Quantitative Analysis of Copper and Sulfate in Multivitamins using Flame AAS and IC

Names of Authors

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Introduction

Usually obtained in food, vitamins and minerals are essential for growth and maintenance of the body. Recently, there has been a large market for multivitamin companies, as it contains a complex array of vitamins and minerals. While synthetic, multivitamin pills have more stability when comparing to its natural food-like component, there is a shelf life for the synthetic pills. [1]

Multivitamins (LOT # 8NA0232C) will be assessed for long-term shelf life by the stability of copper concentration, as cupric sulfate, over a length of time. The amount of copper will be evaluated at a 95% confidence interval to determine if the label on the provided container accurately displays the quantified amount. Copper sulfate, or cupric sulfate, has experimentally shown to decompose in air in isothermal conditions. [2] This phenomenon can affect the multivitamin pills within their storage container.

A way to determine metals in samples is through electrothermal atomic absorption spectrometry; however, that method needs special tools, hindering the repeatability of the method. [3] Another common method of determining the concentration of specific metal cations in a sample is flame atomic absorption spectroscopy (Flame AAS). This instrument nebulizes a solution into an acetylene flame, where it is atomized. The atoms that are absorbed by the flame give characteristic wavelengths of light unique to each element, meaning that flame AAS is useful for probing a single analyte for a sample solution. For this experiment, a copper hollow cathode lamp will shine through the flame, where the light will be absorbed by the sample. The amount of absorption will be linearly proportional to the concentration of the analyte of interest (copper) in the solution.

Comment [AF1]: The atoms don't get absorbed by the flame. Atoms in the flame absorb light from the hollow cathode lamp.

To determine the concentration of sulfates in the multivitamins, we will use ion chromatography (IC). IC is a common method for separating and quantifying ions in solution; the instrument used in this experiment was specifically set to only detect anions. This chromatographic technique separates ions based on a mobile phase and a stationary phase. In this experiment, the stationary phase is a column packed with quaternary amine coated, resin beads and the mobile phase is a sodium carbonate / sodium bicarbonate eluent.

Materials and Methods

All methods are adapted from "CHEM 370 Lab:3A – Cu in Multivitamins by Flame AA" and "CHEM 370 Lab:3B – Sulfate in Multivitamins by IC" Handouts from Dr. Fischer

I. Copper Determination via Flame AAS

Comment [AF2]: Try to avoid headings left by themselves.

A. Standard Preparation

The rinse blank consisted of 5% nitric acid. A stock solution of a 100 ppm copper solution in 5% nitric acid was pre-made by Dr. Fischer. A standard addition spike solution (SASS) was made by adding 10 mL of the stock solution to a 100 mL volumetric flask and bringing to volume with 100 mL 5% nitric acid.

Comment [AF3]: Note that grammatically it should be 100-ppm, as "100-ppm" functions as a compound adjective in this instance (thus the blue underline in Word). This is rarely written as such in scientific writing, though. (And most of my lab-handouts omit the dash.)

B. Sample Preparation

A multivitamin pill with 50 mL of ultrapure water was placed in a 100 mL volumetric flask before sonicating for 5 minutes. The mixture was then brought to volume with ultrapure water before storing it in a plastic storage bottle. The stock sample solution was made via a 1:10 dilution of the previous solution with 5% nitric acid. 70 mL of the sample is filtered through a 0.45 µm syringe filter. The first 5 mL of the filtrate was discarded. A series of samples of varying volume of the 10 ppm Cu SASS was made, ranging from the addition of 0, 1, 2, 3, 4, and 10 mL of the SASS. An aliquot of each sample was used to fill 15 mL conical vials for analysis. All samples were analyzed using the methods described in Table 1.

Table 1: Flame AAS Method

Parameter	Value
Make & Model	Perkin Elmer PinAAcle 900F AAS
Sample	Multivitamin Pill
Solvent	5% Nitric Acid
Measurement per Sample	3
Wavelength	324.75 nm
Hollow-cathode Lamp	Copper

II. Sulfate Determination via IC

A. Standard Preparation

The rinse blank consisted of ultrapure water. A set of standards at 0.5, 1, 5, 10, and 20 ppm of sodium sulfate in ultrapure water was made via serial dilution.

B. Sample Preparation

A multivitamin pill was placed in a 100 mL volumetric flask with 50 mL of ultrapure water before sonicating the mixture for 5 minutes. The sample was dilute to volume using ultrapure water before

transferring to a plastic storage container. Then, a 1:10 dilution was performed using the pill solution with ultrapure water. Lastly, the solution was then filtered with a 0.45 µm syringe filter, while discarding the first 5 mL of filtrate, before placing in an IC sample vial. All samples were analyzed using the methods described in Table 2.

Table 2: IC Method

Parameter	Value
Make & Model	Thermo-Dionex ICS-1600 IC
Solvent	Ultrapure Water
Eluent	4.5 mM Sodium Carbonate/1.4 mM Sodium Bicarbonate
Flow Rate	1.2 mL/min
Injection Volume	25.00 µL
Column	Dionex IonPac AS22 FAST (4 x 150 mm)
Temperature	30 °C
Detection	Suppressed conductivity, Dionex AERS 300 suppressor, 4 mm, AutoSuppression recycle mode

Comment [AF4]: Actually we don't use recycle mode. Recycle mode reuses the same mobile phase over and over (only water is added).

Results and Discussion

Below, Table 3, displays the results obtain for the analysis of the multivitamin pill samples using the Perkin Elmer PinAAcle 900F Atomic Absorption Spectrometer (AAS). The analysis time per sample was 4.0 seconds, with 18 seconds of delay time between each sample. Each blank and samples was replicated 3 times to ensure quality measurements. A baseline correction was executed by subtracting each sample, including the blank, by the blank to correct for the background.

Comment [AF5]: This sentence is overly wordy.

To determine the amount of Cu in the sample solution, a normal calibration curve was made, as shown in Figure 1. Since different volumes of the 10 ppm Cu SASS and the analyte was added, the method of standard addition was to calculate the concentration of Cu within the analyte sample.

Comment [AF6]: What does "normal" mean? External standards are probably the most common way of creating a calibration curve, but for FAAS we used standard addition.

Table 3: **The mean result of the copper in multivitamin analysis via flame AAS (n=3).**

Sample	Volume of SASS Added (mL)	Absorbance	Relative Standard Deviation (%)	Corrected Absorbance
Blank	-	-0.037 ± 0.001	3.66	0 ± 0.002
Sample 0	0	-0.030 ± 0.004	14.52	0.007 ± 0.004
Sample 1	1	-0.013 ± 0.008	61.84	0.024 ± 0.008
Sample 2	2	0.004 ± 0.011	305.12	0.04 ± 0.01
Sample 3	3	0.02 ± 0.02	72.46	0.06 ± 0.02
Sample 4	4	0.04 ± 0.02	47.25	0.08 ± 0.02
Sample 10	10	0.14 ± 0.05	38.67	0.17 ± 0.05

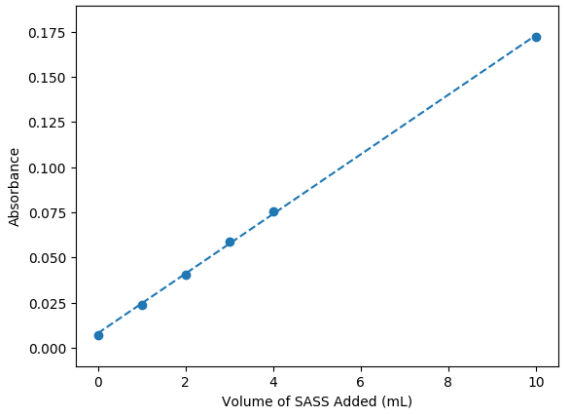

Figure 1: The calibration curve for Copper for the flame AAS assay. The equation of the linear line of best fit is: A = 0.0165051[C] + 0.00803495, with a R^2 value of 0.0999569.*

The x-intercept was found by setting the y-value of the equation of the line of best fit to 0, which was -0.486816 mL. In standard addition methods, the x-intercept equates to $-C_A*V_0/C_{std}$ where C_A is the

Comment [AF7]: This is basic algebra…you don't need to train the reader in algebra, just say you found the x-intercept.

Comment [AF8]: Do you really have this many sig figs?

Comment [AF9]: This should be written using the equation editor, or at least placed in italic font. The variables should be in italic font, too. That would make it stand out as an equation/mathematical variable.

concentration of the analyte, V_0 is the volume of the added analyte (10 mL), and C_{std} is the concentration of the added SASS. The amount of copper in the multivitamin pill was found to be 0.49 ± 0.05. The 95% confidence interval was found to be (0.43, 0.54). A 95% confident z-comparison test from the source of the multivitamin pills found that the amount of copper tested via the standard addition method is significantly different than advertised, with a percent difference of 60 ± 6 %. The amount of copper in the multivitamin pill is found to be less than what is listed on the label of the bottle (0.49 mg vs 0.90 mg).

For the sulfate analysis in the multivitamin pill, Table 4 shows the results obtained via the Thermo-Dionex ICS-1600 Ion Chromatography instrument. A blank was run before standards were established. Then, the analyte sample was run three separate times consecutively to obtain for errors. The sulfate peak's retention time in the standards was found to be 9.533 ± 0.009 min and the retention time in the samples was found to be 9.542 ± 0.004 min, as shown in Figure 2.

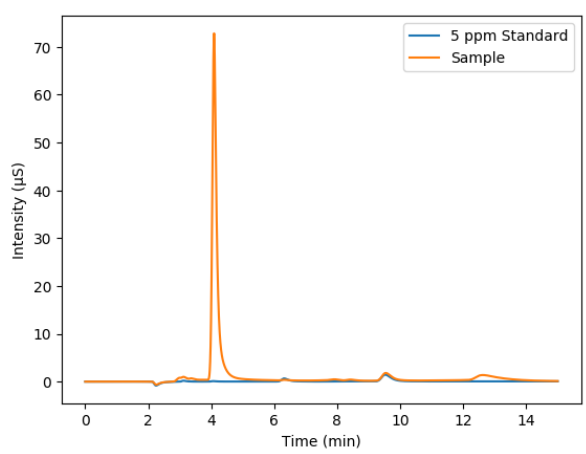

Figure 2: A graph comparing the sample curve to the 5 ppm standard curve. The overlapping peak near 9.5 min indicates the sulfate peak in the sample.

The sulfate peak's retention time overall was found to be 9.536 ± 0.008. Similar to the copper analysis, an external, calibration curve was made to determine the concentration of sulfates in the analyte, as shown in Figure 3.

Table 4: The mean result of the concentration of sulfates in a multivitamin pill analysis via IC.

Sample	Concentration (ppm)	Retention Time (min)	Area (µS*min)
Standard 1	0.5	9.524	0.062
Standard 2	1	9.531	0.129

Comment [AF10]: How did you determine that the retention time has 4 significant figures/is accurate to 3 decimals? This would require a sampling rate of at least 4 Hz.

Comment [AF11]: I would have zoomed in on the y-axis some, probably 0-10 uS or so to more clearly show the sulfate peak. The large peak at 4 minutes isn't relevant to the analysis so there's not real need to show it (although if you do cut it off you should say why you did so). An alternative approach would be to show the whole chromatography as Figure 2A and then show a zoomed version focused just on the sulfate peak as Figure 2B.

Standard 3	5	9.530	0.545
Standard 4	10	9.531	1.075
Standard 5	20	9.547	2.221
Sample 1	Unknown	9.544	0.843
Sample 2	Unknown	9.537	0.670
Sample 3	Unknown	9.544	0.841

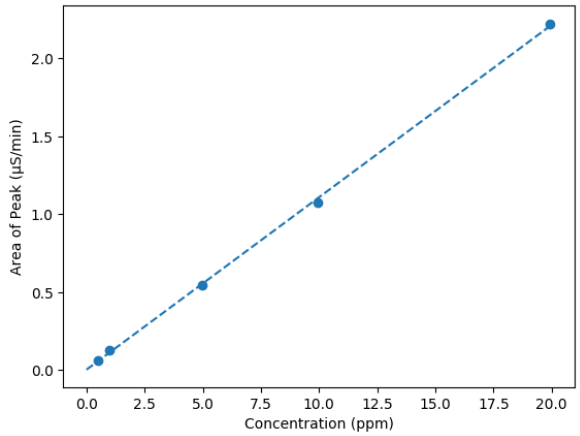

Figure 3: The calibration curve for sulfate analysis for the flame AAS assay. The equation of the linear line of best fit is: Area = 0.110707[C] + 0.00218103, with a R^2 value of 0.099954.*

The concentration of sulfates found in the sample was found to be 0.71 ± 0.09 mg, which help supports the copper analysis. The copper within the multivitamin pills exists as cupric sulfate, with a 1:1 ratio of copper to sulfate. From the found concentration of copper and sulfates, the moles of each substance can be calculated, which was 0.0077 ± 0.0008 mmols and 0.0074 ± 0.0009 mmols, respectively. However, a look at the source of the multivitamin pills revealed that compounds like manganese sulfate and nickelous sulfate exists within the pills. Meaning that, the concentration of sulfates found in our sample must also be paired with nickel and manganese. There are simply not enough sulfates to accommodate for the metals in the pill, suggesting that the sulfates are also decaying with time.

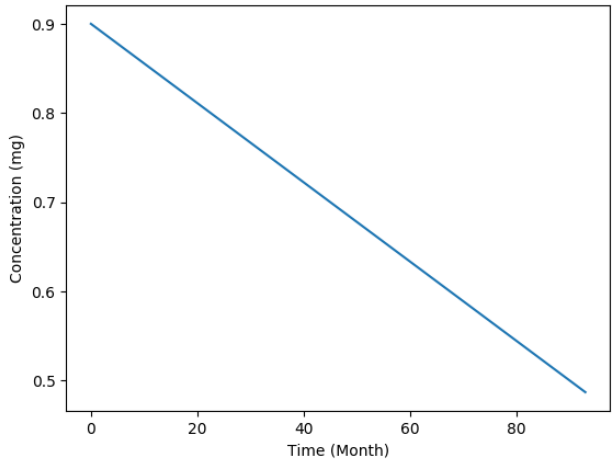

Figure 4: The estimate line of copper concentration decay over time. The equation of the line of best fit is [C] = -0.0044t + 0.09.

Assuming the pill contained exactly 0.9 mg of copper when it was manufactured in June 2011, the decay rate of copper can be estimated by plotting the start time with the starting concentration of copper against the time the sample was analyzed with the experimental concentration of copper. Since the sample was analyzed in March of 2019, the time between the analysis and the manufactured date can be estimated to be 93 months. The decay rate of copper in the multivitamin was estimated to be -0.0044 mg per month, as shown in Figure 4. From the 95% z-comparison test, we can also estimate the long-term shelf life of the copper concentration in the multivitamin pill sample. It is calculated that if the concentration of copper is less than 0.845 mg at 95% confidence interval, it is considered to be significantly different than the listed value of 0.9 mg. Thus, by using our estimated decay rate of copper, we can conclude that the estimated long-term shelf life of the multivitamin pills are around 12.5 months.

Comment [AF12]: This is a good interpretation of the data. However, you should note that you are assuming [Cu] decays linearly with respect to time. What if it's an exponential decay, or is stable for a very long time and them drops very suddenly? You know the endpoints, but not what's in between.

But again, this is a nice interpretation. Just note your assumptions, or – even better – find a source to back them up.

Conclusions

The analysis of copper and sulfates in the multivitamin pills via flame AAS and IC was successful. The experiment and methods were performed up to quality standards. The amount of copper in the sample was found to be 0.49 ± 0.05 mg, with a 95% confidence interval of 0.43 – 0.54 mg. The sulfate concentration in the sample was found to be 0.71 ± 0.09 mg. The analysis of the data obtain in this experiment reveals the decay of copper in the multivitamin pill sample to be -0.0044 mg/month and the estimated long-term shelf life to be 12.5 months for the copper in the pills, assuming that the copper decays linear naturally. Meaning that the copper concentration, as cupric sulfate, is unstable over time. For future analysis, multiple trials of multiple multivitamin pills are needed for a more representative sample size. We need to measure the nature decay of copper sulfate to see if the compound decays linearly to better determine the shelf life of the multivitamins. The pills we have analyzed could have been an outlier, further experimentation is needed. We could also try to

Comment [AF13]: Oh, OK, here it is. It would have been good to also provide a bit about this where it was first discussed.

measure other compounds in the vitamin pills to more accurately determine the long-term shelf life of the multivitamin pills.

Reference(s)

[1] Finocchiaro, E. T.; *Multivitamin and mineral supplements: An overview of key product issues*, 250th ACS National Meeting & Exposition, Boston, MA, August 16-20; American Chemical Society: Washington, DC, **2015**; AGFD-78.

[2] Riad, G.H.; Mahdy, A.N.; Abadir, M.F.; Khattab, I.A. *Trans. Egypt. Soc. Chem. Eng*, **1992**, *18*(2), 442-453.

[3] Tokman, N.; *J. Hazard. Mater,* **2007**, *143*(1-2), 87-94.

Data Availability

Where the data is available.

CHEM 370 Lab Report Rubric

		Vitamin				
Grade	Points earned:	235	30	0	0	0
	Group Report Grade (%):	98				

wt. — 5 = Excellent, 3 = Average, 1 = Lacking

wt.			5	4	3	2	1
1	Title	Is the title descriptive and succinct?	x				
1	Introduction	Does the Introduction clearly state the overall question/purpose of the study?	x				
1		Has relevant background information about the analyte of interest been given?	x				
1		Has relevant background information about the technique been given?	x				
1		Is there anything that could be omitted?	x				
2	Materials & Methods	Are methods detailed enough that the study can be repeated by another trained scientist?	x				
2		Are there any irrelevant details that could be omitted?	x				
2		Are instrument methods clearly detailed, including tables where appropriate?		x			
3	Results & Discussion	Has the main finding been clearly presented?	x				
5		Are there factual, logical, analytical, statistical, or mathematical errors?	x				
3		Are all figures and tables clearly explained, in order?	x				
3		Have the results been related back to the question(s) posed in the Introduction?	x				
5		Is there sufficient data and/or supporting evidence to support the answer?	x				
3		Have the results been put into perspective by relating them to literature libraries, standard data sources, and/or expectations? Have errors been provided?	x				
1		Is there anything that could be omitted?		x			
3	Conclusions	Has the overall interpretation of the results been clearly conveyed?	x				
3		Are the conclusions free from logical errors?	x				
1		Has the study been adequately summarized?	x				
3		Has/have clear conclusion(s) been presented regarding question at hand?	x				
1	References	Have references been cited where needed?	x				
1		Are sources cited adequately, appropriately, accurately, and in the ACS format?	x				
1		Are all the citations in the text listed in the References section, and *vice-versa*?	x				
1	General Style	Is the document written according to the CHEM 370 writing guide?		x			
1		Are sections clearly labelled?		x			
1		Are the transitions between sections and paragraphs logical?	x				
1		Are paragraphs and sentences cohesive?	x				
1	Composition	Are there any grammar, punctuation, or spelling errors?		x			
1		Is the style concise?	x				
1		Is there excess wordiness?		x			