National Initiative on Undergraduate Science - Teacher Development Workshop

(NIUS-TD Workshop)

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Homi Bhabha Centre for Science Education

Tata Institute of Fundamental Research

In collaboration with

Gogate Joglekar College, Ratnagiri

This workshop is organized by Centre for Excellence in Science and Mathematics Education (CESME-HBCSE) and set up under thePandit Madan Mohan Malaviya National Mission for Teachers and Teaching Programme

Exploring estimation of Ca (II)

For tracking colors of solutions					
Glassware	Number	Chemicals (stock solutions)	Concentrations	Quantity	Safety Symbol
Test tubes	12	Ca (II) solution	0.01 M	5 mL	
Test tube stand	1	Mg (II) solution	0.01 M	5mL	
Beakers (25 mL or 50 mL)	2	Indicator solution	0.0027 M	5 mL (to be used for both tracking experiments and Beer Lambert's Law)	
Measuring cylinder (10 mL)	1				
Graduated pipette- 1mL	1	Na_2EDTA solution	0.01 M	3 mL	
Colorimeter	1	Buffer pH 8		30 mL	
Cuvettes for colorimeter	2	Buffer pH 10		70 mL (to be used for both tracking experiments and Beer Lambert's Law)	
		Buffer pH 12		30 mL	

Hazard Symbols and their meanings

Corrosive 

Flammable 

Irritant 

Toxic 

Hazard to environment 

Common items
Distilled water, Micropipettes- 100-1000µL and 10-100µL, parafilm, tissue rolls

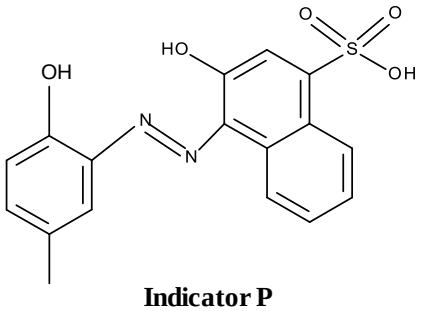
Exploring estimation of Ca (II)

In this experimental module, we will explore one/two indicators that can be used for estimation of Ca(II) and since the solutions are highly coloured, visible spectrophotometer will be used to track the prepared solutions and the likely reactions. This experiment has several subparts. We request you to observe the solutions carefully for their colours by naked eyes in addition to the spectrophotometric measurements.

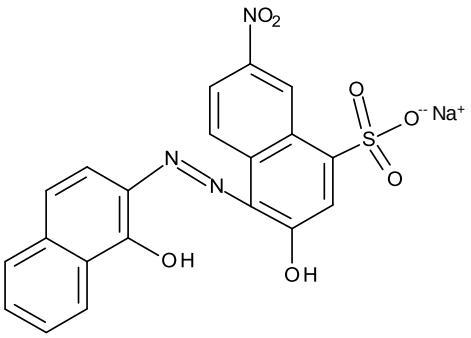
The subparts of the experiment are as follows -

1. Tracking colour of Indicator solutions in buffer medium with different pH and over time.
2. Is Beer- Lambert law valid for indicator/s solutions at pH 10?
3. Tracking colour of Indicator + metal ion(s) solutions in buffer medium with different pH and over time
4. Tracking colour of solution containing Indicator + Ca (II) in presence of Mg (II) at fixed pH

The structures of the two indicators are as follows. However, you have only **one** of these indicators on your table.



Indicator P



Indicator Q

This is a group activity. As a group, all of you are expected to read the given experimental module, discuss and understand what exactly needs to be done in the laboratory. You may have to do some calculations. Each small group will conduct the trial in the laboratory independently. The visible spectrophotometer/colorimeter and how to use it will be demonstrated to you in the lab on Day 2 when you start the lab work

For this experiment, you will be primarily using micropipettes for volume transfer for indicator solution, metal ions solution Na_2EDTA and distilled water

1. Tracking colour of Indicator solution in buffer medium with different pH and over time

In this sub-part, we will explore the solutions of indicators in buffer mediums with different pH (8, 10 and 12) over a short time period. For the same, we need to prepare solutions in test tubes as described below using the supplied stocks on your table. You will note the color of the solution. **In addition, please take photograph of the solutions just prior to the measurement. These photographs will be used for describing colour of the solutions and will be needed for later parts.** You are expected to scan the prepared solutions at time t = 2-3 minutes, 15 minutes and 30 minutes. Note the λ_{max} and the corresponding absorbance (A). The prepared solutions have to be covered using parafilm strips as they have to be stored for the observations for at least 30 mins. The experiment will be performed for only one indicator provided to you on your table.

Note: In some cases you may obtain more than one λ_{max} and you report both in the following table.

Solutions to be prepared – 5 mL buffer of given pH + 0.2 mL indicator P or Q + 0.8 mL distilled water

Table I: Study of indicator solutions at different pH

	Buffer = pH 8			Buffer = pH 10			Buffer = pH 12		
Time (t) in minutes	Color	λ_{max}	A	Color	λ_{max}	A	Color	λ_{max}	A
2-3									
15									
30									

1. Look at the photographs of the solutions at time t = 2-3 minutes for pH= 8, 10 and 12. Do these solutions have same colors when seen by naked eyes ? Yes ☐ No ☐

The following table gives the list of the absorbed/transmitted color and the corresponding wavelengths of absorption. You will need this table to answer the following questions

Absorbed Wavelength (nm)	Absorbed Color	Transmitted Color	Absorbed Wavelength (nm)	Absorbed Color	Transmitted Color
400	violet green	yellow	530	green	purple
450	indigo	yellow	570	yellow-green	dark blue
480	blue	orange	600	orange	blue
490	blue-green	red	650	red	green

2. Using the above table and the colour recorded by you in **Table I**, indicate the approximate wavelength that will be absorbed by the solutions at pH=8,10 and 12 (please answer this question only for solutions w.r.t. t = 2-3 minutes.)

pH 8		pH 10		pH 12	
Color of solution	Wavelength that will be absorbed	Color of solution	Wavelength that will be absorbed	Color of solution	Wavelength that will be absorbed

3. Now look at the observed λ_{max} for these solutions recorded in Table **I**. Whether these λ_{max} match with your answers in question **2**.?

4. From your measurements, what is your conclusion about -

a) Trend in λ_{max} for solutions at different pH.

t = 2-3 mins

t = 15 mins

t = 30 mins

Overall conclusion

b) Using your answers in 4a), comment about the trend in absorbance of the solutions at different pH.

t = 2-3 mins.

t = 15 mins.

t = 30 mins.

Overall conclusion

2. Is Beer- Lambert law valid for indicator/s solutions at pH 10?

We will now investigate whether indicator solutions with varying concentrations follow the Beer – Lambert law.($A = \varepsilon_o bc$, A= absorbance, ε_o = molar absorbance coefficient, b= path length in cm and c= concentration in mol L^{-1}) For this study, we will keep the pH constant, that is, pH=10. Such a study is important before we check suitability of indicator for the metal ion(s) using visible spectrometer.

You will prepare solutions with different concentrations using the stock solution of the given indicator (**P** or **Q**). The concentrations to be prepared are given in the table below. These solutions are prepared in test tubes and the total volume of each solution is **5 mL**. You will calculate the volume of indicator and water required for preparing each solution. Like previous subpart, you need to scan the spectra of each solution. For each solution, note the λ_{max} and the corresponding absorbance.

Table II: Observation Table

Final Conc. of indicator (M)	Volume of 0.0027M indicator (µL)	Volume of buffersolution pH=10 (mL)	Volume of distilled water (µL)	λ_{max}	Absorbance at λ_{max} value
5.40×10^{-5}		2			
4.86×10^{-5}		2			
4.32×10^{-5}		2			
3.78×10^{-5}		2			
3.24×10^{-5}		2			
2.7×10^{-5}		2			
2.16×10^{-5}		2			
1.62×10^{-5}		2			
1.08×10^{-5}		2			
0.80×10^{-5}		2			
0.54×10^{-5}		2			

1. Plot the appropriate a graph by identifying dependent and independent variable and please observe its nature. Briefly write your observations about the plot.

2. What is your conclusion about the question- whether the above concentration range obeys the Beer-Lambart law?

3. Can you calculate the ε_o = molar absorbance coefficientfrom the data obtained by you or using the graph plotted by you. If yes calculate the value ε_o.

3. Tracking colour of Indicator + metal ion(s) solutions in buffer medium with different pH and over time

In this part, you will explore solutions containing indicator and metal ion (only Ca (II) and only Mg (II)) in buffer mediums with different pH (pH 8, 10 and 12). You will take measurements over a short time period of 30 mins. The solutions are prepared in test tubes as given below using the supplied stock solution on your table. **Like subpart 1, you will take photographs of the prepared solutions.** You will note the color of the solution, the λ_{max} and the corresponding absorbance (A). You are expected to do measurements at time t = 2-3 minutes, 15 minutes and 30 minutes.The solutions have to be kept covered using parafilm strips and stored for the observations to be made over a period of time.

Solution:- 5 mL buffer + 0.2 mL indicator P/Q + 0.27 mL Ca (II) + 0.53 mL distilled water

Table III: Study of solutions containing indicator + Ca (II) ions at different pH

Time (t) in minutes	pH 8			pH 10			pH 12		
	Color	λ_{max}	A	Color	λ_{max}	A	Color	λ_{max}	A
t = 2-3									
t = 15									
t = 30									

You will compare the photographs of solutions taken in subpart **1** with those taken in this subpart. This comparison is done only for time t = 2-3 minutes. Remember for subpart **1,** your solution contained only the indicator in buffer medium at different pH**.**

1. For which pH (out of 8, 10 and 12) - the colour of two solutions, that is, (indicator) and (indicator + Ca(II) ions) is different

2. Now compare the λ_{max} of the solutions at pH 8, 10 and 12 for the solutions, that is, (indicator) and (indicator + Ca(II) ions). Do your observations regarding colors match with the λ_{max} change? Comment on your comparisons.

3. Based on your answers in **1** and **2**, indicate in words the possible reaction happening in solutions

Perform similar trial now with Mg(II) solution

Solution: 5 mL buffer + 0.2 mL indicator P/Q + 0.27 mL Mg (II) + 0.53 mL distilled water

Table IV: Study of solutions containing indicator + Mg (II) ions at different pH

Time (t) in minutes	pH 8			pH 10			pH 12		
	Color	λ_{max}	A	Color	λ_{max}	A	Color	λ_{max}	A
t = 2-3									
t = 15									
t = 30									

You will compare the photographs of solutions taken in subpart **1** with those taken in this subpart. This comparison is done only for time t = 2-3 minutes. Remember for subpart **1,** your solution contained only the indicator in buffer medium with different pH.

4. For which pH (out of 8, 10 and 12) - the colour of two solutions, that is, (indicator) and (indicator + Ca(II) ions) are different?

5. Based on your answer in **4**, indicate in words the possible reaction happening in solutions

6. Write your overall conclusion for this experiment.

You will need photographs of **subpart 3** , i.e., solution having indicator (P or Q) + Ca (II) ions at pH 10 for comparing the colors observed for the following **subpart 5**. The comparison will done only for photographs taken for solutions at t = 2-3 minutes only.

4. Tracking colour of solution containing Indicator + Ca (II) in presence of Mg (II) at fixed pH

In this part you will explore solutions containingfixed amount of indicator (P or Q) solutionandCa (II) in presence of different concentrations of Mg (II). We will keep the fixed pH(=10) for these observations and measurements. The solutions to be prepared are indicated in the table. Please carefully read the description of the solutions. **You will observe that each solution also contains Na_2EDTA.** Also check whether you have indicator **P** or **Q** as the solutions composition is different for the indicator**.** The measurements are done only for t = 2-3 mins. Take photographs of these solutions. **Kindly do not alter the sequence of addition of different reagents and keep observing colors.**

	System (Indicator P)	Color	λ_{max}(s)	A (s)
A	5 mL buffer + 0.1 mL indicator P (**observe colour**) + 0.08 mL Na_2EDTA + 0.054 mL Mg (II) (**observe colour**) + 0.27 mL Ca (II) + 0.59 mL distilled water (**observe final colour**)			
B	5 mL buffer + 0.1 mL indicator P (**observe colour**) + 0.13 mL Na_2EDTA + 0.027 mL Mg (II) (**observe colour**) + 0.27 mL Ca (II) + 0.473 mL distilled water (**observe the final colour**)			
C	5 mL buffer + 0.1 mL indicator P (**observe colour**) + 0.13 mL Na_2EDTA + 0.108 mL Mg (II) (**observe colour**) + 0.27 mL Ca (II) + 0.392 mL distilled water (**observe the final colour**)			

	System(Indicator Q)	Color	λ_{max}(s)	A (s)
A	5 mL buffer + 0.1 mL indicator Q (**observe colour**)+ 0.08 mL Na_2EDTA + 0.054 mL Mg (II) (**observe colour**) + 0.27 mL Ca (II) + 0.396 mL distilled water (**observe final colour**)			
B	5 mL buffer + 0.1 mL indicator Q (**observe colour**)+ 0.050 mL Na_2EDTA + 0.027 mL Mg (II) (**observe colour**) + 0.27 mL Ca (II) + 0.553 mL distilled water(**observe the final colour**)			
C	5 mL buffer + 0.1 mLindicator Q (**observe colour**)+ 0.13 mL Na_2EDTA + 0.108 mL Mg (II) (**observe colour**) + 0.27 mL Ca (II) + 0.392 mL distilled water(**observe the final colour**)			

1. Calculate the moles of indicator, Ca(II), Na_2EDTA and Mg(II) for each solution. Also calculate the ratio of these moles for each solution.

Indicator: **P** ☐ **Q** ☐ (Tick in appropriate box)

Solution A

Solution B

Solution C

2. Is observed colour of the above three solutions is almost same or different ?

3. See the obtained data for λ_{max} for all three solutions. Is the observed λ_{max} for all the above three solutions almost same or different ?

4. Can you describe all the reaction/s that have taken place in solution A ?

5. The observed color is due to presence of (you may have to use the photos in **subpart 3** as reference to answer this question)-