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DETERMINATION AND EVALUATION OF FERROUS SULPHATE IN MARKETED PREPARATION (CAPSULE) USING COLOUR DEVELOPMENT UV SPECTROSCOPY

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ABSTRACT

This project focuses on the determination and evaluation of ferrous sulphate content in marketed preparations, specifically capsules, using UV Spectroscopy. The methodology involves colour development as a key parameter for quantitative analysis. The study aims to establish a reliable and efficient approach for assessing the ferrous sulphate concentration in pharmaceutical formulations, contributing to quality control and ensuring product efficacy.

KEYWORD: Ferrous Sulphate, Marketed Preparation (capsule), Determination, Evaluation,
Colour Development, UV Spectroscopy.

INTRODUCTION

Ferrous sulphate

Iron is a medication used in management and treatment of iron deficiency anaemia. This activity illustrates the indications, action, and contraindications for iron supplementation as a valuable agent in management of iron-deficient states such as iron deficiency anaemia, iron deficiency without anaemia, nutritional deficiency, mal absorption, blood loss, or an increase in the body's need for iron. This activity will highlight the mechanism of action, adverse event profile (e.g., off label uses, dosing, pharmacodynamics, pharmacokinetics, and monitoring, relevant interactions) pertinent for members of the healthcare team in the management of patients with iron deficiency and related conditions.[1]

In the UK, iron deficiency anaemia (IDA) affects around 4.7 million people every year Groups mostly at risk include those with increased iron demands, for example children and pregnant women and especially those with increased iron loses, for example premenopausal women and patients with inflammatory bowel disease (IBD).

First-line treatment is oral therapy with ferrous iron (Fe (II)) salts. For example, in 2012 more than 6.8 million prescriptions were filled for oral iron in England and 97.6% of them were for simple Fe (II) salts.[2]

Iron deficiency (ID) and iron-deficiency anaemia (IDA) are a worldwide concern. With 750 million children affected around the world, IDA is the most common nutritional disorder occurring during childhood. Among children in the developing world, ID is the most common single-nutrient deficiency caused by insufficient intake. Despite a decline in prevalence, IDA remains a common cause of anaemia in young children from industrialized countries. In 2011, the World Health Organization (WHO) estimates for children aged 6–59 months indicated a global prevalence of anaemia of approximately 43%, ranging from around 22% in developed regions to around 62% in developing regions. Around 42% of cases of anaemia in children can be attributed to Iron Deficiency.

Iron deficiency and IDA impair the cognitive development and physical growth of infants and children, depress immune function, and increase morbidity from infectious diseases. Usually, ID evolves slowly and is not clinically apparent until the anaemia is severe. Mild or moderate IDA may go unnoticed and is difficult to diagnose because IDA symptoms are infrequent and nonspecific, including pallor, irritability, poor appetite, fatigue, and lethargy. Diagnosis is even more problematic in infants, for whom it is difficult to obtain blood samples in sufficient quantities for hematologic diagnosis.

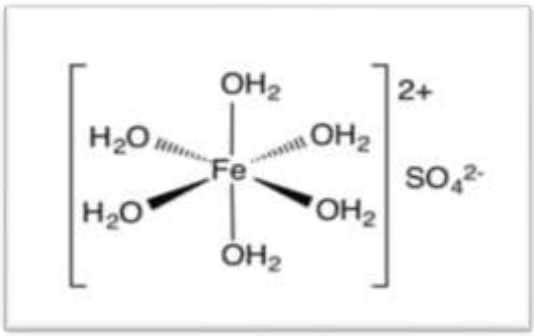
Iron supplementation to improve iron storage is the preferred treatment for IDA. Ferrous salts are preferred over ferric salts because of their absorption, which is

about three times better. They are registered in the WHO model list of essential medicines as the most efficacious, safe, and cost-effective iron supplements for the treatment of anaemia in children and adults. The ferrous sulphate form, which is the most commonly prescribed, constitutes the treatment of choice.[3]

UV-VIS spectrophotometry is probably the most useful tool available for quantitative determinations in diverse areas. It is due to its versatility, accuracy and sensitivity. You have learnt that the UV-VIS spectrum results from the interaction of electromagnetic radiation in the UV-VIS region with molecules, ions or complexes. You would recall that the absorption of radiation in the UV-VIS region by the absorbing species is governed by the Beer-Lambert's law which relates it to the thickness of the absorbing medium and the concentration of the absorbing species. This law forms the basis of a number of methods for the determination of micro and semi-micro quantities of analyte. UV-VIS spectrophotometry can be used for direct determination of a large number of organic, inorganic and biochemical species accurately at fairly low concentrations; viz., 10^{-4} to 10^{-5} M or even lower. A significant feature of UV-VIS spectrophotometry is that it can also be used for the quantitative determination of analyte which do not absorb in the UV-VIS region. It is achieved by making them react with a reagent that gives a product which strongly absorbs in the region.[4]

The amount of soluble iron (II) in each capsule was determined by reaction of Fe^{2+} with 1, 10- phenanthroline to convert weakly coloured iron to a complex that is intensely coloured that can be used in the analysis. When a polychromatic light from the source was passed to these intensely coloured complex, the substance absorbed certain wavelengths. The intensity of the colour of a solution is proportional to the concentration of absorbing species and absorbance is proportional to the concentration of the substance. A different concentration (in ppm) of standards was prepared and absorbance in 508 nm. A calibration curve that follows Beer's Law was constructed.[5]

Structure of ferrous sulphate[6]



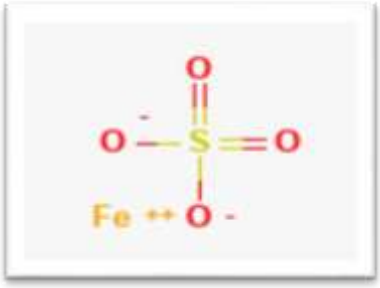
Description[7]

Ferrous sulphate appears as a greenish or yellow-brown crystalline solid. Density 15.0 lb. /gal. Melts at 64 °C and loses the seven waters of hydration at 90 °C. The primary hazard is the threat to the environment. Immediate steps should be taken to limit its spread to the environment. Used for water or sewage treatment, as a fertilizer ingredient.

Iron (2+) sulphate (anhydrous) is a compound of iron and sulphate in which the ratio of iron (2+) to sulphate ions is 1:1. Various hydrates occur naturally - most commonly the heptahydrate, It has a role as a reducing agent. It is a metal sulphate and an iron molecular entity. It contains an iron (2+).

Iron deficiency anaemia is a large public health concern worldwide, especially in young children, infants, and women of childbearing age. This type of anaemia occurs when iron intake, iron stores, and iron loss do not adequately support the formation of erythrocytes, also known as red blood cells. Ferrous sulphate is a synthetic agent used in the treatment of iron deficiency. It is the gold standard of oral iron therapy in the UK and many other countries.

Chemical Structure Depiction



IUPAC Name
Iron (2+) sulphate

Molecular Formula
$FeSO_4$

Synonyms
Iron (II) sulphate, Green vitriol, Iron vitriol.

Molecular Weight:- 151.91 g/mole

Physical Properties
Ferrous sulphate appears as a greenish or yellow-brown crystalline solid. Density 15.0 lb. /gal. Melts at 64 °C and loses the seven waters of hydration at 90 °C. The primary hazard is the threat to the environment. Immediate steps should be taken to limit its spread to the environment. Used for water or sewage treatment, as a fertilizer ingredient.

Pellets or Large Crystals

- **Odour: -** Odourless
- **Appearance:-**Ferrous sulphate appears as a greenish or yellow-brown crystalline solid.
- **Complexity: -** 62.2

- **Hydrogen bond acceptor: -** 4
- **Covalently Bonded unit**: - 2
- **Boiling Point**: - > 300 °C (Greater than 300 °C)
- **Melting Point**: - 64
- **Solubility: -** 25.6g/100 ml (Soluble in water), but negligible in alcohol.
- **Density**

The density of ferrous sulphate (FeSO4) is 2.84 g/cm3.
The density of ferrous sulphate in different forms is
Anhydrous: 3.65g/cm3
Monohydrate: 3g/cm3
Pent hydrate: 2.15g/cm3
Hex hydrate: 1.934g/cm3
The density of ferrous sulphate heptahydrate (FeSO4· 7H2O): 1.898 g/mL at 25°C.

Stability / Shelf Life
In moist air, ferrous sulphate rapidly oxidizes and becomes coated with brownish yellow ferric sulphate. The rate of oxidation is increased by the addition of alkali or by exposure to light.

Decomposition
When heated to decomposition it emit toxic fumes of sulphur oxide.

Refractive Index
INDEX OF REFRACTION: 1.471

Dissociation Constants: - pKa = -3
Drug Indication:- Ferrous sulphate is used for the prevention and treatment of iron deficiency anaemia in adults and children.

Therapeutic Uses
1. Initial response to iron therapy will confirm or negate diagnosis of iron-deficiency anaemia. Ferrous sulphate, 150-300 mg thrice daily, is given for 3 week. Beginning about a week after starting therapy, circulating haemoglobin should rise about 0.1-0.3 g % daily; less severe anaemia, less daily rise.
2. Supplementation with 30-60 mg of iron daily (i.e. 150-300 mg of ferrous sulphate) has been advocated for pregnant women and 0.3-0.6 mL of ferrous sulphate paediatric "drops" daily for low-birth-weight infants from 1 month until 1 year of age. Physician must use his judgement in this regard....
3. Hematinic.
4. MEDICATION (VET): In iron deficiency. Astringent.

Biological Half-Life[8]
The half-life of orally administered iron is not readily available in the literature, with total effects lasting 2-4 months (congruent with the red blood cell life span) with an onset of action of 4 days and peak activity at 7-10 days.

Mechanism of Action
Iron is required to maintain optimal health, particularly for helping to form red blood cells (RBC) that carry oxygen around the body. A deficiency in iron indicates that the body cannot produce enough normal red blood cells. Iron deficiency anaemia occurs when body stores of iron decrease to very low levels, and the stored iron is insufficient to support normal red blood cell (RBC) production. Insufficient dietary iron, impaired iron absorption, bleeding, pregnancy, or loss of iron through the urine can lead to iron deficiency. Symptoms of iron deficiency anaemia include fatigue, breathlessness, palpitations, dizziness, and headache. Taking iron in supplement form, such as ferrous sulphate, allows for more rapid increases in iron levels when dietary supply and stores are not sufficient. Iron is transported by the divalent metal transporter 1 (DMT1) across the endolysosomal membrane to enter the macrophage. It can then can be incorporated into ferritin and be stored in the macrophage or carried of the macrophage by ferroportin. This exported iron is oxidized by the enzyme to ceruloplasmin to Fe3+, followed by sequestration by transferrin for transport in the serum to various sites, including the bone marrow for haemoglobin synthesis or into the liver. Iron combines with porphyrin and globin chains to form haemoglobin, which is critical for oxygen delivery from the lungs to other tissues.

Coagulopathy is a hallmark of severe ferrous sulphate poisoning in humans and lab animals. At iron concentration comparable to those of previous animal investigations, the coagulopathy, in other words, the dose-related prolongation of the prothrombin, thrombin, and partial thromboplastin time, was reproduced in human plasma in vitro. Studies of the mechanism by which iron prevents a normal plasma coagulation revealed that the proenzymes of the coagulation cascade and fibrinogen were not damaged by iron. Fibrinogen coagulability and fibrin monomer aggregation were unaffected by very high iron concentration. Instead, thrombin was markedly inhibited by iron in its clotting effect on fibrinogen and, specifically, in its fibrin peptide A-generating capacity, the inhibitory effect being reversible upon iron removal by ethylene diamine tetra acetic acid chelation and gel filtration. Thrombin generation in the presence of iron was reduced as well, indicating an inhibition of one or several other enzymes of the intrinsic coagulation cascade. Because the amidolytic activity of human thrombin as well as factor Xa, kallikrein, and bovine trypsin was also reversibly suppressed by ferrous sulphate, it is considered likely that coagulopathy occurring in iron poisoning is the consequence of a general, physiologically important phenomenon: the susceptibility of serine proteases to non-transferrin-bound iron (3+).

The mechanism of acute iron cardio toxicity was investigated in isometrically contracting left atrial strips and right ventricular papillary muscles isolated from rabbit hearts. A 90 min exposure to iron (1.8 mm; as

ferrous sulphate) reduced the peak-developed tension and the maximal rate of tension development.

Iron is stored in the liver in the oxidized or ferric state & is tightly bound to protein as ferric ferritin. Xanthine oxidase appears to be involved in the conversion of ferric ferritin to ferrous ferritin. The reduced form of iron is less tightly bound to ferritin and thus is more easily released for utilization. Therefore, a possible inverse relationship between hepatic xanthine oxidase activity & hepatic iron storage exists. Theoretically, the xanthine oxidase inhibitor allopurinol should decrease the activity of xanthine oxidase & increase hepatic iron storage.

Use Classification
Agrochemicals - > Pesticides
Human Drugs - > FDA Approved Drug Products with Therapeutic Equivalence Evaluations
(Orange Book) - > Active Ingredients
Food Additives - > Nutrient supplement
Cosmetics - > Astringent

Industry Uses
Oxidizing agent.

Storage Conditions
Store in tight containers.

Reactivity Profile
Weak inorganic reducing agents, such as FERROUS SULFATE, react with oxidizing agents to generate heat and products that may be flammable, combustible, or otherwise reactive.

Toxicological Information
Effects during Pregnancy and Lactation
Summary of Use during Lactation
Iron is a normal component in human milk. Daily oral iron intake from prenatal vitamins or other multi-mineral supplements does not affect milk iron levels. Higher daily oral iron dosing has a minimal effect on milk iron levels and is not expected to cause harm to the breastfed infant if needed to treat the mother's anaemia, but it is not an adequate substitute for direct infant iron supplementation to prevent or treat infant anaemia. Pasteurization of milk by the Holder method reduces the concentration of iron in milk by about 6.5%.

Effects in Breastfed Infants
One-hundred thirty-one non-anaemic, lactating mothers in Ankara, Turkey randomly received 80 mg elemental iron as ferrous sulphate daily or placebo beginning 10 to 20 days postpartum and continued for 4 months. Haematological indices and biochemical iron status values were no different between the two groups of infants at the end of the study.

Effects on Lactation and Breast milk
Iron-enriched human milk fortifier intended for preterm hospitalized infant's increases milk microbial growth compared to iron-free fortifier under in vitro experimental conditions. The clinical consequences of these findings have not been evaluated. Such studies tested milk iron concentrations in the range of 13 to 14 mg/L which were common for fortifiers in the countries where the studies were conducted. Currently available U.S. premature infant milk fortifier provides a much lower iron supplemental dose resulting in a milk iron level of 3 mg/L above the mother's underlying milk level.

Adverse Effects
Occupational hepatotoxin - Secondary hepatotoxins: the potential for toxic effect in the occupational setting is based on cases of poisoning by human ingestion or animal experimentation.

Interactions
- Simultaneous administration of iron as ferrous sulphate reduced absorption and caused significant decrease in serum concentration of tetracycline, oxytetracycline, methacycline, and doxycycline in man.
- Oral ferrous sulphate appears to impair the GI absorption of various tetracycline, possibly because of chelation or other type of binding in the gut.
- It is suggested that the magnesium trisilicate either changes the ferrous sulphate into less easily absorbed iron salts, or increases its polymerization, thereby rendering it less easily absorbed. Sodium bicarbonate causes the formation of poorly absorbed iron complexes.

Drug-Food Interactions
- Avoid milk and dairy products. Take ferrous sulphate at least 2 hours before or after milk.
- Limit caffeine intake. Food and beverages containing caffeine may reduce iron absorption.
- Take at least 2 hours before or after calcium supplements.
- Take separate from antacids. Take ferrous sulphate at least 2 hours before or after antacids.
- Take with food. This may reduce gastric irritation.
- Take with foods containing vitamin C.
- Foods rich in vitamin C increase the absorption of iron.[8]

METHOD OF EVALUATION
- **Principle[4]**

As mentioned in the introduction, UV-visible absorption spectrophotometry provides a convenient method of determination of concentration of any substance which can be treated to form a coloured solution in which the colour intensity is proportional to the concentration of the substance. This experiment is based on the determination involving the formation of a complex species that absorbs in the visible region. The general procedure usually involves the following basic steps.

- Treatment of the properly prepared sample with a reagent to form a coloured Solution.
- Controlling factors influencing absorption by the coloured species.
- Measurement of absorbance of the coloured solution at the appropriate wavelength.
- Preparation of an absorbance-concentration plot (calibration plot) by measurements of the absorbance of the standard solutions of known concentrations.
- Estimation of concentration in the unknown sample corresponding to the absorbance measured by using the calibration plot.

In the determination of iron (II) in aqueous solutions, a tricyclic nitrogen heterocyclic compound, 1, 10-phenanthroline ($C_{12}H_8N_2$, ortho-phenanthroline or *o*-Phen) is used as the ligand that reacts with metals such as iron, nickel, ruthenium, and silver to form strongly coloured complexes. With ferrous ions (Fe^{2+}), it reacts in a ratio of 1:3 to form an orange red coloured complex $[(C_{12}H_8N_2)_3Fe]^{2+}$ in aqueous medium as per the following equation. $\mathbf{Fe^{2+} + 3\ Phen \longrightarrow Fe\ (Phen)_3^{2+}}$

The ligand is a weak base that reacts to form phenanthrolinium ion, $phenH^+$, in acidic medium. Accordingly, the complex formation may be represented as follows,

$$\mathbf{Fe^{2+} + 3PhenH^+ \rightleftharpoons Fe\ (Phen)_3^{2+} + 3H^+}$$

The molar absorption coefficient (ε) of the ferrous complex, $[(C_{12}H_8N_2)_3Fe]^{2+}$ so obtained is 11,100 $dm^3\ mol^{-1}\ cm^{-1}$ at the wavelength of maximum absorbance, λ_{max} = 508 nm. The large value is indicative of strong absorption by the complex and forms the basis of the quantitative determination of iron (II). The absorbance of the coloured complex is measured at 508 nm using a spectrophotometer or with the help of a filter photometer using a blue-green filter.

The colour intensity is not affected by change of pH over the range 2-9 and is also stable for a long time. However, a pH of about 4.5 is ordinarily recommended to prevent precipitation of iron salts. Further, the cations like $\mathbf{Ag^+}$, $\mathbf{Bi^{3+}}$, $\mathbf{Cu^{2+}}$, $\mathbf{Ni^{2+}}$, $\mathbf{Co^{2+}}$ and anions such as perchlorate, cyanide, molybedate and tungstate interfere significantly in this determination, therefore, these must be absent in the analyte solution.

As we are attempting to determine the concentration of iron (II) ions in the analyte sample, it must be free from any iron (III) ions which may be present due to the partial oxidation of the ferrous ions. This is achieved by adding a reducing agent before the coloured complex is formed. Ferric ion (Fe^{3+}) is reduced to ferrous state (Fe^{2+}) by hydroxylamine before complexation as per the following equation.

$$\mathbf{4Fe^{3+} + 2NH_2OH \rightarrow 4Fe^{2+} + N_2O + 4\ H^+ + H_2O}$$

Under these conditions the complex obeys Beer-Lambert's law in the range of the concentrations being determined (~0.5–2.0 ppm). The range for the validity of Beer Lambert's law can be determined by plotting a calibration curve by measuring the absorbance values for a series of standard solutions of the complex being determined at a fixed wavelength of 508 nm. The curve so obtained is then used for the determination of concentration of unknown solutions from a measurement of its absorbance at the same wavelength.[4]

Requirements
Lab Apparatus
Lab Chemical
Marketed Preparation Details

- **Brand Name: -** R.B. Tone Capsules**.**
- **Batch Number:-** E30814
- **Mfg. lic:-** JK/01/05-06/76
- **Manufactured By: -** Medley Pharmaceutical pvt. Ltd

Fig. Marketed Preparation required for the evaluation.

Sr. No.	Apparatus	Quantity	Sr. No.	Chemical
1.	UV Spectrophotometry	1	1.	Ferrous ammonium sulphate hex hydrate
2.	Volumetric flasks (1 dm^3)	1	2.	1, 10-Phenanthroline
3.	Volumetric flasks (50 cm^3)	6	3.	Hydroxylamine hydrochloride
4.	Graduated pipette, 10 cm^3	1	4.	Sulphuric acid
5.	Pipettes (5, 10 cm^3)	1	5.	Acetic acid
6.	Weighing Balance	1	6.	Sodium acetate
7.	Beaker	4		

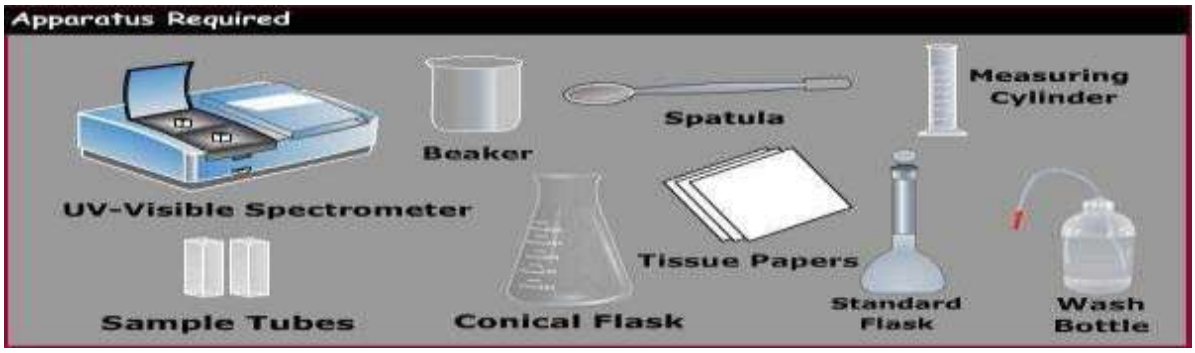


Fig. Apparatus required for the evaluation.

1. Experimental Work

Reagent preparation[4]

1. **Standard ferrous solution:** Accurately weighed and transfer 100mg of ammonium iron sulphate in 250ml of volumetric flask and added 25 ml of diluent (10% HCL) and sonicated for 5 min, after sonication volumetric flask cool at room temperature and volume make up with distilled water and mixed well.
2. **1, 10-phenanthroline**: - Accurately weighted and transfer 0.25g of the 1, 10- phenanthroline in 100 ml of volumetric flask and added 25 ml of distilled water. And heated on water bath at 60^0 C for 1hr ad cool at room temperature. And volume make up with water. The reagent can be stored in a bottle.
3. **Hydroxylamine hydrochloride: -** Accurately weighted and transferred 10g of hydroxylamine HCL in 100ml of volumetric flask and added 25 ml of water, sonicated for 5 min and cool at room temperature and volume make up with water and mixed well.
4. **Acetic acid-sodium acetate buffer (pH = 4.5):-** weighed and transferred 27.5 g of sodium acetate anhydrous added in 1000ml of water and adjusted pH with glacial acetic acid.
5. **Diluent** (10% HCL):- 10ml of HCL in 100ml of water.
6. **Marketed Preparation Details**

- **Brand Name: -** R.B. Tone Capsules**.**
- **Batch Number:-** E30814
- **Mfg. lic:-** JK/01/05-06/76
- **Manufactured By:-** Medley Pharmaceutical pvt. Ltd

Dissolution parameters[9]

a. **Speed** -100RPM
b. **Volume of media**: - 900ml of 0.01N HCL
c. **Time** -30 min, 1hr, 2hr, 3hr,
d. **Apparatus** –USP Type 1- Basket
e. **Temperature** – 37^0 C

Media preparation

0.01N HCL: - weighed and transferred 0.85ml of Conc. HCL added in a 1000 ml of water.

Sample preparation

Withdrawn 10ml adequate sample transferred in 10 ml of test tube and centrifuged the sample at 200 RPM for 10min and filter through a 0.45 µm filtered and proceed for colour development method.

Colour development chart[10]

Chemicals	Blank preparation	Standard preparation	Sample preparation
Solution	-	2ml standard solution	2ml sample solution
10% Hydroxylamine HCL	3ml	3ml	3ml
pH 4.5 Sodium acetate buffer	7ml	7ml	7ml
1,10- phenanthroline indicator	2ml	2ml	2ml
Distilled water	q. s	q. s	q. s
Observation	No colour developed	Orange-Red colour developed	Orange-Red colour developed

Blank preparation

All the chemicals were added in 50 ml of Volumetric Flask and Volume make up with distilled water.

Standard preparation

Added 2ml of standard and added 3 ml of Hydroxylamine HCL and 7 ml of pH 4.5 Acetate Buffer solution and stand for 10 min and after 10 min , added 2ml of 1,10 Phenanthroline indicator in 50 ml of Volumetric flask and the Volume was make up with distilled water.

Sample preparation

Added 2ml of sample and added 3 ml of Hydroxylamine HCL and 7 ml of pH 4.5 Acetate Buffer solution and stand for 10 min and after 10 min, added 2ml of 1,10 Phenanthroline in 50 ml of Volumetric flask and then Volume was make up with distilled water.

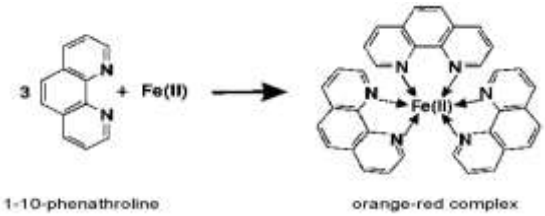
Instrument Details

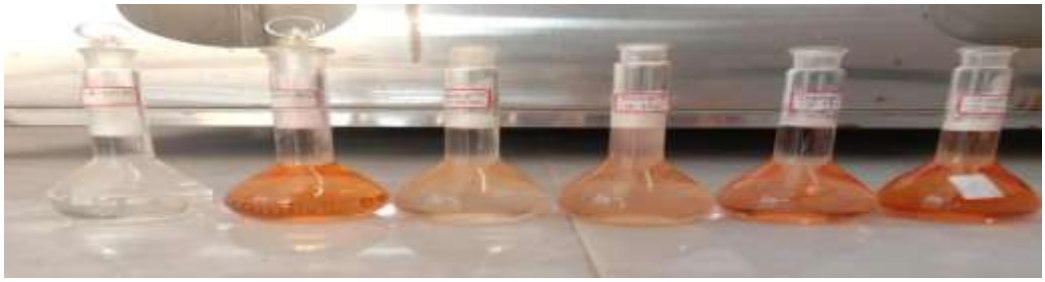
- **Instruments ID**
- **Analytical Weighing Balance ID**: 11812939055
- **PH Meter ID**: ProfiLine pH 3110
- **Basket Type Dissolution Apparatus ID**: 1912
- **UV Spectrophotometer ID**: LJ-2371 UV

2. Observation

Reaction of ferrous sulphate with 1, 10-phenanthroline[11]

By the colour development method following colours was developed.





Solution Stability

1. **Day –** Stable no change
2. **Day -** Stable no change
3. **Day-** Stable no change

Net Content

1. Filled 10 capsule weight = 6,800 Mg
2. Empty 10 capsule weight = 1000 Mg

Net content of 10 capsule = Filled 10 capsule weight - Empty 10 capsule weight ÷10
= 6,800 - 1000
= 5,800 Mg

3. CALCULATION AND RESULT

Standard Absorbance

Sr. No.	Concentration (PPM)	Absorbance
1	6.97	0.458
2	6.97	0.458
3	6.97	0.458
4	6.97	0.458
5	6.97	0.458

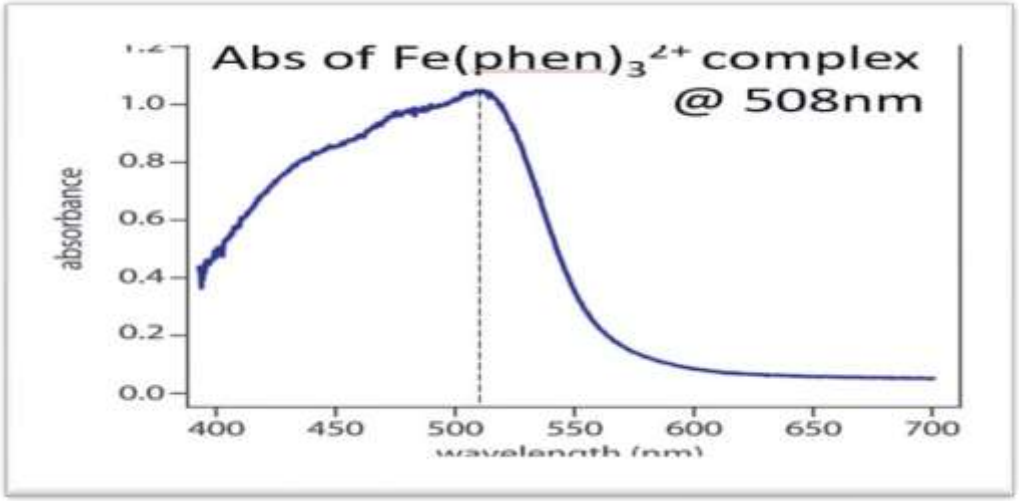
Absorbance of Sample

Sr. No.	30 Min	1 hour	2 hour	3 hour
	Absorbance	Absorbance	Absorbance	Absorbance
1	0.432	0.445	0.447	0.448
2	0.430	0.440	0.448	0.449
3	0.429	0.445	0.446	0.450
4	0.425	0.441	0.445	0.451

Average of Standard

Sr. No	30 Min	1 hour	2 hour	3 hour
1	**94.32**	**97.16**	**97.59**	**97.81**
2	**93.88**	**96.06**	**97.81**	**98.03**
3	**93.66**	**97.16**	**97.37**	**98.25**
4	**92.79**	**96.28**	**97.16**	**98.47**

Wavelength[12]



4. CONCLUSION

Here I have come to the end of the project on the topic

- Determination and Evaluation of Ferrous Sulphate in Marketed Preparation (Capsule) By Colour Development UV Spectroscopy.
- As we have find that ferrous sulphate is an photosensitive molecule which changes its colour on reacting with light and solution like 10% Hydroxylamine HCL, at pH of 4.5 Sodium Acetate buffer solution and 1,10 Phenanthroline Indicator which gives Orange- Red colour on reacting with them.
- We also found that various result of Dissolution and Absorption of our sample solution with Marketed product.
- We have find that after dissolution of Ferrous sulphate molecule at 200 RPM at Various time Intervals like 30min, 1hrs. 2hrs show the development of the Orange-Red Colour'
- As well as we have got different different Absorption result of 4 dilution in UV Spectroscopy at the Wavelength of 508 nm as Compared with Marketed Preparation.

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