

Experiment	Quantitative colorimetric determination of Fe^{2+} metal ions using conventional and smart phone digital-imaging methods
Problem definition	Excess Iron in drinking water cause negative effects like poor skin and hemochromatosis (damage to liver, heart and pancreas). There are plenty of other health concerns associated with too much iron intake, and hence it is important to estimate the amount of iron in waste samples.
Methodology	Fe and 1,10-phenanthroline forms a stable deep-red colored complex. With increasing concentration of Fe in solution, its color intensity also increases, which in turn is a function of color coordinates (Red, Blue and Green, RGB) in the image taken using mobile phone camera.
Solution	Estimation of Fe concentration in different water samples can be found out using colorimetry technique from the calibration graph plotted based on different known Fe concentrations.
Student learning outcomes	Students will learn to perform colorimetric method, perform RGB response analysis and analyze Fe composition in different grades of steel

(i). Principle:**(a). Colorimetric method:**

Photo-sensitive measurements are expressed in terms of absorbance, (A) as given in Eq. (1). Further, the linear relationship between absorbance (A) and concentration of the analyte

$$\epsilon cl = A = \log(I_0/I) \quad \dots (1)$$

Where, I_0 is the incident light power, I the transmitted light power, ϵ = molar absorptivity, c = concentration of analyte and l = thickness of the solution.

(b). Digital-imaging method:

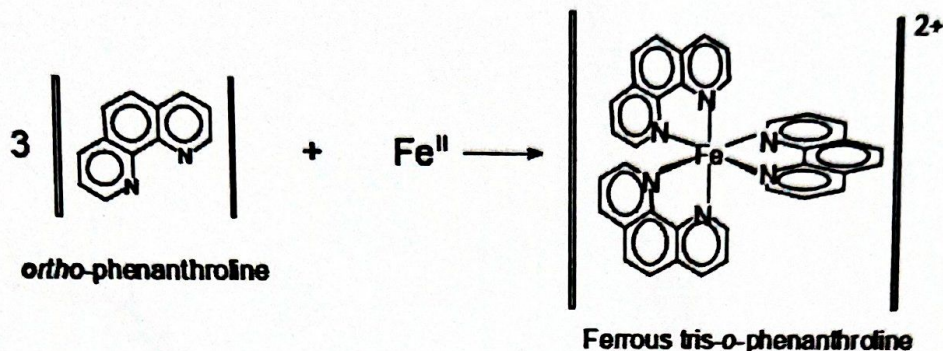
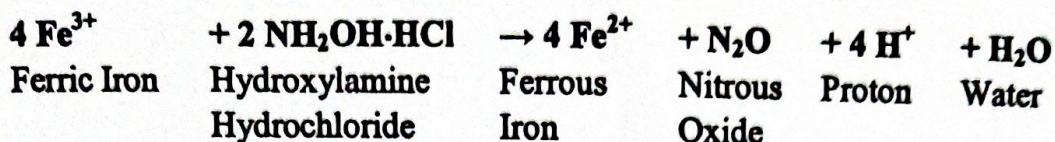
The color and intensity of digital image are usually 24 bit data (8 bit R + 8 bit G + 8 bit B) forming an additive color space, in which R, G and B lights are added together in various combinations to reproduce a broad range of colors. By using combination of R, G and B intensities, many colors can be displayed. The intensity of each color has 256 levels (from 0 to 255). The value of $R = 0$, $G = 0$, $B = 0$ refers to pure black while $R = 255$, $G = 255$, $B = 255$ is pure white. With this system, unique combinations of R, G and B values are allowed, providing for millions of different hue, saturation and lightness shades. These extensive dynamic colors of images provide the database for quantitative analysis. The goal of this study is to employ digital images-based colorimetry for the determination of Ni^{2+} concentration in aqueous samples.

The concentration of analyte is a function of color coordinates: $c = RGB \quad \dots (2)$

(ii) Scheme of the reaction and requirements

A complex of Iron (II) is formed with 1,10-phenanthroline $[\text{Fe}(\text{C}_{12}\text{H}_8\text{N}_2)_3]^{2+}$ and the absorbance of this colored solution is measured with a colorimeter. Hydroxylamine (as the

hydrochloride salt to increase solubility) is added to reduce any Fe^{3+} to Fe^{2+} and to maintain it in that state. The spectrum is plotted to determine the absorption maximum.



Reagents and solutions: Ferrous Ammonium Sulphate (10 ppm), 1,10-phenanthroline, Hydroxylamine hydrochloride and Sodium acetate solutions

Instrument: Colorimetry and smartphone

(iii). Procedure:

(a). Colorimetry method: Take 6 standard 50 mL volumetric flasks (to prepare 5 known and 1 unknown solution). Fill the burette with Fe stock solution (10 ppm). Add 2.5, 5, 10, 15 and 20 mL of the Fe solution in burette to the std. flasks to get 0.5, 1, 2, 3 and 4 ppm of Fe(II) solutions. The unknown sample will be furnished in another 50 ml volumetric flask. Further, add 0.5 mL of hydroxyl ammonium chloride solution followed by 2.5 ml of 1,10-phenanthroline using a burette. The Fe(II) -phenanthroline complex forms at pH 2 to 9. Add 2.5 mL of sodium acetate buffer to neutralize the acid present and adjust the pH to a value at which complex forms. After that, make up the 50 mL mark in std. flask with distilled water. Allow at least 15 minutes before making absorbance measurements so that the color of the complex can fully develop. Once developed, the color is stable for hours. Obtain the absorption spectrum of the Fe solutions by measuring the absorbance from about 400 to 600 nm.

Record these absorbance readings in Table 1. Draw a calibration graph taking concentration of Fe^{2+} (in ppm) as X-axis and absorbance readings as Y-axis. A straight line that passes through the origin is an indication that the measured data obeys Beer's Law. From the calibration plot, measure the concentration of Iron in the given unknown sample.

Digital imaging method: The prepared standard solutions are lined up along with unknown concentration sample and blank. Using a white paper as background, take a photograph of the samples by holding the camera around 50 cm away. The calibration curve will be constructed through the RGB values of analytical response with different concentration of Fe^{2+} ions using smartphone app (RGB Tool). In the plotted graph, RGB response varies linearly vs the analyte concentration. In order to get precise analysis, follow the steps given below:

Transfer prepared standard solution and unknown solution into different colorimetric test tubes

↓
Take image of all test tube solution using smart phone camera

↓
Open the image processing app

↓
Go to gallery, open the image stored in app and extract RGB values for each image/conc.

↓
Process the RGB values (R/G) or (R/B) or (G/B) etc., till to get linear response

↓
Plot the calibration curve using RGB linear response vs concentration

↓
Find the unknown conc using the calibration curve

Table 1: Experimental Data

S. No.	Data collected from Colorimetric device		Data collected from smartphone device*				
	Conc (ppm)	Abs (Y-axis)	R	G	B	G/B	B/G
1.	1	0.70	62	132	91	1.45	0.58
2.	2	0.87	26	13	4	3.25	0.3
3.	3	0.90	60	42	30	1.4	0.71
4.	4	0.95	39	25	19	1.31	0.76
5.							
	Unknown	0.77	20	16	1	10	0.1

*If your solution looks Red or blue or green then the corresponding ratio can be ignored and select RGB data which is linear with concentration of analyte for plotting calibration graph (Y-axis)