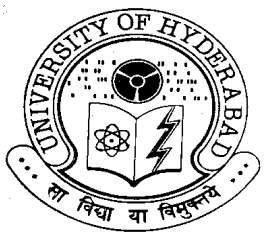
Course Manual

Physical Chemistry Laboratory –I (CY 457)



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Contents

Preparation for the laboratory class and laboratory practices

1. Read the instruction manual carefully; discuss with the instructors any point that is not clear.
2. Read and understand the principles of the experiment before coming to the laboratory class.
3. You must arrive for the laboratory on time; late entry into the laboratory is not allowed.
4. Follow strictly, the instructions given in the laboratory by the instructors.
5. Safe practices in the laboratory are of utmost importance:
   a. Wear shoes that cover your feet properly.
   b. Wear preferably cotton clothing; clothing made of easily inflammable materials such as silk should be avoided.
   c. Do not use your mouth to draw solutions into the pipette or similar equipment.
6. Inside the laboratory, maintain absolute discipline in your behaviour and focus full attention on the experiment you are carrying out. Do not engage in any conversations and discussions. Do not compare your experimental observations and results with those of others; you are supposed to report your own results.
7. Keep your laboratory bench clean and organized; careless and disorderly handling of chemicals, glassware or equipment can lead to major laboratory accidents.
8. Maintain your laboratory notebooks properly. You must have two notebooks; (i) the Observation Notebook for recording the various observations and data during the experiment in the laboratory class and (ii) the Record Notebook for reporting the details of the experiments, observations and conclusions in the following laboratory class.
9. After you finish the experiment, ensure that you obtain the signature of the course instructor on the data/observations recorded in your Observation Notebook. The same observations along with details of the experiment, analysis, results and conclusions must be recorded neatly in the Record Notebook and submitted promptly at the beginning of the following laboratory class. If this is not followed strictly, the grade for the specific experiment will not be awarded.
10. At the end of the laboratory class, hand over to the laboratory staff, the glassware, chemicals, equipment etc. after proper cleaning.
11. Reagent bottles and their stoppers should not be left at the work bench. They should be returned to their proper place upon the shelves immediately after use. If a reagent bottle is empty, bring it to the attention of laboratory staff.
12. Use the chemicals/samples provided to you very carefully, without wasting any material; extra samples will not be given under any circumstance.
13. Handle all the equipment carefully, following strictly the instructions for appropriate usage.

Course evaluation / Grading

Step 1. Continuous assessment (60 marks): All experiments will be given equal weightage. The grading for each experiment will be based on an assessment (for that experiment) of: (a) the performance and experimental work in the laboratory, (b) quality and correctness of the observations and recording of data, (c) presentation in the Record Notebook and (d) responses to questions during the evaluation of the Record Notebook in the following week.

Step 2. End semester written examination (40 marks): This will be a 1 h examination to assess the understanding of the basic theory and conceptual details of all the experiments carried out during the semester.

Experiment 1

Kinetics of Iodine Clock Reaction

Kinetics of the oxidation of iodide by hydrogen peroxide is studied; the clock refers to the periodic cycling between iodide and iodine (visualized by the starch-iodine complex) effected by the hydrogen peroxide in the forward direction and thiosulfate in the reverse

Basic concepts

Kinetics of the oxidation of iodide (I^-, often in the form I_3^-) by hydrogen peroxide (H_2O_2) is studied in this experiment. By an ingenious trick of converting the iodine (I_2) formed, back to I^- using sodium thiosulfate, $Na_2S_2O_3$, the concentration of I^- is maintained constant so that the reaction is controlled essentially by the concentration of H_2O_2 alone which keeps decreasing with each run. By successive addition of small amounts of $Na_2S_2O_3$, several different runs of the experiment are mimicked in a single flask. The reactions involved are:

$$2I^- + H_2O_2 + 2H^+ \rightarrow I_2 + 2H_2O; \qquad I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

Experimental procedure

1. Prepare the following solutions:
   (a) 100 ml 0.01 M H_2O_2 (the solution provided should be diluted appropriately based on the strength indicated on the bottle)
   (b) 100 ml 0.1 M KI
   (c) 100 ml 0.01 M $Na_2S_2O_3.5H_2O$
   (d) 50 ml pH 4.5 buffer solution (mix equal volumes of 0.2 M sodium acetate and 0.2 M acetic acid)
   (e) Fresh starch solution (0.5 g starch made into a paste and dissolved in 50 ml boiling water)
   (f) 100 ml 0.02 M oxalic acid ($H_2C_2O_4.2H_2O$)
   (g) 100 ml 0.004 M KMnO4
   (h) 100 ml 2.5 M H_2SO_4

   (f) – (h) are required for estimating H_2O_2

2. Standardise the potassium permanganate solution using the oxalic acid, and then the hydrogen peroxide solution, using the potassium permanganate solution.

3. In a 250 ml conical flask, mix 20 ml of the buffer, 10 ml of the potassium iodide solution and 50 ml of distilled water; add 2 ml of the starch solution and 1 ml of the sodium thiosulfate solution. Maintain the reaction mixture at a temperature (T).
4. To the mixture prepared in 3. above, add 20 ml of the hydrogen peroxide solution, starting the clock when the solution is half down the pipette.
5. Stir the solution continuously and note the time taken for the appearance of the blue color (indicating the formation of iodine).
6. Add 1 ml of the sodium thiosulfate solution, starting the clock simultaneously; the blue color disappears immediately.
7. Stir the solution continuously and note the time taken for the reappearance of the blue color.
8. Repeat steps 6 and 7 at least twelve times.

Data and analysis

1. The calculations proceed as follows:
   a. Concentration of H_2O_2 prepared = x M
   b. Volume of H_2O_2 taken for the reaction = v ml
   c. Quantity of H_2O_2 taken for the experiment = xv mmol
   d. Initial concentration of H_2O_2 (C_0) = xv/V M
   e. Concentration of $Na_2S_2O_3$ taken = y M
   f. Volume of $Na_2S_2O_3$ added initially = v_1 ml
   g. Quantity of $Na_2S_2O_3$ present initially = yv_1 mmol
   h. Quantity of H_2O_2 consumed in the first step = ½ yv_1 mmol
   i. Final concentration of H_2O_2 in the first step (C) = [xv - ½ yv_1]/V M
   j. ln (C_0/C) = ln{xv/[xv - ½ yv_1]}
   k. Time taken for the step = t s
   l. First order rate constant (k) = [{ln (C_0/C)}/t] s^{-1}

V is the total volume in ml, of the reaction mixture; it need not be known explicitly as will be seen below

The volume change is assumed to be negligible

2. For step 2, C_0 is equal to the C calculated in step 1; the calculations may be repeated for further steps.
3. Tabulate the data as follows:

Step	Volume of $Na_2S_2O_3$ added (ml)	Amount of H_2O_2 (mmol)		ln (C_0/C)	Time taken, t (s)	k (s^{-1})
		Beginning (xv)	End (xv - ½ yv_1)			
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						

4. Estimate the average value of the rate constant, k at temperature T.
5. The experiment may be repeated at different temperatures; Arrhenius plot can be used to determine the activation energy of the reaction, and the pre-exponential factor.

Further reading

http://www.kbcc.cuny.edu/academicDepartments/PHYSCI/PL/chm12/Documents/CHM12_Experiment_5_Kinetics.pdf

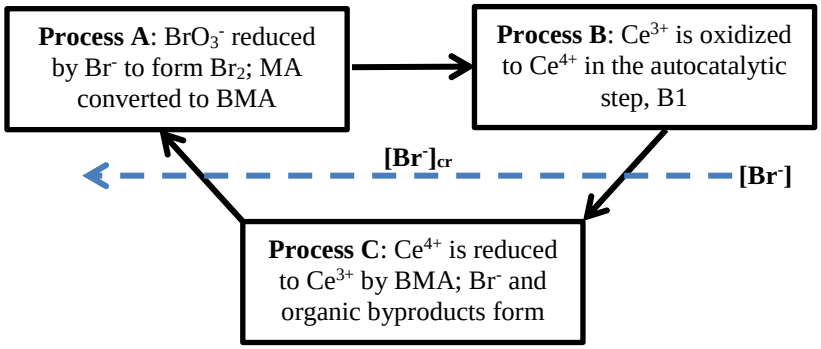
Experiment 2

Study of an Oscillating Reaction

The oscillating reaction (Belousov-Zhabotinsky) is studied using the Ce^{3+}/Ce^{4+} system; dependence of the oscillation period on the metal ion concentration is monitored.

Basic concepts

Several natural phenomena show periodic (oscillatory) behavior, for example night and day or cell division. Oscillatory behavior in chemical reactions is associated with regular and periodic fluctuation in the concentration of a specific chemical component. This occurs when (i) the reaction follows a complex mechanism with at least one auto-catalytic step (where the product catalyzes the reaction step) coupled with the other steps, (ii) the system is far from equilibrium with some components in large excess and some are removed from the system, for example, as gases, (iii) there are large variations in the rate constants of different steps. The most celebrated oscillatory reaction is perhaps, the Belousov-Zhabotinsky reaction; a typical case involves a solution of malonic acid and bromate ions with a metal ion or metal ion complex acting as a catalyst in acidic medium. The metal ion or complex commonly used are Ce^{4+}/Ce^{3+} and $Fe(phen)_3^{3+}/Fe(phen)_3^{2+}$ (ferriin/ferroin). An explanation for the oscillation of the metal ion concentration was provided by several models such as Field-Körös-Noyes (FKN) and the 'Oregonator'. Even though a large number of steps are likely to occur in this complex reaction, the essence of the oscillatory process can be captured using the following cycle which is expanded further in the table below.



Process A		
1	$Br^- + BrO_3^- + 2H^+ \rightarrow HOBr + HBrO_2$	**P** + **S** → **Q** + **R**
2	$Br^- + HBrO_2 + H^+ \rightarrow 2HOBr$	**P** + **R** → 2**Q**
3	$Br^- + HOBr + H^+ \rightarrow Br_2 + H_2O$	
4	$CH_2(COOH)_2 + Br_2 \rightarrow BrCH(COOH)_2 + H^+ + Br^-$	
Process B		
1	$HBrO_2 + BrO_3^- + 2Ce^{3+} + 3H^+ \rightarrow 2HBrO_2 + 2Ce^{4+} + H_2O$	**R** + **S** + 2**X** → 2**R** + 2**Y**
2	$2HBrO_2 \rightarrow HOBr + BrO_3^- + H^+$	2**R** → **Q** + **S**
Process C		
1	$BrCH(COOH)_2 + Ce^{4+} + CH_2(COOH)_2 + H^+ \rightarrow Br^- + Ce^{3+}$ + organic products	**BMA** + **Y** → **P** + **X** + …

P = Br^-; **Q** = HOBr; **R** = $HBrO_2$; **S** = BrO_3^-; **X** = Ce^{3+}; **Y** = Ce^{4+}; **BMA** = bromomalonic acid

Depending on the concentration of bromide ion, whether it is above or below the critical value, $[Br^-]_{cr}$, the processes A and B cycle back and forth; the products of these processes BMA and Ce^{4+} react accordingly. The concurrent oxidation of the organic species in the step C1, reduces Ce^{4+} to Ce^{3+}, and regenerates Br^-; this is accompanied by the color change form blue to red (in presence of the ferroin indicator). Oxidation of Ce^{3+} to Ce^{4+} in the step B1 shows the reverse color change. The experiment can be carried out in a number of ways; we will follow one of them.

Experimental procedure

1. Prepare (or procure) the following solutions:
   (a) 100 ml 0.006 M ceric ammonium sulphate [$Ce(NH_4)_4(SO_4)_4.2H_2O$] in 0.5 M sulfuric acid
   (b) 100 ml 0.09 M potassium bromate ($KBrO_3$) in 0.5 M sulfuric acid
   (c) 100 ml 0.30 M malonic acid ($CH_2(COOH)_2$) in 0.5 M sulfuric acid
   (d) Ferroin indicator solution
   (e) 20 ml each of 0.0045, 0.003, 0.0025 and 0.0015 M ceric ammonium sulphate in 0.5 M sulfuric acid [these may be prepared by appropriate dilution of (a); use 0.5 M sulfuric acid for the dilutions]
2. Prepare reaction mixtures containing equal volumes of the solutions (a), (b) and (c); note down the effective concentration of each in the mixture. Maintain the mixture at a constant temperature. Add a few drops of ferroin; wait for a few minutes for the

color oscillation to start. Note down the oscillation period at least 10 times, and estimate the average value and standard deviation.

3. Repeat step 2. using each of the ceric ammonium sulphate solutions prepared in (e).
4. Prepare a reaction mixture containing equal volumes of the solutions (a), (b) and (c). Record the electronic absorption spectrum when the solution shows a clearly visible color. Note down the peak position (λ_{max}).
5. Record the time variation of the absorbance at λ_{max}.

Data and analysis

1. Plot the oscillation period (with standard deviation) determined from steps 2 and 3, as a function of the concentration of ceric ammonium sulphate solution.
2. Plot the time variation of the absorbance at λ_{max} determined in step 5.
3. Compare the period of the oscillation determined from the visual and the spectral observations.
4. Write down the rate equations for the concentrations, [P], [R] and [Y] using the 5 symbolic equations in the last column of the Table {note that solution of these differential equations provide insight into the oscillatory nature of the reaction}.

Further reading

1. O. Benini, C. Rinaldo, P. Fetto, *J. Chem. Ed.* **1996**, *73*, 865.
2. https://www.rose-hulman.edu/mathjournal/archives/2002/vol3-n1/paper1/v3n1-1pd.pdf

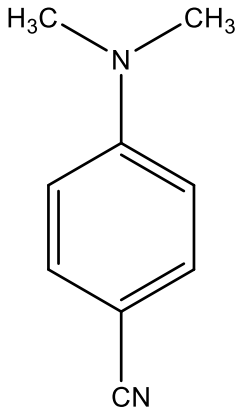
Experiment 3

Determination of the Change in Dipole Moment on Electronic Excitation

Change in the dipole moment on electronic excitation of a push-pull chromophore is determined by studying the solvatochromism of its fluorescence emission Stokes shift.

Basic concepts

UV or visible light excitation of molecules can lead to significant electron density redistribution and hence change in the dipole moment, between the ground and excited electronic states. Energy level of polar ground or excited electronic states can be shifted by solvation effects. For example, the electronic excitation energy can increase markedly, if the molecule has an appreciable ground state dipole moment, and is placed in a solvent with a high dielectric constant. In a similar solvent, the fluorescence emission energy would decrease if the molecule has an excited state with a high dipole moment. These solvatochromic effects therefore provide insight into the dipole moment of the molecule in the relevant electronic states. In this experiment, the change in dipole moment on excitation ($\Delta\mu_{ge}$) of a 'push-pull' molecule (N,N-dimethylamino benzonitrile, DMABN) will be determined by measuring the solvatochromism of its Stokes shift (shift in energy between the excitation and fluorescence emission). Solvent mixtures with varying polarity will be used to observe the solvatochromic effect, and a standard betaine dye (known as the Reichardt's dye) will be used as a reference. Incidentally, DMABN has a complex excited state profile, which leads to interesting consequences in the fluorescence emission spectrum.



DMABN

Some data required for the experiment: solvent polarity value, E_T^N for toluene (T) – acetonitrile (A) mixtures:

Vol. % of A in T-A mixture	20	30	40	50	60	70	80	90
E_T^N	0.286	0.312	0.349	0.360	0.379	0.397	0.410	0.420

Experimental procedure

1. Prepare mixtures of toluene (T) and acetonitrile (A) with 20, 30, 40, 50, 60, 70, 80 and 90% by volume of acetonitrile. The solvent polarity parameter of each of these mixtures denoted by the E_T^N value is provided above.

What is E_T^N ?

2. Dissolve equal weight of DMABN in each of the solvent mixtures prepared in step 1, to make approximately 1 μM solutions.
3. Record the electronic absorption and fluorescence emission spectra of all the solutions prepared in step 2; the wavelength corresponding to the maximum of the absorption spectrum (λ_{max}) may be used as the excitation wavelength for recording the fluorescence spectrum.

What is the basis for choosing λ_{exc} ?

4. Record on paper, the absorption and fluorescence spectrum of only one solution; note down the λ_{max} for both spectra of all the solutions.

Data and analysis

1. Calculate the Stokes shift ($\Delta\bar{\nu}$ in cm^{-1}) for the different DMABN solutions, corresponding to each of the peaks (if more than one is observed) in the fluorescence spectrum.

What is the origin of the peaks in the fluorescence spectrum ?

2. Plot the Stokes shifts against the E_T^N values. Carry out a least square fit analysis and determine the least square fit straight line; note down the slope.
3. $\Delta\mu_{ge}$ for DMABN is given by following equation:

$$\Delta\bar{\nu}\ (cm^{-1}) = 11307.6\left\{\left(\frac{\Delta\mu_{ge}}{\Delta\mu_D}\right)^2\left(\frac{a_D}{a}\right)^3\right\}E_T^N + constant$$

where $\Delta\mu_D$ (9 D) and a_D (6.2 Å) are the dipole moment change on excitation and the Onsager radius respectively, of the betaine dye used for the E_T^N determination. $\Delta\mu_{ge}$ and a are the corresponding quantities for DMABN; a of the molecule may be approximated by half the length of the molecular dipole.

4. Compare the value of $\Delta\mu_{ge}$ you have determined with what is reported in the literature.
5. Discuss the reason for the solvatochromism observed.
6. Find out the structure of the betaine dye; why is it uniquely suited for solvent polarity measurements ?

Further Reading

M. Ravi, A. Samanta, T. P. Radhakrishnan, *J. Phys. Chem.* **1994**, *98*, 9133.

Experiment 4

Adsorption of Acetic Acid on Charcoal

Adsorption of acetic acid on charcoal is studied, and the adsorption isotherm determined.

Basic concepts

Adsorption is the process of adhesion of atoms, molecules or ions from a gaseous or liquid phase on to a surface, usually of a solid material; the interaction is generally weak and reversible, if it is a case of physical adsorption (chemical adsorption involves a bond formation and is stronger). Materials with a large surface area per unit weight (for example, charcoal) is capable of adsorbing significant amount of molecules (like acetic acid or oxalic acid) from an aqueous solution. Adsorption from solution often follows the Freundlich isotherm:

$$\left(\frac{X}{m}\right) = kC_e^n$$

where X is the number of moles of the adsorbate (acetic acid) adsorbed by m g of the adsorbent (charcoal); C_e is the equilibrium concentration of the adsorbate and k and n are constants for a particular adsorbate-adsorbent combination. The equation can be rewritten as :

$$log\left(\frac{X}{m}\right) = constant + (n \times logC_e)$$

implying that a plot of $log\left(\frac{X}{m}\right)$ versus $\log(C_e)$ is linear.

Experimental procedure

1. Prepare (or procure) the following:
   (a) 250 ml 0.5 N acetic acid solution
   (b) 100 ml 0.5 N sodium hydroxide (solution)
   (c) Phenolphthalein indicator
   (d) Powdered charcoal
2. Weigh 2.00 ± 0.01 g charcoal each into 6 stoppered conical flasks or bottles.
3. Add into the flasks, acetic acid solution + water as follows:

Flask No.	**1**	**2**	**3**	**4**	**5**	**6**

0.5 N acetic acid (ml)	50	40	30	20	15	10
Water (ml)	0	10	20	30	35	40

4. Shake thoroughly each sample regularly for a period of ~ 20 min. Allow to stand for ~ 5 min.
5. Filter through a filter paper; take care to use a small filter paper. Collect the filtrate in appropriately labeled flasks; discard the first 5 ml while collecting the filtrate.
6. Titrate 10 ml of each filtrate ~~and titrate~~ against 0.5 N NaOH solution; use phenolphthalein as the indicator.
7. Titrate also 10 ml of the stock 0.5 N acetic acid solution.

Justify the precautions taken during filtration.

Data and analysis

1. Titration value for the 10 ml of stock 0.5 N acetic acid = x ml (NaOH).
2. From x, the concentration of acetic acid in each flask before adsorption can be estimated (in terms of the volume equivalent of 0.5 N NaOH), considering the dilution involved (use the Table in step 3 of the experimental procedure); titrations in step 6 provides the concentrations after adsorption *ie.* at equilibrium.
3. Amount of acetic acid adsorbed (X) on charcoal can be estimated using the initial (before adsorption) and final (equilibrium) amount of acetic acid, in terms of the volume equivalent of 0.5 N NaOH; see the following table:

Flask No.	**Weight of charcoal, m (g)**	**Concentration of acetic acid (volume equivalent in ml of NaOH)**			$\frac{X}{m}$	$\log\left(\frac{X}{m}\right)$	$\log C_e$
		Initial, C	**Final, C_e**	**X = C - C_e**			
1							
2							
3							
4							
5							
6							

4. Plot $\log\left(\frac{X}{m}\right)$ versus $\log C_e$; verify if the Freundlich isotherm is followed.
5. Plot the data appropriately to see if it follows the Langmuir adsorption isotherm.

Further Reading

P. Atkins, J. de Paula, *Atkin's Physical Chemistry*, Oxford University Press, 2010.

Experiment 5

Fluorescence Study of Critical Micellar Concentration

The critical micellar concentration of a surfactant is determined by measuring the steady state fluorescence intensity of an environment-sensitive probe as a function of the concentration of surfactant

Basic concepts

Surfactant molecules aggregate to form a micellar structure only when the concentration exceeds a particular value, which is commonly termed as critical micellar concentration (CMC). In this experiment, the CMC of an anionic surfactant, sodium dodecyl sulfate (SDS), is determined by following the variation of fluorescence intensity of 8-anilino-1-naphthalene sulfonate (ANS), an environment sensitive fluorescence probe, for various concentrations of SDS. As ANS molecules exist in aqueous environment below the CMC, but above it they get incorporated into the micelle, wherein the polarity is very different from that in aqueous medium, the formation of micelle is associated with a sharp change in the plot of fluorescence intensity (I_f) versus [SDS].

Experimental procedure

1. Prepare 100 ml of ~10^{-4} M aqueous solution of ANS.
2. Divide this solution to 10 x10 ml volumetric flasks.
3. Label these solutions from 1 to 10
4. Add appropriate amount of solid SDS to these solutions (except for the solution labelled 1) such that [SDS] in flasks labelled 2-10 are 0.5, 0.8, 1.0, 2.0, 5.0, 8.0, 10.0, 15.0 and 20.0 mM, respectively.
5. Measure the fluorescence spectra of the solutions under identical instrumental settings.
6. Plot the fluorescence intensity (I_f) of the system versus [SDS].
7. Determine CMC of SDS from the plot and compare it with the literature value.

Suggested reading

1. Photochemistry in Microheterogeneous Systems, K. Kalyanasundaram
2. Any other book on fluorescence spectroscopy or photochemistry

Experiment 6

Spectrophotometric Determination of pK_a

The pK_a of a weak acid is estimated by measuring the concentrations of its neutral (acidic) and deprotonated (basic) forms spectrophotometrically at various pH.

Basic concepts

The extent of dissociation of a weak acid can be determined spectrophometrically when the characteristic absorption of its acidic and basic forms are measured. In this experiment, the dissociation constant and pK_a of a weak acid, methyl red, is estimated by determining the concentrations of its acidic (HMR) and basic (MR^-) forms at different pH by spectrophotometric measurements. In the case of methyl red, its two forms have strong characteristic absorption in the visible region with HMR (red) having an absorption peak at ~520 nm and MR^- (yellow) at 430 nm. The transformation between the two forms are observable in the pH interval of 4 – 6 and this pH range can be conveniently obtained by CH_3COONa - CH_3COOH buffer. The determination of pK_a involves three steps: (i) determination of peak wavelengths, λ_A and λ_B, for HMR and MR^-, respectively, (ii) verification of Beer's law for both forms at λ_A and λ_B and (iii) determination of relative amounts of HMR and MR^- as a function of pH of the medium.

The relative amount of HMR and MR^- present in solution can be calculated by relating the measured absorbance, A_A (at λ_A) and A_B (at λ_B) as:

$$A_A = d_{A,HMR}\,[HMR] + d_{A,MR^-}\,[MR^-] \qquad (1)$$

$$A_B = d_{B,HMR}\,[HMR] + d_{B,MR^-}\,[MR^-] \qquad (2)$$

By estimating $d_{A,HMR}$, $d_{B,HMR}$, d_{A,MR^-}, and d_{B,MR^-} from the plots (discussed later), the ratio, $[MR^-]/[HMR]$ in solution can be calculated by solving simultaneous equations (1) and (2), and pK_a can be determined using

$$pK_a = pH - \log[MR^-]/[HMR] \qquad (3)$$

Experimental procedure

1. Prepare a <u>stock</u> solution (in a 50 ml volumetric flask) of methyl red by dissolving 0.1 g in 30 ml ethanol and then diluting it by water to 50 ml.

2. Take 5 ml of this stock solution in a 100 ml volumetric flask. Add 50 ml ethanol and then make up the volume to 100 ml by water. This is your standard solution.
3. Then prepare solution A by diluting a mixture of 10 ml of standard solution and 10 ml of 0.1 M HCl solution to 100 ml. (the pH of this solution is expected to be ~2 and methyl red will be present exclusively as HMR).
4. Prepare solution B by diluting a mixture of 10 ml standard solution and 25 ml 0.04 M CH_3COONa solution to 100 ml. (the pH of this solution is expected to be ~8 and methyl red will be present entirely as MR^-)
5. Measure the absorption spectra of solution A and solution B over the range of 350 – 600 nm and determine λ_A and λ_B.
6. Measure out 40, 25 and 10 ml of solution A into three separate 50 ml volumetric flasks and make up the volume to 50 ml by 0.1 M HCl solution. The solutions will contain 0.8, 0.5 and 0.2 times the initial [HMR].
7. Measure out 40, 25 and 10 ml of solution B into three separate 50 ml volumetric flasks and make up the volume to 50 ml by 0.01 M CH_3COONa solution. The solutions will contain 0.8, 0.5 and 0.2 times the initial $[MR^-]$.
8. Measure the absorbance of this six solutions at λ_A and λ_B.
9. Plot absorbance versus relative concentration of methyl red (Plot 1). In each case, a linear plot is obtained.
10. Prepare the following solutions in four 100 ml volumetric flasks by mixing 10 ml standard solution, 25 ml 0.4 M CH_3COONa solution and
    (i) 50 ml 0.02 M CH_3COOH solution and 15 ml water
    (ii) 25 ml 0.02 M CH_3COOH solution and 40 ml water
    (iii) 10 ml 0.02 M CH_3COOH solution and 55 ml water
    (iv) 5 ml 0.02 M CH_3COOH solution and 60 ml water
11. Measure the pH of each solution.
12. Measure the absorbance of each solution at λ_A and λ_B.
13. For each solution, obtain $d_{A,HMR}$, $d_{B,HMR}$, d_{A,MR^-}, and d_{B,MR^-} at relative concentration of 1.0 from Plot 1.
14. Solve simultaneous equations (1) and (2) to determine $[MR^-]/[HMR]$ in each solution.

15. Now, calculate pK_a using equation (3).

Suggested reading

1. Physical Chemistry, by P.W. Atkins and J. de Paula

Experiment 7

Conductometric Study of Critical Micellar Concentration

The critical micellar concentration of a surfactant is determined by monitoring the change in specific conductivity of the solution as a function of the concentration of surfactant

Basic concepts

Surfactant molecules aggregate to form a micellar structure only when the concentration exceeds a particular value, which is commonly known as its critical micellar concentration (CMC). In this experiment, the CMC of an anionic surfactant, sodium dodecyl sulfate (SDS), is measured by following the variation of specific or/and molar conductivity of the solution as a function of the concentration of SDS. Below the CMC, addition of SDS to an aqueous solution increases the number of change carriers and hence, the conductivity. Above CMC, addition of surfactants increases the micelle concentration maintaining the monomer concentration approximately same. As micelles are much larger than the monomers and diffuse more slowly, the conductivity increases much slowly beyond the CMC. Thus CMC can be determined from the break point in the plot of specific/molar conductivity versus [SDS].

Experimental procedure

1. Use redistilled water for all measurements.
2. Prepare 25 ml of ~0.04 M aqueous stock solution of SDS.
3. Pipette 25 ml of water into the conductance cell. Add 0.5 ml SDS stock solution using a pipette. Stir the solution slowly for a minute or two (without creating too many bubbles) and then read the conductance.
4. Add additional 0.5 ml stock solution and measure the conductance. Continue the process of adding stock solution and measuring conductance until 40-45 aliquots have been added.

5. In order to study the effect of electrolyte, prepare 100 ml of ~0.02 M NaCl solution. Use this as solvent for making up 25 ml of ~0.04 M aqueous stock solution of SDS. Pipette 25 ml of salt solution into the conductance cell and measure the conductance. Add 0.5 ml of the SDS stock solution and record the conductance. Repeat the same for about 30-40 additions.
6. Prepare appropriate Table to record the conductance and [SDS] data.
7. Find out the cell constant of the conductivity cell using standard KCl solution and convert the conductance values into specific conductance (κ) and molar conductance (Λ_m). Then plot κ vs [SDS] and Λ_m vs [SDS] and determine CMC.
8. Compare the estimated CMC of SDS with the literature value.

Suggested reading

Experiment 8

Conductometric Titration of a Charge Transfer System

The formation of charge transfer complex between an electron donor and acceptor is studied and the stoichiometry of the complex is determined by following the variation of conductance of the solution with concentration of the donor and acceptor.

Basic concepts

A charge transfer complex is formed between an electron donor with low oxidation potential and an acceptor having high electron affinity. The complex dissociates into ions in medium of high dielectric constant and contribute to the conductivity. Thus formation of charge transfer complex can be studied by monitoring the conductance of a solution containing electron donor and acceptor. In this experiment, the formation of charge transfer complex between p-phenylenediamine (donor) and phthalic acid (acceptor) is investigated by measuring the conductance of the solution for several concentrations of the donor and acceptor.

Experimental procedure

1. Prepare 250 ml of 3.4 mM solution of p-phenylenediamine in acetone.
2. Prepare 250 ml of 3.4 mM solution of phthalic acid in acetone.
3. 50 ml solution of phthalic acid is taken in a beaker and its conductivity is measured.
4. The phenylenediamine solution is taken in a burette and 1 ml of it is added to the phthalic acid solution. The solution is then stirred and conductivity measured.
5. Repeat the process of addition and measurement of conductivity until the latter reaches its maximum value and then start showing a decreasing trend (140-150 ml may be required).
6. Similarly, 50 ml phenylenediamine is taken in another beaker, phthalic acid solution is then added in small quantities from a burette and the conductivity is measured.

Data and analysis

1. Estimate molar concentrations of the electron donor (D) and acceptor (A) using $M_A = M_A^0.V_A/ (V_A+V_D)$ and $M_D = M_D^0.V_D/ (V_A+V_D)$, where, M_A^0 and M_D^0 are the initial concentrations and V_A and V_D refer to the volume of the stock solution of A and D, respectively.
2. Plot a graph of conductance versus M_D (1^{st} titration of addition of donor) and another one versus M_A (2^{nd} titration of addition of acceptor) on the same graph sheet.
3. Determine the molar concentrations of phenylenediamine and phthalic acid for which maximum conductance is observed in each case and determine the stoichiometry of the complex.

Suggested reading

Experiment 9

Determination of solubility diagram for a three-component liquid system

The triangular phase diagram of a three-component liquid system involving acetic acid, chloroform and water is determined.

Basic Concepts:

According to the phase rule (F = C – P + 2; F: degrees of freedom, C: component and P: no of phases) there are at most four degrees of freedom, for a system containing three liquid components. If the pressure and temperature be kept constant, it follows that the concentrations of only two of the three components can be *independently* varied. When only two of the three parameters are independently variable, all three parameters can be represented in one plane by means of the triangular diagram shown below.\

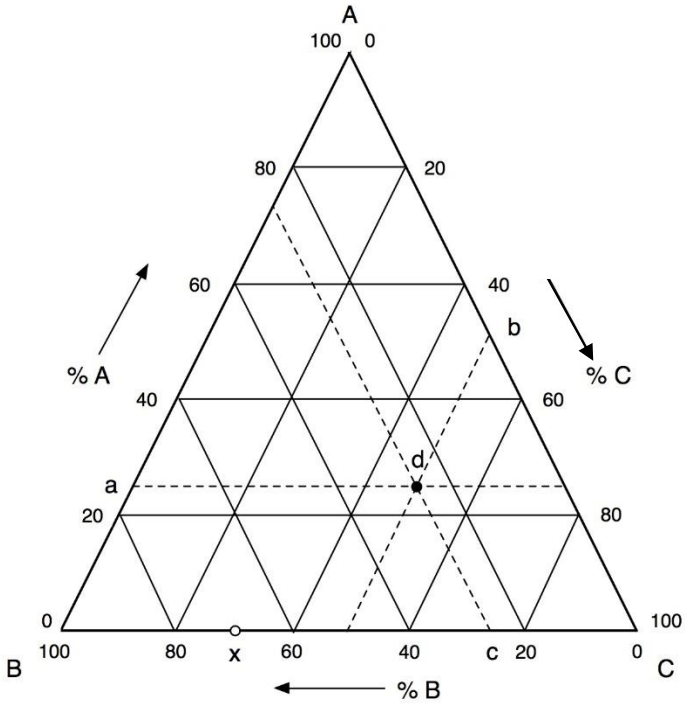


Fig. 1: Three component solubility diagram or phase diagram

In this diagram each side of the equilateral triangle represents 0-100 percent of one of the components. Let us fix the point that represents a mixture containing 25 percent of A, 25 percent of B and 50 percent of C. Let us mark the point ***a*** on side AB, which represents 25 percent of A. Now draw a dotted line from ***a*** to the opposite side (AC) parallel to the side BC. All mixtures containing 25 percent of A will fall on this. Mark a point ***b*** on BC, which represents 25 percent of B. Draw a dotted line from ***b*** to the opposite side (AC) parallel to

AB. All mixture containing 25 percent of B will fall on this. Draw dotted line from ***c*** on AC to BC parallel to AB. The three dotted lines intersects at some ***d*** which represents the composition of the mixture.

It may be noted that only two of the three dotted lines are required to find out ***d***. Any mixture of only two components can be represented on one side of the triangle. For instance, the point ***x*** represents the composition of a mixture containing 30 percent of C and 70 percent of B.

Experimental procedure:

1. Procure the following liquids: glacial acetic acid, chloroform and water
2. Set up three clean and dry burettes and fill them with acetic acid, chloroform and water separately. (caution: acetic acid is corrosive)
3. Make up 7 mixtures of chloroform and acetic acid in clean and dry glass-stoppered bottles (or conical flasks) as shown in the Table 1. The total volume of each mixture should be 20 ml.
4. Add water into each mixture, shaking the mixture well after each addition until the clear solution becomes permanently turbid. Note the volume of water for each experimental set up.

Table 1: Data table for the experiment.

Setup No.	Vol. of $CHCl_3$ (ml)	Vol. of CH_3COOH (ml)	Vol. of added H_2O (ml)	Wt % of $CHCl_3$ (ml)	Wt % of CH_3COOH (ml)	Wt % of added H_2O (ml)
1	1.5	18.5				
2	2.0	18.0				
3	3.5	16.5				
4	6.5	13.5				
5	10.0	10				
6	12.5	7.5				
7	16.0	4.0				
8				99.0		1.0
9				0.8		99.2

Data and analysis:

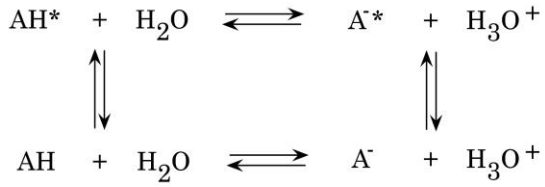
1. Calculate the wt % of the homogeneous mixture from the data table.
2. Plot the three-component phase diagram using the data

Experiment 10
Determination of excited state acidity constant

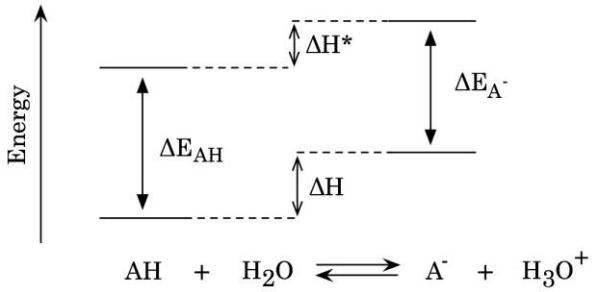
The acidity constant in the excited state is studied using UV-Vis absorption and fluorescence spectral measurements.

Basic concepts:

The acidity constant of a molecule in the excited state can be quite different from that in the ground state because of the difference in the electron distribution in the two states. This experiment provides a simple method of determination of the change in pK_a of 2-naphthol on electronic excitation from UV-Vis absorption and fluorescence spectral measurements. 2-Naphthol is a weak acid in the ground state with pK_a value of 9.5. Therefore, the compound exists almost exclusively in the neutral form (AH) at pH in between 4-5 in the ground state. However, on excitation of AH at this pH, the compound exhibits dual fluorescence that can be assigned to emission from AH* (maximum at around 360 nm) and A^{-}* (maximum at around 420 nm). The fluorescence excitation spectrum obtained on monitoring the long wavelength band is identical with the absorption spectrum of AH indicating that A^{-}* is formed as a result of the excited state deprotonation reaction, not due to direct excitation of A-.



The determination of the change in pK_a of a compound on electronic excitation is based on a method suggested by Forster which can be understood from the following diagram:



It can be seen from the above diagram, $\Delta H + \Delta E_{A^-} = \Delta H^* + \Delta E_{AH}$. Assuming the entropy changes associated with the acid dissociation in the ground and excited states are the same, one can write the free energy change as $\Delta G - \Delta G^* = \Delta E_{AH} - \Delta E_{A^-}$ and which ultimately

can be transformed as $pK_a - pK_a^* = (\Delta E_{\mathrm{AH}} - \Delta E_{\mathrm{A}^-})/2.303RT$ or $pK_a - pK_a^* = 0.625(\bar{\nu}_{\mathrm{AH}} - \bar{\nu}_{\mathrm{A}^-})/T$, where $\bar{\nu}$ is the wavenumber of the associated electronic transition abtained from the absorption and fluorescence spectra.

Experimental procedure:

1) Prepare a stock solution of 100 ml 10^{-3} M 2-naphthol in 20% methanol. Using the stock solution prepare the following solutions:
   a) 25 ml 5×10^{-4} M solution at pH 0
   b) 25 ml 5×10^{-4} M solution at pH 13
   c) 25 ml 5×10^{-4} M solution at pH in between 4-5
2) Record the absorption spectra of all solutions in the range of 300–425 nm.
3) Record fluorescence emission spectra of all solutions. Note that for the emission spectra the excitation wavelength must be at the absorption peak plus 10 nm. Scan till 550 nm
4) Record fluorescence excitation spectrum (300 – 400 nm) of the third solution monitoring the longer wavelength emission.
5) Determine the mean 0-0 transition energies of the neutral and the anionic forms from the spectral data and estimate $\Delta pK_a (= pK_a - pK_a^*)$.

Reference:

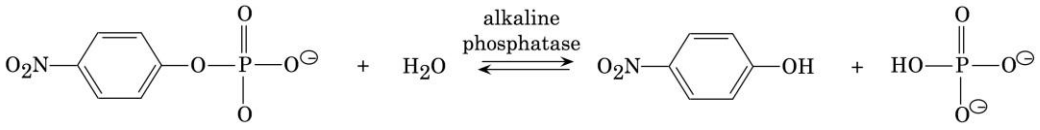
J. F. Ireland and P. A. H. Wyatt, *Adv. Phys. Org. Chem.* **12** (1976) 131 and references therein

Experiment 11
Study of enzyme-substrate catalysis reaction

The catalytic efficiency of a non-specific enzyme is studied by measuring the rate of the enzyme-catalysed reaction.

Basic concepts:

Phosphatases are a class of enzymes that can catalyse the hydrolysis of phosphate monoesters to give inorganic phosphate and an alcohol. An enzyme alkaline phosphatase is a non-specific phosphatase, recognizing a wide variety of molecules as substrates, that is found in bacteria, fungi, and higher animals. In this experiment the catalytic efficiency of an alkaline phosphatase- is investigated by measuring the rate of the enzyme-catalysed reaction under different conditions. To measure the alkaline phosphatase activity a non-biological substrate *p*-nitrophenyl phosphate is chosen as substrate (Scheme 1) that is hyldrolyzed to *p*-nitrophenol by the enzyme.



Scheme 1. The hydrolysis of *p*-nitrophenyl phosphate as catalysed by alkaline phosphatase.

Activity assays of alkaline phosphatase with *p*-nitrophenyl phosphate are terminated by the addition of NaOH. This serves two functions; first, it stops the enzyme-catalysed reaction by changing pH which is unsuitable for the enzyme (the enzyme is denatured at high pH and so unable to function). Second, it deprotonates the *p*-nitrophenol to give the yellow coloured *p*-nitrophenylate. The progress of the reaction is monitored by absorption spectrum of *p*-nitrophenylate (obtained upon deprotonation of *p*-nitrophenol) that has an intense yellow colour.

Experimental procedure:

1. Prepapre or procure the following solutions:
    a) 100 ml of phosphate buffer of pH 6.8
    b) 5 ml 7.5 mM *p*-nitrophenyl phosphate and 5 ml alkaline phosphatse solutions
    c) 20 mL of 1.0 M NaOH

2. Turn on the spectrometer and set the wavelength to the λ_{max} of *p*-nitrophenylate, which you need to determine first.
3. Dispense 6 mL of phosphate buffer (pH 6.8) into a test tube.
4. **Uncatalyzed reaction:** Add 0.2 mL of *p*-nitrophenyl phosphate (7.5 mM). Place a piece of parafilm over the tubes and gently invert 3 times (DO NOT shake the solution!!!). Write down the time at which you added the *p*-nitrophenyl phosphate. At the end of the lab you will measure the absorption of this solution. This will be your uncatalyzed reaction, and will give you a measure of the rate of the non-catalysed reaction.
5. **Control reaction:** Dispense 6 mL of pH 6.8 phosphate buffer into another tube, 0.2 mL of *p*-nitrophenyl phosphate, and 1.0 mL of 1.0 M NaOH. Note the time, and place a piece of parafilm over the tube and gently invert 3 times. Transfer the solution to the absorption cell, insert the cell into the spectrometer and record the absorbance. This is the time 0 measurement, and will provide the absorbance of the solution in the absence of any reaction. This value will need to be subtracted from all other measurements to provide "net" absorption values. Transfer the contents back to the tube and set the tube aside until the end of the lab. This will allow you to measure the rate of hydrolysis in the presence of strong base. This is important because the enzymatic reactions are terminated with NaOH, and the analysis is predicated upon the assumptions that no significant reaction occurs once the NaOH has been added.
6. Now place 6 mL of the buffer into another tube and add 0.2 mL of *p*-nitrophenyl phosphate. Add 0.2 mL of the solution containing alkaline phosphatase to the tube, note the time, place a piece of parafilm over the top, and gently invert 3 times (DO NOT shake the solution!!!). This should all be done in 30-45 seconds.
7. After a total of 2 mins has elapsed add 1.0 mL of NaOH to terminate the reaction. Note: the amount of time that you allow the reaction to progress is not critical, but it is critical to know exactly what the reaction time is (i.e. whether the reaction was run for 1 min 50 sec or 2 min 5 sec doesn't matter, only that you know the actual elapsed time).
8. Take the solution into spectrophotometer cell and record the absorbance.

9. Repeat the rate measurement in duplicate.
10. Repeat Steps 5-8 for 0.4, 0.6, 0.8, 1.2 mL of *p*-nitrophenyl phosphate (7.5 mM).
11. After the enzyme rate measurements have been made, add 1.0 mL NaOH to your control reaction prepared in Step 3 and note the time.
12. Measure the absorption of the control reaction and the NaOH reaction prepared in Step 4.

Data and Analysis:

1. Use the calibration curve of *p*-nitrophenylate to convert all of the absorbance data to concentration using Beer's law.
2. Now divide the concentrations by the various reaction times (in minutes) to get reaction rates in M min^{-1} (mol/L min).
3. Use the Linewaver-Burk plot to find out the maximum rate of the reaction and Michaelis constant.

Further Reading

P. Atkins, J. de Paula, *Atkin's Physical Chemistry*, Oxford University Press, 2010.

Experiment 12
Potentiometric titration of a redox reaction

Potentiometric redox titration of $K_3Fe(CN)_6$ with Co(II) to find out the concentration of the latter in a given solution.

Basic concepts:

Cobalt (II) can be estimated by $K_3Fe(CN)_6$ in strongly alkaline medium in presence of ammonium citrate. $Co^{II}(NH_3)_6$ formed in alkaline medium is oxidised to $Co^{III}(NH_3)_6$ by ferricyanide.

The oxidation potential of any redox system is given by Nenrst equation

$$E = E^0 + \frac{RT}{nF}\ln\frac{[\text{Oxidised from}]}{[\text{Reduced form}]} \quad (1)$$

Therefore, for $Fe(CN)_6^{3-} + e \rightleftharpoons Fe(CN)_6^{4-}$ reaction

$$E = E^0 + \frac{RT}{F}\ln\frac{[Fe(CN)_6^{3-}]}{[Fe(CN)_6^{4-}]} \quad (2)$$

The cell used for the experiment can be represented as

$$\text{Std. Calomel} \parallel Fe(CN)_6^{3-}(aq), Fe(CN)_6^{4-}(aq) \parallel Pt(s)$$

The observed *emf* of such a cell is given by

$$E_{obs} = E - E_{\text{Calomel}} = (E^0 - E_{\text{Calomel}}) + \frac{RT}{F}\ln\frac{[Fe(CN)_6^{3-}]}{[Fe(CN)_6^{4-}]} \quad (3)$$

When Cobalt (II) is added to the system, $Fe(CN)_6^{3-}$ is removed and the concentration of $Fe(CN)_6^{4-}$increases, leading to gradual decrease in observed *emf*, E_{obs}. At the end point there will be a sharp decrease in E_{obs} due to the sudden removal of all ferricyanide ions.

Experimental procedure:

13. Prepapre the following solutions:
    d) 250 ml 0.01 M $K_3Fe(CN)_6$
    e) 250 ml 0.01 M $Co(NO_3)_2$
    f) Ammonium citrate solution: 25 g citric acid + 100 ml water + 67.5 ml liq. ammonia
    g) 250 ml 5N ammonia
    h) Sodium thiosulphate and potassium dichromate solutions
    i) Prepare or procure saturate KCl solution for salt bridge

14. Standardize $Na_2S_2O_3$ solution by potassium dichromate solution. Titrate $K_3Fe(CN)_6$ with standardised $Na_2S_2O_3$ and find out the strength of ferricyanide solution.

15. In a 250 ml beaker take 20 ml of ferricyanide, 20 ml of ammonium citrate and 100 ml of 5 N ammonia. The mixture is covered with sufficient petroleum ether whose function is to prevent aerial oxidation of $Co(NH_3)_6^{2-}$ and to prevent evaporation of NH_3.
16. Setup the cell for the *emf* measurement.
17. Add cobaltous nitrate solution from a burette. Stir the solution and note down the observed *emf*. Note: initially larger amounts (say 1 ml each time) may be added but as the end-point is approached, readings are taken more frequently (0.1 or 0.05 ml) and after the end point amounts in larger steps can be added.
18. Dilute the original cobaltous nitrate solution by de-ionized water so that its concentration is exactly half of that of the original one. Repeat the above procedure with this dilute solution.

Data and analysis

1. Prepare data table as given below

Experiment 1		Experiment 2	
Volume of $K_3Fe(CN)_6$ = 20 ml		Volume of $K_3Fe(CN)_6$ = 20 ml	
Vol. of Co(II) solution (ml)	E_{obs} (volt)	Vol. of Co(II) solution (mL)	E_{obs} (volt)

2. Plot E_{obs} vs Vol. of Co(II) solution. Find out the inflection point to obtain the end point of the titration. You may also calculate the first and second derivatives of your data to find the inflection point.
3. Calculate the strength of the Co(II) solutions from the emf experiments and compare the values with the indicator titration values.