THERMODYNAMICALLY DRIVEN ION EXCHANGE BETWEEN MS (M= FE & ZN) AND THE CU^{2+} ION: TOWARDS A SAFE ORAL COPPER DETOXIFYING DRUG

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Finally, I would like to thank my parents and my friends for their continued supports and love.
CHAPTER I

ABSTRACT

Copper is an essential trace mineral in the body that supports many important functions. However, copper can be poisonous if exists in large amount. In this experiment, we tested FeS and ZnS’s capacity as safe oral copper detoxifying drugs for acute copper poisoning treatment. It turned out FeS was not very selective for Cu\(^{2+}\) but it decreased Cu\(^{2+}\) concentration from 90ppm to 3 ppm in less than eight hours. As a side effect, it emits H\(_2\)S gas that might be considered toxic to human body. ZnS showed significant increase in Zn concentration due to ion exchange; however, copper concentration did not show corresponding decrease. Further work needs to be done to seek explanations of this phenomenon.
CHAPTER II
INTRODUCTION

Copper toxicity is a commonly overlooked cause to many health problems, including anorexia, fatigue, depression, anxiety, migraine headaches, insomnia, allergies, and childhood hyperactivity and learning disorders\(^1\). In fact, when these symptoms appear, people would never suspect they become increasingly copper-toxic.

**Copper utilization and toxicity**

Copper is an essential trace mineral that supports important functions in the body. The Recommended Dietary Allowance (RDA) for adult men and women is 900 µg/day and the median intake of copper from food in the United States is approximately 1.0 to 1.6 mg/day. The Tolerable Upper Intake Level (UL) for adults is 10,000 µg/day (10 mg/day), a value based on not causing liver damage as the critical adverse effect\(^2\).

Copper is a chemical element with the symbol Cu and atomic number 29, with electron configuration of \([\text{Ar}] \, 3d^{10} \, 4s^1\). Copper ion has two oxidation states, \(\text{Cu}^+\) and \(\text{Cu}^{2+}\). \(\text{Cu}^+\) has an electron configuration of \([\text{Ar}]3d^{10}\) while \(\text{Cu}^{2+}\) has an electron configuration of \([\text{Ar}]3d^9\) because of the Jahn-Teller effect. Normally, copper exists in the form of \(\text{Cu}^{2+}\) in the body. However, it can shifts between the \(\text{Cu}^+\) and \(\text{Cu}^{2+}\) form, which makes copper an excellent electron acceptor and donor. Because of the ability to easily accept and donate electrons, copper plays an important role in oxidation-reduction (redox) reactions. It is an essential cofactor for redox reactions involving copper-containing oxidases. Copper enzymes regulate a variety of
physiologic pathways, such as energy production, iron metabolism, connective tissue maturation and neurotransmission.

Copper is needed at the final step of the Krebs cycle (citric acid cycle), in which a series of chemical reactions occur in aerobic organisms to generate energy in the form of ATP from oxidation of acetate. Copper bound enzymes, called cytochrome c oxidase, uses one electron transfers between Cu\(^+\) to Cu\(^{2+}\) oxidation state, to catalyze the reduction of molecular oxygen (O\(_2\)) to water (H\(_2\)O) and therefore creates an electrical gradient used by the mitochondria to create ATP\(^3\).

Four copper containing enzymes known as the multi-copper oxidases (MCO) or ferroxidases oxidize ferrous iron (Fe\(^{2+}\)) to ferric iron (Fe\(^{3+}\)), the form that is in hemoglobin in red blood cell formation. The MCO family comprises the circulating ceruloplasmin, the membrane-bound ceruloplasmin, and two proteins called hephaerstin and zyklopen that are found in intestine and the placenta, respectively\(^4\).

Copper is required to fix calcium in the bone and to build and repair all connective tissues including tendons, ligaments, skin, hair, nails, arteries, veins, and etc\(^1\). Lysyl oxidase, a cuproenzyme, acts on lysine and hydroxylysine side chains of collagen and elastin that present in almost all human connective tissues. It deaminates the lysine of newly formed, immature elastin and collage, after which cross-linkings are formed\(^5\).

Copper stimulates the production of neurotransmitter: epinephirine, norepinephrine and dopamine. A number of reactions involving brain and nervous system functions depend on cuproenzymes. Dopamine beta-hydroxylase catalyzes the conversion of dopamine to norepinephrine\(^6\). The formation of myelin sheath depends on cytochrome c oxidase activity\(^5\). Copper is also required by monoamine oxidase, an enzyme related to serotonin production.
Copperedus refers to the presence of excess copper in the body. They are many sources from which people will obtain excess copper: eating acid food cooked in copper cookware, on high copper diets such as nuts, beans, seeds, meats and chocolates, drinking water exposed to copper pollutant, or using non-sparkling tools. Copper exists in two forms in the body: bound to ceruloplasmin or the “free” form. It is always the free copper ion that causes toxicity. It generates free radical species such as superoxide, hydrogen peroxide and hydroxyl radical that damages proteins, lipids and DNA. Some research also showed that excess copper is associated with aging.

Acute copper poisoning is rare nowadays in developed countries. However, it was a serious problem in third-world country like India. Symptoms of acute copper poisoning by ingestion include vomiting, hematemesis, hypotension, melena, coma, jaundice, and gastrointestinal distress. As reported by Chuttani, H. K., about one-third of the cases of poisoning encountered in the Irwin Hospital, New Delhi were copper poisoning. Because copper sulfate is a cheap, readily available substance used in agriculture as a fungicide, herbicide and fertilizer additive; also in textile, timber, leather industry, photography, emetic and antidote, to name a few, it has commonly been employed as a suicidal poison by people in low-income groups in India. It was reported that ingestion of copper substance greater than 1 gram results in signs and symptoms of acute copper poisoning. Although reliable information regarding the quantities of copper ingested was not available, Chuttani, H. K managed to give a table that reflects the quantity of copper sulfate ingested and its relation to the levels of copper in serum and whole food. In the table, it appeared that with more copper sulfate ingested, the serum level of copper did not increase significantly.
However, it was obvious that once the quantity ingested exceeds 1 gram, copper level in serum almost doubled. Chuttani H. K. reported that 7 deaths occurred out of the 48 patients. Deaths occurred within 24 hours of poisoning were due to shock while deaths occurred after 24 hours were due to hepatic or renal complications. To prevent lethal outcomes, it is important to start an early treatment with substances that can largely reduce the copper concentration.

There are also many diseases that link with copper. Wilson’s disease, a rare autosomal recessive genetic disorder that causes the body to retain copper, if untreated, will lead to brain and liver damage. The disease affects between one in 30,000 and one in 100,000 individuals, and was first described as a syndrome by Kinnier Wilson in 1912\textsuperscript{12}. The diagnosis of Wilson’s disease can be achieved by hepatic copper measurement. The normal copper content of liver is less than 55µg /g dry weight. A hepatic copper concentration greater than 250µg/g dry weight is usually a homozygous in Wilson’s disease. This is so far the best biochemical test for Wilson’s disease\textsuperscript{13}. Urinary excretion of copper measurement can also serve the purpose of disease detection. The method is based on the “free” (non-caeruloplasmin-bound) copper circulating in plasma. However, the interpretation of 24 hours urinary copper excretion test can be difficult to assess because the results might potentially interfere with results in other types of liver diseases. The treatment was based on the use of copper chelators to promote copper excretion from the body or use zinc to reduce copper absorption. In conclusion, reducing free copper concentration in the body will help relieving the Wilson’s disease.

Alzheimer’s disease, or dementia, is a disease that involves loss of brain functions including emotional behavior or personality, language, memory, perception,
thinking and judgment. Butterfield’s group has discovered the role of oxidant radicals generated from Fenton reactions, which depends on iron or copper, to accelerate protein and lipid peroxidation, brain ageing and neuro-degeneration process\textsuperscript{8}. Elevated free copper levels are often observed in Alzheimer’s disease\textsuperscript{7}. This may not as evident as in Wilson’s disease, however, the harmful effects from copper accumulation gradually develops. Brewer GJ argued that by lowering the availability of both iron and copper, the disease might get mitigated and aging process might be slowed.

Some animal studies have been done on the relationship between copper levels and atherosclerosis. Lamb \textit{et al.} have used the cholesterol-fed rabbit of atherosclerosis and compared a copper-deficient and copper-adequate diet. He found that when rabbits are on high level of copper diet, they have greater atherosclerosis than at intermediate levels\textsuperscript{14}. The study also suggested that lowering free copper level could greatly reduce atherosclerosis.

**Cadmium and Lead toxicity**

Under some circumstances, people might get poisoned by cadmium or lead. Cadmium is widely used in industry products such as pigments, electroplating, alloys and batteries. Acute human intoxications with cadmium are due to suicidal oral intake, food contamination with cadmium compounds, or inhalation of cadmium fumes\textsuperscript{15}. Cadmium poisoning is considered acute from 3mg of ingestion. The symptoms include bloody vomiting associated with severe abdominal pain, diarrhea and myalgia\textsuperscript{16}. Cadmium acts as a catalyst in formation of reactive oxygen species. It promotes lipid peroxidation, depletes antioxidants and promotes inflammatory
cytokines. Cadmium sulfide will dissociate in acidic conditions by the following reaction:

\[
\text{CdS} + 2 \text{HCl} \rightarrow \text{CdCl}_2 + \text{H}_2\text{S}
\]

Lead is also an important element widely used in industry. It can be found in alloys, pigments, batteries and many other applications. One common source of Lead that has been causing Lead poisoning is from lead paint in old houses. It was reported by the U.S. Centers of Disease Control that 1 million children in the United States were exposed to childhood lead poisoning. Lead interferes with many physiological processes and is toxic to many organs and tissues. It can hinder nervous system development; cause symptoms that include abdominal pains, confusion, headache, anemia; and in severe cases, cause seizures, coma and death. The U.S. Centers for Disease Control has set the standard blood lead level for adults to be 10 (µg/dl) of the whole blood and lower than 5 (µg/dl) of blood for children.

Lead can form precipitate with S\(^{2-}\), by the following reactions:

\[
\text{Pb}^{2+} + \text{H}_2\text{S} \rightarrow \text{PbS} + 2 \text{H}^+
\]

PbS will not dissociate in acidic solutions.

**Current treatment**

Copper chelators are common chemicals to give to the patients suspected of copper poisoning. Chelation refers to a chemical reaction that ions and molecules bind metal ions to form two or more separate coordinate bonds between a polydentate ligand and a single central atom. Chelating agents form stable complex with the toxic heavy metals and prevent them from attacking other biological targets.
Chelation treatment started about a hundred years ago with Alfred Werner in Zurich and Paul Ehrlich in Frankfurt to find less toxic arsenic compounds for syphilis treatment. From then on, many chelating agents were invented to combat metal intoxication. Dimercaprol (BAL), penicillamine, 2,3-dimercaptopropane-1-sulphonate (DMPS), and EDTA are all options of chelating agents to administer to the patients.

The most common synthetic chelator for copper is penicillamine, sold under the names Cupramine or Depen. It binds to copper intestinally and excretes copper through urination. However, animal tests showed that intestinal absorption of penicillamine is only about 50% and the metabolism is insignificant, therefore a large portion of the systemic dose was excreted rapidly in urine without binding to the copper. It can lead to side effects include kidney damage, blurred vision, B6 deficiency, ringing in the ears, ulcers, jaundice and other liver damage, abdominal pain, bloody urine and more. As we can see, penicillamine is not an ideal treatment for acute copper poisoning.

DMPS and DMSA are also used by some doctors. They were absorbed in the intestinal tract and were distributed predominantly extracellular. 50-60% of the DMPS got absorbed into the intestine and reacted with copper there. At high doses, DMPS can cause motor excitement followed by lethargy, vomiting, hypotension and cramps in animal studies. Clinical evaluation showed both drug could cause gastrointestinal discomfort, skin reactions, mild neutropenia and elevated liver enzymes. Therefore, DMPS is not an ideal treatment either.

Dimercaprol (BAL) is relatively unstable and susceptible to oxidation. Originally BAL was considered a general antidote to treat metal poisoning because of its high efficiency to deplete arsenic and mercury, however, it was then proved to be
of a lower therapeutic efficacy compared to other chelators, partly due to its severe side effects, which made it a drug only for brief acute toxicity treatment for arsenic, gold, mercury and copper poisoning\textsuperscript{20}. BAL can only be injected intramuscularly; there are complaints about its very painful administration and unpleasant odor\textsuperscript{21}. In addition to all the defects, approximately 50\% of the patients treated with 5mg/kg BAL intramuscularly showed side effects\textsuperscript{22}. Common side effects from use of BAL include increase in nausea and vomiting; headache; sweating; high fever; hypertension\textsuperscript{22}. Taken the above information into account, we are convinced that there should exist better alternatives to dimercaprol therapies out there.

EDTA derivatives are also used as arsenic, lead, and copper chelating agents. It is poorly absorbed in the GI tract (<5\%); therefore it is best used for intravenously infusion instead of as an oral drug. The most severe problem with EDTA is that it redistributes Lead (Pb\textsuperscript{2+}) to the brain after both chronic and acute treatment, which has the potential to endanger the patient\textsuperscript{20}. Overall, there are quite a few chelating agents out there that can sequester copper ions, however, they have toxic effects on the body more or less. We need to explore a safer and effective copper sequester at the same time.

One might raise questions about natural copper chelators such as Metal Free, NDF, OSR, chlorella, cilantro, spirulina and other natural products that can chelate metals\textsuperscript{1}. However, there is hardly any scientific evidence of whether these “organic” supplements actually work to remove heavy metals or not. For example, high sulfur food such as onions, garlic, green foods and seaweeds, was once promoted as the “chelating agents”, however, it turned out to have no therapeutic effects.
Cilantro was once introduced as a chelator and was used in medications like “PCA-Rx”, “Metal-free” and “NDF”. It was reported by Aga, M. that reduction of lead-induced inhibition of ALAD activity by cilantro was about 10-20%. Although cilantro did show some heavy-metal removal capability, it remains unclear whether cilantro contains any chelating substances or not. Even if cilantro is a safe medication to give, its metal removal efficiency is too low to be used in acute copper poisoning treatment.

Chlorella, a type of algae that grows in fresh water, and was used to prevent cancer, reduce radiation treatment side effects, improve immune response to bacteria, etc. there were some studies of using chlorella as a metal removal agent. However, when chlorella removes toxic heavy metals, it also removes other beneficial nutrient and minerals at the same time. Dissolved polysaccharide produced by one strand of chlorella, C. stigmatophora can bind to metal ions. Kaplan, D. conducted experiment on the ability of dissolved polysaccharide to bind to Zn$^{2+}$, Cd$^{2+}$, Pb$^{2+}$ and Cu$^{2+}$. The result is shown in Table 1.

**Table 1.** Complexing Capacity of Dissolved Polysaccharide from *C. stigmatophora* for Zn, Cd and Cu as a Function of Polysaccharide Concentration

<table>
<thead>
<tr>
<th>Cation</th>
<th>Complexing capacity (ppb) of polysaccharides (in µg of glucose equivalent ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>None</td>
</tr>
<tr>
<td>Cd$^{2+}$</td>
<td>None</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>None</td>
</tr>
<tr>
<td>Cu$^{2+}$</td>
<td>160</td>
</tr>
</tbody>
</table>
As we can see, chlorella can do a fairly good job of removing copper, however, it also absorbs \( \text{Zn}^{2+} \) (the complexing capacity for \( \text{Zn}^{2+} \) is about 213 ppb) and maybe other beneficial substances that was not tested for.

Besides seemingly organic and nontoxic on the outside, some of these natural chelators can be toxic and very costly. For example, NDF is a heavy metal and chemical detoxifier that is claimed to be able to safely remove toxic heavy metals, chemicals, and pesticides while balancing beneficial nutrient and mineral levels\(^1\). As a supplement, NDF has not been evaluated by the Food and Drug administration (FDA). This means NDF may not be as effective as they claimed. Despite of the risk of breaking internal homeostasis, a small 1 oz. bottle of NDF still costs $71.10.

**Experiment Setup**

Copper absorption occurs primarily in the small intestine. Some absorption may occur in the stomach where the acidic environment promotes copper solubility by dissociation from copper-containing macromolecules derived from dietary sources\(^2\). In order to prevent copper absorption to the largest extent, it is best to start depleting copper while it is still in the stomach. The ideal drug we are looking for should be cheap, nontoxic and can tightly bind to copper without releasing it under any circumstances.

Normally the volume of stomach fluid is 20 to 100ml and the pH is around 1.5 to 3.5\(^2\). All chelating agents that have proven effective nowadays either dissociate in the stomach under high acidic conditions, or in the case of penicillamine, remain inactive in stomach and waits until it gets to intestine to react with copper. Therefore, ion exchange reactions can be an alternative way to deplete copper in the stomach.
Ion exchange is the exchange of ions between two electrolytes or two electrolyte solutions. Similarly, ion exchange reaction is a type of chemical reactions between two substances that involves an exchange of one or more ions.

In metal exchange reactions:

\[ M'L + M \rightarrow ML + M' \]

M and M' represent metals and L represents ligand. Equilibrium constant of this reaction is \( k = [ML][M'] / [M'L][M] \). To drive reaction equilibrium to the right, according to Le Chatelier's principle, one strategy is to let ML precipitate out. With ML precipitating out of the solution, ML concentration in solution greatly decreases and M’L and M further react to generate more ML. Because of the extremely small solubility constant (\( K_{sp} \)) of CuS in solution, sulfide ligand is a good candidate for our purposes.

Iron sulfide (FeS) and Zinc Sulfide (ZnS) are cheap and readily available minerals that exist in large amount in nature. Shown in Table 1 is the \( K_{sp} \) of several metal sulfides. As we can see, CuS has the smallest solubility constant, followed by PbS, CdS, ZnS and FeS.
### Table 2. Solubility Constant for Several Metal Sulfides

| Ksp of Metal sulfide in water (at room temperature) |
|----------------------------------------|-----------------|
| FeS                                    | $8 \times 10^{-19}$ |
| ZnS                                    | $2 \times 10^{-25}$ |
| CuS                                    | $8 \times 10^{-37}$ |
| PbS                                    | $3 \times 10^{-28}$ |
| CdS                                    | $1 \times 10^{-27}$ |

Because the solubility of CuS is 10 magnitudes smaller than ZnS and FeS, we are confident that iron exchange between Fe$^{2+}$, Zn$^{2+}$ with Cu$^{2+}$ will take place.

FeS, Iron (II) sulfide, or ferrous sulfide, exists in many type of iron and sulfur minerals. Iron sulfides are generally non-stoichiometric, but troilite has an empirical formula of FeS, with Fe and S in 1:1 ratio. It is insoluble in water, but can react with acid to undergo dissociation:

$$\text{FeS} + 2 \text{HCl} \rightarrow \text{FeCl}_2 + \text{H}_2\text{S}$$

This reaction emits H$_2$S (hydrogen sulfide), a malodorous gas that can be toxic to human body both intracellularly and extracellularly. However, hydrogen sulfide automatically forms through fermentation in the stomach under some pathological conditions. Most hydrogen sulfide gas can be eliminated from the stomach by burping. It was reported that hydrogen sulfide causes benign ectasias and is an extremely common occurrence$^{26}$. Moreover, evidences have accumulated to suggest that hydrogen sulfide plays an important role as a mediator in several aspects of gastrointestinal and liver functioning. Fiorucci, S. showed that some nonsteroidal
anti-inflammatory drugs could reduce production of hydrogen sulfide in the stomach and this in turn causes mucosal injury.

**Table 3.** Concentration and Rates of Generations of H\(_2\)S in Various Tissues and Cells

<table>
<thead>
<tr>
<th>Species</th>
<th>Tissue</th>
<th>Concentration</th>
<th>Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Whole brain</td>
<td>87.2 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brainstem</td>
<td>68.3 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cerebellum</td>
<td>87.8 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hippocampus</td>
<td>97.8 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Striatum</td>
<td>102.8 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ileum</td>
<td></td>
<td>6.5 nmol/min/g</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>26.0 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>39.9 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>129.3 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>12.5 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lung</td>
<td>30.0 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>0.4 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythrocytes</td>
<td>0.2 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mesenteric aorta</td>
<td>3 nmol/min/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tail artery</td>
<td>8 nmol/min/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aorta</td>
<td>3 nmol/min/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>46 µmol/g</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>Brainstem</td>
<td>38.3 nmol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>1.2 µmol/g</td>
<td></td>
</tr>
</tbody>
</table>

Shown in Table 3, it is evident that hydrogen sulfide present in micro-molar concentrations in blood. Even if burping fails to eliminate hydrogen sulfide in the stomach, which causes subsequently absorption of hydrogen sulfide gas in the body, the concentration should not fluctuate given the relatively high concentration hydrogen sulfide that is already present in the serum.

As for iron, it is an essential element for human body. It is required for the production of red blood cells, hemoglobin, myoglobin and many enzymes. It is also
responsible for conversion of blood sugar to ATP, production of enzymes, new cells, amino acids and hormones. The Recommended Dietary Allowance (RDA) for all age groups of men and women is 8 mg/day, 18 mg/day for premenopausal women. The Tolerable Upper Intake Level (UL) for adults is 45 mg/day of iron, a level based on gastrointestinal distress as an adverse effect. The amount of Fe\(^{2+}\) released from FeS dissociation will not exceed the upper intake limit and therefore will not cause any side effects.

The crystal structure of FeS is shown is Figure 1.

**Figure 1.** The unit cell of FeS crystal structure
Troilite is hexagonal. There is no recorded toxicity effect of ferrous sulfide in the digestive system; the only potential risk may result from the H$_2$S gas emitted when FeS gets dissolved in acidic solution. As we discussed before, hydrogen sulfide gas generated in this reaction will not cause damage to the system given the relatively high concentration of hydrogen sulfide that is already present in the bloodstream.

ZnS, or Zinc Sulfide is the main form of zinc found in nature, mainly occurs in mineral sphalerite. Zinc sulfide can be used as luminescent material, optic material and pigments. It will not dissociate in gastric acid, however with exchange of copper, Zn ions will gradually be released from the ZnS minerals. Zn is an essential element as in a wide variety of enzymes, including ribonucleic polymerase, alcohol dehydrogenase, carbonic anhydrase and alkaline phosphates$^{27}$. The RDA for zinc is 11mg/day for males and 8mg/day for females$^{27}$. Gastrointestinal irritation and vomiting might occur with 2g or more zinc sulfate ingestion. If taken under 2g per day, zinc will do no harm to the human body.
Figure 2. The unit cell of ZnS, sphalerite crystal structure

The structure of sphalerite is shown in Figure 2. ZnS is cubic stacking and is more stable than hexagonal structure. There is no recorded toxicity effect of zinc sulfide in the digestive system. In addition, ZnS does not dissolve in acidic solution, which means it will not emit H$_2$S gas that can be potentially toxic to the human body. Therefore, ZnS is safe to use as an oral drug to deplete copper.

In order to measure ion concentration changes, we need to employ Atomic Absorption Spectroscopy. It is a spectroanalytical procedure to quantitatively determine the concentration of one chemical element using the absorption of optical radiation by free atoms in the gaseous state. The Atomic Absorption Spectroscopy we
used is a flame atomizer. The air-acetylene flame has a temperature of about 2300°C. The solution undergoes several stages: first of all, the solvent got evaporated and dry sample particles remain. Secondly, solid particles are converted into gaseous molecules, after which the molecules are dissociated into free atoms. A standard curve is required to convert absorption into elemental concentration.

The purpose of this experiment is to explore ZnS and FeS’s capacity as a safe oral drug for acute copper poisoning. We explored their capabilities to deplete copper in acidic conditions comparable to gastric acid, measured their ion exchange kinetics with copper alone and in the presence of other heavy metals such as lead and cadmium, and to understand the mechanism behind these experimental results.
CHAPTER III
MATERIAL AND METHODS

The acidic buffer solution was prepared by measuring 11.373g of citric acid along with 1.2896g of sodium carbonate and dissolved in 400ml distilled water. pH of the buffer solution was measured using a pH meter. Two 50ml of the buffer solution were measured and transferred separately to two beakers labeled 1 and 4.

Then 0.0765g of Cu(NO$_3$)$_2$3H$_2$O was measured and dissolved in 200ml of the previous prepared buffer solution. Two 50ml of the resulting solution were measured and transferred to two beakers labeled 2 and 5.

Finally, 0.0185g of Pb(OAc)$_2$3H$_2$O and 0.0276g of Cd(NO$_3$)$_2$4H$_2$O was weighted out and dissolved in 100ml buffer solution containing Cu(NO$_3$)$_2$3H$_2$O from the last step. The resulting 100ml solution was then split in half and transferred to two beakers labeled 3 and 6.

Three 0.2g samples of ZnS were weighed out separately and carefully transferred into three dialysis bags. Dialysis bags were properly sealed and immersed into solution 1, 2, and 3 respectively. Time was noted at the very moment each dialysis bag was immersed into solution. First 0.5ml sample of solutions 1, 2 and 3 was taken at time 0 minute. Then 0.5ml samples of solutions 1, 2, 3 were taken at time point 30 minutes, 90 minutes, 210 minutes, 450 minutes and 960 minutes.

The same process was repeated for FeS in solutions 4, 5 and 6.

After all samples were collected (36 sample total for 6 solutions), they were centrifuged at 10,000 rps for 15 minutes. Only 0.4ml supernatant of each sample was taken and diluted 20 times before taken to Atomic Absorption inspection.
CHAPTER IV
RESULTS

After reactions had taken place, the following changes were observed:

In solution 1, presence of ZnS in blank citric acid buffer (citric acid + sodium carbonate 6:1 molar ratio, pH =3.03) showed no color change in solution outside of the dialysis bag. ZnS powder inside the dialysis bag remained white with no color change.

In solution 2, presence of ZnS in 100ppm Cu(NO$_3$)$_2$ + citric acid buffer showed blue color intensity decrease in solution outside of the dialysis bag. There were black precipitates formed inside the dialysis bag.

In solution 3, presence of ZnS in 100ppm Cu(NO$_3$)$_2$ + 100ppm Pb(OAc)$_2$ + 100ppm Cd(NO$_3$)$_2$ + citric acid buffer showed blue color intensity decrease in solution outside of the dialysis bag. Black precipitates also formed inside the dialysis bag.

In solution 4, presence of FeS in blank citric acid buffer (citric acid + sodium carbonate 6:1 molar ratio, pH =3.03) showed color change from blue to green in solution outside of the dialysis bag. No color change occurred inside the dialysis bag.

In solution 5, presence of FeS in 100ppm Cu(NO$_3$)$_2$ + citric acid buffer showed color change from blue to green in solution outside of the dialysis bag. Black precipitates also formed in the solution outside of the dialysis bag. No color change occurred inside the dialysis bag.

In solution 6, presence of FeS in 100ppm Cu(NO$_3$)$_2$ + 100ppm Pb(OAc)$_2$ + 100ppm Cd(NO$_3$)$_2$ + citric acid buffer showed color change from blue to green in
solution outside of the dialysis bag. Black precipitates also formed in the solution outside of the dialysis bag. No color change occurred inside the dialysis bag.

After analyzing readings from Atomic Absorbance, the following graphs were plotted to show ion exchange kinetics.

**Figure 3.** Graphs of [Fe] vs. time for FeS in the presence of other divalent metal ions and FeS alone
**Figure 4.** Graphs of [Zn] vs. time for ZnS in the presence of other divalent metal ions and ZnS alone

**Figure 5.** Copper exchange kinetics curves with FeS in the absence and presence of other metal ions
Figure 6. Copper exchange kinetics curves with ZnS in the absence and presence of other metal ions

![Copper exchange kinetics curves with ZnS in the absence and presence of other metal ions](image)

- **Cu concentration in the presence of ZnS and in the absence of other metal ions (ppm)**
- **Copper concentration in the presence of ZnS and other metal ions (ppm)**

Figure 7. Comparison of lead exchange kinetics with FeS and ZnS

![Comparison of lead exchange kinetics with FeS and ZnS](image)

- **Pb concentration in the presence ZnS**
- **Pb concentration in the presence of FeS**
**Figure 8.** Comparison of Cadmium exchange kinetics with FeS and ZnS

**Figure 9.** Comparison of Copper exchange kinetics with FeS and ZnS in the absence of other metal ions (lead and Cadmium)
Figure 10. Comparison of Copper exchange kinetics with FeS and ZnS in the presence of other metal ions (lead and Cadmium)

Figure 11. Comparison of Copper, Lead and Cadmium exchange kinetics with ZnS
Figure 12. Comparison of Copper, Lead and Cadmium exchange kinetics with FeS

- Cu concentration in the presence of FeS and other metal ions (ppm)
- Pb concentration in the presence of FeS (ppm)
- Cd concentration in the presence of FeS (ppm)
CHAPTER V
DISCUSSION AND CONCLUSION

In Figure 3, after 16 hours of reactions, Fe concentration in blank buffer increased from zero to 406ppm; Fe concentration in copper nitrate only increased to 471ppm; while Fe concentration in copper nitrate, lead acetate and cadmium nitrate increased to 421ppm.

In Figure 4 as a contrast, after 16 hours of reaction, Zn concentration in blank buffer remained zero while its concentration in copper nitrate solution increased to 80 ppm. In solutions of copper nitrate, lead acetate and cadmium nitrate, Zn concentration increased to 87ppm.

The above results suggest that reaction of FeS with copper nitrate is not driven by ion exchange. In fact, this is a two-step reaction:

\[
\text{FeS} + H^+ \rightarrow \text{Fe}^{2+} + \text{HS}^- \\
\text{HS}^- + \text{Cu}^{2+} \rightarrow \text{CuS} + H^+ 
\]

The first reaction being FeS dissociated in acidic buffer, releasing Fe\(^{2+}\) ion. The second reaction is hydrogen sulfide ion reacting with free copper ions and forming CuS precipitate.

On the contrary, the reaction of ZnS with Cu\(^{2+}\) was driven by ion exchange. With no Zn dissociated in blank buffer, the resulting Zn concentration increase in solution 2 and 3 was due to ZnS + Cu\(^{2+}\) → CuS +Zn\(^{2+}\). Furthermore, we observed a further increase in Zn concentration in solutions consisted of copper nitrate, lead acetate and cadmium nitrate. This suggests that in addition to react with Cu\(^{2+}\), ZnS also reacted with Pb\(^{2+}\) and Cd\(^{2+}\), but to a much smaller extent (7ppm) compared to reaction with Cu\(^{2+}\) (80ppm).
In Figure 5, kinetics curves of Cu\(^{2+}\) reacted with FeS in the presence and absence of Pb\(^{2+}\) and Cd\(^{2+}\) were shown. It is evident that with the presence of other metal ions (lead and cadmium), copper depletion rate was much slower than without the above metal ions. At 450 minutes, Cu concentration in solution without lead and cadmium dropped to 2.5ppm, while Cu concentration in solution with lead and cadmium was at 40ppm. The result also suggests that FeS is not selective for Cu\(^{2+}\) because with dissociation in acidic solution as the first step, HS\(^{-}\) can freely react with metal ions.

In Figure 6, kinetics curves of copper concentration after reacted with ZnS in the presence and absence of Pb\(^{2+}\) and Cd\(^{2+}\) were shown. After 16 hours of reaction, Cu concentration did not show significant decrease as it was supposed to. Only 24ppm decrease was calculated in solution with only copper nitrate, while 17ppm copper decrease was calculated in solution with Cu\(^{2+}\), Pb\(^{2+}\) and Cd\(^{2+}\). This difference in Cu concentration decrease suggests that ZnS also reacted with Pb\(^{2+}\) and Cd\(^{2+}\) but to a smaller extent than with Cu\(^{2+}\).

In Figure 7, lead kinetics in ZnS solution and FeS solution were compared. In ZnS solution, Pb ion did not show significant decrease. In fact, it went down 11.8%. While in FeS solution, Pb ion decreased significantly, from 121.5ppm to 42 ppm, a 65.4% decrease. This disparity was due to the fact that after sulfide ions dissociated in FeS solution, it could freely move around, react with Pb ion without going through ion exchange reaction as it would with ZnS. On the other hand, ZnS did not quite react with Pb\(^{2+}\) and undergo ZnS + Pb\(^{2+}\)→ PbS +Zn\(^{2+}\) reaction. This was due to the fact that K\(_{sp}\), the solubility product, of PbS was not significantly larger than ZnS. The equilibrium was not driven to form the product.
The same trend was observed in Figure 8, cadmium kinetics in ZnS solution vs. FeS solution. Concentration of cadmium in ZnS hardly decreased (from 89ppm to 83ppm) while its concentration in FeS decreased significantly (from 87ppm to 22ppm). The same solubility product explanation of lead concentration decrease also applies to cadmium. CdS formation is favored in the presence of FeS than in the presence of ZnS.

In Figure 9 that showed copper kinetics in ZnS solution vs. FeS solution without the presence of lead and cadmium ions, a greater decrease in copper concentration was observed in FeS solution (from 90ppm to 2ppm) while decrease in copper concentration in ZnS was from 95ppm to 71ppm. It remained unclear why copper concentration did not show significant decrease in ZnS solution. As in Figure 7, $[\text{Zn}^{2+}]$ increased significantly in reaction with Cu$^{2+}$ through ion exchange compared to in blank buffer. Our hypothesis is that colloidal CuS was formed in the outside solution. When atomic absorption was measured, colloidal CuS was also sucked into the machine along with the free Cu$^{2+}$ ions, which accounted for the insignificant Cu concentration decrease.

Similarly, in Figure 10, Cu kinetics showed a same trend despite of the presence of lead and cadmium ions, whose reactions with metal sulfides are negligible.

Figure 11 and 12 compared Cu$^{2+}$, Pb$^{2+}$, and Cd$^{2+}$ concentration in ZnS solution and FeS solution respectively. In Figure 11, kinetics curves of all three ions were relatively flat compared to sharp downward slopes in Figure 12. The reason why ZnS only reacted with limited amount of Cd$^{2+}$ and Pb$^{2+}$ was due to the insignificant $K_{sp}$ difference among ZnS, PbS, and CdS. The explanation of why Cu$^{2+}$ concentration did not show large decrease remains to be explored. All three ions: Cu$^{2+}$, Pb$^{2+}$ and Cd$^{2+}$
greatly reacted with FeS with Cu\(^{2+}\) concentration decreased the most. The reaction of Cu\(^{2+}\) with FeS was most significant is due to the fact that CuS has the smallest solubility product among the three metal sulfides. Reaction of FeS with Cu\(^{2+}\) will form the most stable metal sulfide product. With even a minute amount of free Cu\(^{2+}\) present in solution, the likelihood of cation reacting with S\(^{2-}\) to form precipitate is higher than the cation being Cd\(^{2+}\) or Pb\(^{2+}\).

To sum up, FeS showed vitally important capability to remove free Cu\(^{2+}\) from the stomach, along with lead and cadmium. In the case of cadmium or lead poisoning, FeS can also serve as an excellent oral drug for cadmium or lead depletion. The acidic condition favors the formation of HS\(^-\) ions that resulted in CuS, PbS, and CdS precipitation. However, the drawback is that H\(_2\)S will be generated and emitted during this process. Although H\(_2\)S was considered toxic to human metabolism, in a small amount it would not do damage to the system given the fact that H\(_2\)S is automatically generated in human body and present in micromolar concentration in serum. In the absence of other heavy metal ions, FeS can decrease copper concentration from 95ppm to 3ppm in less than 8 hours, which makes it an effective drug towards acute copper poisoning treatment. ZnS on the other hand, showed significant increase in Zn\(^{2+}\) concentration not due to acidic dissociation, but due to ion exchange with copper. However, because of the insignificant decrease in copper concentration in solution, ZnS’s efficacy in removing Cu\(^{2+}\) remains to be further tested. If further tests could show free copper ions indeed decreased considerably in solution, ZnS would be an ideal drug in acute copper depletion due to its high selectivity to copper relative to other ions, not producing H\(_2\)S gas that possesses unpleasant smell and can potentially be toxic. In addition, in all experiments, bulk particles of ZnS and FeS were used. If
surface areas of these particles can be increased, the reaction efficiency can be further improved. For example, if ZnS and FeS can be made into nanoparticles, they can better function as oral drugs for acute copper poisoning treatment. Furthermore, ZnS nanoparticles can be a promising intravenous injection treatment to give to patients without causing discomfort and side effects.
REFERENCES


2. Food and Nutrition Board, Institute of Medicine. National Academy Press:


