Development of a Colorimetric Test for Uranium

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ABSTRACT

This paper discusses the development of and proposed enhancements to a colorimetric test for the detection of uranium in biological samples such as urine. The goal of this work is to develop a technique for the detection of uranium that could: 1) be conducted rapidly and accurately; 2) would not require extensive sample preparation; 3) would not require expensive or complicated instrumentation; 4) would require little or no technical training to conduct; and 5) could be used in a field situation if needed.

The technique described in this paper involves the following steps. A buffer is added to the sample to maintain the pH of the mixture within an experimentally acceptable range and a quaternary ammonium salt is added to aid in solubilization of the reaction components. A pyridylazo stain, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol, capable of binding a variety of metals, is used to complex the uranium. This interaction has been made specific for uranium through the use of "masking agents." Color development indicating the presence of uranium is monitored and can be quantitated by determining the absorbance of the reaction mixture at 578 nm, using a spectrophotometer or colorimeter.

At present, the limit of sensitivity of the procedure is approximately 30 µg of uranium/L. However, through the incorporation of a sample concentration step in the procedure, we believe we can greatly increase the sensitivity of the technique. The two areas we believe are amenable to our concentration efforts are prior to the addition of the assay components (pre-complexation concentration step) or after the formation of the stain/uranium complex (post-complexation concentration step). Our goal is to make the procedure more applicable while still maintaining technical simplicity and ease of use.

We believe this research will provide the capability to rapidly and accurately screen biological samples for uranium.

1.0 INTRODUCTION

Since the terrorist attacks of September 11, 2001, concern about the potential use of radiological weapons directed against civilians or military personnel has risen dramatically. Concern is no longer limited to nuclear fission devices delivered by rogue states or terrorist groups. Other means of spreading radioactivity, especially use of a radiological dispersion device (RDD)—the so-called dirty bomb—are now thought to be urgent risks. An RDD uses conventional explosives to disperse radioactive material over a wide area. Although a relatively small number of people may be killed or injured by blast effects from such a weapon, a much larger number

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potentially could be exposed to dispersed radionuclides. Exposures could occur by any of several routes, including inhalation, ingestion, embedded fragments, or wound contamination.

Uranium has been thought by some to be a potential component of an RDD. It is a naturally occurring, radioactive, metallic element found in trace amounts in soil and rocks, water, and air. Uranium, as found in nature, consists primarily of three isotopes in the following percentages (by weight): ^{238}U (99.283%); ^{235}U (0.711%); and ^{234}U (0.005%). As produced for power generation and nuclear weapons, uranium contains greater than 0.711% ^{235}U and is considered "enriched" uranium. Uranium containing less than 0.711% ^{235}U is considered "depleted" uranium. Depleted uranium (DU), obtained as a by-product of the enrichment process for nuclear reactor- and weapons-grade uranium, usually contains less than 0.3% ^{235}U and is therefore less than half as radioactive as natural uranium. Because it is extremely dense (1.7 times the density of lead), DU has several applications including the armor plating of military vehicles. Its mass and pyrophoric properties under conditions of extreme temperature and pressure also make it useful for penetrator munitions designed to defeat enemy armor.

After internalization, uranium, no matter what the form, is eventually excreted from the body in the urine. As such, urine uranium levels are excellent indicators of exposure. There are a variety of techniques available to measure uranium levels in fluids, including neutron activation analysis [Zouridakis 2002], kinetic phosphorescence analysis (KPA) [Brina 1995, Ejnik 2000], alpha spectrometry [Ethington 2000], liquid scintillation spectrometry [Salonen 1993], and inductively coupled-plasma mass spectrometry (ICP-MS) [Karpas 1996]. The unifying feature of these techniques is that they either require extensive sample preparation times (sometimes requiring days to complete) or use expensive instrumentation with which to conduct the analysis. Because many individuals in the vicinity of an RDD detonation would not be exposed but would, as "worried well," seek assessment and treatment, a method to rapidly assess and differentiate exposed from unexposed individuals would be useful. Colorimetric or spectrophotometric detection methods are preferable for use in the field.

The pyridylazo dye, 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP), was previously used to detect uranium in organic leach liquors from nuclear fuel reprocessing plants [Johnson 1971, Pakalns 1972] as well as used histochemically to detect a variety of metals in rat liver samples [Sumi 1983]. Its use in aqueous solutions is greatly hindered by its insolubility and nonspecificity. However, recent procedural advancements have enabled its use to specifically bind to uranium in such diverse aqueous environments as water and urine [Kalinich 2000], cells [Kalinich 2001, Kalinich 2002], and buffered extracts of metallic shrapnel [Kalinich 2000].

2.0 PROGRESS TO DATE

Br-PADAP (Figure 1) has been used to bind to a variety of metal ions including cobalt, nickel, zinc, and copper [Sumi 1983], as well as uranium in organic leach liquors from nuclear reprocessing facilities [Johnson 1971, Pakalns 1972].

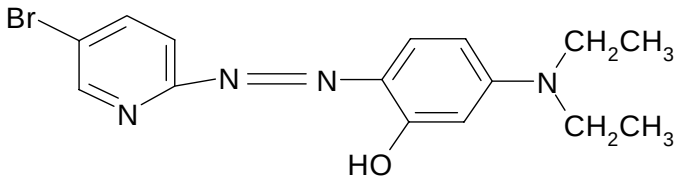

Figure 1: Structure of Br-PADAP

Figure 2 shows our procedure for detecting uranium in fluids.

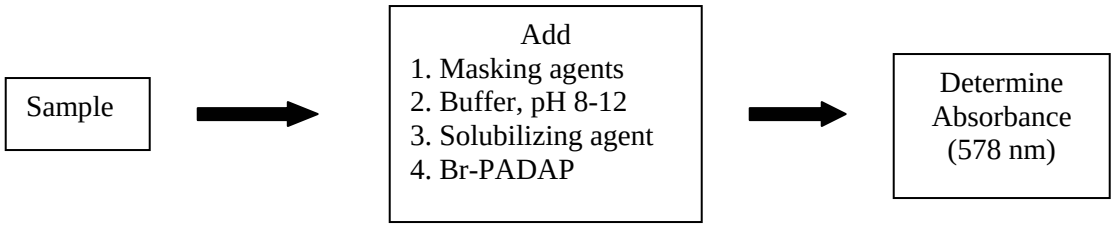

Figure 2: Procedure for the colorimetric detection of uranium

Because Br-PADAP is capable of binding a variety of metals, a procedure was needed to eliminate binding of Br-PADAP to metals other than uranium. We have accomplished that goal through the use of "masking agents," compounds that prevent the binding of the stain to metals not of interest. The addition of a mixture of EDTA and sodium citrate allows the stain to bind to uranium, but not to other metals. Table 1 shows the metals that do not bind Br-PADAP, those that can bind Br-PADAP but can be masked by EDTA/citrate, and those that bind Br-PADAP but are not masked.

Table 1: Metals tested for the ability to bind Br-PADAP

Do not bind		Bind but can be masked		Bind, not masked
Lithium	Potassium	Cobalt	Zinc	Uranium
Cesium	Calcium	Cadmium	Iron	
Tantalum	Molybdenum	Nickel	Copper	
Sodium	Rubidium	Lead		
Magnesium	Barium			
Cerium	Silver			
Chromium	Gadolinium			
Lanthanum	Aluminum			
Tungsten				

The binding of Br-PADAP to uranium is pH dependent, with an optimal pH range of 8–12. Several common laboratory buffers, including phosphate, borate, and CAPS, are capable of maintaining the pH within the ac-

ceptable range. Because of the limited water solubility of Br-PADAP, we have prepared the stain as a stock solution in ethanol or dimethyl sulfoxide (DMSO) prior to addition to the reaction mixture. Also, we have discovered that the addition of a quaternary ammonium salt acts as a "solubilizing agent" to keep the reaction mixture components in solution. After addition of the Br-PADAP, color development is rapid if uranium is present in sufficient quantity. This results in a bathochromic shift of the absorption maximum from 444 nm to 578 nm (Figure 3). This wavelength shift easily can be detected with a visible-light spectrophotometer or colorimeter.

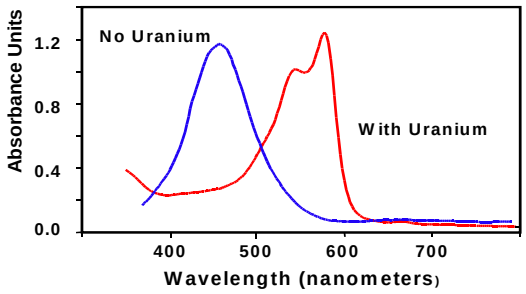

Figure 3: Spectra of Br-PADAP in the absence and presence of uranium

The procedure requires minimal technical training to conduct and can be performed in the field using commercially available, battery-powered colorimeters. Using this procedure we have been able to detect uranium levels in synthetic urine in the range of 30 µg/l. However, this is well above the level most uranium-exposed individuals potentially would be excreting. Therefore, in the next section, we propose several methods we believe will increase the sensitivity of the technique yet still retain its simplicity.

3.0 CURRENT STUDIES

The inclusion of a sample concentration step should greatly increase the sensitivity of the technique. However, for testing samples rapidly in the field, this concentration step should be technically simple to accomplish, not require any complicated equipment, and not generate excessive amounts of chemical waste. There are two areas where such a step might be included in this procedure. The first would be prior to the formation of the Br-PADAP/uranium complex ("pre-complexation concentration" step) while the second area could be a concentration step after complexation of the stain and uranium but prior to measuring the absorbance ("post-complexation concentration" step). The pre-complexation concentration techniques that are being tested include solid-phase extraction procedures with commercially available ion exchange resins such as HYPHAN, UTEVA, TRU, as well as with standard anion exchange resin. HYPHAN, UTEVA, and TRU resins have been used by others to concentrate uranium from dilute samples and may be of utility here [Van Britson 1995, Horowitz 1992]. The use of standard anion exchange resin involves treating the sample with concentrated HCl to convert the uranium to $UO_2Cl_4^{2-}$, a form that will be retained on the resin [Millet 1991]. Both standard column procedures as well as resin-containing filter disks are being tested. Another pre-complexation concentration technique being assessed is metal chelation chromatography using C_{18} filter disks impregnated with various metal chelators. A review of the literature suggests that several compounds may prove useful in this regard, including dipicolinic acid [Shaw 1999, Shaw 2000], and tri-*n*-octylphosphine oxide [Shamsipur 1999]. The final pre-complexation concentration technique under investigation is the recently developed technique of

molecularly imprinted polymers. This procedure involves binding ligands to the uranium then forming an insoluble polymer to lock the uranium recognition site in place. After removing the uranium atom, via an acid wash, the polymer can act as a highly specific uranium-binding resin [Saunders 2000, Bae 1999].

Two post-complexation concentration techniques are being tested, cloud-point extraction and concentration of the Br-PADAP/uranium complex by adsorption to microcrystalline naphthalene. The technique of cloud point extraction attempts to capitalize on the solubility characteristics of Br-PADAP [Silva 1997]. The solubility of many nonionic surfactants/detergents in aqueous systems is greatly reduced above a well-defined temperature called the cloud point. By forming the Br-PADAP/uranium complex, adding a nonionic surfactant, heating then cooling, the mixture should separate into an aqueous layer and a smaller surfactant layer. The Br-PADAP/ uranium complex, due to its limited solubility, should be concentrated in the surfactant layer. This layer then can be resuspended in an appropriate solvent and the uranium concentration determined by measuring the absorbance at 578 nm. The microcrystalline naphthalene technique uses the affinity of pyridylazo compounds for tetraphenylborate-treated microcrystalline naphthalene in order to concentrate uranium from dilute solutions [Taher 2000]. In this procedure, the Br-PADAP/uranium complex will be formed in the test sample. This then will be reacted with a small amount of a slurry of tetraphenylborate and microcrystalline naphthalene. The Br-PADAP/uranium should bind to the naphthalene mixture. After filtration, the precipitate will be dissolved in an appropriate organic solvent and the uranium levels determined by measuring the absorbance at 578 nm.

The purpose for assessing a wide variety of concentration techniques is to find one that can be integrated into the existing protocol for determining uranium levels in biological fluids. The ideal concentration method would be one that helps to increase the sensitivity level of the test but is not cumbersome to conduct (with respect to equipment, technical knowledge, and waste generation).

4.0 CONCLUSION

We are developing a colorimetric technique for the rapid assessment of uranium in biological fluids such as urine. The goal of our work is to develop a technique that can be conducted rapidly and accurately, will not require extensive sample preparation or complicated instrumentation, will require little or no technical training to conduct, and can be used in a field situation if needed. In order to increase the sensitivity of the procedure and increase its applicability, we are characterizing several sample concentration steps for possible integration into the procedure. Our aim is to increase the sensitivity of the assay yet still retain its simplicity.

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