</td>
<td>4.013(0.055)</td>
<td>0.714(0.015)</td>
<td>3.985(0.055)</td>
</tr>
<tr>
<td>D</td>
<td>0.600(0.010)</td>
<td>3.760(0.079)</td>
<td>0.572(0.011)</td>
<td>3.732(0.079)</td>
</tr>
<tr>
<td>E</td>
<td>0.151(0.006)</td>
<td>3.110(0.113)</td>
<td>0.123(0.006)</td>
<td>3.082(0.113)</td>
</tr>
<tr>
<td>F</td>
<td>0.415(0.011)</td>
<td>3.570(0.114)</td>
<td>0.387(0.011)</td>
<td>3.542(0.114)</td>
</tr>
<tr>
<td>BLNK</td>
<td>0.028(0.0025)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>$\bar{Y}' - \bar{X}'$</th>
<th>U CONTENT $\mu g/L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.420(0.113)</td>
</tr>
<tr>
<td>B</td>
<td>3.253(0.085)</td>
</tr>
<tr>
<td>C</td>
<td>3.271(0.057)</td>
</tr>
<tr>
<td>D</td>
<td>3.160(0.080)</td>
</tr>
<tr>
<td>E</td>
<td>2.959(0.113)</td>
</tr>
<tr>
<td>F</td>
<td>3.155(0.114)</td>
</tr>
</tbody>
</table>

The figures in parentheses represent the precision of the sample set to 1σ.
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