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Applicability of a Field-Portable Toxic Heavy Metal Detector, Using a Radioisotope-Tagged Metalloprotein, to DOE Environmental Remediation and Waste Minimization Initiatives

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Abstract Follows

ABSTRACT

A system based on the metal-binding kidney protein, metallothionein, bound with a trace quantity of radioactive metal, has been shown to be capable of detecting parts-per-million (ppm) to parts-per-billion (ppb) concentrations of some heavy metals in liquid solution. The main objective of this study was to determine if this type of system has adequate sensitivity and selectivity for application in detecting a number of metallic species of concern to DOE, such as mercury, lead, and chromium.

An affinity-displacement study is reported here using the heavy metal radiotracers ^{65}Zn and ^{109}Cd bound to metallothionein immobilized on an Affi-Gel 10 filter support. When a heavy metal solution with a greater affinity than the tracer for the protein is poured through the filter the radiotracer is displaced by a mechanism similar to ion exchange. The main objective of this study was to verify previous internal experimental parameters and results, and to determine the specific affinities of metallothionein for the metallic species of most concern to DOE. The previous internal experiments did not yield the same sensitivities as reported by Griffith, et. al[1]. Potential deficiencies in our experiments were investigated and determined not to have influenced our results. The source of the discrepancy is as yet unknown. The threshold sensitivity of the metallothionein-radiotracer system for detecting heavy metals is at the ppm level. This is sensitive enough to be useful as a general positive/negative test for lead, cadmium and chromium. However, greater sensitivity would need to be demonstrated for this system to be employed as a quantitative analytical technique or as a simple detector for the presence of mercury and silver.

The conclusion based on these results is that the detector has sufficient market potential to justify additional spending on its development, in spite of its current engineering issues. The metallothionein-radiotracer system technology is closely related to the technology employed with affinity chromatography. Affinity chromatography has been employed quite successfully to purify proteins, analyze organic molecules, and to detect trace quantities of biologically active molecules, such as antibodies. There still remains much opportunity to enhance the sensitivity of our procedures by drawing on the base technologies and well developed protocols of affinity chromatography. Therefore, it is recommended that additional funding be actively pursued for more rigorous testing and development of the system.

Table of Contents

| | |
|---|----|
| I. INTRODUCTION..... | 1 |
| Problem..... | 1 |
| Project Background..... | 1 |
| Technical Background | 2 |
| Purpose..... | 2 |
| II. MATERIALS AND METHODS..... | 3 |
| Materials | 3 |
| Methods | 5 |
| III. RESULTS AND DISCUSSION | 8 |
| Preface..... | 8 |
| <i>Iron</i> | 8 |
| <i>Mercury</i> | 10 |
| <i>Lead</i> | 11 |
| <i>Cadmium</i> | 12 |
| <i>Chromium</i> | 13 |
| <i>Erroneous Results of Silver</i> | 14 |
| Data Summary | 16 |
| IV. CONCLUSION..... | 18 |
| Appendix A..... | 19 |
| Commercialization Issues | 19 |
| U.S. Department of Energy’s Primary Toxic Metal Concerns..... | 19 |
| Appendix B..... | 21 |
| Recommendations for Future Work | 21 |
| REFERENCES..... | 24 |

I. INTRODUCTION

Problem

The annual U.S. generation of hazardous waste is expected to average 165 million tons by the year 2000, with heavy metals representing approximately 30 percent.²(Freedonia) Additionally, regulatory agencies continue to decrease metal discharge limits due to the growing toxic load, which necessitates industrial waste monitoring and pretreatment programs. Increased public pressure for government and industry to clean up past waste sites exacerbates the problem, and current characterization and treatment methods have reached their limits of capability and cost-effectiveness. The hazardous waste management industry and DOE would benefit from the aggressive development and commercialization of innovative technologies in the waste characterization and treatment fields.

Project Background

In 1995, a small business in Albuquerque approached Sandia National Laboratories (SNL) about the possible development of a heavy metal sensing technology that they had invented and patented, but did not have the capabilities to further develop independently. The U.S. Department of Energy (DOE) agreed to assist them, and a small business Cooperative Research and Development Agreement (CRADA) was formed. The purpose of this CRADA was to ascertain the feasibility of a field-portable unit using the metallothionein protein bound with a radioisotope as an indicator of trace amounts of metals in aqueous solutions, based on the protein's differential affinities for various metals.

The CRADA project became active in the summer of fiscal year 1996 (FY96). A newly built laboratory was selected in Sandia National Laboratories' Technical Area V to do the studies, due to the desire to minimize background from other activities involving radioactive materials. A significant startup effort was required for the completion of necessary Environment, Safety, and Health (ES&H) documentation, the receipt of the necessary reviews and approvals for the activity, and the procurement and assembly of appropriate hardware and chemicals. Once the approvals were obtained, the metallothionein-radiotracer (MT-RT) system was only able to be tested using three of the fifteen heavy metal solutions of interest due to time constraints. The CRADA, which ended in FY96, successfully demonstrated a portable sample analysis system for mercury and lead.

Funding for a follow-up project to focus on DOE applicability was obtained for FY97 through a Laboratory-directed Research and Development (LDRD) proposal. Under this LDRD funding, additional experimentation was completed

on the MT-RT heavy metal sensor, as well as environmental market research into the commercialization potential of this technology.

Technical Background

The MT-RT heavy metal detection system utilizes a kidney protein called metallothionein (MT), which binds to and controls the level of the trace elements Zn and Cu in the body. MT can also bind to Pb, Hg, Cd, Bi, Ag, and Au. It binds with different affinities to the different metals. The MT protein is chemically bound to a trace amount of a radioisotope, and is immobilized on a filter column for support. When the MT-RT complex comes into contact with a heavy metal of greater affinity for the protein than the metal radiotracer, the radiotracer is displaced through a mechanism similar to ion exchange. The displaced radiotracer is readily detected in the filter column's effluent by a gamma-counting scintillator instrument.

The experimental results from FY96 indicated that the system is readily applicable for measurements of metals dissolved in liquids, and that it is compact, relatively inexpensive, and portable. However, the sensitivity was less than anticipated for lead, mercury, and iron. Lead was detectable at 62 ppm, mercury was detectable at 201 ppm, and iron was detectable at 11 ppm.[3] (Bragg and Randles)

These results were vastly different from those of previous independent experiments, both in the order of affinities for the metals and in the concentration required for detectability. However, several possible reasons for the deviating results were identified, including: (1) a very short contact time between the MT and the heavy metal under study (approximately 1 second), and (2) unverified concentrations of heavy metals in the test solution. It had been postulated that some of the metals had precipitated out of solution creating a much lower concentration than expected. These issues were investigated in the experiments reported here.

Purpose

The objective of the current study was to determine whether the MT-RT system has adequate sensitivity and selectivity for application in detecting a number of metallic species of concern to DOE. Specifically, the goal was to determine: (1) the specific affinities of MT for the metallic species of most concern to the DOE, (2) the most promising applications of this detection system to DOE remediation and waste minimization efforts, and (3) possible partners for the commercialization of the toxic metal detection system. Chapters II and III report on the current series of laboratory experiments. Chapter IV examines the potential application of the MT-RT system and its commercialization potential.

II. MATERIALS AND METHODS

Materials

The materials used in the laboratory experiments were:

- (1) metallothionein (MT),
- (2) radioactive isotopes,
- (3) metals in solution,
- (4) general laboratory equipment,
- (5) a scintillator, and
- (6) a rate meter.

The MT (Sigma Chemical Co.) was covalently bonded to a gel-based resin called Affi-Gel10 (BioRad Laboratories), composed mostly of acrylate beads in isopropyl alcohol. The Affi-Gel 10 has an activated ligand which binds spontaneously to the amino complexes of MT. Once the MT had bound to the Affi-Gel 10, the resulting slurry was then suspended in a buffer called tris(hydroxymethyl)aminomethane (referred to as Tris). The MT/Affi-Gel 10 complex was prepared by the CRADA partner prior to shipment to SNL.

The MT was chemically bound to a radioactive isotope to form the MT-RT complex used as the basis for detection, as explained below in "Methods." The two metal isotopes, cadmium-109 (^{109}Cd) and zinc-65 (^{65}Zn), were chosen for their desirable half-lives and gamma energies. The ^{109}Cd was purchased (from Isotope Products Laboratories) in the form of cadmium chloride (CdCl_2) dissolved in 0.1M hydrochloric acid (HCl). It had a volume of 1 milliliter (mL) and a specific activity of 1 milliCurie (mCi). The ^{65}Zn was purchased in the form of ZnCl_2 dissolved in 0.5 M HCl. It had a volume of 0.18332 mL and a specific activity of 1 mCi. Both solutions were diluted to much greater volumes (and much lower activity concentrations) for use in this project.

One-half of the MT/Affigel-10 complex was stored under refrigeration as received. The other half was divided in two to be bound to either ^{109}Cd or ^{65}Zn . The binding process was achieved by mixing the MT/Affigel complex with isotope solution, and incubating overnight at room temperature. Enough isotope solution was added so that 0.1 to 0.3 mL of the mixture would give a count rate approximately 10 to 20 times the background count using the sodium iodide detector and single channel analyzer described below. These two solutions (MT/Affigel/ ^{109}Cd and MT/Affigel/ ^{65}Zn , both suspended in solutions of Tris) were also stored under refrigeration to preserve them.

The toxic metals to be tested using this system were mercury, iron, lead, cadmium, chromium, and silver. These metals were all procured in the form of

solid chlorides. Based on independent data concerning the affinity of MT for these metals⁴, and on the solubility of the metal chlorides, the following stock solutions were prepared:

- 1 mM solution of mercuric chloride (HgCl_2) dissolved in deionized water
- 1 M solution of ferric chloride (FeCl_3) dissolved in deionized water
- 1 mM solution of lead chloride (PbCl_2) dissolved in deionized water
- 1 mM solution of cadmium chloride (PbCl_2) dissolved in deionized water
- 1 M solution of chromic chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$) dissolved in deionized water
- 1 mM solution of silver chloride (AgCl) dissolved in ammonium hydroxide

These metal chloride stock solutions were used in the serial dilution process described in “Methods” to prepare the test samples.

In addition to general laboratory equipment, a gamma scintillation detector and ratemeter, both from Ludlum Instruments Inc., were used in the experiment to measure the gamma activity of samples as described in “Methods.” The scintillator (Ludlum Model 44-12) is a well-type, high efficiency (nominally 60% intrinsic at 88keV) sodium iodide detector with dimensions of 2.5” D X 10.5” L. Lead bricks were stacked around the detector to isolate it from stray radiation. The scaler/ratemeter (Ludlum Model 2221) is a battery-powered, field-portable, single channel analyzer with 6-digit readout and a range of 0-500,000 counts per minute (cpm). The scintillator/ratemeter system was calibrated, and the highest detection efficiency for gamma-rays from both ^{65}Zn and ^{109}Cd was at a voltage of 680 V, a threshold of 85, and a window of 40. This combination yielded a cadmium efficiency of 69% and a zinc efficiency of 56%. A photograph of the scintillator/ratemeter system is shown below in Figure 1.

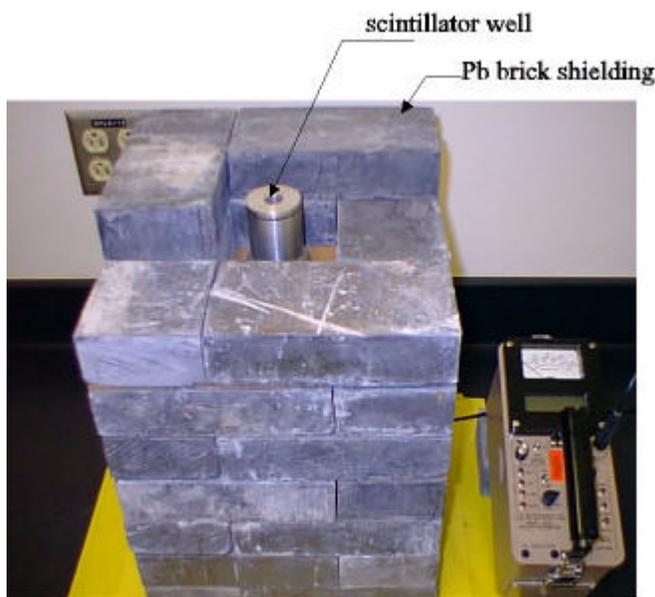


Figure 1. Scintillator and rate meter.

Methods

The experimental setup is shown in Figure 2 on page 7.

Each of the metal stock solutions was used in a serial dilution process that yielded 6 sets of 22 samples each, of increasing concentration. The samples prepared from the 1 mM stock solutions ranged from 1×10^{-10} M for Sample 1 to 1×10^{-3} M for Sample 22 in the following fashion: 1×10^{-10} M, 2×10^{-10} M, 5×10^{-10} M, 1×10^{-9} M, ..., 1×10^{-4} M, 2×10^{-4} M, 5×10^{-4} M, 1×10^{-3} M. When plotted logarithmically, these concentrations are evenly spaced. The samples prepared from the 1 M stock solutions followed the same pattern, but at concentrations a factor of one-thousand higher (thus ranging from 1×10^{-7} M for Sample 1 to 1 M for Sample 22). There were 6 sample sets prepared for each metal, so that 3 could be tested using MT bound with ^{109}Cd and 3 could be tested using MT bound with ^{65}Zn . Each of the samples was 2 mL in volume.

The radiotracers were bound to the MT by adding 8 mL of a ~ 10 $\mu\text{Ci/mL}$ radiotracer solution to 15 mL of a standard MT/Affi-Gel 10 slurry (approximately 1/10 MT/Affi-Gel 10 complex and 9/10 Tris buffer). The mixture was incubated at room temperature for 2 hours and washed with a 0.1 Molar NaCl solution to remove any excess radiotracer.

Filter columns were then loaded with either ^{109}Cd -bound MT or ^{65}Zn -bound MT. This was achieved by thoroughly mixing the buffer solution containing the desired MT-RT slurry, pipetting 1-3 mL of the slurry into the filter column, and allowing the excess buffer fluid to drain through the column into a collection container. The efflux of fluid through the column was approximately 1 second in the original FY96 experiments. It was postulated that this might have affected the results by not allowing enough contact time between the heavy metal ions and the MT for an ion exchange reaction to take place. Therefore, an additional layer of filter paper was placed in the base of the column to decrease the rate of fluid flow. The efflux time was approximately 1-2 minutes in the current set of experiments.

After the MT/Affi-Gel 10 was loaded onto the filter bed of the column, the column was rinsed several times with deionized water to rinse off any unbound radiotracer. After each rinse, the column was placed in a scintillation vial and counted several times for 12 seconds each using the gamma detection system. Once the measured gamma-ray activity remained constant between rinses, the column was ready for testing. There were six filter columns prepared for each metal: three using ^{109}Cd -bound MT and three using ^{65}Zn -bound MT. The background gamma radiation was also determined by placing a scintillation vial

containing 2 mL of deionized water in the detector well, counting at least 5 times for 12 seconds each time, and averaging the results.

The next step in the procedure was to test the MT/Affi-Gel 10 columns using heavy metal dilutions. For each heavy metal study, there were six test tubes containing the most dilute sample of the metal. The contents of each of these six test tubes were pipetted into each of the six prepared columns, and the effluents collected in six scintillation vials. These six scintillation vials were each counted three times (12 seconds each time; except iron which was counted for 6 seconds each time), using the gamma-ray detector. If the activity levels of the samples were approximately the same as the background radiation level, then the next set of dilutions with higher concentration of the heavy metal were pipetted through the columns.

The process was repeated in the same way until the gamma activity of the radiotracer was detected in the effluent (i.e. the measured activity was at least twice the background activity). Prior to detecting any radiotracer in the effluent, the gamma-ray activity of a column was measured after every fifth sample had been pipetted through the column. This double-checked that the radiotracer was not being displaced from the column at a low level with a significant cumulative effect. As soon as the gamma-ray activity in the effluent began to rise, the gamma-ray activity of the column was measured along with the effluent after every test. The procedure was terminated as soon as the radioactivity level of the column had decreased to the background level and/or was no longer decreasing with each sample (or if all samples had been used).

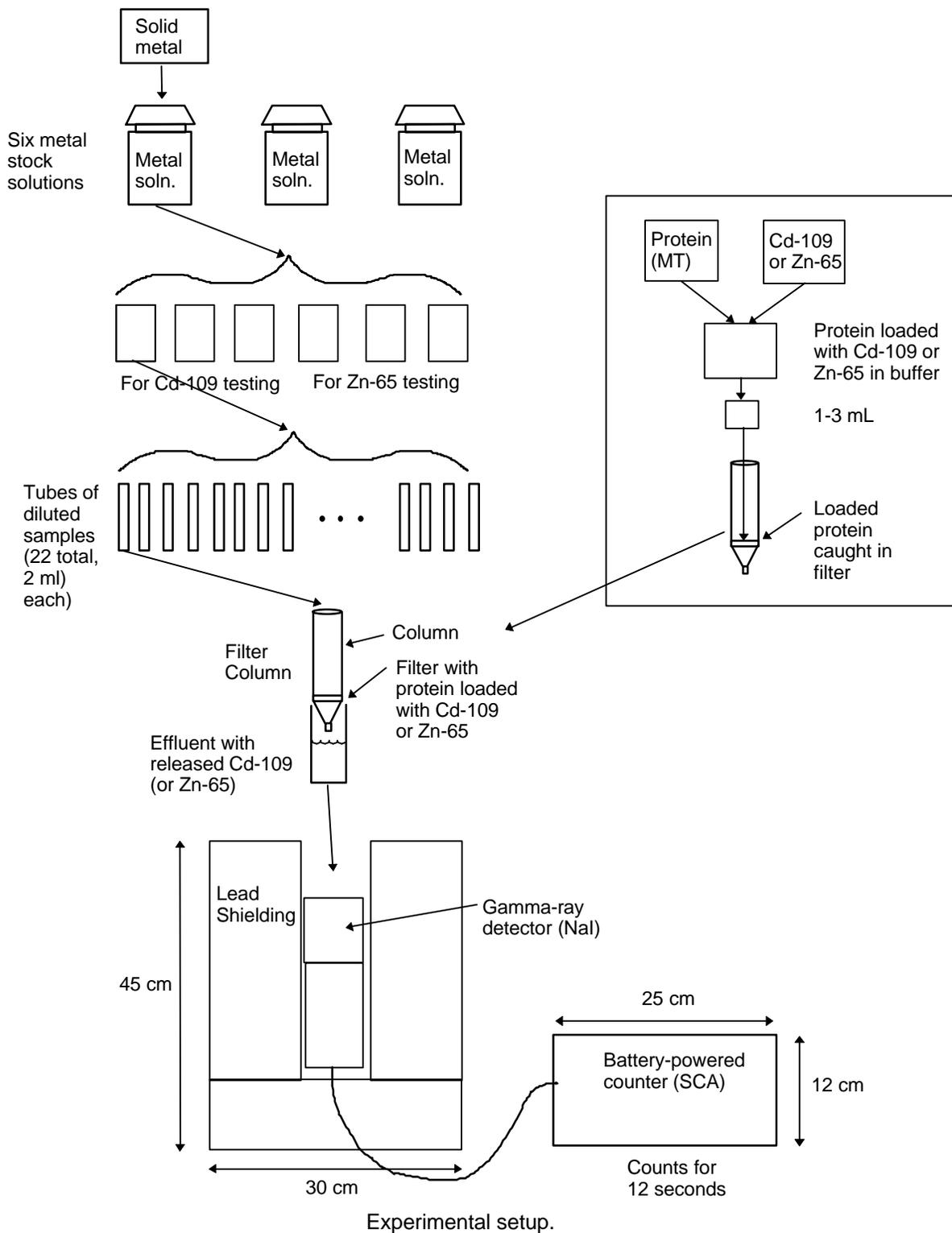


Figure 2

III. RESULTS AND DISCUSSION

Preface

One of the major concerns after the FY96 experiments was that the stock solution concentrations were unverified. It was possible that some of the metal might have precipitated out of a solution, leaving the stock solution concentration much lower than expected. Therefore, in the FY97 experiment series the metal concentration in the stock solutions was verified by an independent lab. The metal concentrations were measured by an Inductively-Coupled Plasma (ICP) technique, which also verified the solutions qualitatively (i.e., what was thought to be a particular metal solution did indeed contain that particular metal ion). The measured concentrations are shown below along with the expected concentrations for comparison. It can be seen that none of the actual concentrations were vastly different from the expected values. The threshold concentrations for detectability, discussed later in this section, have been corrected for the slight differences.

| <u>Metal Solution</u> | <u>Expected Concentration (M)</u> | <u>Measured Conc. (M)</u> |
|-----------------------|-----------------------------------|---------------------------|
| Cadmium | 1×10^{-3} | 9.43×10^{-4} |
| Chromium | 1 | 9.77×10^{-1} |
| Iron | 1 | 9.72×10^{-1} |
| Lead | 1×10^{-3} | 9.56×10^{-4} |
| Mercury | 1×10^{-1} | 9.17×10^{-2} |
| Silver | 1×10^{-3} | 8.35×10^{-4} |

Iron

Iron was the first metal tested, with sample concentrations ranging from 9.72×10^{-8} M to 9.72×10^{-1} M. As Figure 3 shows, the threshold concentration at which iron began to displace both the cadmium and the zinc radiotracers was approximately 1.94×10^{-4} M (10.8 ppm). This verified the result of the FY96 test, in which iron was shown to be detectable at 11.2 ppm.

Three curves are plotted for each radiotracer: a) the measured activity in the filter column's effluent, b) the total (or cumulative) activity displaced into the filter column's effluent, and c) the activity remaining on the filter column. There were three filter columns for each of the two radiotracers, Cd and Zn, and the results of these three columns were simply averaged together to produce the plots. The curves for the cumulative activity displaced into the effluent were arrived at by subtracting out background gamma-ray activity from each of the measurements,

and then integrating the activity displaced in each of the previous serial dilution samples. For example, assuming the measured background activity is 10 counts, if the activity measured for Sample 10 was 60 counts then the net measured activity would have been 50 counts. Had Sample 10 been the first to have any activity measured in the effluent then the cumulative displaced activity would also be 50 counts. Similarly, if 110 counts were displaced by Sample 11 and 210 counts were displaced by Sample 12, then the cumulative displaced activity level for Sample 11 and 12 would have been 150 and 350 counts, respectively. The curves for the average measured activity for each concentration sample, that is “Avg. Activity of Effluent from Cd (or Zn)”, are shown in the figure below to clarify the generation of the “total activity displaced” curves. However, these curves are not on the graphs for the other heavy metals tested.

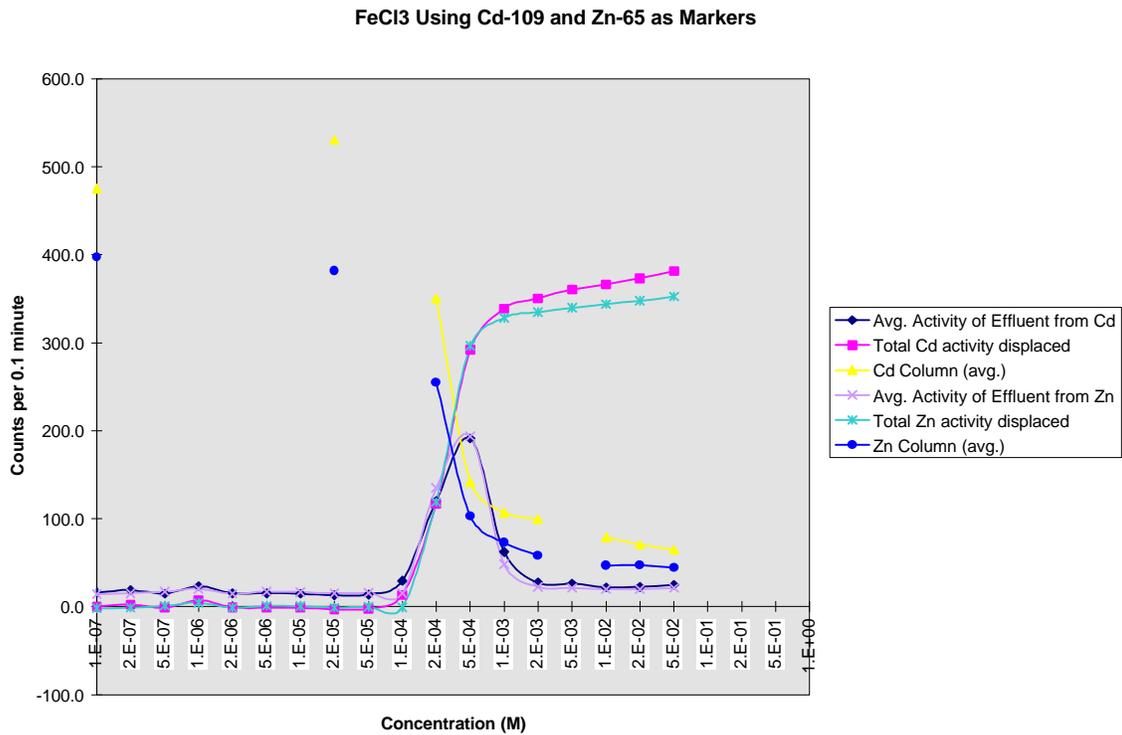


Figure 3

Mercury

Mercury sample concentrations ranged from 9.17×10^{-9} M to 9.17×10^{-2} M¹. Figure 4 below shows that the threshold concentration at which mercury began to displace both the cadmium and the zinc radiotracers was approximately 7.34×10^{-4} M (147 ppm). This was slightly lower than the result of the FY96 test, in which mercury was detectable at approximately 201 ppm. The toxicity characteristic leaching procedure (TCLP) regulatory limit for mercury is 0.2 ppm. Based on our current results, therefore, this detection method would not be acceptable for mercury.

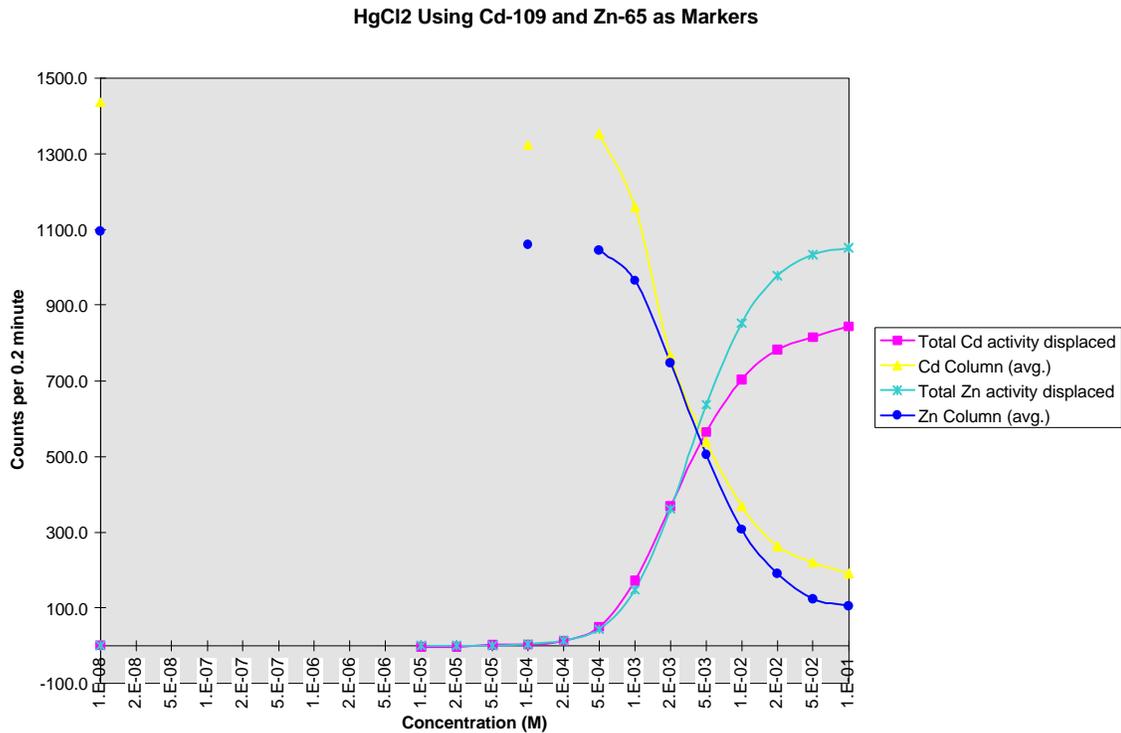


Figure 4

¹. The original dilutions ranged from 1×10^{-10} M to 1×10^{-3} M, but no activity was displaced until the last sample was tested. Therefore, a higher-concentration stock solution was prepared (approximately 0.1 M), and a new set of samples was prepared from it with the dilution range described above.

Lead

Lead sample concentrations ranged from 9.56×10^{-11} M to 9.56×10^{-4} M. The results are shown below. The threshold concentration at which lead began to displace the cadmium was 7.65×10^{-5} M (15.9 ppm). The threshold concentration at which lead began to displace the zinc was 2.87×10^{-4} M (59.5 ppm). The result of the FY96 test indicated a lead detectability of approximately 62 ppm using cadmium and of approximately 104 ppm using zinc. The current results, therefore, are slightly lower than the previous results. Over the range of possible threshold concentrations, however, these numbers should be considered very close, and the current results a verification of the previous ones. The TCLP regulatory limit for lead is 5.0 ppm. Based on our current results, therefore, this detection method might be useful as a general positive/negative test for lead, if cadmium is used as the radioactive tracer.

The fact that cadmium was displaced at a lower concentration than zinc was a bit surprising, because previous independent experimenters[1] had claimed that MT has a higher affinity for cadmium than it does for zinc. This anomaly occurred again in the cadmium test.

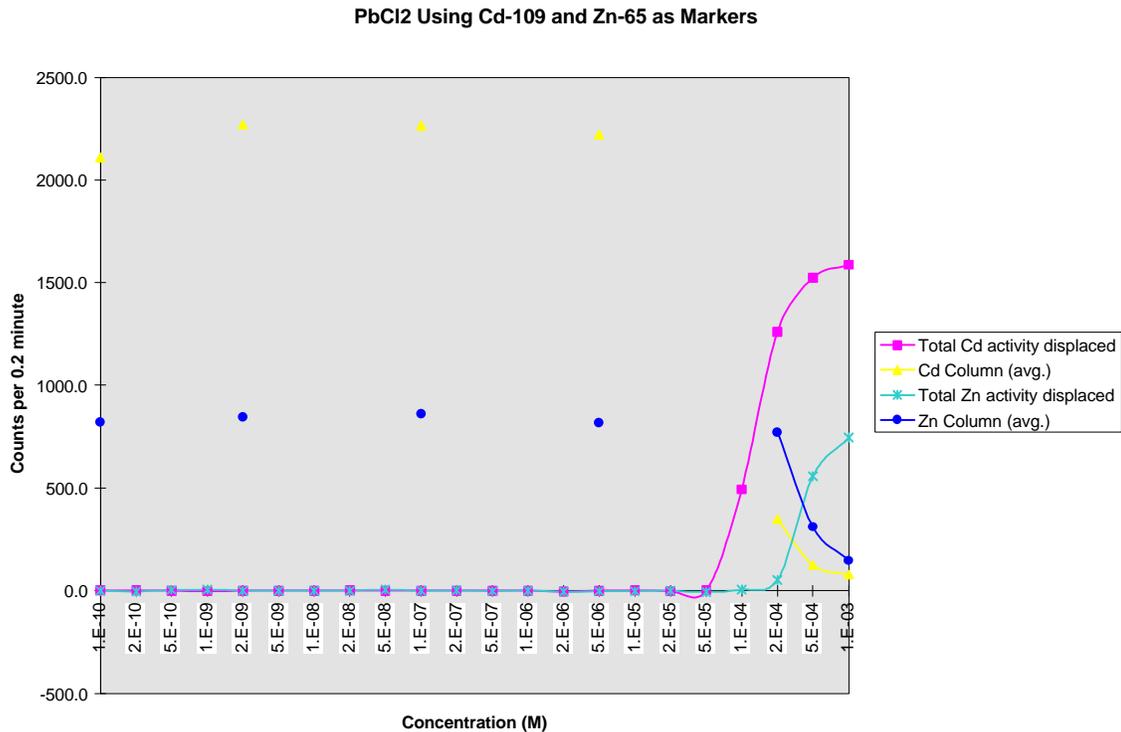


Figure 5

Cadmium

Cadmium was the next metal tested. Its sample concentrations ranged from 9.43×10^{-11} M to 9.43×10^{-4} M. Figure 6 shows the results. The threshold concentration at which cadmium began to displace the cadmium was 9.43×10^{-5} M (10.6 ppm). The threshold concentration at which cadmium began to displace the zinc was 1.89×10^{-4} M (21.2 ppm). The TCLP regulatory limit for cadmium is 1.0 ppm. Based on our results, therefore, this detection method might also be useful as a general positive/negative test for cadmium, if cadmium is used as the radioactive tracer. Again, the cadmium was displaced at a lower concentration than zinc, in disagreement with independent results.

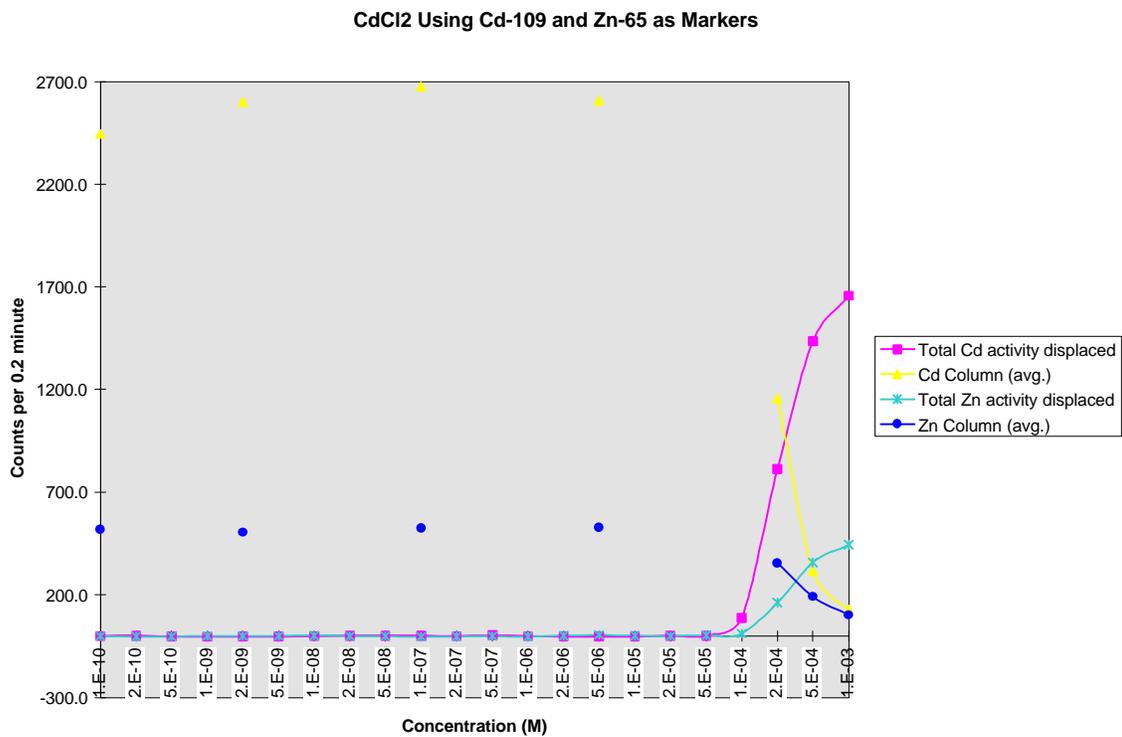


Figure 6

Chromium

The final metal tested was chromium. Its sample concentrations ranged from 9.77×10^{-8} M to 9.77×10^{-1} M. Figure 7 shows the results. The threshold concentration at which chromium began to displace both the cadmium and the zinc radiotracers was 1.95×10^{-4} M (10.1 ppm). The TCLP regulatory limit for chromium is 5.0 ppm. Based on our results this detection method might be useful as a general positive/negative test for chromium, whether cadmium or zinc is used as the radioactive tracer.

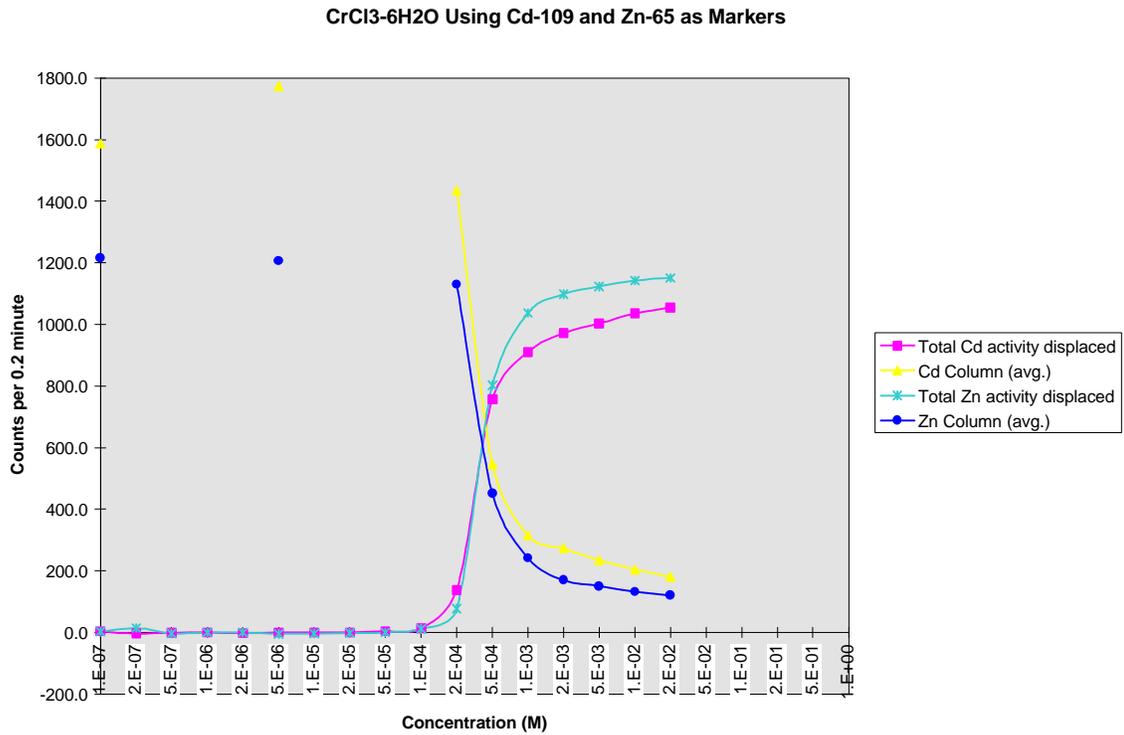


Figure 7

Erroneous Results of Silver

Silver was also tested, with sample concentrations ranging from 8.35×10^{-11} M to 8.35×10^{-4} M. Figure 9 shows that the threshold concentration at which silver began to displace the cadmium radiotracer was 4.18×10^{-5} M (4.5 ppm). The threshold concentration at which silver began to displace the zinc radiotracer was 3.34×10^{-7} M (0.036 ppm or 36 ppb). However, the results for silver were determined to be erroneous, as explained below. There were not sufficient resources to repeat the trials with a corrected protocol for silver.

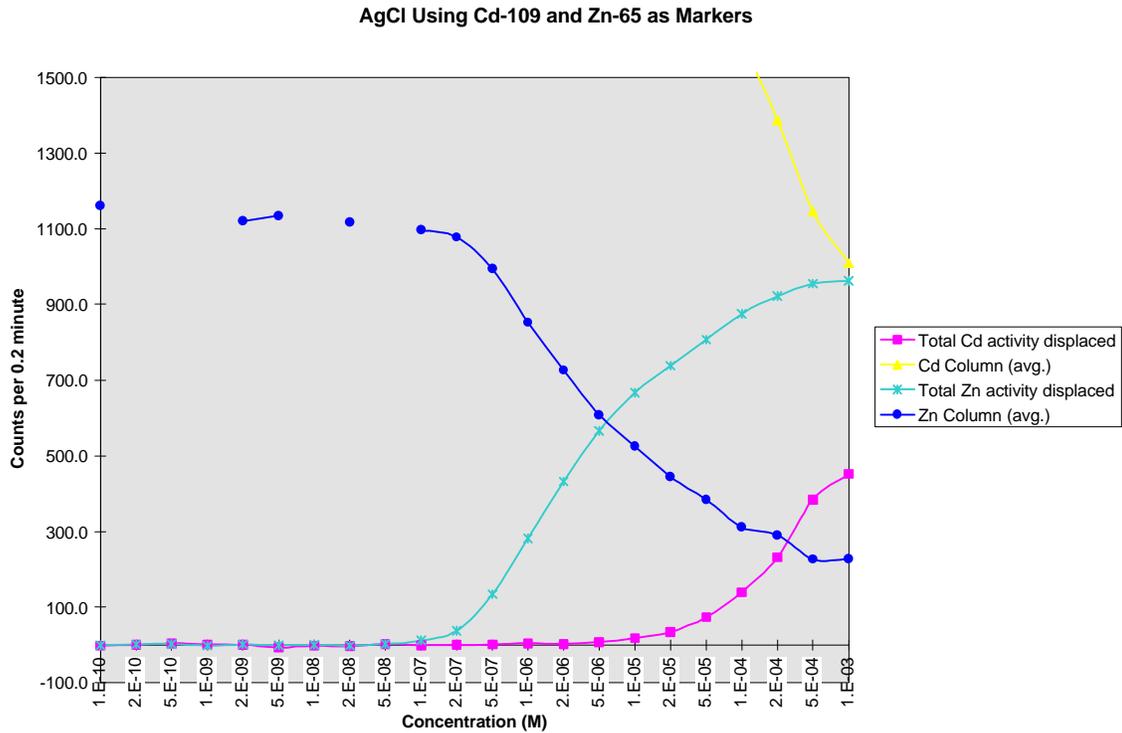


Figure 8

All of the metals tested, except silver, were dissolved and diluted in water. The silver was dissolved in 30% ammonium hydroxide because silver has a very low solubility in water, and then the dilutions were performed in water. The result was that the most dilute samples were dissolved in almost pure water and that the most concentrated samples were dissolved in almost 30% ammonium hydroxide, with a spectrum of water/ammonium hydroxide mixtures in between. Because the silver produced vastly different results from the other metals, it seemed prudent to check the effect of the ammonium hydroxide, even though independent experiments had concluded that caustic solvents would not strip MT.

The silver trials were repeated with a modified protocol. The set of dilutions were prepared using 30% ammonium hydroxide as the solvent and diluent, so that all samples were of the same ammonium hydroxide concentration. The result was that even the most dilute samples displaced radioactivity from the columns. It was therefore apparent that the ammonium hydroxide had affected the silver results. A solution of just 30% ammonium hydroxide (containing no silver) was then tested, and it was found that it too displaced the cadmium and zinc radiotracers from the columns.

Another attempt to assess the effect of the ammonium hydroxide on the silver results was to dilute 30% ammonium hydroxide solution, without silver, in water to levels that matched the levels in the silver samples, and to test these dilutions. The result is shown below in Figure 10. From the fact that the ammonium hydroxide began displacing the zinc at a concentration corresponding to 2×10^{-6} M for the silver (which is quite close to the concentration at which the silver began displacing zinc), it is clear that the low concentration at which silver was detectable was actually due to the ammonium hydroxide, not the silver.

The precise mechanism for the erroneous results has not been determined. It is unknown whether the ammonium hydroxide was attacking the MT or the ligand binding the MT to the Affi-Gel 10 support, or perhaps it is a pH effect or some other unrecognized variable. Until a proper determination is made it is unlikely that a correct protocol can be implemented.

NH4OH Using Zn-65 as Marker

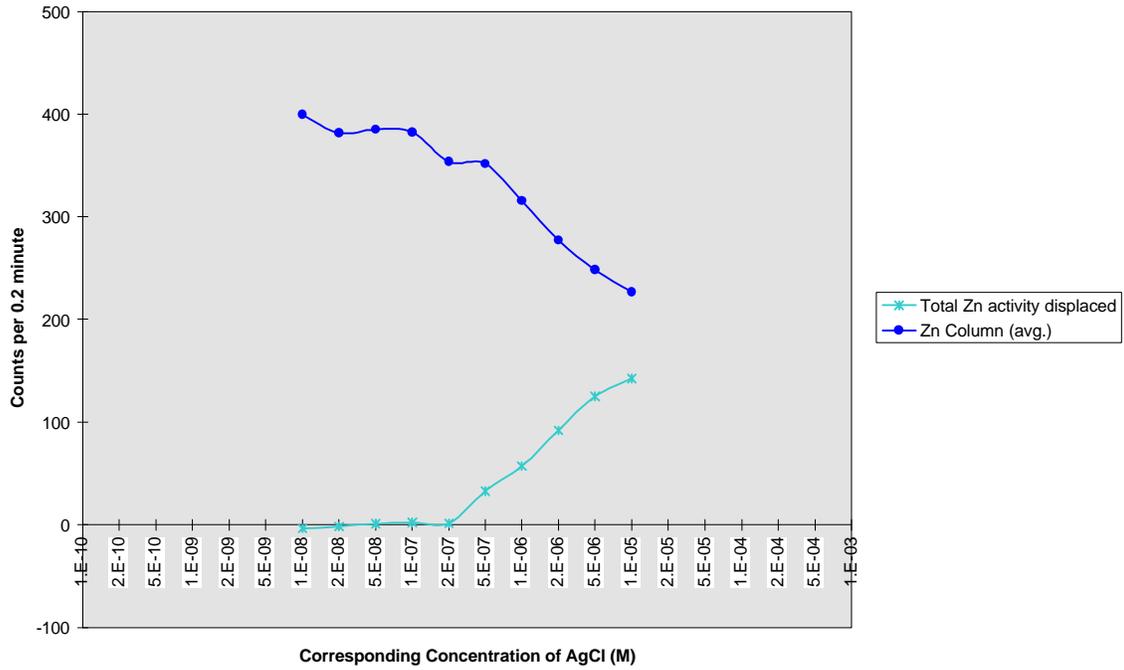


Figure 9

Data Summary

All of the “Total Activity Displaced” curves from Figures 3-7 are plotted together in Figure 10 in order to show their relative affinities for MT. The concentration at which the curves reached approximately 100 counts per 0.2 minute is considered the threshold concentration of detectability. A value of 100 counts was chosen because it was about 3-5 times higher than the background activity, yielding an unambiguous value. The threshold concentration of detectability for Pb is the lowest when using a Cd tracer. The threshold concentrations of detectability for Fe and Cr cluster together whether a Cd or Zn tracer is used. Hg had the highest threshold concentration of detectability.

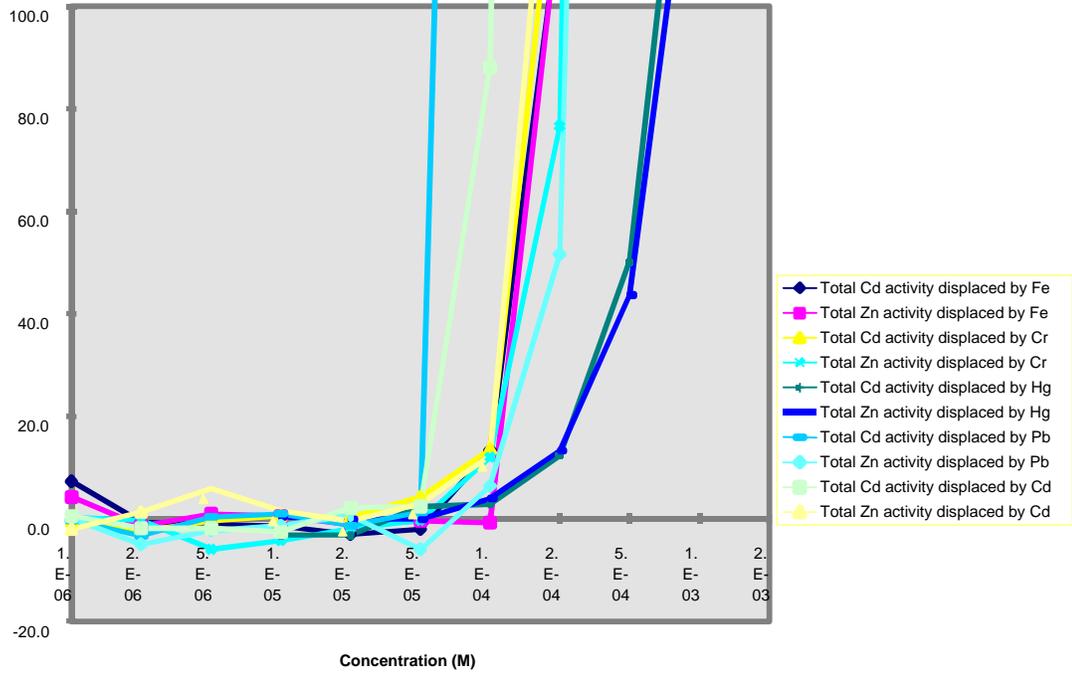


Figure 10 Comparison of Threshold Concentrations

IV. CONCLUSION

The objective of the study was to determine whether a detection system, based on metallothionein bound with a trace quantity of radioactive metal, has adequate sensitivity and selectivity for application in detecting a number of metallic species of concern to DOE. Specifically, the goal was to determine: the specific affinities of MT for the metallic species of most concern to the DOE and the most promising applications of this detection system to DOE remediation and waste minimization efforts.

The toxicity characteristic leaching procedure (TCLP) regulatory limit for mercury is 0.2 ppm, for lead is 5.0 ppm, for cadmium is 1.0 ppm, for silver is 5.0 ppm, and for chromium is 5.0 ppm. Based on our current results, therefore, this detection method is not acceptable for mercury or silver, and is not necessary for iron, but might be useful as a general indicator test for lead, cadmium, and chromium. A significant amount of basic research remains to be done to characterize this technique before commercialization is possible.

Appendix A

Commercialization Issues

U.S. Department of Energy's Primary Toxic Metal Concerns

DOE's primary concern for non-radioactive heavy metals comes from processing of mixed waste in which the non-radioactive toxic waste is separated from the radioactive waste. Mercury is by far the metal of greatest concern, followed by chromium and lead. Beryllium is also a major concern. Regulatory limits for allowable concentrations have been established for cadmium, lead, and silver, which make them prime targets for a highly sensitive detector. Finally, additional metals of concern are antimony, arsenic, barium, cobalt, manganese, nickel, selenium, and thallium. [5, 6, 7]

Instrumentational Needs and Environmental Clients

Discussions with several experts in the fields of environmental characterization, monitoring, and treatment, have revealed that most field characterization technologies are quite sophisticated and costly, and that quick, inexpensive, qualitative screening methods would be of great value in the environmental market. Very few of these quick-screening methods are currently available. Additionally, there are no highly desirable technologies available for automatic sensing applications, such as process effluent monitoring. ⁸(Southwick)

The major client of environmental firms seems to be the government, especially the agencies associated with the Department of Defense and the Department of Energy. Commercial entities and the Environmental Protection Agency (EPA) both require some environmental products and services, but the government represents by far the largest market potential. (Southwick)

Potential Partners for Commercialization

As part of the strategy for identifying potential partners for commercialization of this innovative technology, a Commerce Business Daily (CBD) advertisement was submitted, and solicitation letters were sent to about 50 companies which had been identified as manufacturers of environmental equipment. There have currently been about ten replies to the ad and the letter. Most of the responding companies seem quite excited about the project, and are looking forward to further discussing the possibility of a partnership.

The metals of most concern to DOE are mercury, chromium, lead, beryllium, cadmium, and silver. Quick, inexpensive, qualitative screening methods would

be of great value in the environmental market, because very few of these quick-screening methods are currently available. Additionally, there are no highly desirable technologies available for automatic sensing applications, such as process effluent monitoring.

The major client of environmental firms seems to be the government, especially the agencies associated with the Department of Defense and the Department of Energy, and there are several companies that have shown quite a bit of excitement about the toxic metal detector, and are looking forward to further discussing the possibility of a partnership.

The conclusion based on these responses is that the detector has sufficient market potential to justify additional spending on its development, in spite of its current engineering issues.

Appendix B

Recommendations for Future Work

The results obtained in the current study differ significantly from previously published experimental results [9]. The procedures followed in these previous studies should therefore be closely examined to identify the sources of the discrepancies. This identification will surely aid in optimizing the performance of the system.

The current technique of binding the metallothionein (MT) onto a polyacrylamide bead bed is a standard practice in affinity chromatography. Affinity chromatography (AC) makes use of the fact that proteins preferentially bind to specific ligands. Cellulose, agarose, or similar synthetic beads are coated with a ligand or close analog that binds with a high specificity to the protein of interest. When a mixture of proteins and buffer solution are passed through a column of the activated beads the proteins which have a high affinity for the ligands remain bound in the column. The sequestered proteins can be released from the column by a suitable eluant, frequently a strong acid or base. Several of the large chemical supply houses (e.g. Sigma and Bio-Rad) provide what are called activated supports with spacer ligands for binding to a particular class of proteins. The literature is replete with thousands of references to affinity chromatographic techniques being used for the purification of proteins, enzymes, some even using immobilized metals. The more than two dozen books published on the subject attest to the maturity of these techniques. Yet the actual practice is still more art than science. As explained in one of the manufacturer's application notes for their immunoaffinity supports, "...the choice of an effective eluant often appears to be empirical."

1. The possibility of steric hindrance is an often repeated caution. The folded structure of a protein can be affected by the support beads if they are in too close a proximity. This steric hindrance can limit the binding efficiency of MT to the support beads or reduce the ability of MT to bind to heavy metals. The steric hindrance can be overcome by using a different support or by adding a spacer ligand between the support and the MT. Trying several supports and spacer ligand combinations from the various chemical suppliers may improve the threshold concentration of detectability of MT for heavy metals. One caveat of the spacer ligand approach is the possibility that the introduction of a spacer ligand may be incompatible with the current procedure of eluting the heavy metal and recharging the MT with the radio tracer. The problem appears to be that strong acids strip the heavy metal from the MT and may also strip the MT from the spacer ligand. Indeed, this may be just the problem encountered with the silver trials. Ammonium hydroxide is a caustic

and may have stripped the MT from the column or perhaps it can strip the radiotracer from the MT.

2. The current experiment protocol used Affi-Gel 10 from Bio-Rad Laboratories as the support for MT. Bio-Rad recommends that the coupling of the MT to the Affi-Gel 10 support be done at pH conditions at or below the isoelectric point (pI) of the MT. They show the coupling efficiency varies dramatically with the pH conditions used. Large molecules such as MT can function as cations or anions depending on the pH conditions. The pI is the pH at which MT is functionally neutral. The pI of MT can be determined readily by electrophoretic techniques if it has not been published already. Should the pI of MT be less than 6.5 then Bio-Rad Laboratories would recommend using a different support, Affi-Gel 15.
3. Several authors emphasize that the minimum size column should be used when working with low concentrations of a protein or enzyme. Perhaps this could be a complication with the current experimental protocol. The amount of MT/Affi-Gel 10 complex loaded into the column was determined by the quantity of radiotracer it contained and not on the anticipated concentration or total quantity of heavy metal atoms to be detected. What could be happening is that the radio tracer which is displaced at the top of the column by the incoming heavy metal, gets re-adsorbed by MT lower in the column.
4. Verify that the MT bound to a support retains its full metal binding capability. This is related to the problem identified in 3 above. The act of binding the MT to the support can alter its metal binding characteristics. For instance, the activated support recommended by the Sigma Chemical Company is a thiopropyl/Sepharose which may bind to an amino site which interferes with a metal atom binding to the MT protein with a strong affinity. The repeated washing prior to using the MT/Affi-Gel 10 columns may have stripped some of the radiotracer from the sites with reduced metal binding affinity. Actually verify that the MT binds to the same quantity of metal before and after it is bound to the Affi-Gel 10 support.
5. Heavy metals in a soil sample is measured using a protocol that leaches the metals from the soil. The leachate could be evaporated or subjected to a reverse osmosis process which would raise the metal concentration high enough to be detectable with the current MT column. A standard protocol could be developed to match with the TCLP concentration regulatory limits for soils.
6. Several authors have reported that the binding ability of their columns decreased with repeated use in which an elution with a strong acid or base was required. This could be a potential problem with regenerating our MT columns and needs to be looked at before fielding a commercial product.

Denaturing of the MT may similarly occur when stripping the heavy metals or under routine conditions and protocols should be developed to restore the normal MT functioning.

7. It is also possible that the MT from different animals has differing affinities for particular metals that could be suitably exploited. The 1996 Sigma catalogue lists five variations of MT from horses (1), rabbits (3) and mice(1). There are over 50 companies like Sigma so there may be even more varieties of MT already commercially available.
8. The experiments done to date have employed single metal solutions. The presence of other metal ions could interfere with the MT binding to particularly low concentrations of heavy metals. Although this could be circumvented using a protocol to precipitate offending species or by adjusting the pH of the column.

A related chromatographic technique is solid phase extraction (SPE). Classical SPE used ion exchange techniques, but protein specific binding filters are now commercially available. Recent advances in this area include the development of crystalline silicotitanates and mesoporous silica with sulfur compound coatings which preferentially sequester heavy metals. There are over 50 companies which provide a range of different synthetic supports, MT proteins, and binding ligands to the AC and SPE community. The well developed technologies associated with AC and SPE, if applied to the MT-RT system, could provide significant gains in the sensitivity. Gains are also possible through simple process optimizations, such as, pH and temperature variations. The MT-RT detection method has demonstrated sensitivity at the ppm level to toxic heavy metals and can be turned into a commercial product through a focused research program.

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