DETERMINATION OF IRON IN BROCCOLI VIA FORMING ION COMPLEXES AND SPECTROPHOTOMETRY

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Objectives:

- To determine the amount of iron that can be extracted from a food sample.
- To investigate iron's importance in biological system.

Introduction and theory:

Iron (chemical symbol Fe) is an essential element in human blood as it is the main ingredient of hemoglobin which is a red protein responsible for transporting oxygen in the blood of vertebrates (living creatures with a backbone or spine). Without iron, oxygen cannot attach to the red blood cells and will therefore not be able to be carried through the body, which is problematic. By such fact, it can definitely be said that iron is one of the many vital minerals that the human body needs for function and survival. According to the US National Library of Medicine/National Institutes of Health (NCBI/NLM/NIH), proteins that transport oxygen in the human body, hemoglobin and myoglobin, need iron for their synthesis. Therefore, iron is one of the critical minerals that we need. Moreover, the NIH also dictates that iron deficiency is when the hemoglobin concentration drops below 13 g/dL and 12 g/dL in men and women respectively (NIH n. pg.). The RDA of iron for adults of ages between 19 and 50 years is 8 mg and 18 mg, men and women respectively (NIH n. pg.)

To extract iron from a food sample, a portion of broccoli was heated using a crucible to remove most of the nutrients until ashes remained in which iron is contained in the ashes since iron can withstand high temperatures. After burning the broccoli, the ashes were then deposited in a beaker and HCl in an aqueous solution was added into it to extract all the iron according to the following chemical equation:

$$2Fe_{(s)} + 6HCl_{(aq)} \rightarrow 2FeCl_{3(aq)} + 2H_{2(g)}$$

The solution was then filtered to remove the broccoli ashes to keep as much iron diluted in the HCl solution and separate it from the residue.

Multiple techniques for iron extraction are possible; however, the ones selected for the analysis were through spectrophotometry and from the creation of solutions by dilutions to obtain a standard curve. With these methods, the absorbance of the diluted solutions at different concentrations for the same volume of KSCN was obtained according to the following chemical equation:

$$FeCl_{3(aq)} + KSCN_{(aq)} \rightarrow Fe(SCN)_{3(aq)} + 3KCl_{(aq)}$$

By doing this, the equation for the standard curve of the graph could be obtained. To analyze the iron in our sample, the SpectroVis system was used with the LoggerPro software. A cuvette with the solutions at different concentrations was inserted in the SpectroVis. Thanks to LoggerPro, the absorbance of every different concentration was obtained and was used to create a standard curve.

Equipment and Chemicals:

a. Materials and Equipment:
- 5g of broccoli
- Spectrophotometer (Logger Pro)
- Cuvettes
- 30- & 50-mL beakers (3 each)
- Pasteur pipettes
- Crucible & Lid
- Bunsen Burner
- 50 mL Erlenmeyer flasks, filter paper, funnel (2 each)
- 1.00, 5.00, 10.00 mL pipettes & bulb
- Three-ring stand and clay triangle
- Electronic balance, weighing paper
- Tongs

b. Chemicals:
- 2.0 mol/L Hydrochloric acid
- mol/L iron (III) chloride () stock solutions, acidified in HCl
- 1.5 mol/L KSCN stock solutions
- DI water

Record of work:

1. Standard Curve for Spectrophotometry:
   - Only use 2.0 M HCl for diluting the stock 1.0×10^{-3} mol/L $FeCl_3$ solution. The first dilution was HCl_{aq} with stock $FeCl_{3_{aq}}$. The second dilution was a constant volume of $KSCN_{aq}$ with the diluted $FeCl_{3_{aq}}$ above.
   - A mixture of undiluted stock $FeCl_3$ solution was used as blank for calibration.
   - Optimum wavelength for $FeSCN^{2+}$ is $\lambda_{max} = 421.9nm$.

Refer to the following table for dilution steps and final concentration.

Dilution	$V_{HCl}\ (L)$	$V_{FeCl_3}(L)$	$V_{KSCN}(L)$ **(1.5 mol/L)**	$C_{FeSCN}\left(\frac{mol}{L}\right)$	**Absorbance**
0	0.000	.020	.005	8×10^{-4}	2.056
1	.005	.015	.005	6×10^{-4}	2.054
2	.010	.010	.005	4×10^{-4}	1.909
3	.012	.008	.005	3.2×10^{-4}	n/A
4	.015	.005	.005	2×10^{-4}	1.141

- After measuring Concentration vs. Absorbance for all dilutions, a standard curve was obtained for $FeSCN^{2+}$.

2. Extraction of Iron:
   - 5g of broccoli was used for determining the iron content.
   - The broccoli sample was then heated over a Bunsen Burner to ashes under a fume hood.
   - After the content in the crucible was cooled down, transfer it to a beaker.
   - $10.00mL\ of\ 2.0M\ HCl$ was added to the beaker and the mixture was thoroughly mixed by swirling.
   - The mixture was then filtered and the solution (filtrate) was collected.
3. Analysis of Iron:
   - 5.00mL of the filtrate (containing $FeCl_3$) was transferred to another beaker and 5.00mL of 1.5M KSCN was added to the filtrate.
   - An aliquot of the final mixture was transferred to the cuvette to measure the concentration in accordance to the standard curve in part 3.1.

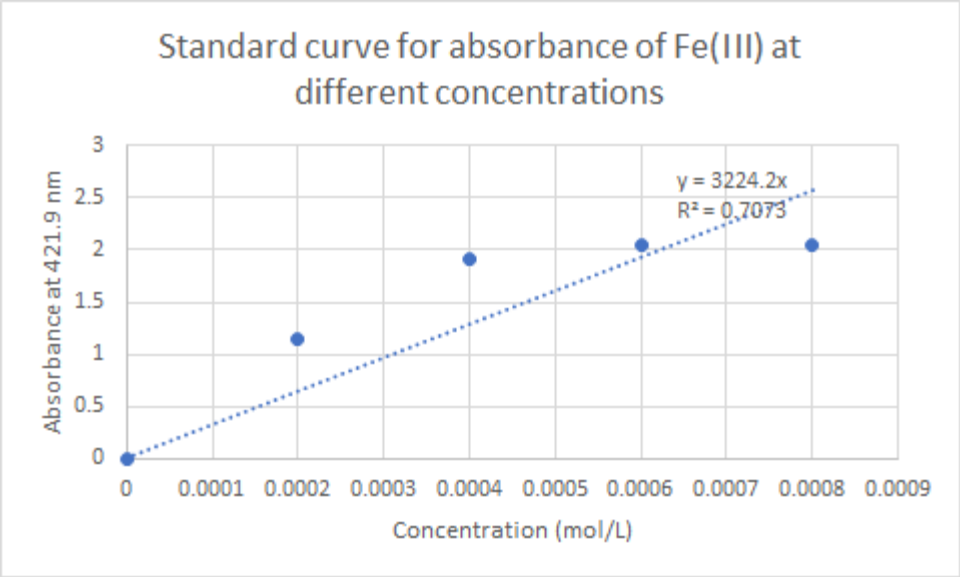
Data collection and results:

Balanced equation of Fe reacting with HCl conc.

$$2Fe_s + 6HCl_{aq} \rightarrow 2FeCl_{3_{aq}} + 3H_{2_g}$$

Balanced equation of $FeCl_3$ reacting with KSCN forming ion complex

$$FeCl_{3_{aq}} + KSCN_{aq} \rightarrow Fe(SCN)_{3_{aq}} + 3KCl_{aq}$$



The final result obtained from the experiment is elaborated as follow:

- Concentration of iron in **aliquot** according to obtained absorbance of sample:

  $0.699 = 3224.2 \times C_{Fe^3+} \rightarrow C_{Fe^3+} = \frac{0.699}{3224.2} = 0.0002 \left(\frac{mol}{L}\right)$

- Concentration of iron in sample **before** KSCN was added: $0.0002 \times 0.01 \div 0.005 = 0.0004 \left(\frac{mol}{L}\right)$ (This gave the wanted concentration).

- Amount of iron (in grams) in the broccoli sample: $0.0004 \left(\frac{mol}{L}\right) \times 0.01\ (L) \times 55.845 \left(\frac{g}{mol}\right) = 0.00022\ (g) = 0.22\ (mg)\ per\ 5g\ of\ broccoli.$ Percentage: $\frac{0.22 \times 10^{-3}}{5} \times 100\% = 0.0044\%$

Discussion:

The results were surprising compared with what was expected, which was theorized that broccoli would contain as much iron as roughly 1% due to its popularity for containing this nutrient amongst health recommendations. The final leachate contained iron Fe^{3+}ions, which when compared to the Fe^{2+} iron ion, produces much less Acetylcholinesterase (AChE) activity in our bodies. AChE is a very important and essential enzyme found in our nervous system playing

a crucial role in cholinergic neurotransmission: the only neurotransmitter that is used for motor movement of somatic cells and are henceforth vital for our survival.

The Recommended Daily Amount (RDA) of iron, according to the Canadian Government, summarizes the males between the ages of 19-50 years of age, should have a daily intake of iron of roughly 8.00mg, and women in the same age group should ingest 18.00mg. The reason for women's increased need for iron is due to the great loss of blood that they experience during their menstruation. Values also increase with younger children under 18 and vegetarians alike. Compared to the RDA's recommendation, a small intake of broccoli only contains 0.22mg per 5.00grams of broccoli (~0.0044%) and is therefore responsible for 2.75% of men's daily intake and 1.22% of women's daily intake per 5 grams. If broccoli was the only food source for iron, men would have to ingest around 181.81 grams of broccoli (~1/2 a medium head of broccoli) and for women, 409.09 grams of broccoli (almost 2 medium heads of broccoli).

Conclusion:

In conclusion, we found five different absorbances at different concentrations of FeSCN so we could then find the slope of the standard curve. Although we did not have time to repeat the dilution and determine the absorbance for the 3.2x10^-4 mol/L concentration of FeSCN due to some errors we made during the handling of the liquids during the dilution, the experiment was still able to draw five results including the data point at the origin which makes sense overall. Nevertheless, we found that a concentration of $8.0x10^{-4}$M of FeSCN had an absorbance of 2.056, $6.0x10^{-4}$M: 2.054, $4.0x10^{-4}$M: 1.909 and $2.0x10^{-4}$M: 1.141. The slope of the standard curve graph, 3224.2, was deciphered and then 0.699 was divided by 3224.2 (the slope of the graph) to obtain the concentration of Fe3+ in the aliquot: 0.0002 mol/L. We then calculated the concentration of iron in sample before the KSCN was added by multiplying 0.0002 by 0.01 L/0.005 L to get 0.0004 mol/L. After this, we multiplied 0.0004 mol/L by 0.01 L and by 55.845 g/mol (molar mass of iron) to finally obtain the number of grams of iron in the broccoli: 0.00022 g. This means that in the five grams of broccoli, there was only 0.22 mg of iron. The percentage of iron in the broccoli was then finally calculated by dividing the amount of iron in the sample (0.00022g) by the total mass of broccoli (5.00 grams) and multiplying it by 100% to produce the percentage 0.0044%.

The experiment encountered some major sources of error including the fact that the ashes from the broccoli were still present in the final solution. The solution was filtrated to our fullest capability, but the equipment was clearly not best suited for filtration, since after a few minutes the ashes would very slowly start to accumulate at the bottom and there would still be some just floating around in the solution. We would wait for as much as ashes as possible to get to the bottom to put the cuvette with the solution in the SpectroVis to get the least experimental error as possible for the values of the absorbance. The fact that the ashes were floating around the solution in the cuvette, blocked the SpectroVis' complete view of the solution for measuring a

completely accurate absorbance. If there were better equipment at our disposal for the filtration, the results would have been much more accurate and reliable. Also, the precision of our dilutions could have been better if we took more time to be extremely accurate in the volume measurements and also if we had more precise equipment to get more exact volumes.

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