Investigating the effects of concentration, time and temperature on the absorptivity of the iron using photometry

Chemistry Internal Assessment

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Introduction:

My passion for this investigation began when I started learning about one of the serious problems in Azerbaijan. According to Weatherall DJ, Clegg JB 2001[1] Azerbaijan is one of the countries in the world distribution of Thalassemia. According to the Ministry of Health of the Republic of Azerbaijan, nearly 300 children are born with thalassemia every year. Patients with thalassemia are characterized with their bone marrow producing few and abnormal forms of hemoglobin. Thalassemia patients cannot live without blood transfusion. However, when the blood is being transferred to a patient iron overloading occurs. Humans are also not able to actively get rid of excess iron [2], causing disease to get worse. The issue of blood transfusions can be solved by transplanting bone marrow to the patient. However, there is also a chance of unsuccessful bone marrow transplantations which result in a loss of patient's life.

I was interested in the method doctors used to determine the concentration of iron. After conducting some research, I learned that excess iron in the body was determined by special scientific method called spectrophotometry. Spectrophotometry is a method of determining the absorbance of a molecule by passing a beam of light and measuring its intensity [3]. Due to the availability of the limited resources only photometer was the best option available instead of spectrophotometer, however, this does not have any significant effect on the results. The principal difference between the two equipment is that photometry is used exclusively for the visible wavelengths, while the other is used for whole electromagnetic spectrum [4]. I decided to determine the concentration of the iron based on its absorption using photometry.

Background

It is impossible to determine the absorptivity of iron on itself, therefore scientists use a reagent to create iron solution and then measure the absorptivity of iron in spectrophotometry. In order to make successful experiment, I used the reagent developed by The Baku State University specifically for the determination of iron. I could not make the reagent myself since it exceeds my understanding of topic. This reagent has formula 2,4 diphenilhexadion -2,6. Here is the structure of the reagent designed by me using the software called chemsketch [5]. After getting the reagent, I had to make an iron Fe(III) since Fe(II) is unstable. Therefore Fe(II) can oxidize very fast and become Fe(III) leaving both Fe(III) and Fe(II) in the solution. Therefore, I added nitric acid(HNO_3) to my solution to make Fe(III). Then I added them to 10 volumetric flasks

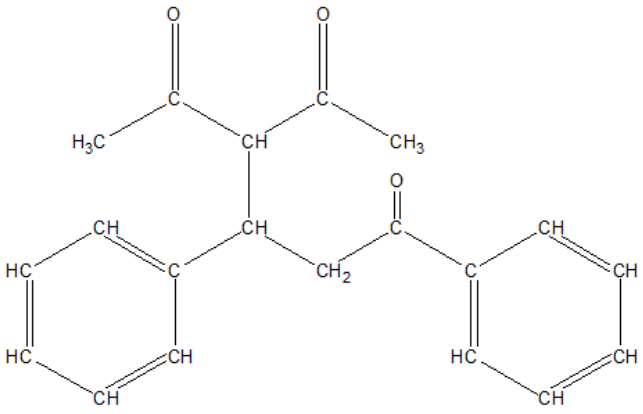

Figure 1: 2D structure of 2,4 diphenilhexadion -2,6 using Chemsketch

and poured solutions whose pH ranging from 1-10 to each of them and then using cuvette, I calculated their absorptivity (more briefly explained in procedure section). After calculating absorptivity at different wavelengths, I found the optimum pH and wavelength and proceeded to investigating the effects of time, temperature and concentration on absorption. Then I used Beer-Lambert's law to measure the absorptivity coefficient.

Beer-Lamberts law

The Beer-Lambert law relates the attenuation of light to the properties of the material through which the light is traveling. Beer-Lambert law can be expressed as:

$$\boldsymbol{A = \varepsilon C l}$$

Where:

- ε -Greek letter epsilon is the molar absorption coefficient. An absorbance of 0 at some wavelength means that no light of that wavelength has been absorbed
- c is the concentration of the substance
- l is the length of the light path
- A is the absorption of the substance

I employed the above equation for the calculation of absorptivity coefficient.

Hypothesis:

My hypothesis is based on three variables: concentration, temperature and time.

- During our chemistry class, I learned that, concentration is proportional to the number of molecules. Therefore, if the concentration is high, there will be more number of molecules that interact with light. This means that, as the concentration increases, absorptivity increases as well. As we can see from the Beer-Lamberts law, I predict a direct proportionality between concentration and absorptivity.

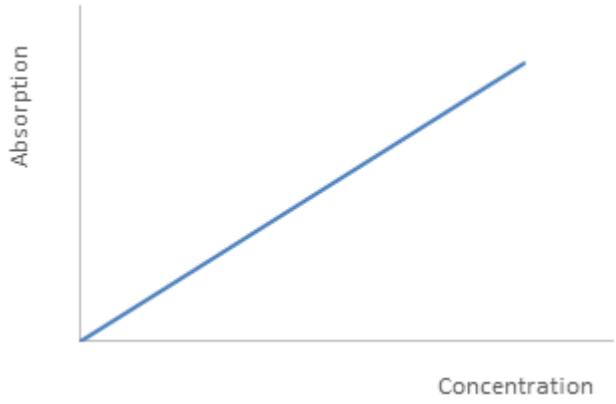

Figure 2: Graph of Absorption on Concentration created by paint software

- In terms of temperature, I think that as the temperature increases, the kinetic energy of the particles will increase which will cause more collisions and at some point, the average speed of particles will be at the very high level that light will encounter fewer molecules, causing absorptivity to decrease.
- For time, I don't predict any change since the particles will not be affected, but I am interested in investigating to see if anything happens beyond my knowledge.

Methodology:

Variables:

This section introduces the list of dependent and independent variables (table 1), and controlled variables (table 2) used during experiment respectively.

Independent Variables	***pH levels:*** By adding pH from 1 to 10 to volumetric flask, absorption values of the solution containing complex were measured. ***Time:*** Using clock watch, absorption values for every 10 min were measured. ***Temperature:*** Using hot plate, solution containing complex and different pH values were heated and then its absorptivity measured ***Concentration:*** Using electronic balance, I added volume of reagent to the solution to increase concentration, and absorption was measured.
Dependent Variable	***Absorptivity of the complex:*** Using KFK-2 photo-electric photometer absorption values of the complex solution measured. ***Wavelength:*** Using KFK-2 photo-electric photometer at different wavelengths (364-590), absorption values were measured.

Table 1: Dependent and independent variables used in the experiment.

Controlled Variable	Effect on my results	Method of Control
Mass of iron used- 0.56 g	Using electronic balance, 10 g of iron were measured, and transferred to 200cm^3 glass beaker.	Changing the mass of the iron will change the concentration of the complex which may result in different absorption values.
Time used after heating volumetric flask containing Fe(III) solution, reagent and pH	Since the temperature of the heated solution will be different from background temperature, it immediately begins to decrease after moving it away from the hot plate.	Try to put the solution to the cuvette and then to the photometer immediately.
pH values	This will result in an incorrect absorption values for the assumed pH values.	I measured their pH levels using PHS-25 pH meter before starting the experiment to make sure they have right pH values.
Volume of the distilled water added to the iron solution- until the graduation line	Distilled water was added to the iron solution until the line of graduation.	Changing the volume of the distilled water added may result incorrect ratios.

Volume of hydrochloric acid used- 15 cm^3	Using 5 cm^3 pipette, 15 cm^3 of hydrochloric acid measured and added to glass beaker containing $FeCl_2$ solution.	Changing the volume of the hydrochloric acid will change the concentration of the iron solution which may result in different absorption values.
Volume of nitric acid used-2 cm^3	Using 2 cm^3 pipette, 2 cm^3 of nitric acid measured and added to 0.56g iron in the glass beaker	Changing the volume of the nitric acid will affect the concentration of the complex which may result different absorption values
Mass of the reagent (3-acetyl-4,6 diphenilhexadion-2,6) used- 0.0308 g	Using an electronic balance, 0.0308 g of reagent were measured and transferred to 100 cm^3 volumetric flask.	Changing the mass of the reagent will affect the concentration of the complex which may result in different absorption values.
Volume of the ethanol - until the graduation line.	Ethanol was added to the reagent and brought until the graduation line.	Changing the volume of the ethanol added may result incorrect ratios of reagent and iron solution.
Volume of the different pH solutions added to the iron solution- until the graduation mark.	Different pH solutions were added to 10 different volumetric flasks containing Fe(III) solution and reagent solution.	Altering the volume of the added pH solutions may result incorrect ratios.

Table 2: Controlled variables, the effect that this variable may create and the method of control

Apparatus and materials:

- 25 cm^3 volumetric flask
- 100 cm^3 volumetric flask
- 200 cm^3 glass beaker
- KFK-2 photo-electric photometer ($\pm$0.005)
- 5 cm^3 Electronic pipette ($\pm$0.01 cm^3)
- 1 cm^3 Electronic pipette ($\pm$0.01 cm^3)
- 2 X 2 cm^3 Electronic pipette ($\pm$0.01 cm^3)
- 3 cm^3 Electronic pipette ($\pm$0.01 cm^3)
- Stopwatch ($\pm$1 s)
- Electronic balance ($\pm$0.001 g)
- PHS-25 pH meter ($\pm$0.005)
- 2 X Cuvette (1cm width)
- Thermometer ($\pm$0.05^{o}C)
- Hot Plate ($\pm$ 0.5^{o}C)
- Funnel

- Stirring rod
- Distilled Water
- pH solutions from 1 -10
- 37% hydrochloric acid solution
- 25% nitric acid solution
- 0.56 g Iron
- 0.0308 gram reactive (3-acetyl-4,6 diphenilhexadion-2,6)

Procedure

Making the iron solution:

1. Measure 0.56 gram of iron using the electronic balance and add it to the glass beaker.
2. Pour 15 ml Hydrochloric acid to the glass beaker and put it on the hot plate.
3. While heating, using pipette add 3 ml Nitric Acid to the glass beaker.
4. Using a funnel, pour the solution into one of 100 ml volumetric flask.
5. Add distilled water until it reaches the graduation line.
6. Take 5 ml from Fe(III) solution and add it to another 100 ml volumetric flask and pour distilled water until it reaches the graduation line.

Steps 2 and 3 are used to make Fe(III) solution since Fe(II) solution is weak and unstable and can oxidize very fast

Preparing the reagent solution

1. Measure 0.0308 gram of reagent using electronic balance and add it to 100mL volumetric flask.
2. Add very little ethanol and shake the flask until it dissolves.
3. After dissolving the iron, add more ethanol until the 100mL line.

Preparing complex and calculating the absorption

1. Take 10 different 25 ml volumetric flask.
2. Measure the pH levels using pH meter to make sure all solutions have correct pH values.
3. By using two different pipettes, (one for reagent and one for iron solution), add 1 ml of Fe(III) and 2 ml reagent to every volumetric flask.
4. Add different pH to every volumetric flask until it reaches the graduation line.
5. Shake them carefully and add them to the cuvette.
6. Put cuvette with complex to one side of photometer and cuvette with distilled water on the other side of the photometer.
7. First putting cuvette with pH 1, change wavelength and take down the absorption values, then change the cuvette from one with pH 1 to one with pH 2 and repeat the steps for all of the pH values ranging from 1-10.

Safety Measures

Risk	Prevention Measure
Safety: 15 ml Hydrochloric acid used. Its contact with skin produces burns, and tissue damage on eyes. Inhalation may result shortness of breath.	Wear eye protection and safety gloves especially when measuring the volume and pouring into volumetric flask is advised. Laboratory must be well-ventilated for work. Avoiding inhaling vapor. Wear lab coat to protect skin.
Safety: 3 ml of nitric acid used. It is hazardous with skin and eye contact. Prolonged exposure may result with skin burn. Inhalation may produce soreness of respiratory track.	Use of gloves and googles when measuring the volume and pouring into volumetric flask is advised. Make sure that there are no other substances which can react with nitric acid. Laboratory must be well-ventilated for work. Avoiding inhaling vapor and wear lab coat to protect skin.
Safety: Broken Lab equipment especially glass may cut people's skin.	It is advisable to keep experimental space tidy to minimize accidents. In the case if any flask or lab equipment made of glass breaks, use brush and dustpan and put it in a bin.
Safety: The amount of ethanol poured to reactive until the graduation line. Ethanol causes skin and eye irritation. May cause vomiting when ingested.	Wear gloves and googles and lab coat and avoid ingestion.
Ethical	No living organisms were used during experiment, therefore no ethical issues.

Table 3: Safety measures that were taken into the account during experiment.

Results:

pH / λ (nm)	1	2	3	4	5	6	7	8	9	10
364	0.14	0.2	0.29	0.43	0.55	0.43	0.28	0.15	0.09	0.06
400	0.12	0.19	0.35	0.48	0.58	0.45	0.3	0.18	0.14	0.11
440	0.11	0.17	0.27	0.41	0.56	0.4	0.27	0.17	0.12	0.09
490	0.09	0.14	0.24	0.34	0.48	0.37	0.25	0.15	0.1	0.08
540	0.06	0.13	0.23	0.29	0.39	0.33	0.2	0.13	0.07	0.05
590	0.04	0.07	0.15	0.22	0.26	0.24	0.19	0.1	0.04	0.03

Table 4: The absorption values obtained from photometer. The table containing absorption values (± 0.005) that arises from different pH levels (± 0.005) and wavelength values.

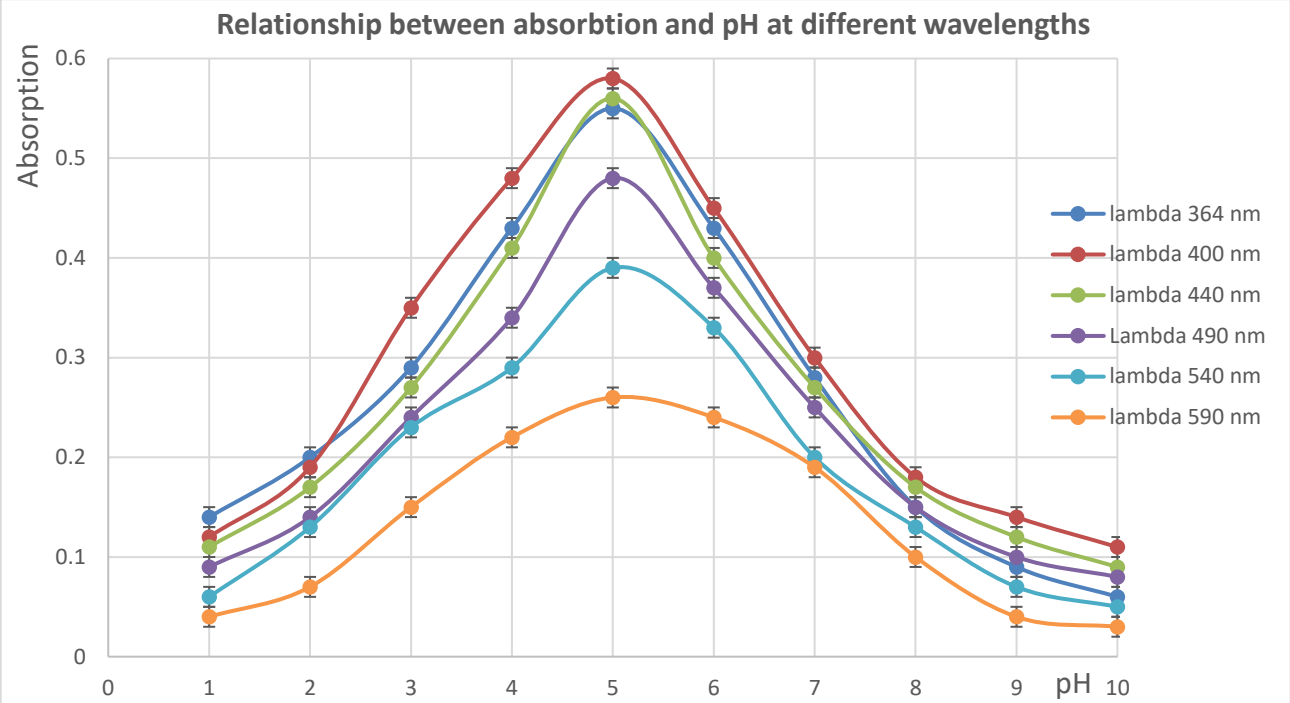

Figure 3: Dependency of absorption at different pH levels according to table 4. Each curve represents different values of wavelength

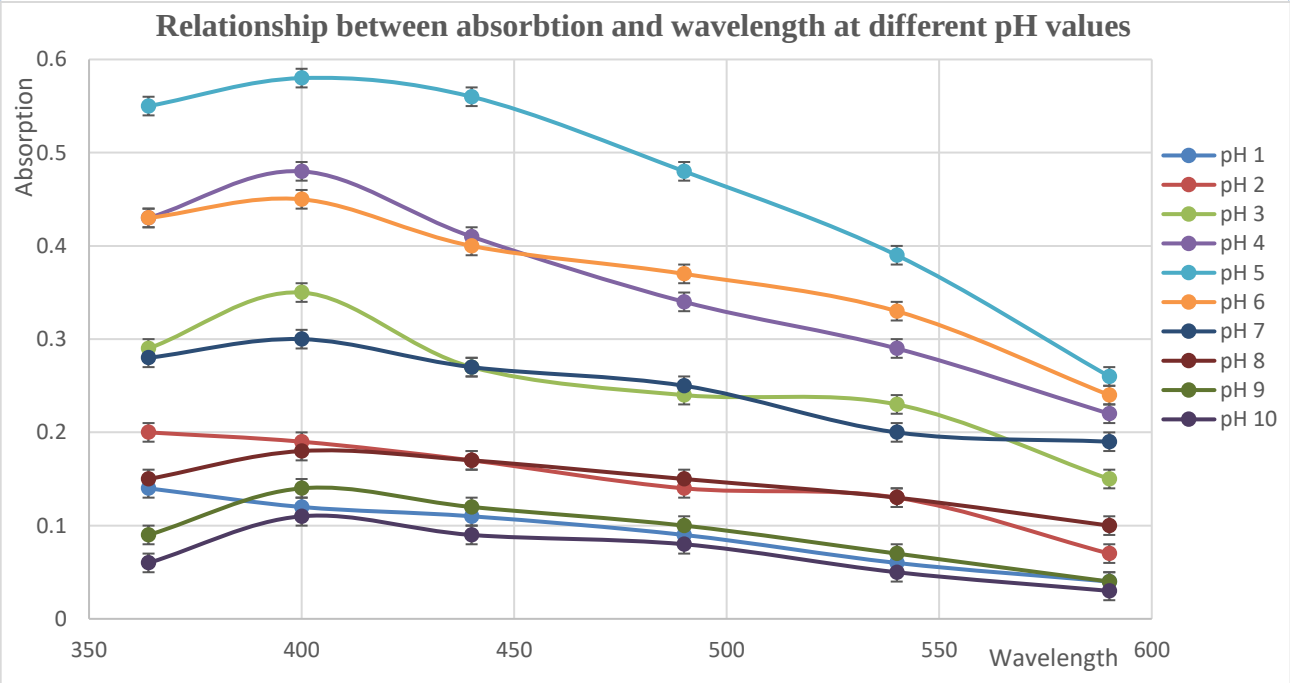

Figure 4: Dependency of absorption at different wavelengths according to table 4. Each curve represents different pH levels

From the figure 3, we can see that the highest absorption is at 400 nm and the highest absorption value is achieved at pH 5. We obtain the same results when we plot the graph of absorption on wavelength at figure 4 indicating that the optimum pH is 5 at 400 nm wavelength.

Investigating the factors affecting the absorption

Time

After finding the optimum pH, I decided to look for possible factors on the absorption value. The factors I thought would affect were time, temperature and concentration. First, I started with time. I used the stopwatch to record the time to see whether the absorption level changed or not.

Time (min)	**0**	**10**	**20**	**30**	**40**	**50**	**60**
A(± 0.005)	0.7	0.7	0.7	0.7	0.7	0.7	0.7

Table 5: Absorption values at every 10 minutes.

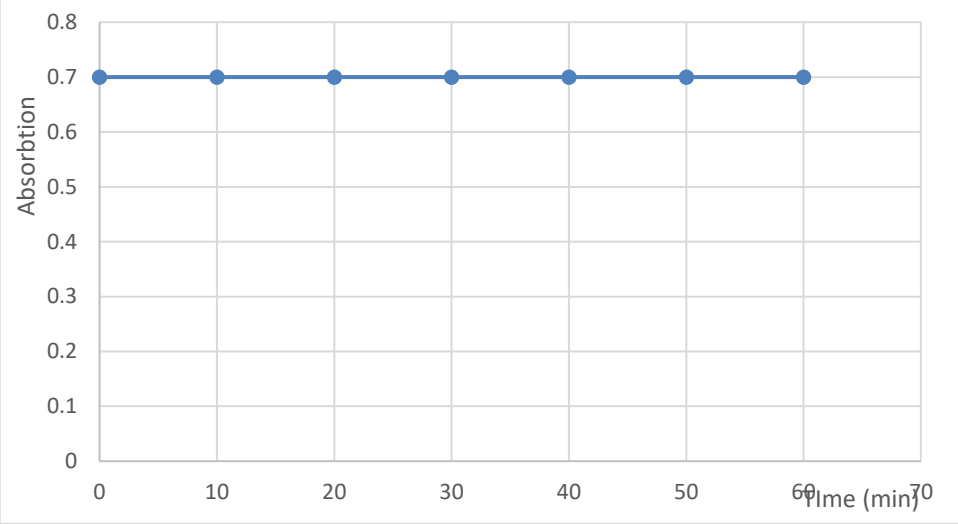

Figure 5: The effect of time on absorption values.

From the table 5 and figure 5, it is obvious that absorption does not change from the time. Therefore, I can conclude that time has no effect on the absorption which is exactly as I have predicted in my hypothesis.

Temperature:

I decided to measure temperature starting from 20^{o}C until 80^{o}C, as shown in the table 3. I heated the volumetric flask containing complex and different pH solutions to specific temperatures before pouring them into cuvette.

Temperature ($\pm 0.05^{o}C$)	**20**	**30**	**40**	**50**	**60**	**70**	**80**
A (± 0.005)	0.7	0.7	0.7	0.7	0.43	0.17	0.03

Table 6: Absorption values at different temperatures.

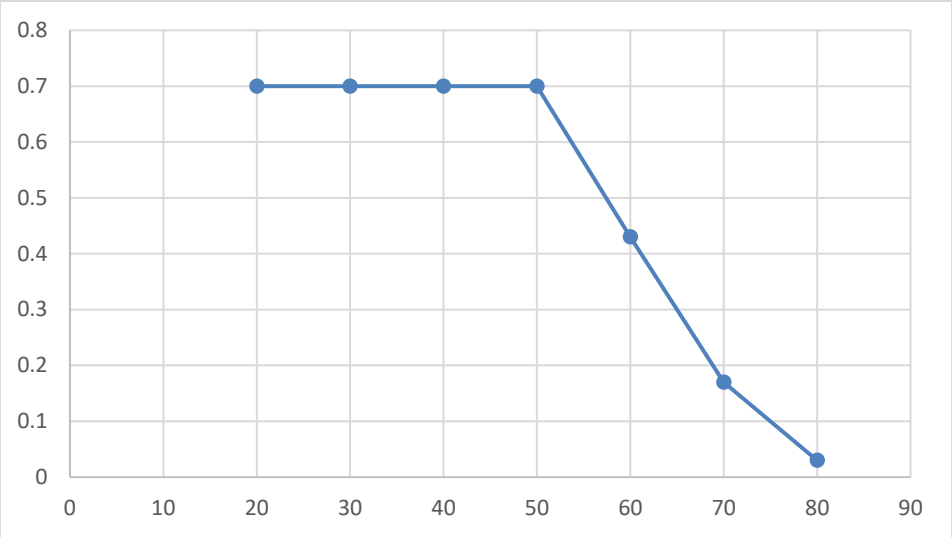

Figure 6: The effect of temperature on absorption values.

From the table 6 and figure 6, we can see that absorption does not change between 20-50 ^{o}C but after 50°C, the temperature decreases almost exponentially. Therefore, I can say that the temperature between 20-50°C is the optimum temperature to measure the absorption of this complex. This is very similar to how I predicted the result according to my hypothesis.

Concentration:

In order to alter the concentration, I added more volume of my reagent. From my understanding of stoichiometry, concentration is proportional to the molar mass of the substance, therefore when I add more volume of my reagent, molar mass will increase, leading increase in concentration.

V $(\pm 0.05\ ml)$	**0.5**	**1**	**1.5**	**2**	**2.5**	**3**
C mol/l	0.00002	0.00004	0.00006	0.00008	0.0001	0.00012
A (± 0.005)	0.13	0.31	0.47	0.6	0.7	0.7

Table 7: Absorption values at different concentration levels.

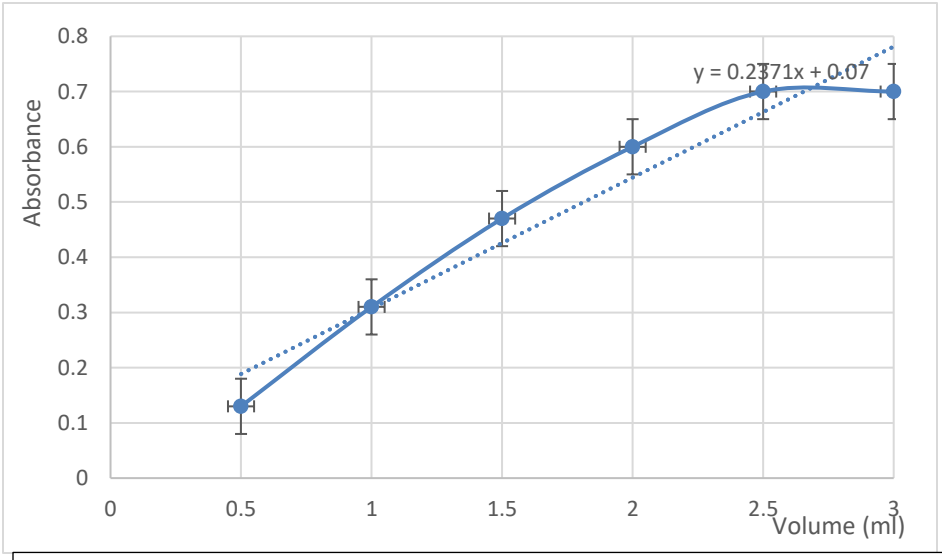

Figure 7: The effect of concentration on absorption values.

According to the data from table 7 and figure 7, I can see that between the values of concentration between 0.5 cm^3 and 2.5 cm^3 the absorbance value increases. This is very similar to how I have predicted this result according to my hypothesis. Concentration threshold was found to be at 2.5 cm^3

Determination of the molar absorptivity constant

Concentration of the substance can be calculated as follows:

$$C = \frac{V_i * M}{V_{tube}}$$

Where V_i^- is the initial volume taken which is 1 cm^3, V_{tube} is the volume of the tube which is 25cm^3 and M is the molar mass of the substance which is $5*10^{-3}$ mole. Then, we can use the equations above to calculate the concentration and the absorption coefficient.

$$C = \frac{1 * 5 * 10^{-3}}{25} = 2 * 10^{-4} mol/dm^3$$

Now I can put the variables to find the value of the absorptivity constant. According to the Beer-Lamberts law, the equation for the absorptivity is:

$$\boldsymbol{\varepsilon = \frac{A}{Cl}}$$

From the experiment, the width of the cuvette was measured to be 1 cm. The absorptivity value is 0.7 which is the absorptivity value for pH 5 and wavelength 400nm. The concentration was calculated to be $2*10^{-4}\, mol/dm^3$. Therefore, when I put the values for the concentration, length and absorption the coefficient of absorption is:

$$\varepsilon = \frac{0.7}{2 * 10^{-4} * 1} = 3.5 * 10^3$$

From this investigation, I found out that the absorption coefficient is equal to $3.5 *10^3$ at 20ºC.

Conclusion

Overall, I enjoyed every moment of my investigation. Focusing around one of the most serious problems in my country was a very important for me since it will be related to my future career. The aim of this investigation was to explore the factors affecting the absorptivity of iron. First, I started to find pH level and wavelength value where absorptivity was the most (optimal). Then, I started investigating factors using the highest pH and wavelength levels; time, temperature and concentration. During the experiment, I also considered the safety conditions and followed every one of them. I tried to control maximum number of variables which represented in table 1 and 2. I also took down the results from experiment and presented them in table 4, table 5, table 6 and table 7. I also used graphs (figure 3, figure 4, figure 5, figure 6 and figure 7) to see the changes in absorption value more clearly.

The correlation between absorption and wavelength represented in Figure 3 and the correlation between absorption and pH represented in Figure 4 suggests that this iron solution has the highest pH value 5 and at wavelength 400 nm. This result suggests that iron solution has the optimum pH of iron solution at 5 and optimum wavelength at 400 nm.

As predicted, there is no correlation between absorption and time, so the absorption value does not change with respect to time. This was expected from my hypothesis since time has no effect on the number of particles that are being exposed to light. Therefore, the absorption does not change, and it is highest during the whole process.

Positive correlation between concentration and absorption supported my hypothesis: as the concentration increases, more molecules are being exposed to light; causing an increase in absorption. However, from figure 7 we can see that after the value of 0.0001 mol/dm^3 concentration of the solution does not change. Therefore, I can say that any value of concentration after 0.0001 mol/dm^3 will not affect absorption and this value is the concentration threshold. Negative exponential correlation between absorption and temperature suggests that after 50^{o}C, the absorption decreases exponentially. Since the temperature values below 20^{o}C hasn't been investigated, I can only conclude that values of temperature between 20-50^{o}C is highest and it is advised to conduct experiment around this temperature to see noticeable effects. Investigations around the Beer-Lamberts law showed that the absorption coefficient is $3.5 * 10^3$ with no units.

<u>Calculating Uncertainty:</u>

% uncertainty in the electronic balance for iron: $\frac{0.001(electronic\ balance\ uncertainty)}{0.56(mass\ of\ iron)} * 100 = 1.78\%$

% uncertainty in the electronic balance for reagent: $\frac{0.001(electronic\ balance\ uncertainty)}{0.0308(mass\ of\ reagent)} * 100 = 3.24\%$

% uncertainty in 5 cm^3 pipette for HCl adding: $\frac{0.01}{5} * 100 = \ 0.2\%$

% uncertainty in 2 cm^3 pipette which used for HNO_3 and reagent: $\frac{0.01}{2} * 100 = \ 0.5\%$

% uncertainty in 1 cm^3 pipette for Fe(III): $\frac{0.01}{1} * 100 = \ 1\%$

% uncertainty in volumetric flask volume (1) 25 cm^3 (2)100 cm^3: (1) $\frac{0.5}{25} * 100 = 2\%$

(2)$\frac{0.5}{100} * 100 = 0.05\%$

The results obtained supported my initial hypothesis which predicted that time will have no impact, concentration will have positive and temperature will have negative effect on absorption. My evaluation will follow possible problems that might have influenced my experiments and can possibly lead to limitations. In addition, I will be addressing the possible improvements for this investigation.

Evaluation

There were no safety issues since the safety measurements were followed. However, looking back to my experiment, I can still see a lot of limitations that could have provided more accurate and precise results.

The first limitation was the heat loss when the dependence between temperature and absorption was investigated. I heated the volumetric flask containing iron and pH using hot plate. After taking away from hot plate and pouring to the cuvette and putting into polarimeter takes time and during this time, the heat from the solution might be transferred to the surroundings. At the same time, temperature of the solution is also likely to drop even inside photometer. I tried to take absorption values as fast as possible but knowing that temperature starts to drop at a high rate due to temperature difference between surrounding, this limitation could have had significant effect on the experiment. This limitation could be reduced by heating the cuvette itself instead of flask to reduce time taken. However, this also casted doubt whether the cuvette will shatter or not.The second limitation was the background pressure. I tried to keep the pressure constant for the whole experiment however, the fact that pressure changes every day and this entire experiment was conducted in three weeks, different pressure values could have affected the experiment in a very minor way. This problem can be decreased by reducing total amount of days the experiment has conducted and if possible, doing the whole experiment in one day.

The third limitation was that the concentration of the ethanol was not taken into the consideration during the experiment. Higher concentration of the ethanol could have resulted in a greater concentration of the solution therefore higher absorption values and on the other hand, lower concentration of the ethanol could have resulted in a lower concentration of the solution, therefore lower absorption values. If the concentration of the ethanol was too high, the part where I calculated the dependence of concentration on absorption loses its quality because by considering the high concentration of the ethanol, higher concentration values could have corresponded for the same absorption values.

In addition, to improve the efficiency of this experiment, a better photometer could have used. This would decrease the overall uncertainty. This experiment can be extended by using actual spectrophotometer to determine the absorption of iron using the same reagent. I also wanted to determine the absorptivity of daily fruits, vegetables and meals and even water that we drink using the same reagent. Controlling their diet is very necessary for thalassemia patients to make sure they have low iron intakes. Furthermore, investigating tablet containing iron content can also be a better way to extend this investigation. In Azerbaijan it is very frequent that person might but tablet that doesn't contain any iron. Investigating these types of tablets, can be very good way to decrease scammers in the country.

In addition, I found that the level of iron in a person's saliva is positively correlated with the level of iron in person's blood [6]. Therefore, determining iron from saliva instead of from blood using spectrophotometry could be very interesting experiment to determine iron deficiency and iron overload.

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