INDIAN INSTITUTE OF TECHNOLOGY, BOMBAY

DEPARTMENT OF CHEMISTRY

MANUAL

CH-117 L

CH117L: Activities

Sr. No.	Date	Activity
1	08-01-2024 to 12-01-2024	Manual Distribution
2	15-01-2024 to 08-03-2024	Regular lab experiments (Expt.1 to Expt.7)
3	11-03-2024 to 15-03-2024	Compensation Lab
4	26-03-2024 to 05-04-2024	CH117L Practical Examination
5	09-05-2024	Last Date for Uploading Grades

For any queries contact below:

Email Id: sushantc@iitb.ac.in

(please mention "CH117L" in the subject, while sending emails).

Lab intercom number: 4183

General rules and regulations to be observed in Chemistry Ist Year U.G. laboratory.

1. **Full sleeves white lab coat** (preferably cotton), **safety goggles,** and **shoes** are mandatory while working in Chemistry Laboratory.
2. Only formal dresses (full) covering from neck to toe are allowed in the Chemistry laboratory. Students with shorts, three-fourths etc., will not be allowed.
3. **Punctuality:** Punctuality & discipline is a must in the Chemistry laboratory.
4. You should reach 5 mins before the scheduled time.
5. Penalties for late coming are as follows:

a) 5 minutes late, 1-mark penalty
b) 10 minutes late, 2-mark penalty
c) 15 minutes late, 5-mark penalty
d) 30 minutes late, experiment not allowed.

6. **Chemicals & apparatus:** Safety should be practiced while performing the experiments. You may come across some new instruments, chemicals, acids, etc.., please handle them carefully and contact the instructor for any special precautions. Handle the glass wares gently.
7. **Lab report:** You are supposed to submit the lab report in a flat cardboard file, regularly. You can purchase this file from the IITB consumer society shop located at the basement of the Main bldg. Also, buy a bunch of loose sheets (for write-up) and 5-6 graph papers along with the file. The file will remain in the laboratory until all experiments are completed.
8. Do not write anything in the file before coming to the lab.
9. TA will explain to you, what you should write in the journal [Objective, theory, principle, calculation, result, etc.] Please do not write procedures in the journal (file).
10. **Viva:** A viva session for each experiment will be conducted by the respective TA during laboratory hours. You should read and prepare for the experiment before coming to the laboratory to have a proper understanding of that experiment.
11. **Crib session:** In case of discrepancy a student can clarify the marks from the TA before the next lab i.e. within 7 days only.
12. **Compensation:**
    a. Only one compensation lab will be conducted towards the end of the semester i.e. after all experiments are completed. If the student fails to attend any of the experiments due to medical reasons and has a pink slip from IITB hospital, he/she should inform the staff in charge and submit a photocopy of the pink slip/medical certificate.
    b. Compensation will not be provided <u>for any personal reasons</u> such as family functions, brother's/sister's marriage or festival celebrations etc.
    c. Compensation for more than one experiment (if, on medical grounds) will require approval from the course In-Charge.
    d. Apart from medical reasons, the compensatory lab can be offered to a student who participates in an Academic activity (from IIT Bombay) that is scheduled on the day of his/her regular lab. The student should provide a Dean's recommendation letter and get prior permission from the Instructor in charge.
13. In case of insufficient attendance, a DX grade will be allotted.
14. Change of practical slot is not permitted.
15. **Mobile phones:** Mobile phones should be switched OFF and kept aside in the bag while working in the laboratory.
16. For calculations, please use calculators instead of mobile phones.
17. **Bags:** You can keep your bags or belongings in the drawers of the laboratory table.

18. **Feedback:** Feedback will be collected from you at the time of your last experiment for the improvisation of the course structure.
19. Please carry this Lab manual every time you come to the lab.

Marks distribution

1. The Lab course contains seven experiments.
2. Semester marks are out of 100.
3. 70 marks for regular experiment and 30 marks for final exam.
4. 70 marks are further distributed in seven experiments with 10 marks for every experiment.
5. These 10 marks are split into further 3 parts as below:

a) **Performance** carries 5 marks, this includes your performance, punctuality, conduct, discipline, etc.
b) **Viva** carries 2 marks. The TA will ask questions based on the experiment (not confined to the manual), marks will be allotted based on your answers.
c) **Journal writing** (3 marks) will be explained by the respective TA at the time of the experiment.

CH 117L Exam: Instructions for students

Dear Students,

You are requested to carefully read the following instructions and understand the modalities

The practical examination is for **one and a half hours only**. You are requested to come 10 mins before the commencement of the examination. The timetable is available on Moodle.

1) Students should wear Lab-coat, shoes, and safety glasses for the entire duration.
2) You should bring only a pen, pencil, eraser, scale, calculator, and ID card for the examination. You should produce your ID card on demand, by the invigilator.
3) Mobile phones and smartwatches are not allowed during the examination.
4) **If we find that the student is having a mobile/smartwatch with him/her during the exam an FR grade will be awarded (even if not using it)**.
5) You should not talk with your friend or neighbor during the examination till the time you are inside the laboratory.
6) **If you are found talking or communicating with other students by any means, it will be treated as academic misconduct and FR Grade will be awarded.**
7) If you are facing any difficulty or if you wish to talk about something, then you should contact the **TA/Staff only**. He/she will resolve the problem.
8) A question slip is attached to the answer sheet, do not detach the question slip from the answer sheet.
9) A number is printed on the question slip, kindly ensure that the number on the question slip and the given solutions are the same. You can copy the same number to your answer sheet as well.
10) The readings which you observe during the experiment should be written in the answer sheet and initialized by the TA assigned to your table.
11) If you have two sets of experiments then you should take at least one signature in each set.

12) Extra solutions/ apparatus will not be provided during the examination.
13) You should write observation, observation table, calculation, and results only in the given answer sheet. Please do not write any theory, principle, or procedure for the experiment.
14) Viva session will not be there for the examination.
15) Examination marks are based on performance/ conduct during the exam (10 Marks) and Readings + Calculations (20 Marks).
16) In case during the experiment you forget and are not able to perform the experiment, the **Staff** will provide the hint for the experiment by deducting 5 marks. If you need more help then more marks will be deducted.
17) No help will be provided in the calculation part.
18) Graph paper if required will be provided by the TA.
19) Bio-breaks are not allowed.
20) You should ensure that your workplace and all apparatus are cleaned before you leave the lab.

All the best!

Instructors CH-117L

CH-117L 2023-2024: Spring semester

Course Instructors assigned to the respective slots of CH117L are as below:

Day/Div	Time(hrs)	Name of Faculty	Contact No.	Email ID
MON (P_{21})	**0930-1230**	Prof. Gopalan Rajaraman	7183	rajaraman@iitb.ac.in
MON (P_{15})	**1400-1700**	Prof. Chidambar Kulkarni	7186	chidambark@iitb.ac.in
TUE (P_{19})	***0830-1130**	Prof. Rahul Maitra	7185	rmaitra@iitb.ac.in
TUE (P_{13})	**1400-1700**	Prof. Arindam Chowdhury	7154	achowdhury@iitb.ac.in
THURS (P_{22})	**0930-1230**	Prof. Ramaswamy Murugavel	7163	muruks@iitb.ac.in
THURS (P_{16})	**1400-1700**	Prof. Rodney Fernandes	7174	rfernand@iitb.ac.in
FRI (P_{20})	**0930-1230**	Prof. Maheswaran S.	7187	eswaran@iitb.ac.in
FRI (P_{14})	**1400-1700**	Prof. Sandip Kar	7193	sandipkar@iitb.ac.in

(Prof. Rajarshi Chakrabarti)

Course In-charge

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1. Electrochemical Cells

Objectives:

(A) To measure the standard electrode potential of Zn^{2+} / Zn couple.
(B) To determine the concentration of Fe^{2+} by potentiometric titration.

Introduction:

Electrochemical techniques can be broadly classified as equilibrium or dynamic techniques. In the present exercise we shall be using equilibrium techniques. These techniques are based on the Nernst equation:

$$E = E° + 2.303\ (RT/nF)\ \log a_{ox}/a_{red}$$

Which defines the electrochemical system at equilibrium. E is the reversible electrode potential measured against a suitable reference electrode. E° is the standard electrode potential measured against the same reference electrode is the number of electrons transferred in the red-ox processes; a_{ox} and a_{red} are the activities of the oxidized and reduced forms respectively. Thus, by measuring the electrode potential (against a suitable reference) one can determine E°.

In part (I) of this exercise, we will determine the standard electrode potential E° for red-ox couple Zn^{2+}/Zn

In part (II) we will make use of the Nernst equation to determine the concentration of Fe^{2+} in a solution of ferrous ammonium sulfate. The inert electrode or indicator electrode (Pt in this case) is used to sense the red-ox potential of the solution as it is being titrated against an oxidizing agent (0.5N $K_2Cr_2O_7$ in this experiment).

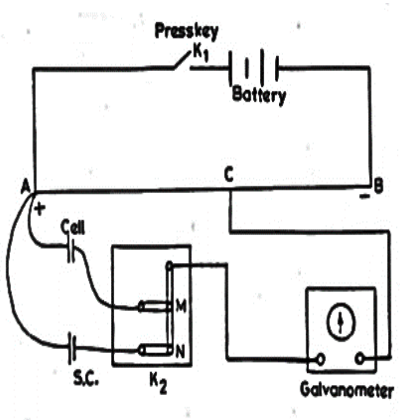
Procedure:

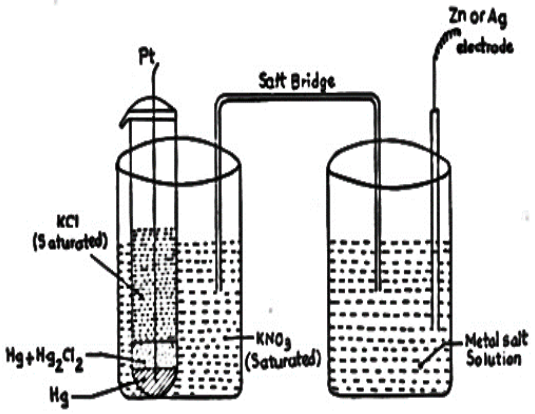
(A) To determine the standard reduction potential of the reaction

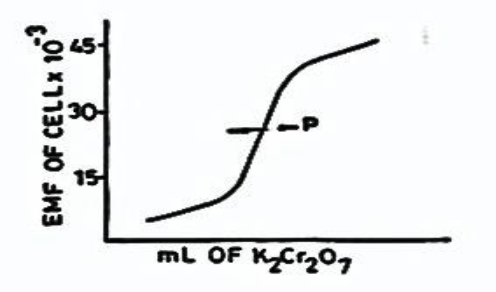
$Zn^{++} + 2e^- = Zn$:

Take sufficient amount of 0.1 M zinc sulphate solution in a beaker to partially immerse the zinc foil in this solution. This forms the Zinc half-cell. Connect this half-cell by means of a salt bridge (as shown in figure) with the saturated calomel half-cell to form the following galvanic cell:

(-) Zn | Zn^{++} (0.1 M) || KCl (satd.) | Hg_2Cl_2(s) | Hg (+)







Calculations:

(A) Determination of $E°Zn^{++}/Zn$:

Cell set up: (-) Zn/Zn^{++} (0.1 M) || KCl (satd.) | Hg_2Cl_2(s) |Hg

The cell potential E°cell = E°R – E°L

E°R= Reversible potential of SCE (= 0.2420 V), of Ag/AgCl (=0.22249V).

E°L = Reversible potential EZn/Zn^{++}

$EZn/Zn^{++} = E°Zn/Zn^{++} - RT/2F \ln (aZn^{++}/aZn)$

Since, aZn = unity and aZn^{++} = 0.1 x 0.15 (activity coefficient = 0.15), we get

$EZn/Zn^{++} = E°Zn/Zn^{++} - (0.0592/2) \log (0.015.)$

$Ecell = E°Zn/Zn^{++} - 0.0296 * \log 0.015 + 0.2420$ volts, and therefore,

$E°Zn/Zn^{++} = Ecell + 0.0296 * \log 0.015 - 0.2420$ volts

$E°Zn^{++}/Zn = - E°Zn/Zn^{++}$

(B) To determine the normality of the ferrous ammonium sulphate solution by titrating potentiometrically with 0.5 N $K_2Cr_2O_7$ solution.

Pipette out 25mL of ferrous solution into a 100mL beaker. Dip a platinum electrode into the solution. Connect this with the saturated calomel half-cell through the salt bridge to form the following galvanic cell:

(-) Hg | Hg_2Cl_2(s) | KCl (satd.) || Fe^{++}, Fe^{+++} |Pt (+)

Connect the +ve and -ve half cells to +ve and -ve ends of a digital voltmeter. Measure the EMF of this cell as a function of $[Fe^{2+}]$ (i.e. vol. of $K_2Cr_2O_7$) directly using the digital voltmeter in the circuit.

Add by means of a burette, small quantities of 0.5N $K_2Cr_2O_7$ solution. Stir well and note the reading on the voltmeter after each addition. Record your results as per the format of Table I. Plot the e.m.f. against mL of $K_2Cr_2O_7$ solution added. It may be noted that after the equivalence point, the galvanic cell will be

(-) Hg | Hg_2Cl_2(s) | KCl (satd) || $Cr_2O_7^{-2}$, Cr^{+++} |Pt (+)

Note the point (mL of $K_2Cr_2O_7$ solution, say V mL) where there is a relatively large change of potential. That will be the equivalence point and it has to be obtained as shown in figure. On another graph, plot the first derivative of e.m.f. $(\Delta E)/(\Delta V)$ against V_{avg} of $K_2Cr_2O_7$ added by recording your data as per the format of Table II. The inflexion point corresponding to peak of the curve gives a more reliable equivalence point.

The normality of the ferrous solution may be evaluated, using the relation:

Normality of the Ferrous solution = 0.5×V/25 (N)

The red-ox reaction is:

$$K_2Cr_2O_7 + 6\ FeSO_4 + 7\ H_2SO_4 \rightarrow K_2SO_4 + 3\ Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + 7H_2O$$

Two model tables for recording your results follow. The actual end point will vary and therefore, the range in which titration should be done with smaller volume change has to be determined individually. The format of these tables is only to serve as an example.

Table I:

Volume of 0.5N $K_2Cr_2O_7$ (mL)	**E.M.F (volts)**	**Volume of 0.5N $K_2Cr_2O_7$ (mL)**	**E.M.F (volts)**
1.0		4.6	
1.5		4.8	
2.0		5.0	
2.5		5.2	
3.0		5.4	
3.5		5.6	
4.0		6.0	
4.2		7.0	
4.4		8.0	

Table II:

Volume of $K_2Cr_2O_7$ (V)	**E.M.F (volts)**	**ΔE**	**ΔV**	**$\Delta E/\Delta V$**	**V_{avg}**
1 mL	0.32				
2 mL	0.40	0.08	1.0	0.08	1.5
3 mL	0.53	0.13	1.0	0.13	2.5

Suggested Reading:

1. Physical Chemistry, P.W. Atkins, 5th Edition (ELBS/OUP) 1994, Pages: 324-348.
2. Vogel's Textbook of Quantitative Analysis revised by G. H. Jeffery, J. Basset J. Mendham and R. C. Denny, 5th edition, Pages: 573-582.

Hazards and Preventions:

- As with all chemistry laboratories, lab coats, goggles or safety glasses and closed-toe shoes are essential.
- Make sure connections of wires are proper, otherwise there are chances of short circuit.
- Sulphuric acid is very **hazardous**. Read MSDS for the use of Sulphuric Acid properly. It is provided here https://www.finarchemicals.com/msds/Sulphuric%20acid.pdf
- Make sure no water or solvent is spilled on the batteries.
- No discarding of chemicals in the sink. Ask Lab Staff/supervisor for the procedure for proper disposal.
- Report any incident with promptness to the lab supervisor/staff.

Additional questions related to Electrochemistry:

1. What do you mean by an electrochemical interface?
2. What is an Electric double layer?
3. What is a Zeta Potential?
4. Does the sign of Zeta potential differ from the sign of the actual surface charge?
5. In your opinion, why do you think electrochemistry is important for the modern science?
6. What is an overpotential?
7. What is the difference between Power Density and Energy Density of a Battery?
8. In electrochemical cell, why is a salt bridge required? And how does it work?
9. If the Zn/Zn^{2+} is the cathode and Mg/Mg^{2+} is anode in the setup of an electrochemical assembly, and the E^0_{cell} is +0.65 V. The reactions occurring at both the electrodes are:
10. Standard Electrode potential for the half reactions, Fe^{2+}/Fe is -0.44 V and Cu^{2+}/Cu is 0.34 V. E^0_{cell} is given by _____ V
11. If you are given the value of the Standard Electrode potential of a cell, E^0_{cell}, the reaction occurring under standard conditions will be spontaneous is E^0_{cell} is _____.
12. Choose the correct option:
13. For a reaction having Cu/Cu^{2+} ($E^{cu/cu2+}$ =-0.34 V) and Zn/Zn^{2+} ($E^{zn/zn2+}$ =0.76 V) as half cells, Cu^{2+} will be *product/reactant*.
14. State whether True or False

    Salt bridge is a glass tube filled with agar jelly containing electrolytes such as KCl and KNO3, their role is to ensure that the solutions in the cell remain electrically neutral and electrons flow from one half cell to the other.

15. State whether True or False

(a) In a potentiometric titration, the emf of the cell is equal to zero at equivalence point.
(b) Potentiometric titrations have an added advantage over volumetric titrations, in the fact that they can be easily performed for turbid or colored solutions.

16. The Nernst equation for reaction of Ferrous ammonium sulphate with Potassium Dichromate, $Cr_2O_7^{2-} + 6Fe^{2+} + 14H^+ \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O$ is:

$$E = E^0_{cell} - \frac{RT}{nF}\frac{[Cr^{3+}]^2[Fe^{3+}]}{[Cr_2O_7^{2-}][Fe^{2+}]},$$

The value of n is _____.

17. State whether True or False

    For the reaction: $Mg^{2+}(aq) + 2e^- \rightarrow Mg(s)$, E^o = -2.37 V, The electrode potential varies linearly with the activity/concentration of Mg^{2+}.

18. State whether True or False

    In a Galvanic cell, electrons flow in the external circuit from anode to cathode while releasing electrical energy.

19. In the potentiometric titration of Ferrous Ammonium sulphate with Potassium Dichromate, after the equivalence point, addition of Potassium dichromate increases the EMF. This increase is due:

    Excess of $Cr_2O_7^{2-}$ ions

    Reduction of Fe^{3+} to Fe^{2+} ions

    Oxidation of Cr^{3+} ions

Additional Learning Sources:

1. Li-ion batteries: https://pubs.acs.org/doi/10.1021/acsenergylett.0c02584 https://iopscience.iop.org/article/10.1149/2.0251701jes/pdf
2. Initiatives by GoI on Renewable Energy: https://mnre.gov.in/new-technologies/hydrogen-energy (Not all of these are related to Electrochemistry, though)
3. Fuel cell Technologies and progress in India: https://www.nasa.gov/centers/glenn/technology/fuel_cells.html#:~:text=Glenn%20is%20investigating%20three%20types,were%20used%20through%20the%201990s and https://www.fchea.org/in-transition/2020/6/9/fuel-cell-and-hydrogen-development-in-india
4. Future of Electrochemistry: https://link.springer.com/content/pdf/10.1007/s10008-020-04585-3.pdf
5. Modern Batteries: Metal-Air Electrochemical Cell

2. Preparation of A Fluorescent Dye and Its Analysis by Thin Layer Chromatography (TLC)

Objectives:

(A) To prepare a fluorescent dye in microscale using a one pot sequential amide formation – nucleophilic aromatic substitution reactions.
(B) To understand the principles and application of Thin Layer Chromatography.
(C) Analysis of TLC using 'ImageJ'.
(D) Determination of molecular weight by using TLC-MS.

Introduction:

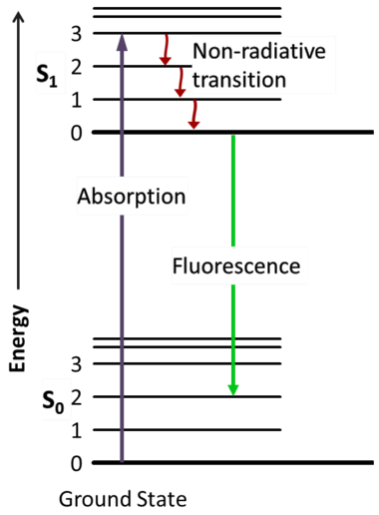
Chromatography is a technique which is widely used in chemistry and biology for the separation of components of a mixture of compounds. The TLC technique consists of applying the mixture to be separated onto stationary phase (such as a thin layer of silica gel coated on a glass plate or preloaded silica gel on aluminium plate) and allowing a solvent (mobile phase) to flow over the adsorbent.

Once the components are separated, they are detected and visualized using colour reactions or UV source. The detection using colour reactions normally involves spraying the TLC plates with an appropriate reagent to obtain characteristic colours. For example, non-selective visualization can be effected by exposure to iodine vapors, which are absorbed by the organic compounds resulting in brown coloured spots marking the positions of the individual components. Visualization under UV illumination is another common method whereby some compounds can be distinguished by characteristic fluorescent spots.

The most important criterion in qualitative identification is the Rf value, which is defined as

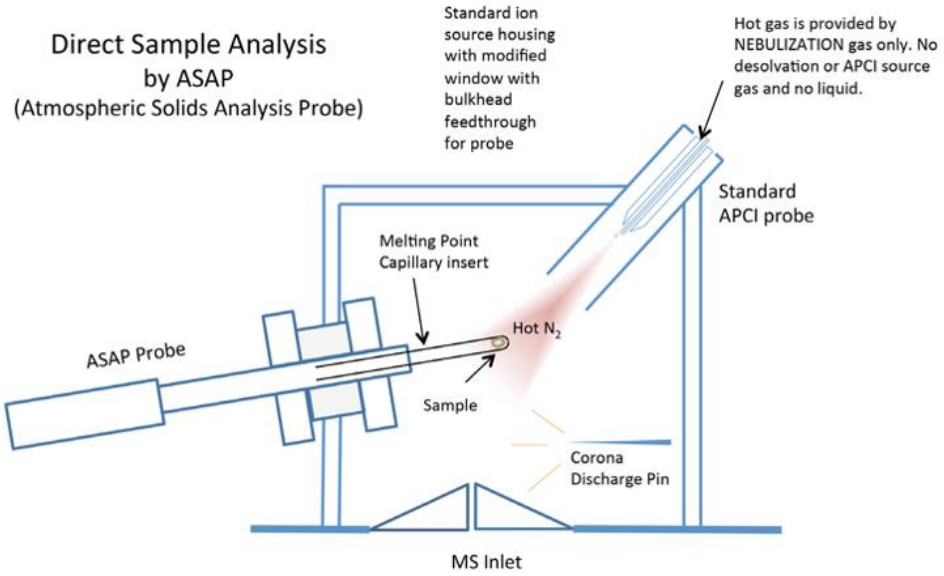
$$Rf = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$$

Fluorophores are the molecules, which can be excited and relax efficiently in a radiative pathway. On absorption of a photon, a molecule in the singlet ground state (S_0) gets excited to the first excited state (S_1). It can relax back to the ground state (S_0) by emission of a photon of lower energy than the incident one (Fluorescence) as depicted below.



In this experiment, we are going to prepare the highly fluorescent dye 4-hexylamino-N-hexyl-1,8-naphthalimide from 4-chloro-1,8-naphthalic anhydride. The reaction can be monitored using TLC with visualization using UV lamp. You would also observe the variation of R_f with the nature of the eluting solvent.

Mass spectrometry



A schematic of the atmospheric solids analysis probe (ASAP).

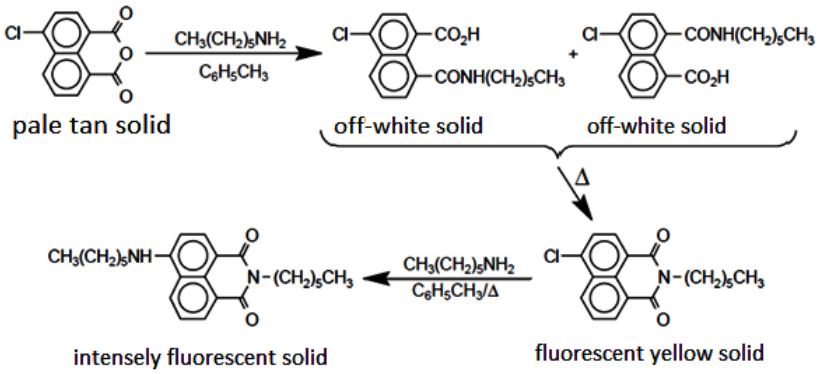
Advion expression compact mass spectrometer (CMS) and a direct analysis probe, known as the ASAP (atmospheric solids analysis probe). The probe uses the instrument's atmospheric pressure chemical ionization (APCI), eliminating the need for students to carry out any sample preparation before analysis. The APCI ion source with an ASAP sampling probe allows for direct insertion of liquids and solids, making for very quick analyses. Each analysis typically takes 30 s or less.

Preparation of fluorescent dye:

In a reaction vial (8 ml), weigh 5-10 mg of 4-chloro-1,8-naphthalic anhydride and add 1 ml of toluene. The mixture is then stirred with a spin bar on a magnetic stirrer. To this, add 1-hexylamine (500µL) by means of a syringe/micropipette. The reaction mixture is then heated and allowed to stir for further 45 minutes under reflux or until all the solid has dissolved. At the end of the heating period, the reaction mixture is allowed to cool. It is then analyzed by using two solvent systems as follows:

1. 20% ethyl acetate in hexane
2. 100% toluene.

The reaction progresses by several readily observable changes during the formation of the final product. The chemical reactions are shown as below:



Following observations can be made during the experiment:
A transient intense orange colour is observed on addition of the amine to the stirred suspension of the anhydride in toluene and the reaction mixture becomes noticeably warmer. With fading of the transient colour, the pale tan of the anhydride gets replaced with the grayish off-white of the acid amide. On heating the reaction mixture a gradual cyclization of the acid amide to the imide takes place to give a yellow solution. When pure, the imide is a yellow solid which exhibits a moderately intense yellow fluorescence. Once the imide is formed, the activating influence of both carbonyl groups is brought to bear on the chlorine. In the presence of the large excess of hexylamine, displacement of the halogen occurs just slightly more slowly than closure of the imide ring to give the bright yellow, intensely fluorescent 4-hexylamine –N-hexyl-1,8- naphthalimide.

On performing a TLC with eluting solvent 20% ethyl acetate in hexane you will observe four to six spots evenly spaced and easily visible, with Rf values falling between 0.8 and 0.3. Using toluene as eluting solvent you will observe four bands with Rf values between 0.5 and 0.05. All the Rf values for the four universally observed spots are collected in observation table.

TLC Procedure:
With a pencil gently mark a base line from the bottom edge of the preloaded TLC plate. The sample should be applied as tiny dots on the base line and well-spaced from each other. Draw a “finish line” a couple of centimetres from the top edge of the silica layer.

The trick in doing a good TLC is the spotting. For good separation (i.e. no streaking or mixing) loading of the sample onto the TLC plate must be neat and compact. Draw a small amount of the sample solution into a capillary, say 1 or 2 cm and lightly touch the capillary to the silica surface so that the sample is soaked up by the silica and forms a tiny spot. It is important to keep the spot small, apply a little at a time. Make about three applications so that there is sufficient amount of the sample loaded to give a distinctly visible coloration in the visualization step. Do not reuse the capillary for another sample to avoid contamination.

After completing the spotting of all the samples, the plate is transferred to a glass chamber containing the solvent to be used as the mobile phase. The solvent level must be a few mm below the starting line. The inside walls of the chamber are lined with filter paper, leaving a gap for viewing the plate. The filter paper is allowed to saturate with the solvent so as to enable the atmosphere in the jar to become saturated with solvent vapour. Allow the solvent to rise along the silica surface and as soon as the solvent front reaches the finish line, remove the plate from the solvent chamber and allow the solvent to dry out. Now capture the image using your smartphone/digital camera as a jpeg file for loading in ImageJ software. Send the pictures you took of the TLC plate to your e-mail or plug in the camera and load the pictures onto the

desktop. Instructions on how to use ImageJ are provided in text at the end of this handout. Calculate relative % of each spot using the following formula:

$$\text{Composition (\%) of a particular spot} = \frac{\text{Integrated value (area) of the particular spot}}{\sum \text{Integrated values of all the spots}} \text{ X } 100$$

Observation Table:

Solvent	Colour of the spot	R_f	Composition (%) of each spots
20% Ethyl acetate in Hexane			
Toluene			

Procedure for ImageJ software:
ImageJ (free download from http://imagej.nih.gov/ij/) is a program that can be used to compare the density of bands or spots. It is most commonly used for biochemistry techniques. This handout assumes that your picture from your TLC plate is a jpeg file.

1. Open the ImageJ program.
2. Open the image file using File > Open in ImageJ. Your TLC plate should be vertical in your image. Rotate it if necessary using Image > Transform.
3. Make sure the rectangle selections tool is clicked.

4. Draw a rectangle around the first lane.
5. After drawing the rectangle around your first lane, press 1. This will leave lane 1 in place while creating a second rectangle directly on top of it (Figure 1).
6. Use your arrow keys to move the second rectangle over the second lane.
7. Press 3. This will automatically bring up a window that has integrated the lanes for density. This window represents the relative density of the contents of the rectangle over each lane.
8. Now, go to the Image menu and select Transform, and do a vertical and horizontal flip. The rectangles are arranged top to bottom on the profile plot (Figure 2).
   A copy of the plate used for ImageJ should be included in your report (Save the image using File > Save As. The image format should be Jpeg).
8. Use the Line tool to draw a line across the baseline for each peak in the TLC lanes. Images will have some background signal, so the peaks likely won't reach down to the baseline of the profile plot. This may make the peak appear to float above the baseline. It is necessary to make sure to close off the peak in order to measure its size.

9. Use the Wand tool to click inside of each of the peaks in the TLC lanes. As you click on a peak, it will be highlighted in yellow. There will also be a window that pops up to give area

values for each of the peaks. Keep track of the order that you click on the peaks, as that is the order they will show up on the Results window.

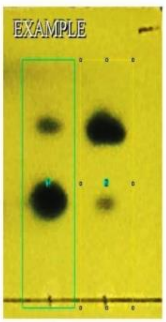


Figure 1.

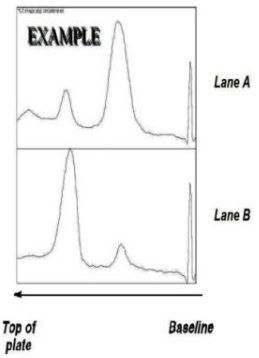
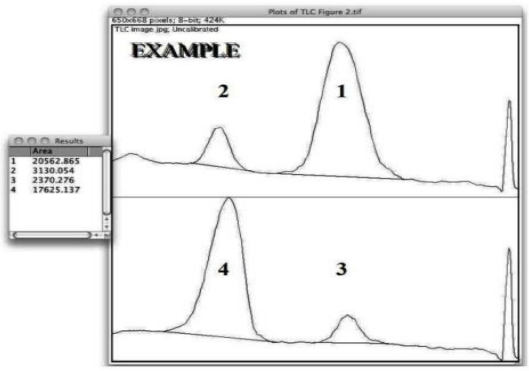


Figure 2.



TLC-MS procedure:

The TLC/CMS analysis of the reaction mixture was performed on the Advion express**ion** CMS and Plate Express. A solvent composed of 0.1% formic in methanol was used for the elution of the analytes from the TLC plate. The eluted analytes were directed to the CMS for acquisition of the corresponding mass spectra for the reactants and products.

Report your result and list some applications possible with this technique.

REFERENCES

(1) Calimente, S.; Strand, S. M.; Chang, S-C.; Lewis, D. E. J. Chem. Ed. 1999, 76, 82-83.
(2) Wagner, A.J.; Miller, S.M.; Naguyen, S.; Lee, G. Y.; Rychnovsky, S.; Link, R.D. J. Chem. Ed. 2014, 91, 716-721.

3. Determination of phosphoric acid in cola drinks

Introduction:

Excessive consumption of cola drinks is detrimental for human health due to their acidity, high caffeine content, high phosphoric acid content etc. This laboratory experiment describes the quantification of phosphoric acid in a popular colourless cola drink using the molybdenum blue method.

Theory:

$7\ H_3PO_4 + 12\ (NH_4)_6Mo_7O_{24}.4H_2O \rightarrow 7\ (NH_4)_3[PO_4(MoO_3)_{12}] + 51\ NH_4^+ + 51\ OH^- + 33\ H_2O$

In this experiment, ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ forms a colourless hexavalent molybdenum phosphate complex with phosphate ion PO_4^{3-} . In acidic conditions, this colourless complex is reduced to a blue pentavalent form by ascorbic acid. The concentration of the pentavalent form can be determined spectrophotometrically by measuring its absorption at 830 nm.

Method:

1. Preparation of standard curve:

a. Preparation of standard stock solution

1.9412 g of KH_2PO_4 is dissolved in deionized water in a 1L volumetric flask and the volume is made up to 1 L (standard A)

Calculation of P content in standard stock solution:

P content in standard A= $\frac{31}{136.086} \times 1.9412 = .44\ g/L$

P content in P_2O_5 = $\frac{62}{141.94} = .44$

P content in standard A (in ppm units) = 1 g/L in P_2O_5 units = 1000 ppm P_2O_5

b. Preparation of Working Standard Solution

Dilute the standard stock solution A 20 times by adding 2.5 ml of standard A to a 50 ml volumetric flask and making up the volume with deionized water (standard B).
Dilute standard B 12.5 times by adding 4 ml of standard B to a 50 ml volumetric flask and making up the volume with deionized water (standard C).
The concentration of standard C is 4 ppm P_2O_5. This is the working standard solution.

c. Preparation of reducing solution R:

To prepare the reducing solution (solution R)
(i) dissolve 1.00 g of ammonium molybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ in 50 ml deionized water (solution M)

(ii) dissolve 1.76 g of ascorbic acid in 100 ml of deionized water (solution A)
(iii) Dilute 17 mL of concentrated sulfuric acid in 200 mL of deionized water (solution S)
(iv) Mix 39 ml of solution M, 60 ml of solution A, 125 ml of solution S and make up the volume to 250 ml in a volumetric flask with deionized water.

d. Sample Preparation and Measurement

To eliminate all gases from the sample (cola drink), it was allowed to stand at atmospheric pressure for 24 h before analysis.

(i) Transfer 2.5 ml of the overnight sample to a 50 ml volumetric flask and make up the volume with deionized water (Solution X)
(ii) Dispense appropriate volumes of the solutions C, X, R and deionized water into test tubes according to Table 1.
(iii) Warm the tubes at 50 °C in a water bath.
(iv) Read the absorbance against a blank at 830 nm. Repeat the measurement three times
(v) Record the values in Table 2.

Table 1. Sample and Calibration Curve Preparation

	Tube No.1	**Tube No.2**	**Tube No. 3**	**Tube no. 4**	**Tube No. 5**	**Tube No. 6**	**Sample Tube**
Volume of Standard C to be added (ml)	0	0.5	1	1.5	2	2.5	
Volume of Solution X to be added (ml)							0.25
Volume of reducing solution R to be added (ml)	2	2	2	2	2	2	2
Volume of deionized water to be added (ml)	3	2.5	2	1.5	1	0.5	2.75
Final volume	5	5	5	5	5	5	5
Final concentration expressed as P_2O_5 (ppm)	0	0.4	0.8	1.2	1.6	2	

Table 2: Absorbance values for Sample and Standard solution tubes 1-5

Solution	**A_{830}**
Standard solution Tube 1 (0 ppm)	
Standard solution Tube 2 (0.4 ppm)	
Standard solution Tube 3 (0.8 ppm)	
Standard solution Tube 4 (1.2 ppm)	
Standard solution Tube 5 (1.6 ppm)	
Standard solution Tube 6 (2.0 ppm)	
Sample solution (trial 1)	
Sample solution (trial 2)	
Sample solution (trial 3)	

e. Plot A_{830} nm values versus concentration in ppm for standard solutions 1-6 (Table 2) to obtain a calibration curve. Fit the points to a linear equation y = mx+c. From the value of the slope "m" and the A_{830} of the sample solution, determine concentration of the diluted sample solution in expressed as P_2O_5 (ppm). Record these values in Table 1.

f. Calculation of phosphorus content expressed as P (ppm) in sample solution.

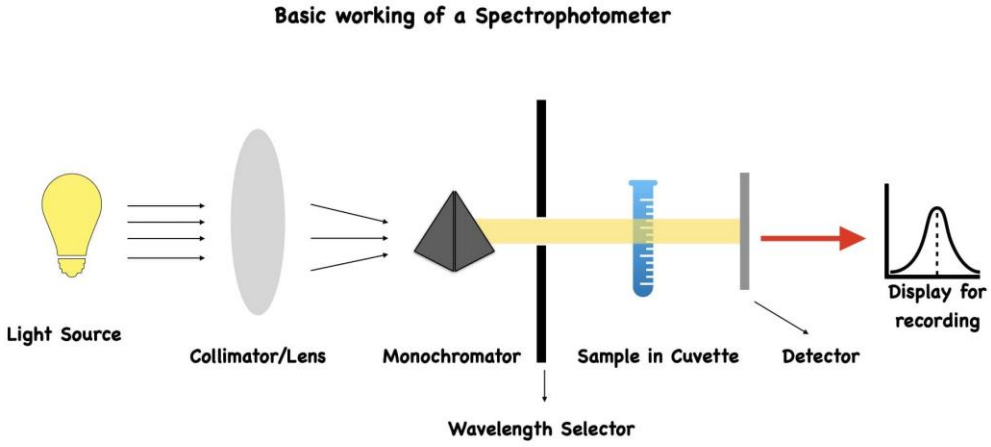
Considering the dilutions made on the original sample and the transformation from P_2O_5 to P content, the phosphorus present in sample (in ppm) = _____ ppm $P_2O_5 \times 20 \times 20 \times \frac{62\ mg\ P}{142\ mg\ P_2O_5}$

References:

1. Murphy, J. Determination of phosphoric acid in cola beverages a colorimetric and pH titration experiment for general chemistry. *J. Chem. Educ.* **60**, 420–421 (1983).
2. Lozano-Calero, D., Martín-Palomeque, P. & Madueño-Loriguillo, S. Determination of phosphorus in cola drinks. *J. Chem. Educ.* **73**, 1173–1174 (1996).

Additional Questions:

1. Below is given a basic working diagram of Spectrophotometer, The output spectrum gives a plot between _____ vs _____.



2. The absorbance of a sample recorded in a spectrophotometric analysis is 4, the percentage Transmittance is _____.
3. Choose the appropriate options:
   Absorbance of a sample depends on:

a) The path length of the sample, the concentration of sample and is independent of molar absorption coefficient (ε).

b) The path length of the sample, the concentration of sample and molar absorption coefficient of the sample (ε).
c) The value of ε remains constant for a particular sample at all wavelengths.
d) ε depends on the chemical structure of the sample under investigation.

Additional Learning Sources:

1. https://www.sciencedirect.com/book/9780128017739/food-safety-in-the-21st-century
2. https://www.dfda.goa.gov.in/images/PDF-DOCUMENTS/quciktestforsomeadullterantsinfood-fssaiinitiative.pdf
3. https://www.cambridge.org/core/services/aop-cambridge-core/content/view/S0007114551000546

4. Oscillatory Chemical Reaction

Objectives:

a) To introduce the students to a fascinating example of an oscillating chemical reaction.
b) To introduce the concept of non-equilibrium thermodynamics.
c) To speculate on the possible causes and applications of oscillating chemical phenomena.

Introduction:

The heartbeat, the tick-tock of a grandpa clock and the motion of waves at a beach are fascinating, and with history repeating itself, periodic phenomena have a certain appeal. At first these reactions were treated with some scepticism because it was felt that true chemical oscillation would be contra-thermodynamic. But now there are numerous indisputable experimental examples and a theory of non-equilibrium thermodynamics. The theory was developed by Lars Onsager of Yale University and Ilya Prigogine of the Free University, Brussels. The theory showed that although chemical oscillation about equilibrium, analogous to the motion of a pendulum, was indeed an impossibility, oscillation far from equilibrium is perfectly consistent with physical laws.

Some well-known oscillating chemical reactions

Bray-Liebhafsky (BL) Reaction:

The first example of a homogenous oscillating chemical reaction was discovered in 1921 by William C. Bray at the University of California, Berkeley and later studied exhaustively by Herman Liebhafsky at Texas A & M University. The reaction is the iodate catalyzed decomposition of hydrogen peroxide. The rate of oxygen evolution and intensity of the iodine color vary in a periodic fashion.

Belusov-Zhabotinskii (BZ) Reaction:

This reaction was discovered by B. P. Belusov at the Institute of Biophysics in the USSR in 1951. The discovery was initially ignored but later taken up by A M Zhabotinskii at Moscow State University. The reaction mixture consists of malonic acid, acidified bromate and a ceric salt and shows oscillations in bromine concentration, going from yellow to colorless and back.

Briggs-Rauscher (BR) Reaction:

Two San Francisco high school teachers, T.S. Briggs and W. C. Rauscher, developed a hybrid of the BZ and BL reactions that provides a visually striking lecture demonstration that oscillates for 20 minutes or more before settling down to thermodynamic equilibrium. The reaction mixture consists of KIO_3, H_2O_2, $HClO_4$, malonic acid, $MnSO_4$ and starch to visualize the oscillations in I_2 concentration.

Mechanisms for chemical oscillators:

Mechanisms for oscillatory reactions are rarely, if ever, simple. They generally involve 10 or more stoichiometrically independent species and perhaps twice as many elementary steps. Although they are less elaborate than the mechanisms that describe combustion and atmospheric processes, they are nonetheless too complex to be grasped intuitively. Development of a mechanism for an oscillating reaction usually requires both extensive computer simulation and a variety of kinetic experiments aimed at establishing the rate laws and rate constants of as many elementary steps as possible. The remainder are estimated either by comparison with related reactions or by fitting the calculated and experimental data.

The earliest model of oscillatory chemical behaviour proposed by A. J. Lotka in 1920 consists of three reactions, two of them autocatalytic. The precursor A is taken to be in large excess so that its concentration has a constant value, say a.

Representing the concentrations of the autocatalytic species X and Y by x and y, respectively, it can be shown that for any constant C the curve given by

$$k2(x+y) - k3\ln x - k1\, a \ln y = C$$

is a solution of the three rate equations. The solution equation represents a closed curve, almost an ellipse, in x-y space. (Try plotting it). Therefore, as the system follows the curve, the concentrations of X and Y vary in a periodic fashion.

Application of chemical oscillators:

Does chemical oscillation have any practical applications, or is it merely a source of attractive, But frivolous lecture demonstrations? Many of the potential practical applications of the knowledge gleaned from the study of chemical oscillators lie in biology. It has been suggested that certain processes might operate more efficiently in an oscillatory than a steady-state fashion. Model calculations show that glycolysis may have evolved as an oscillatory chemical process because this mode of operation is a few percent more efficient than the steady-state alternative. By an examination of what happens when chemical oscillators are perturbed at different points in their cycles, a plausible explanation of the onset.

There may be important analogies between dynamical processes in chemistry and geology. Theories used in explaining periodic Liesegang rings (the periodic precipitation phenomena that occur when ions like silver and chromate, that from a sparingly soluble salt, diffuse towards each other from opposite ends of a gel filled tube) have been adapted to the study of the origin of layering patterns in certain rock formations. Although the time and length scales are quite different, the fundamental physical principles remain the same.

In today's exercise we are going to perform a variation of the Briggs-Rauscher reaction.

Safety:

1. Wear goggles at all times. Use gloves when handling the chemicals.
2. Hydrogen peroxide solutions (3.579 M) are prepared from 30% hydrogen peroxide, which can cause skin and eye irritation on contact, and abdominal pain if ingested. The 0.2 M sulfuric acid may cause harm to the eyes or skin and is harmful to the digestive system if ingested. Dilute product solutions of iodide/iodine may stain the skin.
3. Check with the TA for disposal of the waste liquid and materials. DO NOT DUMP ALL CHEMICALS IN THE SINK.

Procedure:

Part1: Observation of patterns in a diffusion controlled oscillating reactions

For certain reactions, the reactants (and intermediates) diffuse slowly through the solution. Interesting patterns emerge when such a reaction is also oscillatory. We will observe and play with such a reaction:

1. In a 50 mL beaker, mix the 6 solutions in the following order: $MnSO_4$ (0.005 M, 5 mL), H_2SO_4 (0.2M, 5 mL), malonic acid (0.03 M, 5 mL), KIO_3 (0.15M, 5 mL), H_2O_2 (3.579 M, 10 mL), and 2 mL 0.5% starch indicator for visualizing the patterns.
2. Transfer the above solution quickly to the petri dish.
3. Observe any changes in colour and any pattern formation.
   Start with step 2, while the patterns are emerging.

Part 2: Observation and measurement of an oscillatory reaction

Now we will look at an example where the entire solution changes colour periodically and measure the timings.

1. Mix the following solutions in the order given below so that in the mixture each component has the concentration mentioned:

KIO_3	0.07 M
H_2SO_4	0.05 M
Malonic acid	0.05 M
$MnSO_4$	0.007 M
Starch	0.01%
H_2O_2	0.06 M

   Watch the fun. Report your observations carefully.
2. Note the timings of the oscillations using the provided stop-watch.
3. Consider the mechanism provided above. Write the rate law corresponding to [*X*] and [*Y*]. If initially, [X]=[y]=0, qualitatively sketch [X] and [Y] as a function of time.
4. Change concentrations of the different solutions and record the timings of oscillations.
5. Interpret any changes in oscillation timescales based on the above mechanism.

Suggested reading:

1. The above write-up has been adapted from, "Patterns in Time and Space - Generated by Chemistry", I. R. Epstein, C and E News, March 1987.
2. Part 1 of the procedure taken from https://pubs.acs.org/doi/abs/10.1021/acs.jchemed.0c00892
3. "An Oscillating Iodine Clock", T. S. Brigg and W.C. Rauischer, Journal of chemical Education., Vol no. 50, Issue no 7,Page no 496,year 1973
4. "Oscillating Chemical Reactions",I.R. Epstein, K. Kustin, P. DeKepper and M. Orban, Scientific American, Vol no.248, Page no.112,year 1983
5. More on oscillatory reactions: https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Map%3A_Physical_Chemistry_for_the_Biosciences_(Chang)/09%3A_Chemical_Kinetics/9.11%3A_Oscillating_Reactions
6. Video of pattern formation in oscillating reaction: https://www.youtube.com/watch?v=jRQAndvF4sM

Moodle questions:

1. For the elementary reaction A+X -> 2X, propose the rate law with respect to concentration of A.
   a. d[X]/dt=k[A][X]
   b. -d[X]/dt=k[A][X]
   c. ½ d[X]/dt=k[A][X]
   d. -d[X]/dt=½ d[X]/dt k[A][X]
2. Which of the following curves will best represent the concentration of X with time?
3. Which of the following best represents the mechanism for an oscillatory reaction:
   a. The products keep converting back to reactants, leading to oscillations.
   b. The rate determining step keeps changing with time depending on concentrations of different species.

c. Excess of reactant concentration leads to oscillations in the concentration of intermediates.
d. The autocatalytic step in the mechanism leads to oscillations as this step can go both in the forward as well as the backward direction.

4. What safety features are correct for this experiment (select all that apply):
   a. Dispose of chemicals in the sink when used
   b. Concentrated H2O2 can cause skin and eye irritation and must be used with care.
   c. Dilute sulfuric acid can harm the eyes and skin if come in direct contact.
   d. Iodine solutions can stain the skin.
5. How does Briggs-Rauscher work?
6. What is origin of amber color in Briggs-Rauscher reaction?
7. What will be the effect on oscillation time in Briggs-Rauscher reaction if acetone is used as a solvent?
8. Is Briggs-Rauscher reaction reversible?
9. What is the role H2O2 in the oscillatory reaction?
10. When does Briggs-Rauscher reaction stop?
11. What are the main elements of Belousov-Zhabotinsky reaction?
12. What is the role of malonic acid in Belousov-Zhabotinsky reaction?
13. Do oscillating reactions go on forever?
14. Does the frequency of oscillation remain same throughout the process?
15. How does temperature effect the oscillation?

Sr. No.	Vol. of KIO_3 (mL)	Vol. of H_2SO_4 (mL)	Vol. of Malonic acid (mL)	Vol. of $MnSO_4$ (mL)	Starch	Vol. of H_2O_2 (mL)	Time for first appearance of blue colour	No. of Oscillations	Time period of oscillation	Observations
1	3	3	3	3	6 drops	3				
2	3	4*	3	3	6 drops	3				
3	3	1*	3	3	6 drops	3				
4	3	3	0*	3	6 drops	3				
5	3	3	3	0	6 drops	3				

Interpretation of observation marked with*

5. Estimation of Iron

Objective: To estimate the amount of ferrous and ferric ions in a solution containing both.

Introduction:

Iron belongs to the 3d transition series of metals with electronic configuration $3d^6 4s^2$ and forms several oxides with different oxidation states and crystal structures. Of these oxidation states, +2 and +3 states are commonly encountered, the oxides being abundant in nature. Common examples are magnetite (Fe_3O_4) and hematite (Fe_2O_3).

Iron oxide is a raw material for producing iron and steel and a catalyst for many industrial processes (eg., the manufacture of NH_3). The general importance of iron oxides in other disciplines of technology besides metallurgy lies in the fact that these oxides serve as raw materials for the synthesis of magnetic materials. For instance, γFe_2O_3 is used as a magnetic material in recording tapes. Iron oxides mixed with other metallic oxides are used in magnetic materials such as ferrites, perovskites etc., which are useful in semiconductor technology, microwave devices, ferroelectrics etc.

The properties of these iron-containing materials depend on the purity and the required stoichiometry. The presence of impurities and deviation from the stochiometry adversely affect the properties and hence their technological importance. It is therefore obvious that strict quality control is of importance which involves the estimation of the constituent elements. Further, these elements (eg., Fe_3O_4) invariably contain iron in different oxidation states namely +2 (Ferrous) and + 3 (Ferric) and their accurate quantitative determination is of considerable importance.

The quantitative estimation of ferrous and ferric either separately or in a mixture containing both, can be carried out by conventional volumetric and gravimetric methods or using instrumental methods such as spectrophotometric methods. The choice of method depends on the percentage of iron present. The present exercise deals with the volumetric estimation of iron in +2 and +3 oxidation states as the required titrations are relatively simple and less time-consuming.

Principle:

The quantitative estimation of Fe^{2+} either separately or in a mixture containing Fe^{3+} can be carried out by volumetric titration involving an oxidising agent (either $KMnO_4$ or $K_2Cr_2O_7$).
The estimation of Ferric generally involves the quantitative reduction of ferric to ferrous state and subsequent estimation of Ferrous by titration with a standard oxidizing agent such as $KMnO_4$ or $K_2Cr_2O_7$.The reduction of Fe^{3+} to Fe^{2+} can be brought about by one of the following reagents (a) Stannous chloride (b) Amalgamated Zinc (c) Sulphurous acid (d) Titanous salts (e) Hydrogen Sulfide and (f) Zn and dil H_2SO_4.

Procedure:

Make up the solution supplied in 100 mL standard flask by adding small amounts of distilled water very carefully to bring it upto the 100 mL mark (lower meniscus coinciding with the mark) using distilled water. When nearing the 100 mL mark add additional distilled water, a drop at a time. Shake thoroughly to make the solution homogenous. Record your flask No.

(a) Estimation of Ferrous: Pipette out 10 mL of diluted solution into a clean (need not be dry) conical flask. Add 1 test tube full of 2N H_2SO_4.Titrate with $KMnO_4$ taken in the burette. Do

not add more than 0.5 mL of $KMnO_4$ at a time. Continue the titration till the solution changes from colorless to light pink. Repeat the titration till two successive burette readings do not differ by more than 0.1 mL.

(b) Estimation of Ferric: Pipette out 10 mL of the diluted solution into a clean conical flask. Add about 150 mg of Zn powder. Heat the solution on a water bath with continuous swirling till the solution turns almost colorless. Add 1 test tube full of 2N H_2SO_4 and allow it to stand for some time till all excess Zn has reacted. (If some Zn is left, add some more H_2SO_4). Cool the conical flask and titrate with $KMnO_4$ as in (a).

Calculations:

1000 mL= 1N $KMnO_4$ =56 gm. Fe^{2+}

(a) $V_{solution}$ = 10 mL. N_{KMnO4} = a = 0.1N, V_{KMnO4} = b mL.

$$\text{Amount of } Fe^{2+} \text{ in the given solution} = \frac{10 \times a \times b \times 56}{1000} = x \text{ g}$$

(b) Vsolution = 10 mL. N_{KMnO4} = a = 0.1N, V_{KMnO4} = c mL.

$$\text{Total } Fe^{2+} \text{ in the given solution} = \frac{10 \times a \times c \times 56}{1000} = y \text{ g}$$

Amount of Ferric Iron = (y - x) g

Suggested Reading:

Vogel's Textbook of Quantitative Analysis Revised by G.H.Jeffery, J.Basset, J.Mendham and R.C.Denny, 5th edition, Pages: 261, 368-372, 409-416

6. Investigating chemical kinetics using a self-constructed polarimeter

Objective: i) To construct a working polarimeter using the parts given, and ii) To determine the rate constant for the inversion of sucrose using the self-constructed polarimeter.

Principle: The reaction for the inversion of sucrose is as follows:

$$C_{12}H_{22}O_{11} + H_2O \rightarrow C_6H_{12}O_6 + C_6H_{12}O_6$$

This reaction is catalysed by H_3O^+ ions.

The rate of this reaction depends on the concentrations of sucrose, water and H_3O^+. Since a large excess of water is needed, its concentration may be considered to be a constant and thus making the rate independent of water concentration. Therefore, for a given concentration of H_3O^+, the rate of the reaction depends only on the concentration of sucrose at a given temperature. If the reaction is of first order, the differential rate expression is

$$\frac{-d\,[\text{ sucrose }]}{dt} = k\,[\text{ sucrose }]$$

Where k is the specific rate constant (or simply the rate constant).In the integral form, the equation becomes:

$$\ln [\text{Sucrose}] = kt + \text{Constant.}$$

With the limits [Sucrose] at t = O and [Sucrose]t at time t, the equation is

$$k = \frac{2.303^{\log 10}}{t} \quad \frac{[\text{ sucrose }]_0}{[\text{ sucrose }]_t}$$

The rate of a chemical reaction can be followed by measuring the concentration of either the reactant or product as a function of time. This can be done by either chemical analysis or by choosing a physical property which is a linear function of concentration. Physical methods are often more rapid than chemical analysis and one can obtain a large amount of data within a given (short) time. One such physical property is optical activity.

Typically, optical activity is measured by an instrument called polarimeter. In such an experiment, a plane polarized light of known polarization direction is passed through a solution under study and the angle by which the plane polarized is rotated by the solution is measured. The extent of rotation of the plane polarized light is a measure of the optical activity of the solution.

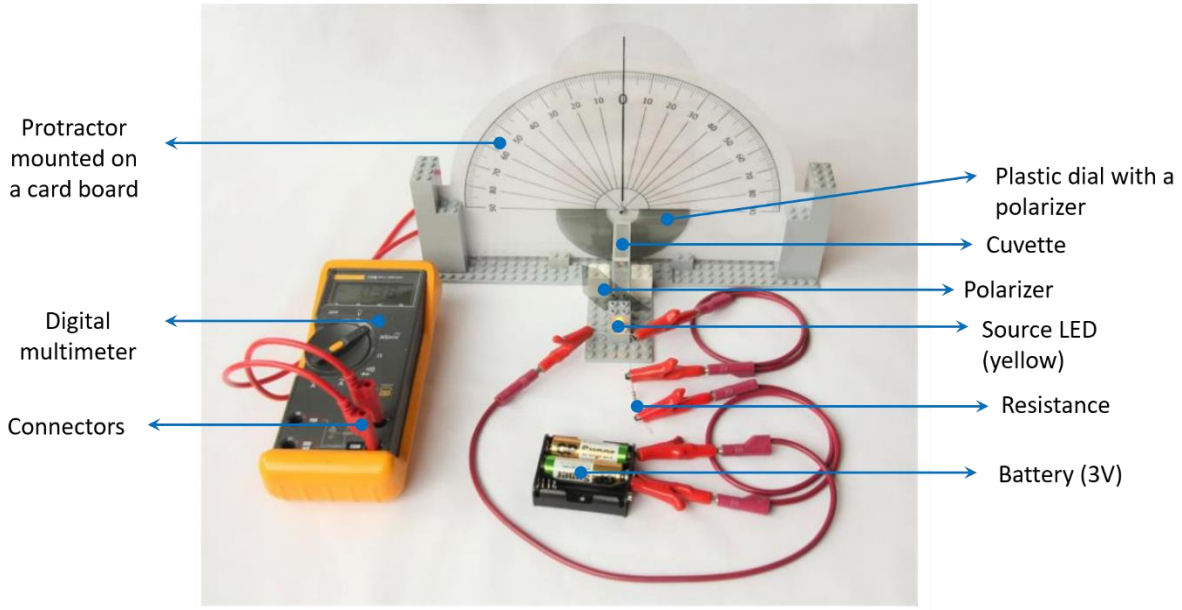
Reagents required: HCl (2M), sugar solution (40%)

Apparatus required: Appropriately cut linear polarizing film (1), Lego brick base (1), protractor mounted on a card board, a plastic dial with an attached polarizer, LEDs (yellow and red), crocodile clips, resistors (10 – 60 Ohms), battery (3V), digital multimeter, Cuvette, 10 mL pipette, beaker.

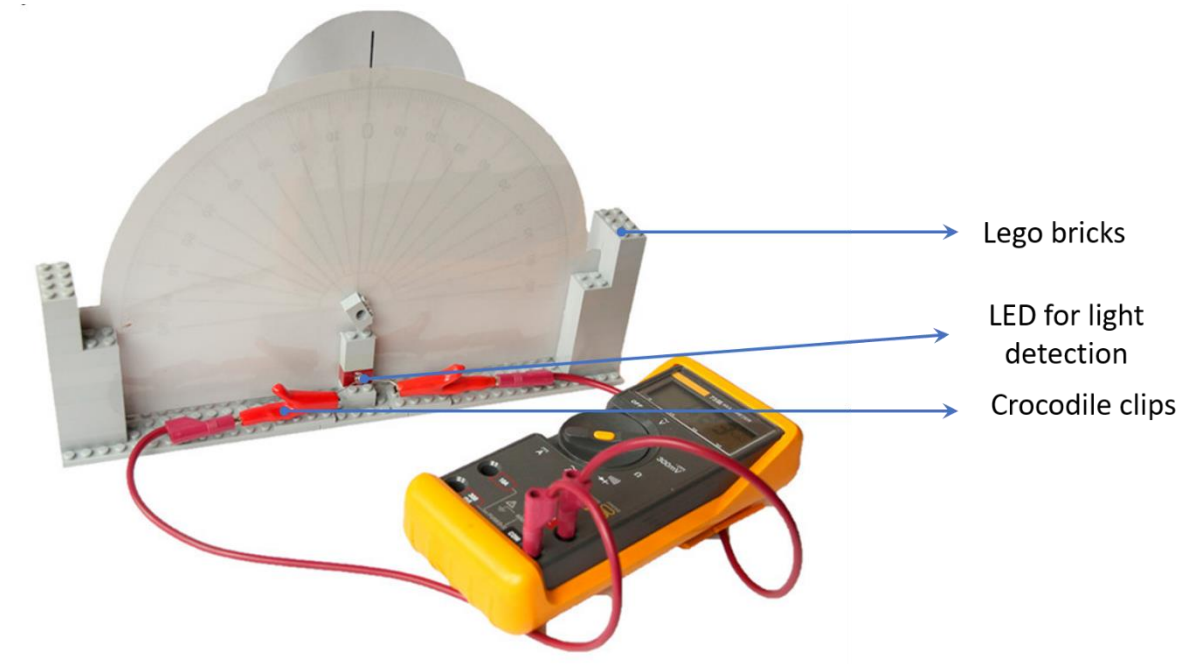
Procedure:

Construction/assembly of a polarimeter:

Front view of self-constructed polarimeter



Rear view of self-constructed polarimeter:



1) Using crocodile clips connect the battery to the source LED (yellow) via a suitable resistor (10 – 60 Ohms)
2) Insert the dial with an attached polarizer onto the dial, as shown in the figure. The direction of polarization of this polarizer is orthogonal to the base

3) Insert the appropriately cut polarizer with its polarization axis perpendicular* to the polarizer mounted on the dial
4) Connect the back LED to the multimeter via crocodile clips

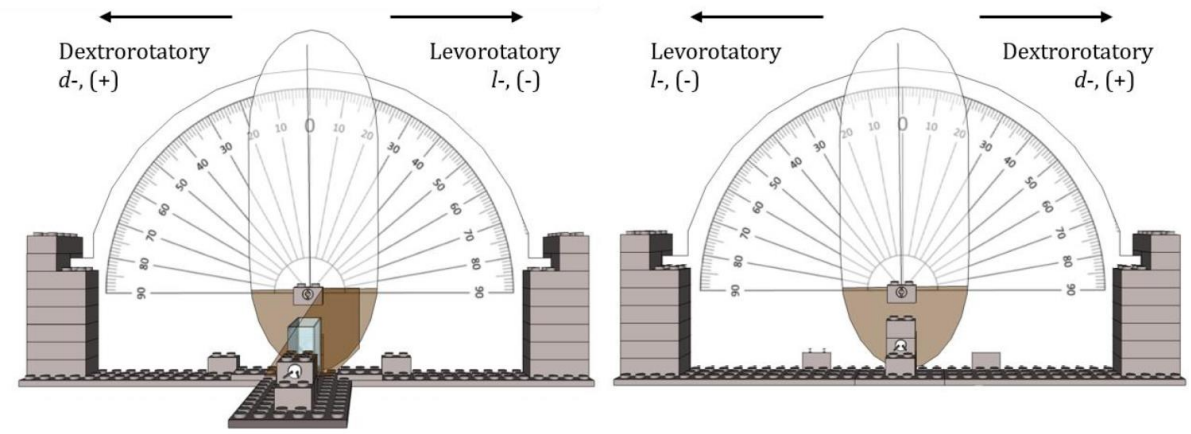
*In principle, the polarization of both the polarizers can be parallel. This only affects the way in which the readings are recorded (i.e., rotating the dial to obtain maximum voltage in multimeter, rather the minimum, as explained below), but not the actual readings.

For more details about the set-up check out the supporting information of the following paper *J. Chem. Educ.* **2020**, *97*, 2196.

Measuring optical rotation using the above constructed polarimeter:

Recording calibration reading: Note the voltage at zero degree on the dial. Move the dial in steps of 0.5° on either side of zero degree on the dial while observing the voltage on the multimeter attached to the detector LED. The lowest reading (mV) in the multimeter represents the point of minimum light transmittance. Check the point of minimum light transmittance by moving the dial on either side to obtain a stable zero transmittance point. Note the degrees on dial for minimum transmittance (θ_0). This is the calibrated zero. At this point both the polarizers are very close orthogonal with respect to each other.

Measuring optical activity with the self-constructed polarimeter: Placing a cuvette with an optical active solution in the instrument will alter the optical zero of the system due to the rotation of the plane polarized light by the optically active solution. To estimate the extent of rotation, move the dial slowly to each side of zero to determine if the rotation is dextro- or levorotatory (see the below picture for the convention), and to locate the new optical zero as described above. Move the dial in smaller steps around the optical zero to obtain accurate results of rotation. Note the degrees on the dial for minimum light transmittance (θ_x). The magnitude of net rotation = $\theta_x - \theta_0$. The direction of rotation can be ascertained based on the below convention.
Cross check the magnitude and direction of rotation with a commercial polarimeter available in the laboratory.



Observer facing the detector LED **Observer facing the light source LED**

To measure A∞ (optical rotation at t =∞).

Fill the cuvette with given sucrose solution (at t=∞) taking care to avoid air bubbles. Place the cuvette in the appropriate position of the self-constructed polarimeter and measure the angle of rotation, as described above.

To measure A0 (optical rotation at t = 0).

Pipette out 20 mL of 40% sucrose (supplied) into a clean beaker. To this add 20 mL distilled water (by means of pipette). Make the resulting solution homogeneous. Fill the cuvette with the solution and measure the angle of rotation.

To measure At (optical rotation at different time intervals).

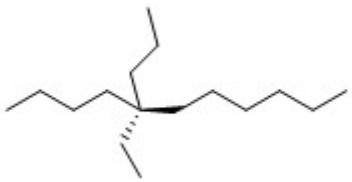
Pipette out 20 mL of 40% sucrose solution in a beaker. To this, add 20 mL (by a pipette) of 2M HCl. Note the time when HCl is added to the sucrose solution. Fill the cuvette with this solution and measure the angle of rotation at different time intervals (see table).

Time (t) minutes	**Optical Rotation (At)**	**(At-A∞)**	**Log** $(\frac{Ao-A\infty}{At-A\infty})$	**k**
5				
10				
20				
30				
40				
60				

Precaution: Wipe the outer surface of the cuvette free of sugar solution with tissue paper before placing inside the polarimeter.

Additional questions:

1. In a polarimeter experiment, the rotation of the plane polarized light by the sample is due to – absorption, scattering, reflection of light or any other process taking place? Explain your answer.
2. Why is it preferred to use a sodium D-line as the source of light for polarimeter experiment? Can other sources of light be used?
3. Although optical rotation is taken as a characteristic signature of a chiral system or molecule, the below shown molecule does not show optical activity in spite of it being chiral (non-racemic). Explain why?



4. Why a circular cuvette is not used for hosting the sucrose solution and measuring the inversion process?
5. Suggest two possible sources of error in – i) construction of the polarimeter, and ii) measurement of the optical activity
6. If the time scale of inversion process in a carbohydrate/molecule is similar to the time scale of the polarimeter measurement, should polarimeter be used to understand such a process? Justify your answer.
7. Does the optical activity of a given molecule change if any of the following conditions are changed – i) solvent in which the measurement is carried out, ii) temperature, and iii) concentration. Justify your answer.

Additional resources:

1. A you tube demonstration of the building a polarimeter, https://www.youtube.com/watch?v=3v1nmc7gu7k&t=391s and https://www.youtube.com/watch?v=AZL7lgWG6DI&t=2s&ab_channel=CuriositasScience
2. Polarization of light (unpolarized, linearly polarized and circularly polarized light)

a. https://www.youtube.com/watch?v=8YkfEft4p-w&t=186s&ab_channel=PhysicsVideosbyEugeneKhutoryansky,
b. https://www.youtube.com/watch?v=PJHCADY-Bio&ab_channel=EricMickelsenEricMickelsen
c. https://www.youtube.com/watch?v=mjwQTL6G8Fs&ab_channel=MITOpenCourseWare

References:

1. Kvittingen, L.; Sjursnes, B. J., *J. Chem. Educ.* **2020**, *97*, 2196.
2. Organic Chemistry, Morrison and Boyd, 6th Edition, Pages: 128-138 and 1191.

7. Complexometric Titration

Objective: Determination of total hardness of water using complexometric titration with Ethylenediaminetetraacetic Acid (EDTA).

Introduction:

Hard water contains high dissolved mineral content—usually, the higher the water hardness, the lower the toxicity of heavy metals to aquatic life. However, hard water is unsuitable for most industrial purposes. Laundries require soft water of zero hardness to avoid precipitation of soaps on textiles. Hard water removes soap from solution as an insoluble precipitate and is not available for detergent action until a sufficient amount is consumed for rendering the water soft. The presence of iron in hard water may lead to staining of fabrics. Boiler feed water should be softened in order to avoid scale formation on boiler walls and tubes leading to pitting corrosion and poor heat transfer. The dyeing industry needs water free from iron which can react with dyes to form insoluble lakes of undesirable shades. A number of chemical methods are available for softening water by precipitation as insoluble carbonates and hydroxides but these do not remove all mineral content since they introduce Na^+ and Cl^- ions. Demineralization can be achieved by distillation. Extent of hardness can be measured in terms of $CaCO_3$ concentration through titration with EDTA as detailed below.

Principle:

EDTA forms stable complexes with bivalent metal ions in basic or slightly acidic medium and it is widely used as a complexing agent for metal ions. If we represent EDTA by the formula H_4Y, its disodium salt will be Na_2H_2Y. The later yields the complex-forming anion H_2Y^{2-} in aqueous solution. The reaction of EDTA with a bivalent cation M^{++} may therefore be written as:

$$M^{++} + H_2Y^{2-} = MY^{2-} + 2H^+$$

The structural formula of H_2Y^{2-} is depicted below (Figure 1):

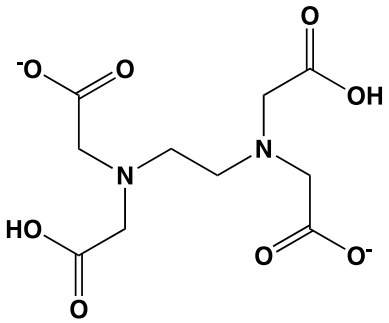


Figure 1. Structure of EDTA (H_2Y^{2-})

In titrating the metal ion solution with EDTA, the indicator usually chosen is Eriochrome Black T (EBT) (Figure 2). The EBT indicator forms a wine-red complex with the metal ion which breaks up giving way to the metal - EDTA complex. At the end point, plus a slight excess of EDTA makes the wine-red color disappear completely, leaving the metal-EDTA complex in solution and the blue color of free indicator appears. The color change at the end point is from wine red to blue-violet.

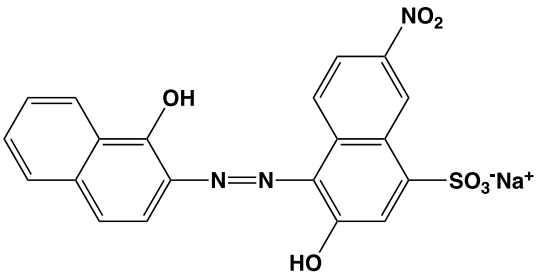


Figure 2. Structure of EBT

The color changes in the solution containing M^{++} ion and EBT in the presence of EDTA can be followed to comprehend the formation of different complexes during the titration. An optical spectroscopy experiment will be performed (mentioned as part B in the following) to monitor the serial changes in the solution. The EBT molecule contains a highly conjugated azo (-N=N-) group that exhibits an absorbance feature in the visible region. This band shifts significantly upon the formation of M^{++} -EBT complex. The EDTA is a better chelating ligand (hexadentate) compared to EBT (tridentate). Hence, Addition of EDTA strips the M^{++} ion from the EBT to regenerate the free EBT ligand. The final step will revert the optical spectra back to the original condition.

Procedure:

Experiment A.

I) Standardization of EDTA.

Pipette out 10 mL of 0 .02 M $MgSO_4$ soln. in a clean conical flask. Add ½ test tube of buffer (pH = 10) and 2 drops of the EBT indicator. Titrate with EDTA in a burette till the color changes from wine red to blue. Repeat the titration till two successive readings do not differ by more than 0.1 ml. Let the volume of EDTA required be 'a' mL.

Calculations:

Standardization:

Molarity of $MgSO_4$ = 0.02 M

$$\text{Molarity of EDTA} = \frac{10 \times 0.02}{a} = M1$$

II) Estimation of total hardness of water.

Make up the given solution to 100 mL in the standard flask by carefully diluting up to the mark using distilled water. Pipette out 10 mL of this solution and then follow the same procedure as in Part- I.

Estimation:

Molarity of EDTA = M1

Volume of EDTA = b mL

Hardness of water in the given solution in terms of

$$CaCO_3 = \frac{M1 \times b}{10} \times \frac{100}{10}\ g = M1 \times b \text{ grams.} = X \text{ grams.}$$

Results:

1. Molarity of EDTA : .....M1. .....M.

2. Amount of $CaCO_3$ in the given hard water sample.....X .....gm.

Experiment B.

Procedure:
1. Take the EBT solution prepared in deionized water (supplied by the TA), and split it into three portions in the 50 mL beakers.
2. Leave the first beaker as it is.
3. Add three drops of Mg(II) salt solution to the second beaker. Notice the color change.
4. In the third beaker, add Mg(II) salt solution (three drops) followed by five drops of EDTA solution. Notice the sequential color change.
5. Record the final colors of the solutions present in the three beakers.
6. The TA will provide the optical spectra recorded for these three solutions.

Analysis
1. Explain the color change of the EBT solution following the periodic addition of Mg(II) and EDTA. Use the supplied optical spectra to support your hypothesis.

Suggested Reading:

Vogel's Textbook of Quantitative Analysis Revised by G.H.Jeffery, J.Basset, J.Mendham and R.C.Denny, 5th edition, Pages: 309-333

Questions:

1. Why metal-EDTA complex is pale coloured compared to metal-EBT? (Discussion on the conjugation of the ligand on metal complex absorbance properties).
2. Why the metal binding is preferred to EDTA vs EBT? (Discussion on the chelation effect, ligand denticity, and the thermodynamic stability).
3. What is the role of the azo group in EBT? (The role of azo group indifferent varieties of regular dyes can be discussed).

x-x-x-x- x-x-x-x-x- x-x-x-x-x- x-x-x-x-x- x-x-x-x-x- x-x-x-x-x- x-x-x-x-x- x-x-x-x-x- x-x-x-x