Metal Remediation and Molecular Imprinting Using Immobilized Glutathione

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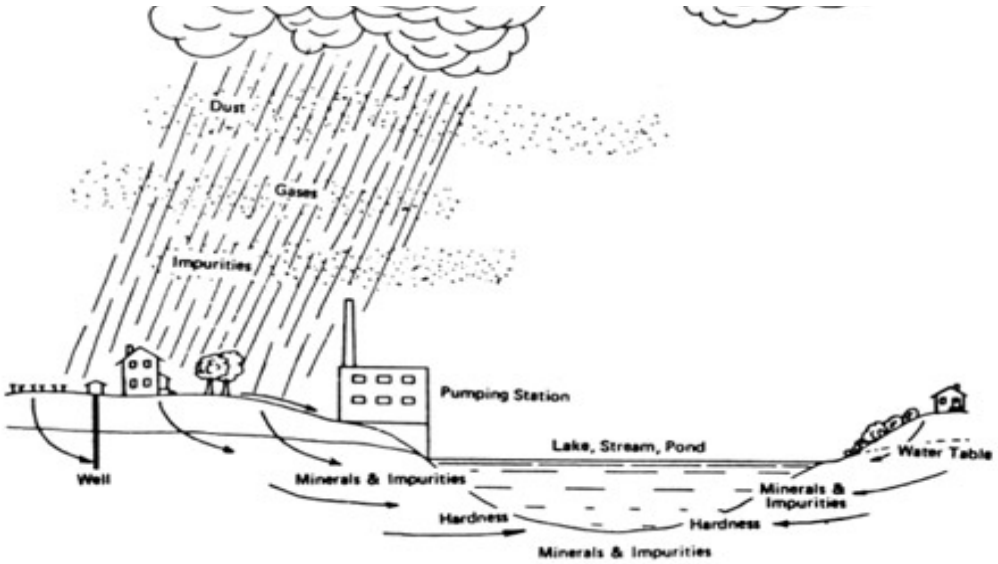
Abstract

To assess the efficiency with which glutathione binds to heavy metals (Zn, Co, Cd, Pb, Cu, Mg), glutathione was immobilized to Controlled Pore Glass (CPG) and packed into mirco-columns. The influent and effluent volumes of heavy metal solutions were then analyzed using Flame Atomic Absorption Spectrometry (FAAS). Through Breakthrough curve analysis, potential for glutathione to act as a selective metal binding chelator was evaluated. By reducing and oxidizing the glutathione immobilized to CPG in the columns, the results show select heavy metals have an affinity exhibited by various receptor sites located along glutathione.

Introduction

In the heyday of modern society, human consumption and waste management remains a top priority among all who seek a clean and productive future for the young of tomorrow. Yet, the world is still at the mercy of many problems that have stemmed from the billions of people who use these planetary resources to carry on with their daily lives. One such crisis is the lack of uncontaminated water. Specifically, the growing amount of heavy metal wastes in both fresh and salt water reservoirs holds much potential to stem the well-being and advancement of society: without any significant and natural filtration system in the environment, heavy metals remain in the water cycle (Figure 1.1) and can disrupt many biological processes vital to many species by displacing elements necessary to carry out vital metabolic functions. Each living organism, however, requires a trace amount of certain heavy metals in order to carry out biological functions. Unfortunately, not all heavy metals are required to live, such as mercury, lead, and cadmium and an abundance of these metals can cause serious biological damage. Thus, humans have already countered this threat with precipitation filtration systems, but to reduce heavy metal levels in freshwater to a "safe" degree, an additional purification step will be needed. Therefore, the use of innovative materials for metal remediation and extraction from water has become a significant sphere of interest to the sciences. One potentially successful area involves using biopolymers such as amino acids and peptide chains.

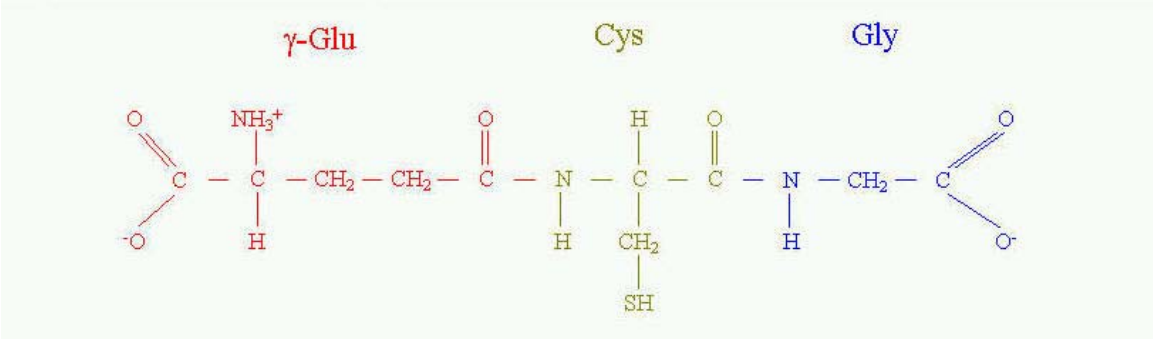
Figure 1.1: The water cycle



Specifically, a "model" chelator would exhibit certain characteristics crucial to the metal remediation process: high selectivity, strong binding with a large capacity, an on-demand metal release mechanism, and minimal costs for the initiation and maintenance of such a system. Unfortunately, inorganic ion exchangers often require harsh and highly expensive treatment for reclamation and exhibit lower efficiency in salty matrices. Using short chains of biopolymers attached to immobilized support systems would, therefore, provide a much larger surface area for extraction and a much easier process by which to separate

the metals from the polymers. Fortunately, proteins such as metallothioneins are natural metal chelators and may provide a solution to the needs of metal remediation. For instance, each protein relies on its own unique tertiary structure and ligands to create binding "pockets" to capture the metal cation. By manipulating the size and shape of the tertiary structure, one could enhance the selectivity of the protein for the heavy metal. Changing the pH level of the environment in which they are located also alters the entire structure of the protein itself. Lowering the pH to an acidic level pronates the protein, resulting in the expulsion of the cation. Likewise, raising the pH to a basic level causes it to be more receptive to metal binding.

Figure 1.2: Structural drawing of Glutathione (GSH)



One such metal-chelating protein is glutathione (gamma-glutamylcysteinylglycine, Figure 1.2), a naturally occurring tripeptide in the human body that functions as an antioxidant. Glutathione is usually found concentrated in the mitochondria of cells where it can protect both the energy production and the regulation of biological functions from toxic compounds, oxidative damage and even radiation within the cell. Studies have shown that glutathione levels decline as a direct result to higher toxicity levels mainly since, while export of glutathione by human cells remains unaffected, glutathione synthesis is greatly inhibited. One study done on Wolf Spiders (*Pardosa lugubris*) studied the relations between glutathione-dependent detoxifying enzymes and heavy metal concentrations. The resulting data revealed the correlation between a high cellular glutathione level and strong resistance to various metallic toxins in the environment. This knowledge that glutathione is of high significance in the body's detoxifying mechanisms makes it a point of great interest in its applied usage to more macroscopic conditions such as that of water purification.

Significantly, glutathione's chemical makeup makes it ideal for use as an immobilized metal chelator. Its unusual peptide linkage between the cysteine and the carboxyl group of the glutamate side chain through the sulfhydryl group allows it to have a large redox potential ($E^o = 0.33U$) and readily create metal-thiol complexes. In this case, the highly reactive sulfhydryl group is able to set the redox stage for metallothioneins to bond to reactive oxygen species. It also serves to alkylate the electrophilic metabolites or reduce free radicals that may catalyze unwanted side reactions. In addition, both terminal portions of the structure of glutathione serve useful purposes in this research. Interestingly, one end is hydrophilic while the other is hydrophobic, which bonds to

naturally occurring lipophilic toxins in the environment. The fact that each of the three amino residues that make up glutathione are covalently bonded to each other generates a great degree of pronation and mobility to form more than one coordinating site per cation exposed.

In order to assess the metal concentrations of the influent and effluent volumes of solution, Flame Atomic Absorption Spectrometry (FAAS) was employed. Significantly, Atomic Absorption Spectrometry has become one of the most widely used methods of assessing single element concentrations in solutions by interpreting the absorption of characteristic wavelengths. It is based on the absorption of radiation by ground-state atoms using a flame to atomize the particles in solution. Since its development, AAS has proven itself a very resourceful and applicable tool for data acquisition, ranging in its uses from mining, soil component analysis, to metal remediation techniques and pharmaceuticals. Its flexibility and diverse usage comes from its high selectivity for specific frequencies (can read changes in parts per billion per gram of solution), ease and simplicity, and the ability to quickly distinguish one element over another.

There are several unique processes involved in obtaining an AAS reading (Figure 1.4):

Atomization: The flame causes the atomization of the solution into its constituent particles. In the beginning, the solution is drawn into a pneumatic nebulizer by the rapid flow of the oxidant through the tubing. The liquid is then broken up into a fine mist as it is directed at a small glass bead. By now, the aerosol is broken down into even more elementary parts by flowing through baffles which prevent large droplet from passing and nebulizing. Excess liquid collects at the bottom of the spray and is drained out. The actual process is very inefficient: only 5% of the actual solution reaches the flames. The flame then evaporates and disperses the remaining particles into atoms for accurate readings. Usually, the most common fuel for the flame is made of acetylene and air (Table 1.1), producing a flame temperature of 2400K-2700K. Other metals with higher boiling temperatures need a higher flame temperature in order to be atomized. Depending on the element, a “lean” or a “rich” flame is needed. A “rich” flame contains excess carbon that would reduce metal oxides and hydroxides, giving a more sensitive reading. A “lean” flame with excess oxidant is much hotter.

Table 1.1: Maximum flame temperatures

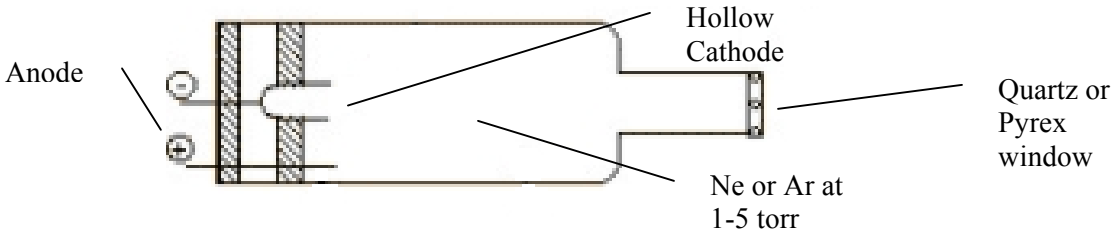
Fuel	Oxidant	Temperature (K)
Acetylene	Air	2400-2700
Acetylene	Nitrous Oxide	2900-3100
Acetylene	Oxygen	3300-3400
Hydrogen	Air	2300-2400
Hydrogen	Oxygen	2800-3000
Cyanogen	Oxygen	4800

Flow Injection Pump: In order to actually test the performance of glutathione immobilized to CPG columns, a flow injection pump was needed to pump metal solutions

at a flow rate of 1ml/min to provide a constant flow of solution through the columns rather than a variable one by directly aspirating it into the nebulizer. By letting the pump flow solutions directly through the columns, it was also possible to analyze the various metal binding properties and capacities through concentrations of the effluent and influent volumes of solution. The one used in this research was an eight column Carter Cassette Pump, made by Monostat.

Hollow Cathode Lamp (HLC): A hollow cathode lamp (Figure 1.3) emits a narrow beam of light carrying the frequency that creates the specific wavelength absorbed by the particles because the monochromator cannot isolate wavelengths of 10^{-2} to 10^{-3} nm. Inside, the cathode is made of the element being assessed while the anode is made of unreactive metal such as tungsten or platinum. Inert gas such as neon or argon fills the lamp so that when high voltages are run through, the filler gas atoms become excited and begin to bombard and knockout cations in the cathode in a process called sputtering. Once this happens, energy is transferred from the neon or argon atoms to the cations in the cathode, causing them to vaporize and emit light with the same frequency that would be absorbed by analyte atoms in the solution as they go back to their ground state. For example, if one were to assess the concentration of copper in a copper solution, then a hollow cathode lamp with a copper cathode would be used to generate the same frequencies that would be absorbed by other copper atoms in the solution. However, the very shape of the HLC causes the cation emission to be directed in a very narrow beam straight through the quartz window. The design of the HLC also prevents disintegration of the cathode in that it forces the cations to return to their ground states and become reabsorbed by the rest of the cathode.

Figure 1.3: Diagram of Hollow Cathode

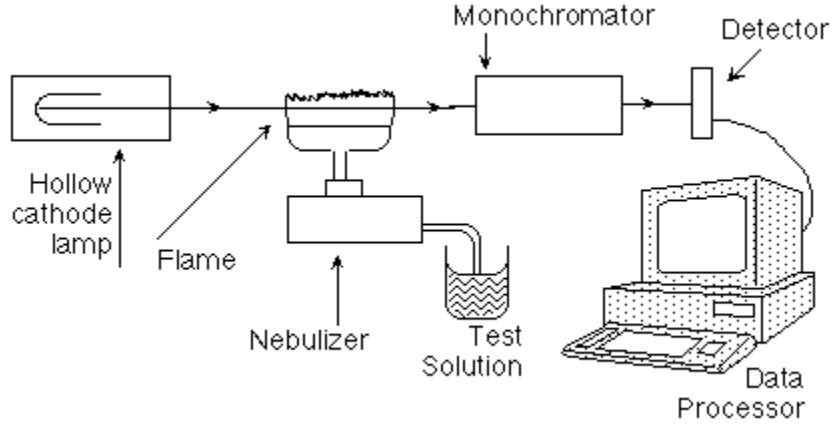


Background Correction: Many problems can potentially arise by the vast possibilities of minute errors. There are two possible types of errors that can occur: spectral and chemical interference. Spectral interference involves the inability of the machine to distinguish the absorbance readings of two elements due to their near overlap and proximity in analyte absorption such that resolution of the two species is impossible. On the other hand, chemical interference results from other chemical reactions that alter the absorbance of the atoms in the analyte solution. In many cases, spectral interference develops via unexpected scattering or broadband absorption caused by reactive species. The presence of products caused by combustion of the oxidants in the flame leads to particulates that have the potential to exhibit scattering or absorption. One source of highly troublesome errors is a resultant of the sample matrix itself. Inside the analyte solution, aggregates created through unwanted reactions could contaminate the

absorption properties of the solution. In many cases, free radicals present in the environment can also result in scattering of the signal. In addition to errors caused by spectral interference, chemical interferences also add to the list of possibilities for error. More commonly, chemical errors are a result of formation of compounds with low volatility, disassociation constants and ionization. Compounds with low volatility tend to slow the flow rate of the analyte solution into the aspirator. As a result, all readings taken from a FAAS are lower than usual. Low disassociation constants also lead to formations of compounds that will not easily atomize in the nebulizer, resulting in more absorption than should actually be occurring. To correct these errors in calculation, several methods such as background correction based on the Zeeman effect have come into play. For instance, strong magnetic fields produced within the lab building may cause various electronic energy levels in different atoms, resulting in several absorption lines for each electronic transition. Each line is about 0.01 nm apart from the original line. Taken into account that the sum of these lines is of the exact value as the original reading, a correct value is possible.

Process of Data Acquisition: At first, the sample solution is drawn into the pneumatic nebulizer by the rapid flow of the oxidant. The liquid is then broken into a fine mist as it leaves the capillary and is directed against the small glass bead that further breaks down the solution. The aerosol is then purified into small droplets by baffles that trap large droplets and causes them to condense and drain back into a waste bucket. As the remaining 5% of the solution reaches the actual flames, the particles are then atomized into separate atoms. Meanwhile, the HCL (Hollow Cathode Lamp) produces a very narrow beam of a single wavelength that is focused through the flames to measure the amount of absorbance of the signal. Because the wavelength of the signal is emitted by the same element being assessed in the experiment, only atoms of the same element would be able to absorb it. From there, it is easy to quantify the data based on Beer's Law, the direct, linear relationship between signal absorbance by the particles and the analyte concentration in the solution. The higher the signal absorbance, the more atoms there are in the analyte solution absorbing the same signal. The data is then recorded by using "LabView Data Acquisition" made by National Laboratories.

Figure 1.4: Schematics of a Flame Atomic Absorption Spectrometry reading.



2. Experimental

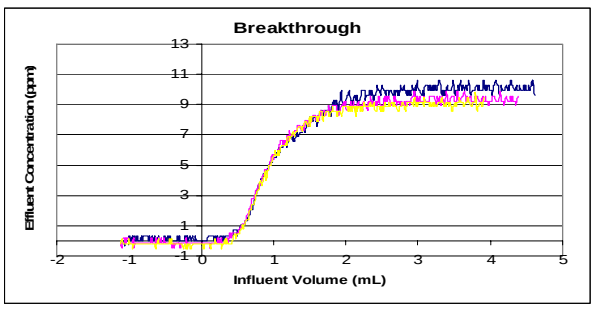
2.1 Materials

All metal solutions were provided by SCP Science (Co, Cu, Pb) and Acros Organics (Cd, Ni, Mg) in 4% HNO_3 with 1000 ppm concentration levels. To dilute the metal solutions to 1 ppm, 5 ppm, and 10 ppm standards for FAAS readings, 0.01M HNO_3 was employed. Standard safety precautions were taken also to ensure the safety and enjoyment of the research. Buffers that were used in the lab settings were 0.02M HEPES and 0.02M Ammonium Acetate, both from Sigma Laboratories, diluted with DI water, run through Chelex 100 column to extract trace metals and pH adjusted to pH 7.0 using HNO_3, NH_4OH, CH_3COOH, concentrated HCl and NaOH. The glutathione, gluteraldehyde and the CPG were also provided by Sigma Laboratories. The 98 % pure glutathione was stored in its reduced form. Gluteraldehyde was of Grade 11 in 25% aqueous solution. The CPG (PG 240-120) had a mean pore diameter of 22.6 nm and a mesh size of 80-120. FAAS readings were obtained by Varian model AA-875.

2.2 Breakthrough Curve and Strip Analysis

Breakthrough analysis can be used to calculate the metal binding capacity of the ion-exchange material packed into columns of GSH and CPG. Strip analysis is also used for calculating metal binding and determines on-demand metal release. FAAS was used to determine the metal binding capacity of GSH-Glu-CPG and Glue-CPG and gave a chance to record by observing the rise in concentration of the effluent volume of solution from 0 ppm to the concentration of the influent volume, 10ppm. In a graph representation of the data, the effluent concentration rises until it reaches equilibrium capacity with the influent concentration. The flow injection machine pumped metal solutions with concentrations of 10 ppm of metal through the column to test how well the glutathione and gluteraldehyde would bind to the metal cations present in the solution. At first, the effluent volume would contain very low concentrations of metal, creating a “baseline” effect. This baseline suggests quantitative binding (100% of metals introduced are being bound) and chelation to strong sites. But as the binding sites in the column on the CPG are saturated, the effluent concentration would rise to that of the influent volume, 10ppm. The sloped regions show weak binding where only a certain percentage of metal is being bound. Once that has occurred, “breakthrough” of metal capacity has also occurred. Through FAAS readings, a graph of the concentration versus a period of time enabled us to quantify the metal binding capacity and the ease with which the column released the metals. Figure 2.1, Equation 2.1.

Figure 2.1: Sample Breakthrough Curve for Gluteraldehyde-CPG column using 10 ppm Cadmium solution.



Equation 2.1: Formula to obtain metal binding capacity based on a breakthrough curve.

$$C_S = (C^oV - \int C_m dV)/m$$

C_s = metal bound to stationary phase after volume of solution is passed
C^o = influent concentration
C_m = mobile phase effluent concentration.
m = mass of GSH-CPG in column

Dead Volume: Because it took a certain amount of time for the actual metal solution to travel from its container to the FAAS machine, a "dead volume", or time the solution took to reach the signal, was recorded and will consequently be deleted from the overall time it took for the metal binding capacity of the column to be reached.

Calibration: In order to calibrate the absorbance signals (Figure 2.2) to their actual concentration readings, standard solutions of the metals must be made before hand to compare with the concentrations of the effluent volume coming out of the columns. These "calibration curve" should be ideally linear (Figure 2.3).

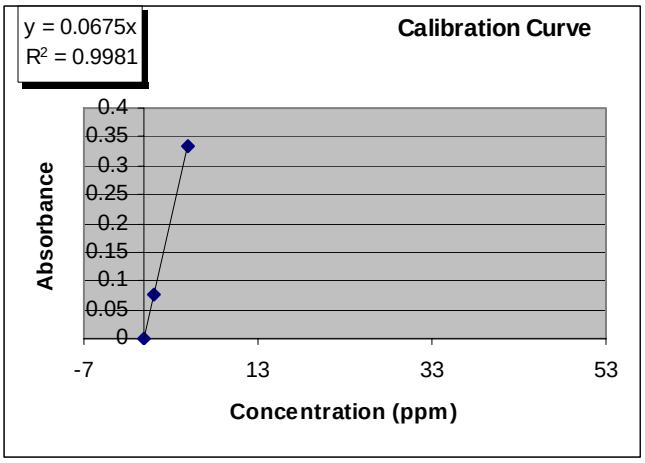
Figure 2.2: AA Instrument Settings for each metal:

Element	Lamp Current (in mA)	Wavelength (nm)	Slit (nm)
Cd	3.5	228.8	0.5
Co	7	240.7	0.2
Cu	3.5	324.8	0.5
Mg	3.5	285.2	0.5
Ni	3.5	232	0.2
Pb	5	217	1

Figure 2.3: Sample calibration curve for 10 ppm oxidized Cadmium solution.

Calibration Standards

ppm	A
0	0
1	0.078164286
5	0.33552
Slope	0.0675
Intercept	0



2.3 Processes

GSH Immobilization onto CPG:

I. Acid Activation of CPG
   a. Take 1 gram of CPG and boil it in 100mL of 5% v/v HNO_3 for thirty minutes.
   b. Filter on sintered glass filter and rinse with DI water.
   c. Dry in an oven (95ºC) for three hours or overnight.

II. Preparing aminoalkylating agent
   a. 5 mL of 3-aminoprpyltriethoxysilane diluted to 50 mL with DI water.
   b. Adjust to pH 3.4-3.5 (will be around 10: adjust with concentrated HCL and 1M HCL).

III. Silanization of the CPG with 3-aminopropyltriethoxysilane (3-APS)
   a. Add the dried, activated glass to 3-APS solution
   b. Check the pH level (usually increases by 0.5 pH units)
   c. Heat mixture to 75ºC for 150 minutes and swirl the flask every 15 minutes.
   d. Filter and wash the glass as before.
   e. Repeat aminoalkylating procedure to ensure complete activation of CPG.
   f. Dry in overnight oven at 80ºC.

IV. Immobilization
   a. Prepare 5 mL of 25% gluteraldehyde diluted to 50 mL with 0.1M NaH_2PO_4.
   b. Place 0.5 gram (up to 1 gram) of activated CPG in a 100 mL round bottom flask.
   c. Add 5 mL gluteraldehyde solution (or add all 50 mL).
   d. React under inert N_2 gas for one hour in stoppered flask (will turn dark yellow or brown).
   e. Meanwhile, dissolve 100 mg of glutathione in 20 mL of pH 7.0-8.0 phosphate buffer (0.1M).
   f. Filter and rinse glutathione CPG with DI water.
   g. Add glutathione solution in 50 mL round bottom flask.
   h. Purge solution and container with N_2.
   i. Swirl solution every 15 minutes for 2 hours.
   j. Let solution react for an additional day if possible (or overnight).
   k. Rinse, filter and dry under N_2 for two days.

In order to effectively attach glutathione to the Control Pore Glass (CPG), the immobilization procedure was necessary. The first section, the acid activation of the CPG, promoted reduction of silicates that make up the overall glass structure. Reacting with the acid caused the oxygen pair in a silicate group to be ejected, leaving only a hydroxide group to take the place of the oxygen pair. Because of the tendency for silicon hydroxide molecules ($Si(OH)_4$) to be unstable, the glass pores on the CPG will readily bind to the 3-aminopropyltriethoxysilane, creating an amine terminus for which gluteraldehyde can bind as a functional linker to glutathione. In the actual immobilization of the glutathione, reacting the GSH-CPG compound caused the nitrogen located on glutathione to react with the oxygen species of gluteraldehyde, thereby preventing any unnecessary reactions that would decrease the binding process of CPG with glutathione.

Atomic Absorption Analysis of Metals:

I. Startup

1. Packed columns (weight before CPG and then after loaded with CPG)
2. Make 10 ppm Metal solutions in 0.02 M HEPES buffer (adjusted to pH 7.0 with NaOH and HCl)
3. Make Metal standards in Acid (0.1 M HNO3) at 1, 5, 10 ppm.
4. Have solutions of 0.02 M HEPES buffer (pH 7.0) and 0.1 M HNO3 ready to use.
5. First flow acid through the column to solvate the CPG.
6. Turn on the Air and fuel, turn on the instrument and warm up (10 min) the lamp (includes adjusting the lamp current and wavelength).
7. When ready, turn on the flame and let warm-up for about 5min.
8. The check solutions by directly aspirating into the flame to see if the absorbance is correct.(Yes-then continue)(No-You may need to remake some solutions)

II. Collecting Breakthrough Curves

1. Make sure pump is running at 1 ml/min (*record flowrate).
2. Pass buffer through the column (*at this point determine the dead volume) for 2 min to allow the peptides to be exposed (equilibrate) to pH 7.0 solution. Zero the instrument.
    a. For reduction: Run 0.01M DTT in pH 8.0 solution for five minutes. Then run HEPES buffer for an additional 3 minutes.
    b. For oxidation: Run 0.01M DTT in pH 8.0 solution for five minutes. Run HEPES buffer for 3 minutes afterwards. Then flow through 0.01M Hydrogen Peroxide solution for five minutes. Then run HEPES buffer for an additional 3 minutes.
3. Breakthrough Data: Pass 10ppm Metal solution in HEPES buffer through the column and into the flame **at the same time start the computer acquisition**. Only stop the data files when Breakthrough occurs (If it's taking over 20 min, something is probably wrong!)
4. Once breakthrough occurs stop the data collection and stop the pump.

III. Collecting Strips after each Breakthrough Curve

1. Pass a dead volume (X seconds) worth of buffer through the column to flush out the metal that is not bonded to the peptide. Stop the pump.
2. Now collect the strip with 5-10ml volumetric flasks and passing acid through the column.
3. Collect for 3min and then fill to the line with Acid. This is your first strip sample.
4. *[Next Repeat Steps 2&3 two more time to have Three of each]

IV. Analyzing and Recording Strip Data

1. Start data acquisition and zero the instrument.
2. Aspirate the strip solutions directly into the flame in this order: 1 ppm, 5 ppm, 10 ppm, acid, sample, acid, sample, acid, sample, acid.
3. Stop and save data collection.

In actually starting the data acquisition, we made solutions of 0.02M HEPES buffer and metal at pH 7.0 in order to promote binding. Running the solutions at an acidic pH would prevent the glutathione from actually binding to the metal cations. For the GSH-CPG based column, reduced and oxidized glutathione runs were taken to gain understanding of the functions of the disulfide bonds during the metal binding process. In order to reduce the ligands, a 0.01M DTT solution at pH 8.0 was run through the column for five minutes to maximize reducing capabilities of the thiol groups which serve as a key receptor site for metal cations. The oxidized run involved running hydrogen peroxide through the column for five minutes also to promote oxidation throughout the entire GSH-CPG compounds, as studies have shown. Once the breakthrough curves were established, we stripped off the metal from the glutathione or gluteraldehyde by running a dead volume of buffer solution at pH 7.0 to disperse any unbonded cations in the column. The actual bonded cations were then released from the column by running nitric acid through the column for three minutes. The solution was collected in 5 and 10 mL volumetric flasks so that the concentration in the flasks could be checked with the influent concentration of metal to confirm the binding capacity of the columns.

3. Results and Discussion

All final metal concentrations coming from the effluent volume of solution from the columns while obtaining breakthrough curves were calibrated according to set metal standards prepared beforehand in the lab. These standards were based on simple calculations and are liable to error based on minute differences in actually preparing the standard solutions and implementing the calculations.

In short, the column containing only gluteraldehyde and CPG was ineffective in its attempt to bind large amounts of heavy metal cations (Table 3.1). Because one of the terminal aldehyde groups on gluteraldehyde is already attached to 3-APS on the glass silicate, it only leaves the other terminal end to bind to the cations. Meanwhile, each carbon atom has its entire valence shell filled, providing a stable bond energy to the rest of the molecule. As expected, few, if any of the heavy metal cations, were successful in binding decently to gluteraldehyde. Only two heavy metals, copper and magnesium, had enough binding with the gluteraldehyde column to give column capacity readings above 1 μmol per gram. However, the binding capacity of the gluteraldehyde column for magnesium is inconclusive due to flow rate problems that were not established until the end of the breakthrough curves. In addition, there was no gluteraldehyde-based CPG to replace the gluteraldehyde column after it leaked out all the gluteraldehyde-CPG from the column itself. The fact that copper showed some affinity for gluteraldehyde can also be traced to the exceedingly well binding achieved by copper cations with the glutathione, which was the best out of the entire group of metals selected. The gluteraldehyde column was not reduced nor oxidized because of the lack of any tertiary structures or disulfide bonds that would have been affected. In any case, there were no thiol groups or free ligands on gluteraldehyde that would have been susceptible to any major changes in binding characteristics to heavy metals as result of complete oxidation and reduction to the column. Instead, the gluteraldehyde column served to create a comparison of the

actual abilities of glutathione to efficiently bind metal cations *ex situ*. The binding capabilities of gluteraldehyde should not be mistaken for the potential of glutathione to bind heavy metal cations. Therefore, compiling the data from gluteraldehyde-based metal chelating activities revealed the actual abilities for glutathione as a metal chelator.

Table 3.1: Binding capacities of Gluteraldehyde-CPG column in 10 ppm heavy metal solutions.

	Cd^{2+}	Co^{2+}	Mg^{2+}	Ni^{2+}	Cu^{2+}
Avg Capacity (in µmol/g)	1.6196	1.2957	3.1107	1.6604	5.4944
Avg Strip Analysis (in µmol/g)	1.4045	1.1246	4.0559	1.5461	4.8763

Despite the inefficiency of the gluteraldehyde-CPG column to bind with heavy metal cations, copper even performed better on this column than certain other metals with the GSH-CPG column. These results can be attributed to the affinities each metal has for the hydroxyl groups located on gluteraldehyde molecules. For instance, copper has a tendency to form copper oxides or cuprite. Because it is a mild oxide, stable copper II ions used in this research would have no trouble binding with the available hydroxyl groups on gluteraldehyde.

Regardless of the results of the gluteraldehyde-CPG column, the GSH-CPG column shared comparable binding capacity when compared to the data from the gluteraldehyde-CPG column (Table 3.2). In almost all instances, the GSH-CPG column performed better when compared with results within each respective metal cations. The results also show that, in general, reduced glutathione exhibited better binding capabilities when compared to it being oxidized. Thus, the disulfide bonds play a major role in the chelating properties of glutathione. However, copper, the only metal to exceed a binding capacity of 10 µmol/g is hardly affected by the reduction or formation of cross-links between thiol groups on the cysteines of glutathione. This may be due to carboxylate side chains along the glutamic acid located on glutathione. While oxidizing and reducing glutathione may only affect the thiol groups along the cysteine chain, it would not alter the binding capabilities of carboxylate groups. It is clear then that glutathione exhibited selectivity for hard and moderately hard metal acids such as Cu^{2+}, consistent with the hard donor ligand character of the carboxylate side chain.

Interestingly, with oxidation of the glutathione-CPG column, glutathione binds more Ni^{2+} than Cd^{2+}. The data that resulted reveals a wide range in the binding capability of cadmium compared to the tight range exhibited by nickel in general. Nickel, as a borderline soft acid metal, would be unaffected by the change in polarization caused by reduction and oxidation because it binds to the carboxyl group located on glycine of glutathione. Nickel also tends to exhibit similar binding characteristics of copper, which explains the lack of affinity for an oxidated or reduced column. Cadmium, however, is a highly soft acid metal, exhibiting strong preference for the sulfhydryl group located on

the cysteine chain of glutathione. Cadmium, like copper, also tends to bind well to cysteine, resulting in the high binding capacity exhibited in the reduced GSH-CPG column.

Table 3.2: Binding capacities of GSH-CPG column in 10 ppm heavy metal solutions.

	Cd^{2+}	Co^{2+}	Mg^{2+}	Ni^{2+}	Cu^{2+}
Reduced GSH (in µmol/g)					
Avg Capacity	7.3814	2.9756	0.9304	4.2950	12.8891
Avg Strip Analysis	6.8290	1.9944	0.8324	4.6426	9.2332
Oxidized GSH (in µmol/g)					
Avg Capacity	1.6724	1.2957	1.8029	4.87	12.5211
Avg Strip Analysis	1.4389	1.1246	1.8117	4.184	12.373

4. Conclusion

Utilizing Flame Injection Analysis in conjunction with Flame Atomic Absorption Spectrometry made it possible to confirm metal chelating selectivity exhibited by glutathione immobilized to CPG using gluteraldehyde as a functional linker. To enhance the consistency and the binding capacities of glutathione as a metal chelator, GSH was immobilized to CPG for its chemical inertness below pH 10 and its high surface porosity (96 m^2/g). Flowing hydrogen peroxide and 0.01M DTT solutions also enabled for greater understanding of the sulfhydryl group and its role in metal binding through their oxidation and reduction properties. From the results, it is easy to see that in all metals but Magnesium, the glutathione exhibited stronger binding to metal cations compared to gluteraldehyde. When comparing these results, gluteraldehyde does contribute to the binding capacity of the oxidized glutathione column. When the glutathione was reduced, however, there were significant increases in the binding capacity of glutathione immobilized to CPG. It has also been shown from the results that glutathione is selective towards Copper II cations. Specifically, it seems to exhibit an affinity for harder acid metals, which may prove why there was little competition stemming from Cobalt and Nickel for binding sites and it instead readily formed complexes with Copper. The noticeably high binding capacity of the GSH-CPG column for Cadmium can likewise be explained by its chemical similarities to Copper. Like Copper, Cadmium shares a tendency to form complexes with a tetrahedral geometry. For instance, it is commonly known that Cadmium can completely replace certain Copper binding sites located on metallothioneins in the human body.

In conclusion, glutathione has potential to better the search for a selective and cost-effective metal chelator to remove dangerous heavy metals from the water cycle. It has been shown to exhibit an affinity for copper II ions and low binding capacities for other metals tested. Despite its comparatively high ability to form bonds with copper cations, similarities between the chemical properties of cadmium and copper cations may hinder its effectiveness for future applications.

5. References

Barrett, G. C, and D. T. Elmore. Amino Acids and Peptides. New York City: Press Syndicate of the University of Cambridge , 1998.

Berthon, Guy, ed. Handbook of Metal-Ligand Interactions in Biological Fluids. 1st ed. Vol. 1. New York: Marcel Dekker, Inc., 1995. 109-129.

Glutathione Conjugation. Ed. Brian Ketterer, and Helmut Sies. San Diego: Academic P Limited, 1988. 96-173.

"Glutathione." Wikipedia. 12 June 2005 <http://en.wikipedia.org/wiki/Glutathione>.

Harris, Daniel C. Quantitative Chemical Analysis. New York: W.H. Freeman and Company, 2003. 494-509.

Harris, Daniel C. Quantitative Chemical Analysis. New York: W.H. Freeman and Company, 2003. 494-509.

Holcombe, James A. Adjustable Biopolymer Chelators for Cadmium, Lead and Mercury. Diss. U of Texas at Austing,

Kidd, Parris M. Glutathione: Systemic Protectant Against Oxidative and Free Radical Damage. 17 Dec. 1999. Thorne Research Inc. 14 July 2005 <http://www.thorne.com/altmedrev/fulltext/glut.html>.

Larsson, Agne, et al., eds. Functions of Glutathione. New York City: Raven Press, 1983.
Nikos, Dracos , and Moore Ross. Aluminum III and Magnesium II Interactions with Aminoacid Chains. 1993. Marquarie University. 2 July 2005 <http://www.sc.ehu.es/powgep99/dcytp/teoricos/txema/tesia/node59.html>.

Taniguchi, Naoyuki, et al. Glutathione Centennial. San Diego: Academic Press Inc., 1989.

"Thiol." Wikipedia. 13 June 2005 <http://en.wikipedia.org/wiki/Thiol>.

Voet, Donald, and Judith Voet. Biochemistry. New York City: John Wiley & Sons, Inc., 1995.

Wilczek, Grazyna, Agnieszka Babczynska, Maria Augustyniak, and Pawel Migula. "Relations between metals and glutathione-dependent detoxifying enzymes in spiders from a heavy metal pollution gradient." Elsevier 132 (2004): 453-461.

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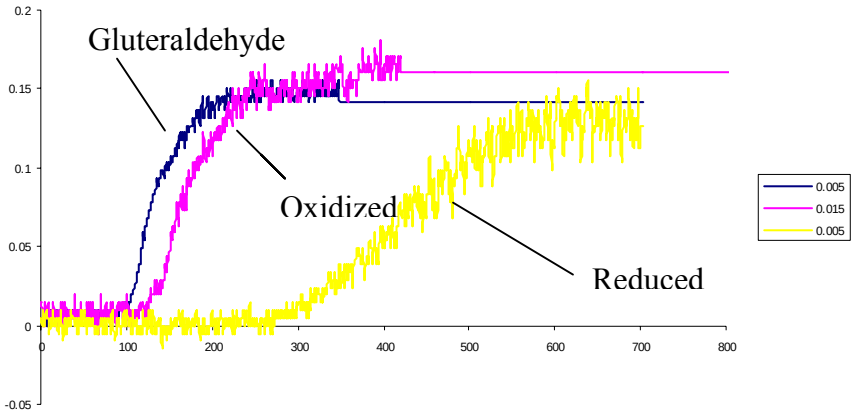
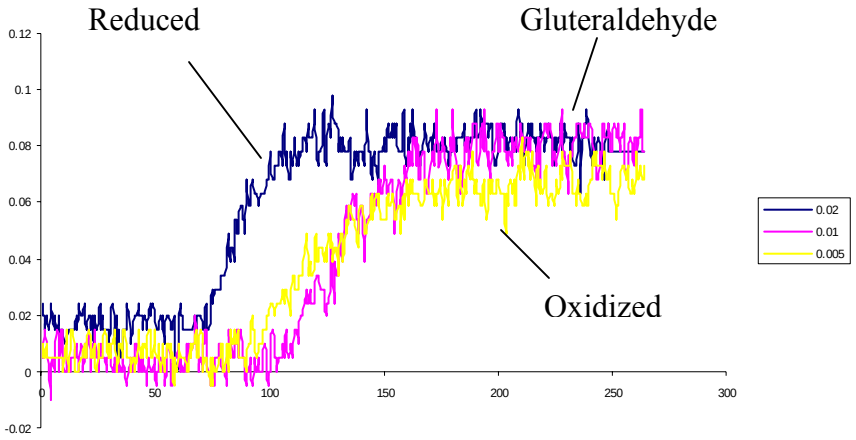
7. Appendices

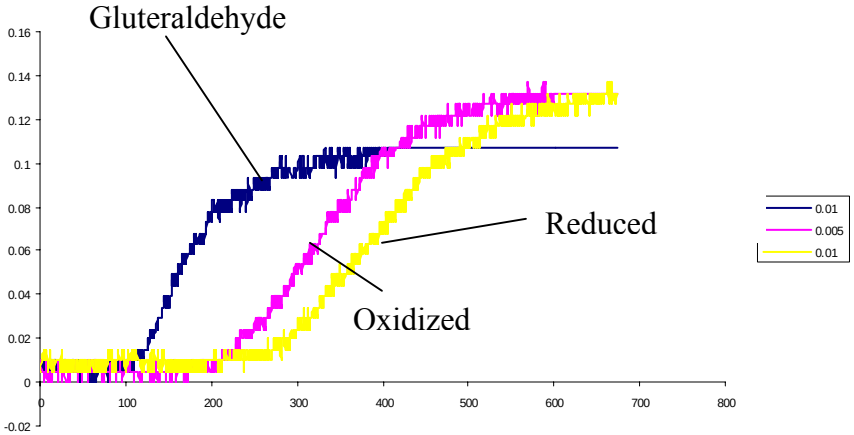
Appendix A: Cadmium Breakthrough Curves



Appendix B: Cobalt Breakthrough Curves



Appendix C: Copper Breakthrough Curves



Appendix D: Nickel Breakthrough Curves