An investigation of dietary factors on supplementary iron

Introduction

When I was nine years old, my doctor prescribed me iron tablets due to iron deficiency. I was first consulted to receive iron supplements. Therefore, I had to change my diet to enhance iron absorption in my body. My doctor advised me to take my tablets with orange juice and avoid milk or coffee when consuming iron tablets. However, I always wondered why I should strictly consume my iron supplements with orange juice and how dietary factors affect the iron absorption in the human body. This internal assessment provided me with the opportunity to investigate this question. Therefore, I decided to determine the concentration of reduced iron (Fe^{2+}) in the tablets which I consume daily, Ferromax, and mix the tablets with orange juice, milk, water, and coffee to observe the degree of oxidation of iron by doing a redox titration.

Background information

Iron is an essential trace metal in human metabolism. Iron absorption is controlled by enterocytes located in the gut and regulated by dietary and systemic factors. Dietary iron is predominantly non-heme found in plant-based alimentary substances such as grains, vegetables, and nuts, while heme iron is found in animal based dietary products such as meat, poultry, and seafood. Despite the lower bioavailability of dietary heme iron, 20%-30% is absorbed, in contrast to 1%-10% non-heme absorption with significantly reduced bioavailability and affected by other components of the diet (Beck, 2014). Iron in the environment is predominantly ferric ion, Fe^{3+}, which is insoluble and not bioavailable. For the gut to absorb iron, ferric ion must be reduced to ferrous ion, Fe^{2+}, by ascorbic acid, ferrireductases or diet reducing agents. Ascorbic acid is the most effective enhancer of non-heme iron absorption, followed by meat and poultry which stimulate gastric acid production (Beck, 2014). Absorption of non-heme iron is inhibited by phytic acids found in cereals and grains. Polyphenols similarly inhibit iron absorption found in certain vegetables, coffee, and tea.

To determine the iron concentration in Ferromax iron tablets, redox titration will be used. The iron in the tablets is found as Fe^{2+}, however iron naturally oxidizes to Fe^{3+}. Therefore, to prevent the iron from oxidizing to Fe^{3+}, the tablets will be crushed and dissolved into sulfuric acid, H_2SO_4. In the following procedure, potassium permanganate $KMnO_4$ is an oxidizing agent used in reduction-oxidation titrations. The oxidation potential of $KMnO_4$ is used to

determine the concentration of Fe in the tablets. In acidic solutions, the permanganate ions undergo reduction to form manganous ion, Mn^{2+}. Redox titration involves the transfer of electrons, thus the oxidizing agent $KMnO_4$ can be used as an indicator for this titration as they have distinctive colours in their reduced and oxidized states. In this redox reaction, Fe^{2+} is oxidized into Fe^{3+} and MnO_4^- is reduced into Mn^{2+}. Potassium permanganate is deeply purple in colour and becomes colourless when reduced to Mn^{2+}. The endpoint of the reaction is reached when the iron sample turns the solution pink, indicating there is no Fe^{2+} to react with $KMnO_4$.

Oxidation reaction: $5Fe^{2+}\ (aq) \rightarrow 5Fe^{3+}\ (aq) + 5e^-$

Reduction reaction: $MnO_4^-\ (aq) + 5e^- \rightarrow Mn^{2+}\ (aq)$

Overall reaction: $5Fe^{2+}\ (aq) + MnO_4^-\ (aq) + 8H^+\ (aq) \rightarrow 5Fe^{3+}\ (aq) + Mn^{2+}\ (aq) + 4H_2O\ (l)$

In humans, iron absorption can be greatly influenced by the presence of enhancers or reducers of iron absorption in the human diet. To investigate how dietary factors contribute to the absorption of iron, the tablets will be added into different solutions of water, milk, orange juice, and coffee. To enhance iron absorption, the iron found in the tablets is Fe^{2+}, however dietary factors could contribute to the oxidation of iron, which inhibits its absorption in the gut. Several factors present in cow milk may reduce iron availability, such as proteins, phosphate, calcium, while lactose, ascorbic acid, and amino acids may enhance iron absorption (Jackson, 1992). Polyphenol compounds are widely available in human diet, present in fruits, vegetables, spices, cereals, and significantly high in tea, red wine, and coffee (Hurell, 1999). Ascorbic acid present in orange juice has been found to increase iron absorption in the human diet (Shah, 2003). Therefore, this internal assessment will investigate to what extent iron (II) is oxidized to iron (III) in the different solutions of coffee, milk, water, and orange juice.

To observe the effect of the beverages on the iron concentration, the mixtures are titrated using an iodine solution. The titration will demonstrate the concentration of an oxidizing agent used to react with the iodide ions to form iodine I_2. As the tablets are mixed with the any of the beverages mentioned above, the titration will demonstrate whether the iron (II) ions in the tablet will oxidize to iron (III). Starch is used as an indicator for the redox titration. In the presence of starch, the solution will turn deep blue, indicating that iodide ions have been reduced and iron (II) ions have been oxidized.

Oxidation reaction: $Fe^{2+}\ (aq) \rightarrow Fe^{3+}\ (aq) + e^-$

Reduction reaction: $I_2\ (aq) + 2e^- \rightarrow 2I^-\ (aq)$

Overall reaction: I_2 (l) + Fe^{2+} (aq) → Fe^{3+} (aq) + $2I^-$ (aq)

Design

Research question: How do dietary factors (milk, water, orange juice, coffee) affect the oxidation of supplementary iron (Fe^{2+}) in tablets?

Dependent variable: Milk, water, orange juice, and coffee are beverages consumed during breakfast, and iron supplements are advised to be taken during the morning. The beverages will allow for comparison and determination of the effect of the dietary factors on iron.

Independent variable: The oxidation of iron from Fe^{2+} to Fe^{3+} when titrated against I_2

Controlled variables

- Temperature: Room temperature, 20°C (±1°C), was kept constant throughout the whole experiment to remove any external factors affecting the results. Conditioning units could not be turned off; however, the tests were run in the same temperature and environment, thus eliminating the possibility of results being modified.
- Volume of iron solutions: The volume of iron solutions was kept constant at 25 cm^3 throughout the experiment to limit variations in the data and allow for comparison between the data sets.
- I_2 concentration: The concentration of iodine was kept constant to ensure the same total number of moles was reacting in all trials.
- $KMnO_4$ concentration: The concentration of iodine was kept constant to ensure the same total number of moles was reacting in all trials.
- Iron tablets: Five tablets from the same brand of iron supplements were used in each solution to ensure equal number of moles reacting in all trials throughout the experiment.
- Equipment: The equipment used was reused for all tests to reduce inaccuracy and variation in the results.

Materials

Apparatus	Reagents
Electronic balance (± 0.001 g) Mortar and pestle Erlenmeyer flasks Funnels 250 cm^3 graduated cylinder (± 1.0 cm^3) 1000 cm^3 graduated cylinder (± 5.0 cm^3)	100 cm^3 0.1 Sulfuric acid H_2SO_4 1000 cm^3 0.005 Iodine I_2 250 cm^3 0.2 standardized Lugol's Iodine solution Distilled water
50 cm^3 Burette (± 0.05 cm^3) Burette stand and clamp Magnetic stir and magnet	1000 cm^3 standardized solution of 0.02 Potassium permanganate $KMnO_4$
25 cm^3 volumetric pipette (± 0.03 cm^3) 50 cm^3 volumetric pipette (± 0.05 cm^3) 5 cm^3 volumetric pipette (± 0.01 cm^3) Pipette pump	25 Iron tablets (Ferromax) Fe^{2+} Starch indicator

Safety and risk assessment

Potassium permanganate ($KMnO_4$) is a strong irritant and stains skin and clothing. Sulfuric acid (H_2SO_4) is highly corrosive and should not meet skin, eyes, or clothing. Therefore, safety precautions were taken into consideration during the experiment, by using safety goggles, apron, and gloves. Excess $KMnO_4$ was poured into a specified container and all chemicals were disposed into acidic inorganic waste containers. Equipment was washed with generous amounts of water.

Procedure

Method 1 - Finding the Fe^{2+} concentration in iron supplements

1. Standardisation of potassium permanganate $KMnO_4$: 3.161g of potassium permanganate concentrate ampoule was emptied using a funnel into a 1000 cm^3 graduated cylinder and diluted with distilled water to the mark of the bottle, resulting in a 0.02mol potassium permanganate solution.
2. Five iron tablets were weighed and grounded using a mortar and pestle. The powdered tablets were washed out using 100 cm^3 of sulfuric acid H_2SO_4 and transferred to a 250 cm^3 graduated cylinder using a funnel. This was repeated several times until the iron tablets

have been completely washed out from the pestle. The iron and sulfuric acid solution was diluted with distilled water to the mark on the bottle. The bottle was stirred to properly mix the solution.

3. A 25 cm^3 pipette was washed using distilled water and then, an iron solution sample. The pipette was used to transfer 25 cm^3 of the iron solution to an Erlenmeyer flask.
4. A 50 cm^3 burette was stabilized on a burette stand held by the clamp and washed using distilled water and a potassium permanganate solution sample. The pipette was fully filled up with 0.02 $KMnO_4$ using a funnel.
5. The flask containing the iron solution was placed on the magnetic stirrer and under the burette. A magnet was placed in the flask.
6. The solution was titrated until it reached an endpoint of turning from colourless to light pink. The reading was taken from the burette and recorded on a table.

Method 2 - Determining dietary factors on iron (II)

1. Standardization of iodine I_2: 2g of potassium iodide and 1.3g of iodine were weighed into a 100ml beaker and dissolved in distilled water. The solution was transferred in a 1000ml graduated cylinder and diluted up to the 1L mark with distilled water.
2. Five iron tablets were weighed and grounded using a mortar and pestle for each solution prepared (milk, orange juice, water, and coffee). The powdered tablets were transferred to 250 cm^3 graduated cylinders and diluted to the mark of the bottle. The solutions were left to react overnight.
3. A 25ml pipette was washed using distilled water and then, an iron solution sample. The solutions of coffee, orange juice, and milk were diluted by 1:5 with distilled water. The 5cm^3 pipette was used to transfer 5ml of the iron solution to an Erlenmeyer flask.
4. A 50ml burette was stabilized on a burette stand held by the clamp and washed using distilled water and an iodine solution sample. The pipette was fully filled up with 0.005 I_2 using a funnel.
5. The flask containing the iron solution was placed on the magnetic stirrer and under the burette. A magnet was placed in the flask.
6. The solution was first titrated without the starch indicator. The solution turned slightly yellow and after the starch indicator was added and the titration continued, a deep blue colour persisted.

Data analysis and processing

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial volume / cm^3 (± 0.05 cm^3)	0.00	5.80	11.70	17.60	23.40
Final volume / cm^3 (± 0.05 cm^3)	5.80	11.70	17.60	23.40	29.30
Total volume of $KMnO_4$ required / cm^3 (± 0.10 cm^3)	5.80	5.90	5.90	5.80	5.90

Table 1: Titration of 25$cm^3$of dissolved iron tablets in H_2SO_4 titrated against $KMnO_4$

The solution was initially light pink due to the coloured coat of the iron supplements. The solution became colourless, and as it reached the endpoint, the purple colour of $KMnO_4$ dissolved slower. At the endpoint of the reaction, the solution turned pink, and the colour persisted.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial volume / cm^3 (± 0.05 cm^3)	0.00	0.00	0.00	0.00	0.00
Final volume / cm^3 (± 0.05 cm^3)	32.60	32.40	32.20	32.20	32.10
Total volume of I_2 required / cm^3 (± 0.10 cm^3)	32.60	32.40	32.20	32.20	32.10

Table 2: Titration of 25cm^3 of dissolved iron tablets in orange juice titrated against I_2

The rough trial was unsuccessful; therefore, the solution was diluted 1:5. After titrating 4 cm^3, the starch indicator was added. The reaction reached the endpoint when the solution turned dark blue.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial volume / cm^3 (± 0.05 cm^3)	0.00	9.50	19.10	28.60	38.10
Final volume / cm^3 (± 0.05 cm^3)	9.50	19.10	28.60	38.10	47.70
Total volume of I_2 required / cm^3 (± 0.10 cm^3)	9.50	9.60	9.50	9.50	9.60

Table 3: Titration of 25cm^3 of iron tablets dissolved in 4% full fat milk titrated against I_2

The rough trial was unsuccessful; therefore, the solution was diluted 1:5. After titrating 4 cm^3, the starch indicator was added. The reaction reached the endpoint when the solution turned light grey.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial volume / cm^3 (± 0.05 cm^3)	0.00	6.40	12.90	19.40	25.90
Final volume / cm^3 (± 0.05 cm^3)	6.40	12.90	19.40	25.90	32.20
Total volume of I_2 required / cm^3 (± 0.10 cm^3)	6.40	6.50	6.50	6.50	6.40

Table 4: Titration of 25cm^3 of dissolved iron tablet in distilled water titrated against I_2

Since the solution simply contained a faint pink colour, it was diluted by 1:5 like the other solutions. Similarly, to the other titrations 4 cm^3 of I_2 was titrated first before adding the starch solution to give a result of a dark blue colour indicating the reaction's endpoint.

The coffee solution was diluted by 1:5, however due to its brown colour, the endpoint was difficult to identify. All solutions were left to react overnight, thus mixing a new coffee solution would limit comparison of data. Therefore, the coffee solution was not included in the experiment.

	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial volume / cm^3 (± 0.05 cm^3)	0.00	0.00	0.00	0.00	0.00
Final volume / cm^3 (± 0.05 cm^3)	22.00	21.80	21.90	21.90	21.80
Total volume of I_2 required / cm^3 (± 0.10 cm^3)	22.00	21.80	21.90	21.90	21.80

Table 5: Titration of 25 cm^3 of diluted orange juice with distilled water against I_2

As orange juice contains ascorbic acid (vitamin C), orange juice was diluted by 1:5 to find the moles of vitamin C in the solution. After titrating 7 cm^3, the starch indicator was added. The solution turned dark blue after the reaction reached its endpoint.

Processed data

To find the amount of moles of Fe^{2+} dissolved in H_2SO_4 in the solution used for the experiment, the average titre was calculated, 5.86 cm^3. The $KMnO_4$ concentration was 0.02 in the solution that was titrated against the iron (II). According to the chemical equation, there is a 5:1 ratio as seen below:

$$5Fe^{2+}\,(aq) + MnO_4^-\,(aq) + 8H^+\,(aq) \rightarrow 5Fe^{3+}\,(aq) + Mn^{2+}\,(aq) + 4H_2O\,(l)$$

Therefore, to find the moles of iron, the following calculations were done:

$$0.02 \times \frac{5.86}{1000} = 1.172 \times 10^{-4}$$

$$1.172 \times 10^{-4} \times 5 = 5.86 \times 10^{-4}$$

$$5.86 \times 10^{-4} \times 10 = 0.00586 \text{ moles of } Fe^{2+}$$

The 5 iron tablets were dissolved in ±100 cm^3 of H_2SO_4 and the rest was diluted to the 250 cm^3 margin of the flask. The sample taken for each titration was 25 cm^3, therefore the moles of Fe^{2+} is 0.00586.

To find the amount of moles of iron (II) that oxidized in the titration against I_2, the average titre was calculated for all titrations. The I_2 concentration was 0.005 in the solution titrated against iron (II) dissolved in either water, milk, orange juice, and coffee. According to the chemical equation, there is 1:1 ratio as seen below:

$$I_2\,(aq) + 2Fe^{2+}\,(aq) \rightarrow 2I^-\,(aq) + 2Fe^{3+}\,(aq)$$

For the solution of five tablets dissolved in water, the average titre was found to be 6.46 cm^3. The following calculations were done to find the moles of iron:

$$0.005 \times \frac{6.46}{1000} = 3.23 \times 10^{-5} \text{ moles of } I_2$$

$$3.23 \times 10^{-5} \times 2 = 6.46 \times 10^{-5}$$

$$6.46 \times 10^{-5} \times 50 = 0.00323 \text{ moles of } Fe^{2+}$$

Each sample used in the titration was diluted by 1:5 diluted with distilled water. Thus, the iron moles were multiplied by 50 to give the final moles of 0.00323.

For the solution of five tablets dissolved in milk, the average titre was found to be 9.54 cm^3. The following calculations were done to find the moles of iron:

$$0.005 \times \frac{9.54}{1000} = 4.77 \times 10^{-5} \text{ moles of } I_2$$

$$4.77 \times 10^{-5} \times 2 = 9.54 \times 10^{-5}$$

$$9.54 \times 10^{-5} \times 50 = 0.00477 \text{ moles of } Fe^{2+}$$

Similarly, to the iron tablet and water solution, the samples were diluted by 1:5 with distilled water. Thus, the moles were multiplied by 50 to give the final moles of 0.00477.

For the solution of five tablets dissolved in orange juice, the average titre was found to be 32.3 cm^3. The following calculations were done to find the moles of iron:

$$0.005 \times \frac{32.3}{1000} = 1.1615 \times 10^{-4} \text{ moles of } I_2$$

$$1.1615 \times 10^{-4} \times 2 = 3.23 \times 10^{-4}$$

$$3.23 \times 10^{-4} \times 50 = 0.0165 \text{ moles}$$

Similarly, to the previous two solutions, the five tablets and orange juice were dissolved by 1:5 with distilled water. Therefore, the moles were multiplied by 50. However, upon calculating the difference between the initial moles of iron found in the first part of the experiment, the amount of moles of iron was found to be significantly greater. Orange juice contains vitamin C, which itself is an antioxidant and readily oxidizes in a titration with I_2. Therefore, both Fe^{2+} and vitamin C can oxidise iodine, requiring a requiring a greater amount of titre before the reaction reaches the endpoint. Therefore, a titration was conducted where orange juice was titrated against I_2 to find the amount of moles of vitamin C oxidized in the reaction. The I_2

solution used in the titration was diluted from a Lugol's Iodine solution containing 10g of KI and 5g of I_2 (BAM R40: Lugol's Iodine Solution). To find the moles of vitamin C oxidized in the titration, the average titre of the diluted Lugol's iodine solution was calculated as 21.9 cm^3, thus the following calculations were done:

$$C_6H_8O_6\ (aq) + I_2\ (aq) \rightarrow C_6H_6O_6\ (aq) + 2I^-\ (aq) + 2H^+\ (aq)$$

$$\text{Moles of Iodine} = \frac{5g}{253.8\ g/mol} = 0.0197 \text{ moles}$$

$$\text{Concentration of } I_2 = \frac{0.0197}{0.1} = 0.197 \text{ mol/dm}^{-3}$$

$$\text{Lugol's Iodine Solution was diluted by 1:20, therefore, } \frac{0.197}{20} = 0.00985 \text{ mol/dm}^{-3}$$

$$\text{Moles of vitamin C} = 0.00985 \times \frac{21.9}{1000} = 2.157 \times 10^{-4} \text{ moles in } 5cm^3 \text{ of orange juice}$$

$$\text{The moles of vitamin C in } 250\ cm^3 = 2.157 \times 10^{-4} \times 50 = 0.0107 \text{ moles}$$

The nutrition facts label on the orange juice bottle states that there is 30mg of vitamin C per 100ml. Therefore, in 250 cm^3 there is 75mg of vitamin C.

$$\text{Moles of vitamin C} = \frac{0.075g}{176.12\ g/mol} = 4.258 \times 10^{-4} \text{ of in } 250\ cm^3$$

	Milk	***Water***	***Orange juice***
$\boldsymbol{Fe^{2+}}$	0.00477 moles	0.00323 moles	0.0058 moles
$\boldsymbol{Fe^{3+}}$	0.00109 moles	0.00263 moles	6×10^{-5} moles

Table 6: Calculations of reduced and oxidized Fe in the beverages

The moles of Fe^{2+} in the titration with $KMnO_4$ were calculated to be 0.00586 moles of Fe^{2+}. Using the moles of Fe^{2+}, the moles of iron remaining as Fe^{2+} in a solution of each dietary factor were calculated as seen above. Subtracting the amount of moles of vitamin C calculated stoichiometrically, the amount of moles of Fe^{2+} found in the orange juice solution is 0.0058.

$$0.0165 - 0.0107 = 0.0058 \text{ moles of } Fe^{2+}$$

When calculating the moles of iron, a 50 cm^3 burette with an absolute uncertainty of ± 0.05, a 50 cm^3 volumetric pipette with an absolute uncertainty of ± 0.05, a 25 cm^3 volumetric pipette with an absolute uncertainty of ± 0.03 and a 5 cm^3 volumetric pipette with an absolute

uncertainty of ± 0.01 were used. These materials were calculated to have a percentage of uncertainty as seen below:

Burette **2.5%**	**Volumetric pipette** **0.75%**	**Volumetric pipette** **2.5%**	**Volumetric pipette** **0.05%**

Table 7: Uncertainty percentage calculations of materials

$$\% \text{ uncertainty} = 2.5 + 0.75 + 2.5 + 0.05 = 5.8\%$$

Using the absolute percentage uncertainty, the absolute percentage can be found by calculating, i.e., 5.8% of Fe^{2+}in pills is 0.00586 moles of iron, thus 0.00586 ± 0.00034.

Evaluation

The results obtained from the experiment were consistent. The amount of moles calculated in the first method of the experiment was 0.00586 moles of Fe^{2+}. According to the packaging of the iron tablets, 65mg of Fe^{2+} is present in each pill. Thus, 0.325g of Fe^{2+} in five pills. The moles of Fe^{2+} in the tablets were calculated to be 0.00582. To measure the discrepancy between the experimental and theoretical value, the percentage error will be calculated below:

$$\% \text{ error} = \frac{(0.00582 - 0.00586)}{0.00582} \times 100\% = 0.7\% \text{ error}$$

The first method of the experiment involved $KMnO_4$ which has a deep purple colour making burette readings difficult. To reduce this systematic error that could affect measurements, a blank paper was used to give a clear background facilitating the final readings. However, the colour could still influence the accuracy of the results which could be eliminated through repeated trials and calculations of the average titre. Furthermore, the iron tablets used for the purposes of the experiment had a thin pink foil coating the tablets. When dissolved in H_2SO_4 and distilled water, the solution was slightly pink. The endpoint of the reaction with $KMnO_4$ also has a bright pink colour. While distinguishable, the human error involved in the identification of the endpoint could affect the burette readings and potential inaccuracies. To minimize such errors, different tablets could be used as well as repeated trials to remove random errors.

The second method of the experiment involved four different sets of titrations. An alternative method tested for the purpose of this experiment, was with Na_2SO_3 and KI. However, the rough

trials were unsuccessful and therefore, I_2 was used instead. The coffee solution was impossible to identify the endpoint due to its brown colour. To investigate the effect of coffee on iron, the coffee could be made weaker and diluted more to observe the endpoint. In addition, the milk had a thick consistency and turned grey when the reaction reached the endpoint. When the Erlenmeyer flasks rested, the grey colour fainted. Furthermore, despite the titration of orange juice against I_2 to measure the amount of moles of vitamin C, there is a significantly large discrepancy in the calculations of the vitamin C using the titration values and the nutrition facts label. Systematic errors could have influenced the results as orange juice was refrigerated for 2 weeks after its opening and used for the titration. Upon further investigation, juice in high oxygen permeability containers showed a faster decrease in ascorbic acid content, independent of initial dissolved oxygen content (Soares, 1999). In addition, both package barrier properties and de-aeration are major factors in maintaining ascorbic acid in refrigerated orange juice (Soares, 1999). Thus, there are several variables that could contribute to the oxidization of orange juice and vitamin C, which is beyond the scope of this internal assessment.

Conclusion

To answer the research question; "How do dietary factors (milk, water, orange juice, coffee) affect the supplementary iron in tablets?". This internal assessment provided empirical evidence on the potential dietary effects on supplementary iron. The purpose of this experiment was to find the quantity of reduced iron in supplementary iron tablets and understand how iron can be kept reduced or oxidized according to the breakfast beverage it is swallowed with. Furthermore, the data were precise as observed from the closeness of the values. The Fe^{2+} mass found by titration was 0.327g ± 0.01897 with a percentage error of 0.7% compared to the manufacturer's declaration. Orange juice kept Fe^{2+} reduced, while milk permitted 18.6% oxidation and water permitted 44.9% oxidation. The experiment displayed how dietary factors may inhibit or promote iron redox reactions, and thus iron absorption in the human body. Further investigation could explore whether pH affects the oxidation or reduction of iron.

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