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Evaluating Potassium Permanganate: A Colorimetric Assay

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ABSTRACT
This quantitative colorimetric assay of potassium permanganate is carried out to determine simple, safe, effective method to evaluate the wavelength of maximum absorbance and to verify the Beer's Lambert's Law. The study involves evaluating the biochemical constituents present in potassium permanganate which can be further implied for therapeutic purposes.
Keywords: Colorimeter, absorbance, Beer's Lambert's law

I. INTRODUCTION:

A Colorimeter involves the measurement of Color and is the widely used method for finding the concentration of biochemical compounds. It Measures absorbance and wavelength between 400 to 700 nm (nanometer) i.e. from the visible spectrum of light of the electromagnetic spectrum. Light falling on a colored solution is either absorbed or transmitted. [1] A colored solution absorbs all the colors of white light and selectively transmits only one color. This is its own color. A colorimeter is based on the photometric technique which states that when a beam of incident light of intensity I_0 passes through a solution, a part of the incident light is reflected (I_r), a part is absorbed (I_a) and rest of the light is transmitted (I_t). [2]

Thus,
$I_0 = I_r + I_a + I_t$

In colorimeter, (I_r) is eliminated because of the measurement of (I_0) and It is sufficient to determine the (I_a). For this purpose, the amount of light reflected (I_r) is kept constant by using cells that have identical properties. (I_0) & (I_t) is then measured.

The mathematical relationship between the amount of light absorbed and the concentration of the substance can be shown by the two fundamental laws of photometry on which the colorimeter is based.

Beer's Law

This law states that the amount of light absorbed is directly proportional to the concentration of the solute in the solution.

$Log_{10}\ I_0/I_t = ab$

where, a_s = Absorbency index c = Concentration of Solution

Lambert's Law

The Lambert's law states that the amount of light absorbed is directly proportional to the length and thickness of the solution under analysis.

$A = \log_{10}\ I_0/I_t = a_s b$

where,

A = Absorbance of test a_s=Absorbance of standard b=length/thickness of the solution
In simplified form,

The working principle of the colorimeter is based on Beer-Lambert's law which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution.[3] [4]

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II. PARTS OF COLORIMETER

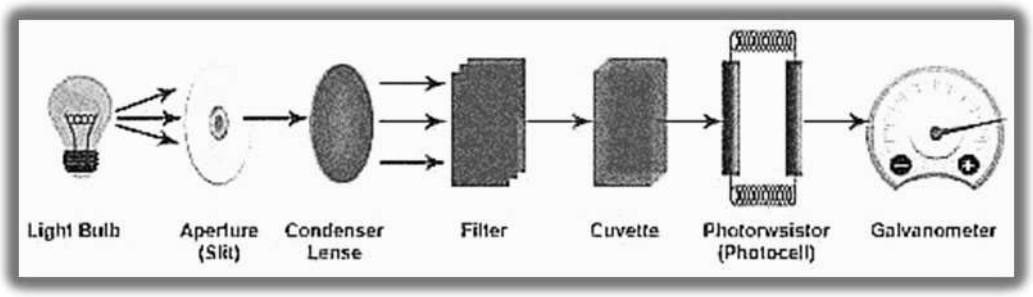

Fig:1 Parts of Colorimeter

There are 5 essential parts in a calorimeter

i. **Light Source** – The most common source of light used in colorimeter is a tungsten filament.
ii. **Monochromator** – To select the particular wavelength filter or monochromators are used to split the light from the light source.
iii. **Sample holder** – Test tube or Cuvettes are used to hold the color solutions they are made up of Glass at the visible wavelength.
iv. **Photo Detector System** – when light falls on the detector system, an electric current is generated, this reflects the Galvanometer reading.
v. **Measuring device** – The current from the detector is fed to the measuring device, the Galvanometer, shows the meter reading that is directly proportional to the intensity of light.[5] [6]

III. WORKING OF COLORIMETER

When using a colorimeter, it requires being calibrated first which is done by using the standard solutions of the known concentration of the solute that has to be determined in the test solution. For this, the standard solutions are filled in the cuvettes and placed in the cuvette holder in the colorimeter. There is a ray of light with a certain wavelength that is specific for the assay is directed towards the solution. Before reaching the solution the ray of light passes through a series of different filters and lenses. These lenses are used for navigation of the colored light in the colorimeter and the filter splits the beam of light into different wavelengths and allows the required wavelength to pass through it and reaches the cuvette containing the standard or test solutions. It analyzes the reflected light and compares it with a predetermined standard solution.[7] [8]

When the monochromatic light (light of one wavelength) reaches the cuvette some of the light is reflected, some part of the light is absorbed by the solution and the remaining part is transmitted through the solution which falls on the photodetector system. The photodetector system measures the intensity of transmitted light and converts it into the electrical signals that are sent to the galvanometer.[9] [10]

The galvanometer measures the electrical signals and displays them in the digital form. That digital representation of the electrical signals is the absorbance or optical density of the solution analyzed. If the absorption of the solution is higher than there will be more light absorbed by the solution and if the absorption of the solution is low then more lights will be transmitted through the solution which affects the galvanometer reading and corresponds to the concentration of the solute in the solution. By putting all the values in the formula given in the below section one can easily determine the concentration of the solution.

Here is the formula used for determining the concentration of a substance in the test solution.

$A = €cl$
For two solutions i.e. Test and standard,
$€$ = Constant
l = Constant (using the same Cuvette or Standard cell) $AT = CT$ ….. (i)
$AS = CS$….. (ii)
From (i) & (ii),
$AT \times CS = AS \times CT$
$CT = (AT/AS) \times CS$
Where,
CT = Concentration of the Test solution
AT = Absorbance/ Optical density of the test solution

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IV. HANDLING OF UV-VISIBLE SPECTROPHOTOMETER

Preparation of SOP for handling of UV-VISIBLE Spectrophotometer

i. Fill 2 of the same cuvettes each with about 2mL of blank solution. Hold the cuvette from the top to prevent tampering with the measurements, and wipe the sides with a lab tissue.
ii. Open panel door and place the cuvettes with blank solution in the cuvette holders. Make sure to use the appropriate orientation for the cuvettes you're using. Also make sure that the cuvettes used for the auto zeroing are the same cuvette you use for the sample reading. If using a standard cuvette, see any orientation of the cuvette in the holder is acceptable, just make sure you wipe the cuvette's sides. If using a micro cuvetter, see figure 9.3, the microcuvette MUST be oriented in the holder so the the 1cm path length goes from left to right.
iii. Press the AUTO ZERO key, then press ENTER.
iv. When the the Auto Zero is complete, open the panel door and remove the front cuvette.
v. Do not replace cuvette in rear holder.
vi. Using the same cuvette style, fill an empty cuvette with about 2-ml of the sample.
vii. Clean the cuvette with a lab tissue.
viii. Place in front cuvette holder, using the appropriate orientation and close the panel door.
ix. Press START to take a reading.
x. Record the results or press COPY for a hard copy printout. Note: If the initial sample OD reading is greater than 1.0, the sample should be diluted until it reads below 1.0 and then multiply by the dilution factor to obtain the absorbance value.
xi. Open panel door and remove test sample from front cuvette holder.
xii. To test additional samples: Place cuvettes in front holder and press start for a reading.
xiii. Record results, or press COPY for a hard copy printout.
xiv. Press RETURN to bring you back to step 8.9. Note: This will erase your old data.
xv. Press FILE to return to the original screen.
xvi. Remove cuvettes remaining in holders.
xvii. Flip power switch located on the side, to turn off the machine.[11] [12]

V. ESTIMATION OF SPECTROPHOTOMETRIC ASSAY OF KMNO4 SOLUTION

i. Potassium permanganate is used to treat variety of skin wounds and infections. It is used topically and is excellent oxidizing agent with healing properties. Procedure for the assay includes preparation of 100 mL of a stock standard solution of 0.001M KMnO4. Accurately weigh 126 mg solid KMnO4. Transfer quantitatively to a 100 mL volumetric flask and fill to the mark with water. This is the stock solution.
ii. Prepare six standards in 100 mL volumetric flask with concentrations of 0.0004 M (solution #1), 0.0002 M (solution #2), 0.0001 M (solution #3) and 0.00005 M (solution #4) by diluting the stock solution prepared in Step I and this is termed as serial dilution.
iii. Rinse one of the cuvettes with distilled water and fill it with water. Put the cuvette in the sample: Compartment. This is the reference solution. Set the wavelength to 400 nm
iv. Rinse a second cuvette once with distilled water and once with standard solution #1, then fill it with standard solution #1 (0.001M KMnO4). Place the cell in the sample compartment, measure the absorbance at 410.
v. Repeat this procedure (steps 3 and 4 above) for the two cuvettes at wavelengths 420, 430,440, 450. 600 nm. First setting A=0 for the cuvette with water, then measuring A for the cuvette with 0.001 M KMnO4, recording the absorbance at each wavelength. Record in data table.
vi. Prepare a graph of absorbance A vs. wavelength and determine max (maximum wavelength).
vii. Set the wavelength at 550 nm (max). Place the cuvette with distilled water in the cell compartment and again set the absorbance to zero.
viii. Measure and record the absorbance of each of the six standard solutions, starting with the most Dilute standard. After each measurement, rinse the cuvette with the next standard, not with distilled Water.
ix. Draw a plot having X-axis as concentration (mole/L) and Y-axis as Absorbance at max (550nm).

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x. Use Beer's law to calculate e for KMnO4, given the cell width (path length 1) to be 1 cm.

VI. OBSERVATIONS & CALCULATIONS

Sr.no	Wavelength (nm)	Absorbance
1.	410	0.056
2.	420	0.033
3.	430	0.037
4.	440	0.077
5.	450	0.237
6.	460	0.239
7.	470	0.401
8.	480	0.585
9.	490	0.940
10.	500	1.173
11.	510	1.616
12.	520	1.751
13.	530	2.12
14.	540	1.81
15.	550	2.019
16.	560	1.287
17.	570	1.168
18.	580	0.686
19.	590	0.311
20.	600	0.217

Absorbance of solution at different concentrations:
At λmax 550nm

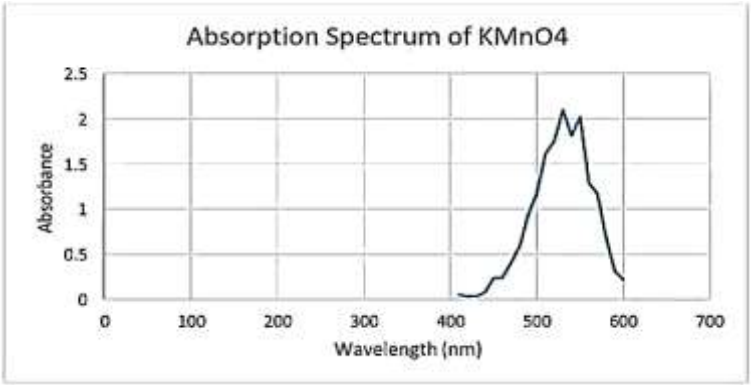

Fig:2 Absorption Spectrum of KMnO4

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Sr.no	Concentration (M)	Absorbance
1.	0.000025	0.033
2.	0.00005	0.186
3.	0.0001	0.352
4.	0.0002	0.531
5.	0.0004	0.665
6.	0.001	2.018

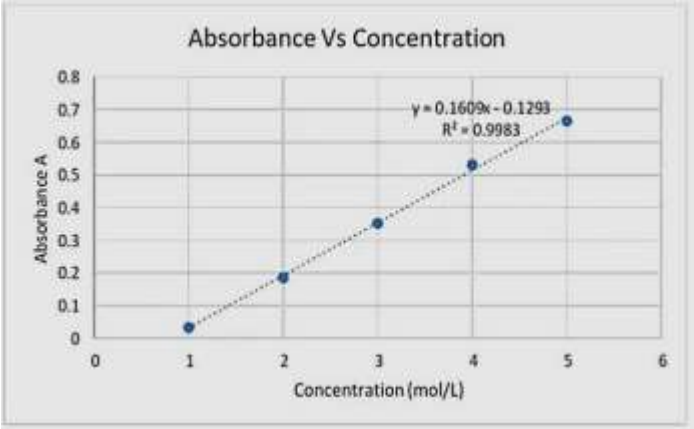

Fig:3 Absorption Vs Concentration

VII. RESULT

The result of the experiment shows greater the concentration greater will be the absorbance. The value of absorbance increases as we increase the concentration of KMnO4 at λmax i.e. is equal to 550nm. Result of the experiment verify Beer Lambert's law.

VIII. CONCLUSION

On the graph, trend line does not pass exactly through zero. This is as expected it may be due to the statistics of the data point, which are not exactly on the straight line due to random errors in the concentration and/or the absorbance reading, or to the fact that there is a remaining solution absorbance (relative to the blank) for the standards. Finally, trend line slope is the Molar extinction coefficient (Molar Absorptivity). The equation y=0.1609x -0.1293 for this application should be read as A= E.C+constant, with the constant intercept.

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