Indian Institute of Science Education and Research Kolkata

Advanced Physical Chemistry Laboratory CH3105

Laboratory Manual

2024

CH3105

3rd year Practical

Autumn Semester, 2024

GENERAL INSTRUCTIONS

1. Attendance is mandatory. In case of illness etc. the student must contact the instructor and fix a schedule for making up the missed lab. All labs must be completed in order to get a passing grade.
2. All data and results should be recorded directly in the lab notebook. The recording should include, title of the experiment, date of experiment, working formula, data in tabulated forms, results and calculations.
3. The instructor should sign the data before the student leaves the lab.
4. Graph papers and computer print-outs may be directly pasted on the lab notebook.

Grading:

The marking scheme in the lab will be as follows:

1. Mid-semester examination 20
2. Lab notebook 20
3. Attendance 10
4. Continuous assessment & Viva 20
5. End Sem Evaluation 30

Lab Safety is not-negotiable!

To help you realize how important it is, **2 marks will be deducted every time** you are found not wearing the lab coat, proper footwear, and so on.

List of Experiments

Total number of students: 54 (tentatively)- made into two groups (A&B).

No.	Experiments	Page	Instr.	TAs	Tentative Date
1.	Conductometric Determination of the Critical Micellar Concentration (CMC) of Sodium Dodecyl Sulphate (SDS)	5	RV	SR, BR (4 days)	Gr. A: 05 Aug & 12 Aug Gr. B: 08 Aug & 19 Aug
2.	Determination of activation energy of a clock reaction	9	DC	BR, ARK (2 days)	Gr. A: 22 Aug Gr. B: 29 Aug
3.	The Reduction of Cr (VI) by Glutathione (Consecutive Reaction Kinetics)	11	RV	AjK BR (4 days)	Gr. A1: 02 Sept /A2:09 Sep Gr. B1: 05 Sept/B2:12 Sept
4.	Potentiometric Titration of Strong and Weak Acid Mixture Using a Strong Base	16	RV		Gr. A1: 09 Sept /A2:02 Sep Gr. B1: 12 Sept/B2:05 Sept
5.	Phase Diagram for a Three Component System	18	DC	ARK, NK (2 days)	Gr. A: 19 Sept Gr. B: 23 Sept
6.	Ground and excited state ionization of 2-napthol using spectroscopic techniques.	22	DC	ArK SR, NK, AjK (6 days)	Gr. A1: 14 Oct & 21 Oct Gr. A2: 21 Oct & 28 Oct Gr. B1: 17 Oct & 24 Oct Gr. B2: 24 Oct & 04 Nov
7.	Verification of Ostwald's Dilution Law by Conductometric Method	27	DC		Gr. A2: 14 Oct & A1: 28 Oct Gr. B2: 17 Oct & B1: 04 Nov
8.	Measurement of Rate-Constant of Fluorescence Quenching: Stern Volmer Method	30	RV	NK, BR (2 days)	Gr. A: 07 Nov Gr. B: 11 Nov
	Make-up Experiments				14 Nov

Mid Sem Quiz: 15 Oct 2024

End Sem (any 3 days): 18, 21, 25, 28 November (Experiments and Viva??)

RV-Instruct for 10 classes

DC-Instruct for 10 Classes

SR (Sampurna Roy)	BR (Bishes Ray)	NK (Nitish Kumar)	ArK (Arya K)	AjK (Amjed)
10 days	12 days	10 days	10 days	10 days

EXPERIMENT 1

Conductometric Determination of the Critical Micellar Concentration (CMC) of Sodium Dodecyl Sulphate (SDS)

THEORY:

Surfactants are amphiphilic molecules that possess both hydrophobic and hydrophilic properties. A typical surfactant molecule consists of a long hydrocarbon 'tail' that dissolves in hydrocarbon and other non-polar solvents, and a hydrophilic 'headgroup' that dissolves in polar solvents (typically water). One example of a dual character molecule having a head-group and a non-polar tail is sodium dodecyl sulphate (SDS), $NaOSO_3C_{12}H_{25}$.

When a sufficient amount of SDS is dissolved in water, several bulk solution properties are significantly changed, particularly the surface tension (which decreases) and the ability of the solution to solubilise hydrocarbons (which increases). These changes do not occur until a minimum bulk SDS concentration is reached. This concentration is called the *critical micelle concentration* (*CMC*).

Several experiments, including light scattering and NMR, show that below the *CMC*, the surfactant exists mainly as solvated monomeric species, whereas above the *CMC* these monomers undergo self-assembly to form roughly spherical structures (having an overall diameter of ~5 nm) known as micelles. Micelles are the simplest of all self-assembly structures.

Technically, a micellar solution is a colloidal dispersion of organised surfactant molecules. Non-ionic surfactant molecules can cluster together in micelles of 1000 molecules or more, but ionic species tend to form micelles of between 10 and about 100 molecules because of electrostatic repulsions between head-groups.

One of the key aspects of micelle structure is that the interior of the micelle consists of an associated arrangement of hydrocarbon chains (an 'oil droplet'). The exterior coat is constructed of the polar, ionic moieties (the SO_4^- groups in the case of SDS). This ionic surface (which also contains associated water of hydration) is called the Stern layer. Surrounding this ionic mantle is a region that contains both counterions and oriented water molecules – the Gouy-Chapman layer. Together the Stern and Gouy-Chapman layers are known as the *electrical double layer*.

But it is the oil-like interior of the micelle that gives it its many diverse and interesting properties. The hydrocarbon core (~3 nm in diameter) has the capacity to accommodate guest molecules. The most common application of micelles is as detergents but they can also act as micro-reaction vessels for organic syntheses and drug delivery agents. In this experiment you will determine some fundamental properties of the SDS micelle: the *CMC* and the free energy, enthalpy and entropy of micellisation. You will measure the *CMC* by measuring the conductivity of the system as a function

of SDS concentration. The thermodynamic properties are obtained by determining the *CMC* at various temperatures.

Conductivity of Electrolyte Solutions

Because SDS is charged it acts as an electrolyte and so obeys Ohm's Law. This means that when a voltage (V) is applied across a cell containing the SDS solution the current (I) that flows is proportional to V. Ohm's Law can be written in two equivalent ways:

$$V = IR \text{ or } I = VG \quad \text{……………………………………………..1}$$

where R is the resistance and G (=1/R) the conductance of the solution. The conductance of the solution depends upon the dimensions of the cell and the nature of the solution. The conductance of a solution contained in a cell of length l and area A is

$$G = (A / l)\ \kappa \quad \text{……………………………………..………2}$$

where κ is the conductivity of the solution and is independent of the shape of the cell.
Conductivity has the units Ω^{-1} m^{-1} or S m^{-1} where S is the SI unit of conductance – the Siemen.
The glass cell used with this experiment has fixed electrodes, and the ratio l/A is a constant – the cell constant. An approximate value can be obtained from the geometry of the cell. Equation 2 can be written as

$$\kappa = G \times \text{(cell constant)} \quad \text{……………………………………3}$$

The cell constant is usually obtained by calibrating the cell using a 0.01 mol kg^{-1} KCl solution at 298 K. For accurate work a correction must be made for the conductance of the solvent (water) so that

$$\kappa = [G(solution) - G(water)] \times \text{(cell constant)} \quad \text{……………………….....4}$$

Having determined the cell constant, the conductivity of any electrolyte solution can be determined from Equation 4. Most conductivity bridges give values of G directly, although it can also be obtained from a measurement of the resistance R since

$$G = 1/R \quad \text{……………………………………………………5}$$

We now introduce a very useful quantity called the *molar conductivity*. For a solution of concentration c mol m^{-3} the molar conductivity, Λ (with units S m^2 mol^{-1}), is given by

$$\Lambda = \kappa\ c \quad \text{…………………………………..………...6}$$

Molar conductivity is a very convenient way of quantifying conductivity because it highlights the properties of the electrolyte. For instance, doubling the concentration of an electrolyte solution would be expected to double the number of ions and thus to double the conductivity. In this case the molar conductivity would be unchanged. In most cases, however, the molar conductivity actually decreases

with increasing concentration owing to the influence of concentration on interactions between electrolyte ions or on an ionic dissociation process.

The molar conductivity of an electrolyte depends upon the extent to which the electrolyte dissociates into ions. Strong electrolytes (e.g. KCl) are almost completely ionised, whilst weak electrolytes (e.g. CH_3COOH) are ionised to only a small extent. Dilution of an electrolyte solution increases the extent of dissociation, and at infinite dilution the molar conductivity reaches a maximum value Λ_o (sometimes written Λ_∞).

For strong 1:1 electrolytes, Λ_o can be obtained from a plot of Λ against $c^{1/2}$ and extrapolating to c = 0.

$$\Lambda = \Lambda_o - Ac^{1/2} \text{……..7}$$

where, *A* is a constant.

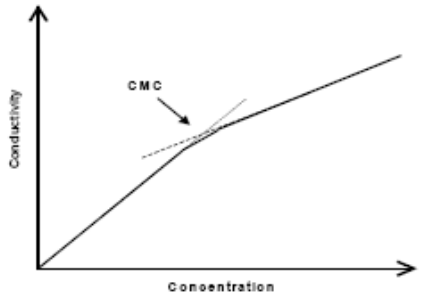
Kohlrausch Law of Independent Migration of Ions

At infinite dilution ion-ion interactions are eliminated, the anions and cations travel independently. Kohlrauch found that the molar conductivity of a salt could be separated into a component from each, called ionic conductivities (λ_o).

$$\Lambda = \lambda_o^+ + \lambda_o^- \text{……..8}$$

So for example, the molar conductivity of sodium chloride is the simply the sum of the ionic conductivities of the sodium and chloride ions. The ionic conductivity as a function of temperature (θ, in °C) is given by the equation:

$$\lambda^{\pm}_o = \lambda^{\pm}_o \text{ (at 25°C)} + a(\theta - 25) + b(\theta - 25)^2 + c'(\theta - 25)^3 \text{ ……9}$$



Below the *CMC*, the addition of surfactant to an aqueous solution causes an increase in the number of charge carriers (Na^+ (aq) and $^-OSO_3C_{12}H_{25}$ (aq)) and consequently, an increase in the conductivity. Above the *CMC*, further addition of surfactant increases the micelle concentration while the monomer concentration remains approximately constant (at the *CMC* level). Since a micelle is much larger than a SDS monomer it diffuses more slowly through solution and so is a less efficient charge carrier. A plot of conductivity against surfactant concentration is, thus expected to show a break at the *CMC.*

PROCEDURE:

1. Turn on the thermostatted bath and conductivity meter.
2. For all measurements use conductivity water.
3. The conductance cell should have been left full with purified water in a 100 mL beaker. Empty it and rinse it twice with purified water, then fill it with purified water and place it in the thermostatted bath, allow 5 min for the cell to reach equilibrium, measure the conductance of purified water. Wash out the cell with purified water, and repeat the measurement until you have three similar readings.
4. Prepare 100 ml of an approximately 0.04 M aqueous stock solution of SDS.
5. Transfer 60 ml of purified water into a 100 mL beaker with a burette and place the conductance cell into the beaker. Check if the two electrodes of the conductance cell are immersed in water. If the electrodes are not completely immersed in water, add more water. Remove the conductance cell from the beaker. Using a burette add 0.5 ml of the SDS stock solution to the water and gently stir the solution with a glass rod making sure you do not create too many bubbles. Place the conductance cell into the beaker and read the conductance which should stabilise after 1-2 min; record the value in your notebook. Add additional 0.5 ml volumes of the SDS stock until 40-45 aliquots have been added.

Added Electrolyte

6. Prepare 250 ml of an approximately 0.02 M aqueous NaCl solution. Use this as the solvent for making up 100 ml of an approximately 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 and record the conductance after the reading has stabilised (1-2 min). About 30-40 additions are appropriate.

Measuring the Cell Constant

7. Prepare by accurate weighing, a 0.01 molal solution of KCl. (N.B. A *molal* solution contains 1 mole of solute in 1 kg of solution.) Rinse out the conductance cell with water, and then with some of the KCl solution. Fill the cell with KCl solution and measure its conductance. Empty the cell, and repeat until conductance measurements are consistent (three readings). Finally wash out the cell with conductivity water twice, and leave it filled with water.

DATA ANALYSIS

Plot concentration of SDS in the X- axis and conductivity in the Y-axis and determine CMC.

EXPERIMENT 2

Determination of Activation Energy of a Clock Reaction

Theory: In this experiment we will determine the effect of temperature on the rate of a chemical reaction. A reaction is chosen which proceeds conveniently slowly near room temperature and which can be measured easily by a dramatic color change. This reaction is the oxidation of iodide ion (I^-) to molecular iodine (I_2) by hydrogen peroxide (H_2O_2) in acidic medium. The reaction is as follows:

$$2I^- + H_2O_2 + 2H^+ = I_2 + 2H_2O \qquad \text{(slow)} \qquad (1)$$

As this reaction proceeds, the colorless reactants gradually develop a brown color due to the product I_2. Because of the difficulty of timing the appearance of the I_2, we make use of another much faster reaction in the same solution to mark the progress of the slow reaction:

$$I_2 + 2\ S_2O_3^{2-} = 2\ I^- + S_4O_6^{2-} \qquad \text{(fast)} \qquad (2)$$

Reaction (2) is so fast that I_2 produced by reaction (1) is consumed instantaneously by the thiosulfate ($S_2O_3^{2-}$), so that the I_2 color cannot develop. Because both $S_2O_3^{2-}$ and $S_4O_6^{2-}$ are colorless, the solution remains colorless. However, we do not add enough thiosulfate to react with all the I_2 that will be formed from reaction (1). By this device the reaction solution stays colorless until the instant at which all the thiosulfate is consumed, and then free I_2 begins to appear. We time the reaction from the initial mixing until the appearance of I_2. In order to help see this appearance we add starch indicator which forms an intensely blue dark complex with I_2 and signals the appearance of I_2 by a dramatic color change.

We know the initial concentration of $S_2O_3^{2-}$ and measure the time interval for this $S_2O_3^{2-}$ to be consumed. These quantities determine the rate of the slow reaction (1). By repeating the experiment with different temperatures, we can determine the effect of temperature on the rate of reaction (1). In this procedure the concentration of I^- is kept fixed and the reaction becomes pseudo-unimolecular. Thus,

$$-\frac{dc}{dt} = kc \text{; c = concentration of } H_2O_2 \qquad (3)$$

So,
$$k = \frac{1}{t}\ln\frac{c_0}{c} = \frac{1}{t_2 - t_1}\ln\frac{T_\infty - T_1}{T_\infty - T_2} \qquad (4)$$

Where T_∞ = titre value at infinite time, T_1 = titre value at time t_1, and T_2 = titre value at time t_2.

At a fixed acid concentration, the reaction is studied at different temperatures. Actual specific rate constant k is

$$k' = \frac{k}{[H^+]} \qquad (5)$$

Now,
$$\ln k' = \ln A - \frac{E_a}{RT} \qquad (6)$$

From which the activation energy, E_a, is determined graphically.

Procedure: Prepare the following solutions.

(1) 500 mL 0.4% KI

(2) 50 mL 12 N H_2SO_4

(3) 50 mL 2 vol H_2O_2

(4) 100 mL 0.2 N $Na_2S_2O_3$

(5) 100 mL 0.1% starch solution

(6) 25 mL catalyst solution

(7) For each set kept in a bath, take 100 mL 0.4% KI + 6 mL 12 N H_2SO_4 + 2 mL starch + 1 mL 0.2 N $Na_2S_2O_3$ + 4 mL 2 vol H_2O_2 (stop-watch is started during H_2O_2 addition). At the time of appearance of iodine color, note the time and add 1 mL 0.2 N $Na_2S_2O_3$ and shake well. Take 5-6 such readings.

Plot $\ln \frac{T_\infty - T_1}{T_\infty - T_i}$ versus (t_i-t_1). A straight line would be obtained according to Eq (4). Determine the value of rate constant from the slope.

(8) Determine T_∞, i.e., the titre value at infinite time (i.e., complete reaction) as follows: Take 100 mL 0.4% KI + 6 mL 12 N H_2SO_4 + 2 mL starch + 1 mL of 1% ammonium molybdate solution + 4 mL 2 vol H_2O_2 and titrate the liberated I_2 against 0.2 N $Na_2S_2O_3$ solution.

(9) Perform the experiment at 20, 25 30, and 35 ^oC.

(10) Plot ln *k*' *versus* 1/*T* and determine the activation energy from the slope. (slope = $-E_a/R$)

Note:

E_a ~ 16 k cal/mol.

Calculate $\Delta S^{\#}$ from the plot.

Dependence of the Reaction Rate on the Presence of Catalyst: Some ions have a pronounced catalytic effect on the rates of many reactions in water solution. Homogenous catalyst $[NH_4]_2MoO_4$, ammonium molybdate is used in the reaction. The activation energy of the catalyzed reaction is smaller than the E_a of an uncatalyzed reaction. Since the function of the catalyst is to lower the activation energy, the reactions moves forward a lot quicker in comparison to the uncatalyzed reaction. Although the catalyst doesn't get consumed in the reaction, it creates new transition states and elementary steps to allow the reaction to precede a lot faster. Hence, the catalyst lowers the E_a for the sodium thiosulfate and iodine reaction and this experiment demonstrated the function of a catalyst in chemical kinetics.

EXPERIMENT 3

The Reduction of Cr (VI) by Glutathione (Consecutive Reaction Kinetics)

Objective To study the first order decay kinetics of the reactant Cr(VI) which absorbs at 370 nm. To study the first-order evolution and first-order decay kinetics of the thioester intermediate which absorbs at 430 nm. To estimate all rate constants graphically and then use those values as best guess values to initiate the analytical nonlinear regression calculations.

Introduction Most of the rate processes that take place in biochemical systems cannot be described by the fundamental, textbook-type kinetic models, such as simple first order or second- order reactions. Recognizing that fact, many physical chemistry textbooks devote a separate section to the kinetics of complex reactions. Reversible, multistep, consecutive reactions are examples of such kinetic models. They are often relevant to biological reactions; moreover, they exhibit fascinating kinetic behavior. In addition, the experimental data are amenable to rigorous interpretation if straightforward computer-assisted data acquisition and analysis techniques are used.

Consider the reaction mechanism in which the reactant, R, reversibly forms an intermediate, I,that in turn, is irreversibly converted to the product, P. The **mechanism** is shown in the followingscheme:

$$\mathrm{R} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{I} \xrightarrow{k_2} \mathrm{P} \qquad (1)$$

We will assume that each elementary step in the mechanism is first order in the corresponding reactant species. The coupled **differential equations** that account for the rate of change in the concentrations of the three species are as follows:

$$\frac{d[R]}{dt} = -k_1[R] + k_{-1}[I] \qquad (2)$$

$$\frac{d[I]}{dt} = k_1[R] - (k_{-1} + k_2)[I] \qquad (3)$$

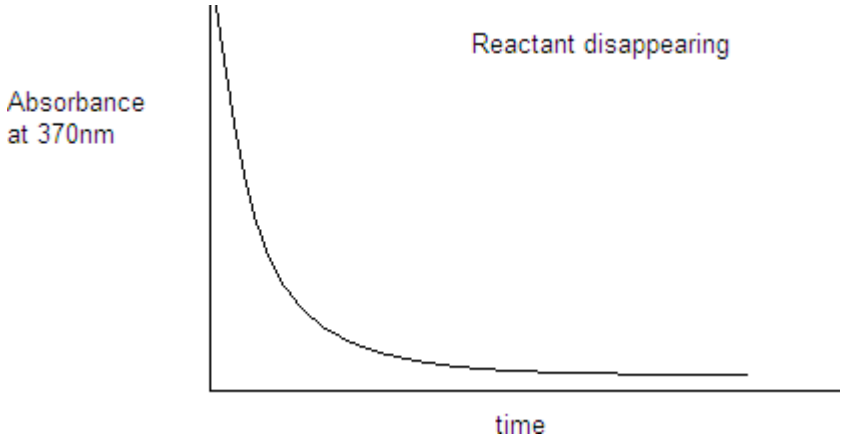
$$\frac{d[P]}{dt} = k_2[I] \qquad (4)$$

When these differential equations are solved exactly, the resulting **integrated equations** for the reactant, R, and intermediate, I, are:

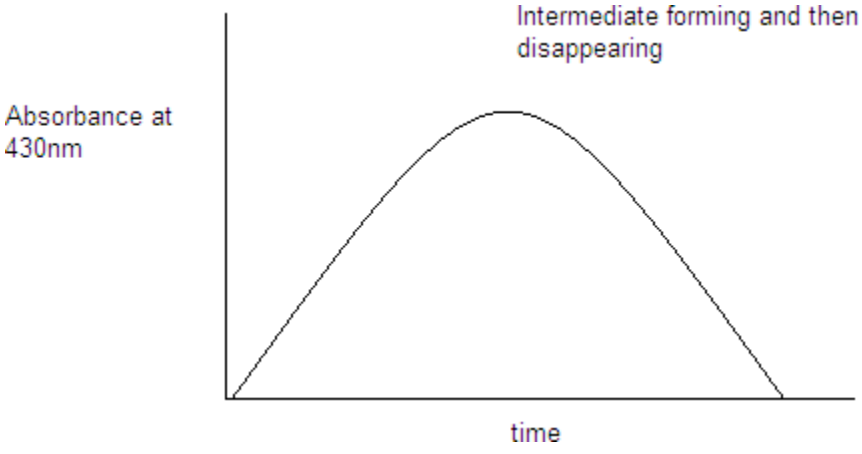
$$[R] = \underbrace{A\left(e^{-k_1 t}\right)}_{decay} + \underbrace{B\left(e^{-k_{-1} t}\right)}_{rise} \qquad (5)$$

$$[I] = \underbrace{C\left(e^{-k_2 t}\right)}_{decay} - \underbrace{D\left(e^{-k_1}\right)}_{rise} \qquad (6)$$

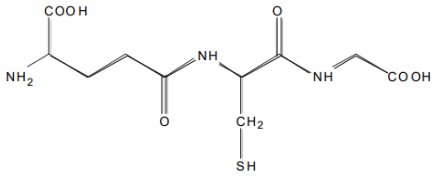
The reactant follows two simple first-order decay processes associated with the forward and reverses reactions and the intermediate exhibits a rise term and a decay term. The magnitude of the decay rate constant of the reactant should match the magnitude of the rise constant of the intermediate, since the intermediate evolves from the reactant. However, the decay constant of the reactant will have a negative sign and the rise constant of the intermediate will have a positive sign.



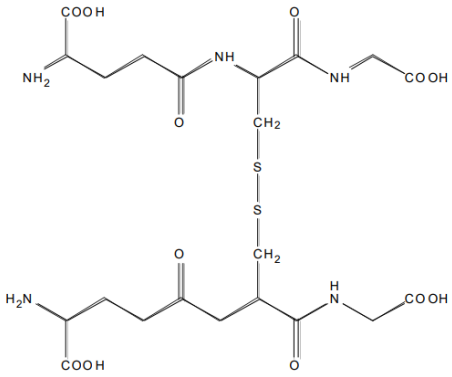
Graph of the evolution (rise) and decay of the intermediate



The Reaction In this experiment, the kinetic behavior of the redox reaction that takes place between the tripeptide glutathione, y-L-glutamyl-Lcysteinylglycine (or GSH) and Cr(VI) at near- neutral pH is studied. Two GSH units are coupled together through the thiol groups, thus being oxidized to glutathionyl disulfide, GSSG. In the process, Cr(VI), which symbolizes the aquated chromium ion in the +6 oxidation state, is reduced to Cr(III).



GSH



GSSG

The reaction is described by the following stoichiometric equation:

$$2CrO_4^{2-} + 6GSH + 10H^+ \rightarrow 2Cr^{3+} + 3GSSG + 8H_2O$$

This reaction is believed to account, in part, for the toxicity and carcinogenicity of chromium (VI). GSH and GSSH function as a redox couple, both in intracellular and plasma environments. An enzyme regulates the appropriate proportion of the oxidized GSH to the reduced GSSH species, both of which are involved in other intracellular redox reactions. GSH also functions as a detoxifying agent that scavenges reactive species, such as free radicals and peroxides. Thus Cr(VI) has the ability to interfere with these processes by causing a depletion of GSH.

The reaction mechanism is believed to involve the reversible formation of a chromium (VI) thioester intermediate. There is a subsequent redox step between this intermediate and a second molecule of GSH, resulting in the ultimate products, Cr(III) and GSSG.

$$CrO_4^{2-} + GSH \rightleftharpoons CrO_4^{2-} + GSH\ (\text{thioester})$$
$$\text{Thioester} + GSH \rightarrow\rightarrow GSSG + Cr^{3+}$$

Thus R, I and P are the symbols of Cr(VI), the Cr(VI)-GSH thioester intermediate, and Cr(III), respectively.

Experimental Method A nice feature of this reaction is that reactant Cr(VI) and the thioester intermediate have reasonably different absorption spectra, rendering the spectroscopic study of the reaction very easy and convenient. This very common experimental strategy is based on the linear relationship between the absorbance, A, of a specie and its molar concentration, C. At a given wavelength, λ, we may write

$$A = \varepsilon bC$$

where ε is the molar absorptivity coefficient, and b is the pathlength of the absorption cell (usually 1 cm.)

The time dependence of the Cr(VI) concentration can be followed by monitoring its absorbance at 370 nm. The evolution and decay of the thioester intermediate can be followed at 430 nm. The reaction rate is highly dependent on pH, the nature of the buffer, as well as the buffer concentration. Hence, the reaction conditions have to be chosen carefully in order for the system to exhibit well-resolved kinetics.

Safety Precautions Hand protection must be used when working with chromium compounds. After the experiment, dispose of all chromium-containing solutions in a heavy-metals waste container.

Procedure You will be given the following aqueous stock solutions:

$0.40\,M\ K_2HPO_4$	(The buffer)
$5.0\times10^{-3}\,M$ HCl	(To adjust the pH)
1 M HCl and 1 M NaOH	(To trim the pH)
$8.0\times10^{-3}\,M$ GSH	(The reductant)
$1.6\times10^{-3}\,M\ K_2Cr_2O_7$	(The oxidant)

Note: Since GSH solutions undergo slow oxidative degradation in air, prepare the stock solution in a 50 mL volumetric flask on the day of the experiment and store it in a refrigerator if necessary. Small volumes (<10 mL) of the first three solutions are needed; 20 mL of the GSH solution is required.

1. Trim the pH. The pH of the reaction medium must be brought to a value of 6.0. Pipet 20 mL of the GSH solution into a test tube or other convenient vessel, such as a small beaker or flask into

which a pH electrode can be inserted. Into that vessel pipet 4 mL of the K_2HPO_4 buffer and 6 mL of the HCl solution. Mix thoroughly, and measure the pH. Add dropwise sufficient 1 M HCl(or NaOH) to bring the pH to 6.0.

2. Turn on the Hewlett-Packard Diode Array UV-vis absorption spectrometer.

3.Set the wavelength at 430 nm, the absorbance at 0.0 and 0.3, the time at 2400 sec (or 1800 if time is short), and the cycle at 5 sec. Click OK.

4.Pipet 3 mL of the pH trimmed reaction solution into a stopper-fitted 1-cm path length spectrophotometer sample cell. Insert into the sample compartment of the Hewlett-Packard Diode Array spectrometer. Push down the lever. When the instrument is set to display 430 nm time behavior, the 370 nm decay of the reactant will be recorded simultaneously, and can be viewed later. Click on BLANK and take the background spectrum. Pull the lever up, remove the cell, Click on TIME BASED MEASUREMENT push START and name the spectrum, (e.g. SC370r1.kd). Now inject 200 µL of the $K_2Cr_2O_7$ solution into the cell solution, stopper it, invert it several times, and quickly place it into the cell compartment. Quickly press START again. Take data for approximately 40 min.

EXPERIMENT 4

Potentiometric Titration of Strong and Weak Acid Mixture Using a Strong Base

Aim: To determine the concentrations of a strong acid and a weak acid present in a mixture by potentiometric titration.

Apparatus: Potentiometer assembly, KCl salt bridge, Pt electrode and saturated calomel electrode

Principle: When a electrode is dipped in an electrolyte solution, its potential depends on the concentration of the ions to which the electrode is reversible. In an acid-base titration, quinhydrone (QH) which is an equimolar mixture of quinone and hydroquinone is used as an H^+ reversible system, and the following cell is constructed:

Pt | acid mixture+QH || calomel

The reduction potential of QH and EMF of the cell will depend on the pH of the solution. On adding small aliquots of base the EMF of the cell drops gradually; but near the equivalence point a large decrease in EMF occurs. Beyond the equivalence point, the change in EMF again becomes gradual. For a mixture of strong and weak acids, first the strong acid will be neutralized followed by the weak acid. Consequently, two equivalence points will be seen.

Procedure:

1. Prepare 100 ml ~0.1 N NaOH solution and standardize against 0.1 N oxalic acid.
2. Pipette out 20 ml of the acid mixture (provided) into a clean beaker and add a pinch of QH to it.
3. Place a Pt electrode in the solution and connect it to a calomel electrode through a salt bridge.
4. Connect the Pt to the positive and SCE to the negative terminals of the potentiometer and measure the initial EMF.
5. Keep adding 1 ml of standardized NaOH solution from the burette to the acid mixture and record the cell EMF after every addition.

6. The EMF decreases gradually, and then dips suddenly, continues to decrease gradually again. Note the volume when the decrease is sudden as V_A.
7. The points when the EMF measurement becomes difficult, reverse the polarity, add few more 1 ml aliquots of NaOH and measure the EMF. Note the volume at which pole reversal occurs as V_B.
8. Repeat the experiment by taking fresh 20 ml of the acid mixture solution and titrating with 0.1 ml of NaOH in the neighborhood of V_A and V_B.
9. Plot E vs volume of NaOH and dE/dV vs. volume of NaOH for both runs. V_A corresponds to the neutralization of the strong acid, and V_B–V_A corresponds to the that of the weak acid. Use these values to calculate the respective acid concentrations.

EXPERIMENT 5

PHASE DIAGRAM FOR A THREE COMPONENT SYSTEM

PURPOSE

The ternary system butanol-water-acetic acid will be investigated. The phase diagram for the system will be constructed and examined for conformity to the phase rule.

THEORY

Information regarding phase equilibria can be predicted by a simple rule that was originally formulated by Gibbs:

$$f = c - p + 2$$

where **c** is the number of components and **p** is the number of phases present in the system. The degrees of freedom, **f**, or variance, gives the number of variables (pressure, temperature, composition, etc..) that must be fixed to completely describe the system, or locate the state of the system on the phase diagram. Gibbs defined the components of a system as *the minimum number of independent species necessary to define the composition of all phases present in the system* and he defined a phase as *a state of matter that is uniform throughout, not only in chemical composition but also in physical state*.

In a one component system such as a pure gas for example, we have c = 1 and p = 1 so that $f = 2$. This means that in order to completely describe the system, we only have to know two out of the three variables P, V, or T to completely describe the 'state' of the system. This fact becomes clear when these variables are considered in conjunction with a phase diagram, but in this case we know that we can calculate the third variable from equations of state such as the Ideal Gas Equation.

As a second example, consider water. Most physical and general chemistry books give its phase diagram. In the pure phase regions (S, L, or V) we have that f = 2, meaning that pressure can be varied independently of temperature. Along the S-L, L-V boundary, however, p = 2, and f

= 1. Thus for every value of P, there can only be one specific value of T. Finally at the triple point, p = 3 and f = 0. Under these conditions the system is totally fixed, and no

variation in temperature or pressure is possible without changing the 'state' of the system.

There are many examples in nature of phase diagrams and the phase rule. This experiment and the liquid-vapor experiment described in this manual are just two examples.

PROCEDURE

ALL PARTS OF THE EXPERIMENT SHOULD BE DONE IN THE HOOD

PART I

1. Place 20 ml of water in a 50 mL Erlenmeyer.flask. Cover the flask with parafilm. Poke the buret containing butanol through the parafilm. Add butanol to the water drop by drop with continuous stirring until the last drop does not dissolve in the water after five minutes of stirring.It will take less than 2 ml to reach this point.

2. Place 20 ml of butanol in a 50 mL parafilm-covered Erlenmeyer flask. Add water to the butanol drop by drop with continuous stirring until the last drop does not dissolve after five minutes of stirring. It will take more than 2 ml to reach this point. Record your results on the data sheet provided.

3a. Place 20 ml of butanol and 5 ml of water in a 200 mL parafilm-covered Erlenmeyer flask. Add acetic acid drop by drop with continuous stirring until the turbidity disappears. Record the volume of acetic acid added.

3b. Add an additional 5 ml aliquot of water to the mixture from step 3a. Again titrate with acetic acid dropwise with stirring until the turbidity disappears.

3c. Continue adding water in 5 ml aliquots and titrating to the turbid point with acetic acid as indicated on the data sheet until a total of 30 ml of water have been added, then 10 ml aliquots of water are added.

PART II

1. Mix 25 ml of water and 25 ml of butanol. Stir for five minutes and let settle. Add 3 ml of acetic acid and again stir for five minutes and let settle. Place in a separating funnel and separate. Determine the mass of each phase. Titrate the bottom phase with standardized 1.0 M NaOH. Thestandardization of NaOH must be done during the period that the bottom -phase titration is done since the NaOH concentration changes rapidly when carbon dioxide is absorbed from the atmosphere. Use the HCL solution provided. High precision is not crucial here. Obtain an average of three runs.

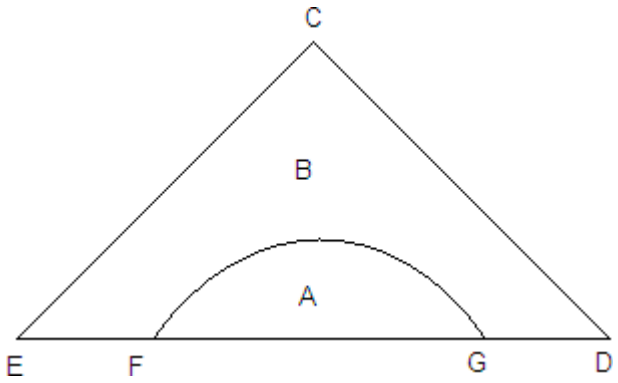
CALCULATIONS

1. For each solution determine the weight percent of each component. Take the densities of H_2O, butanol, and acetic acid to be 0.9970, 0.8098, and 1.046 g/ml, respectively. Plot the dataon the triangular graph paper provided. Show one set of sample calculations.

2. From the results of Part II determine the weight percent of the three components in each phase. Put these two points on the curve and draw a line between them.

FURTHER CALCULATIONS AND QUESTIONS

1. For the two solutions with no acetic acid calculate the mole fraction of water and butanol in each. Give a qualitative explanation as to why butanol is rather insoluble in water while wateris rather soluble in butanol.
2. If two moles of water and one mole of butanol are mixed, will two phases result? If so, what will be the mole fractions of water and butanol in each of the two phases? Which has a lower free energy, a single solution containing two moles of water and one mole of butanol or a two phase system resulting from mixing two moles of water with one mole of butanol?
3. If two moles of butanol and one mole of water are mixed, will two phases result? If so, what will be the mole fraction of water and butanol in the two phases? Which has a lower free energy, a single solution containing two moles of butanol and one mole of water or a two phase system resulting from mixing two moles of butanol with one mole of water?
4. Qualitatively explain why the addition of acetic acid makes a two phase mixture of butanol and water into a one phase system. Answer this question in terms of "like dissolves like" instead of free energy.
5. Determine the C (# of components), p (# of phases), and v (variance) of the system in each region of the phase diagram.



Region A
Region B
Line EC
Line CD
Line EF
Line GD
Line FG
Points C, D, E

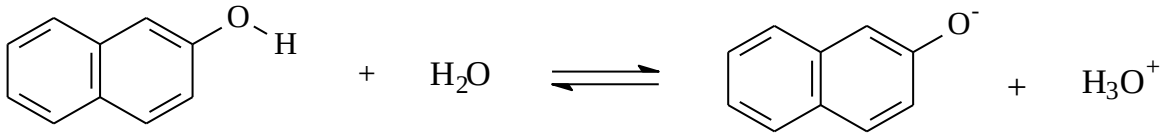
BuOH Total Volume	H_2O Total Volume	HAc Total Volume	BuOH Mass, g	H_2O Mass, g	HAc Mass, g	% BuOH	% H_2O	% HAc
20.00 mL	0 mL	0 mL						
20.00	5.00							
20.00	10.00							
20.00	15.00							
20.00	20.00							
20.00	25.00							
20.00	30.00							
20.00	40.00							
20.00	50.00							
20.00	60.00							
20.00	70.00							
20.00	80.00							
20.00	90.00							
20.00	100.00							
20.00	110.00							

EXPERIMENT 6
GROUND AND EXCITED STATE IONIZATION CONSTANTS OF 2-NAPHTHOL

This experiment has two parts. In Part I of this experiment, the ground state ionization constants and in part II-excited-state, using asorption and fluorescence spectroscopy.

A. Determination of the ground state ionization constant

This analysis is based on the fact that 2-naphthol and its conjugate base, the 2-naphthoxide ion, have different absorption spectra. The ionization equilibrium and ionization equilibrium constant are shown below:



2-Naphthol (2-NOH) 2-Naphthoxide ion (2-NO^-)

$$K_a = \frac{[H_3O^+][NO^-]}{[NOH]} \qquad \text{Equation (1)}$$

In acidic solution (low pH) the equilibrium favors the free acid form of 2-naphthol and in basic solution (high pH) it favors the conjugate base form. At some intermediate pH range, both species will be present. The equilibrium constant depends on concentrations of H_3O^+, NO^-, and NOH. The concentration of H_3O^+ will be determined from the pH and the NO^- and NOH concentrations will be determined from the UV absorption spectra. Combination of these quantities will yield the ground-state dissociation constant, K_a.

Procedure for Part I

1. Measure the absorption spectrum of solutions 1 - 4 over the range of 400 – 250 nm. Display the spectra of solutions 1-3 on the same graph and that of solution 4 on a separate graph.

Examine the absorption spectra. Note that solution 1 is basic and solution 3 is acidic. Therefore, it should be clear to you that the spectrum of solution 3 is the absorption spectrum of the free acid form of 2-naphthol (2-NOH). It should also be clear that the spectrum of solution 1 is the absorption spectrum of the 2-naphthoxide ion (2-NO^-), the conjugate base of 2-naphthol. It should also be clear to you that the buffered solution spectrum (solution 2) contains a mixture of the free acid and the conjugate base in solution. From an analysis of the spectra, you should be able to determine the relative amounts of 2-NOH and 2-NO^- in the buffered solution. More details will be provided later.

Examine the spectrum of solution 4. This solution contains NH_4OAc and is expected to have a pH of about 7. What is the form of 2-naphthol (2-NOH or $2\text{-}NO^-$) in this solution? A comparison of the solution 2 and solution 4 spectra should make it clear that 2-naphthol exists in its free acid form when it is in its ground state at a pH of 7, but it exists in both forms in the 9-10 pH range. Before discarding solution 2, measure its pH.

In order to determine the excited state pKa of 2-naphthol, you will need to know ν_{NOH} and ν_{NO^-}, the transition frequencies for 2-NOH and $2\text{-}NO^-$, respectively. These will be determined from the wavelengths of maximum absorbance and emission intensity of 2-NOH and $2\text{-}NO^-$. Determine the wavelength of maximum absorbance for the first electronic transition of NOH from its absorption spectrum (Solution 3 spectrum). This is the wavelength of maximum absorbance between 300 and 340 nm. Call this $\lambda_{Ab\ NOH}$. Determine the wavelength of maximum absorbance for NO^- from its absorption spectrum (Solution 1 spectrum). This is the wavelength of maximum absorbance between 300 and 380 nm. Call this $\lambda_{Ab\ NO^-}$.

2. Next, record the fluorescence emission spectra of solutions 1 and 3. These are the emission spectra of $2\text{-}NO^-$ and 2-NOH, respectively. Use an excitation wavelength of 323 nm and set the excitation and emission slits at 2 nm and 1 nm bandpass, respectively. Determine the wavelength of maximum emission intensity for 2-NOH and $2\text{-}NO^-$. Call these λ_{EmNOH} and λ_{EmNO^-}, respectively.

Calculate the transition wavelengths for 2-NOH and $2\text{-}NO^-$ from the average of the absorption and emission wavelengths.

$$\lambda_{NOH} = \lambda_{AB\text{-}NOH} + \lambda_{EM\text{-}NOH} \text{ and } \nu_{NOH} = c/\lambda_{NOH} \quad \text{Equation (3)}$$

$$\lambda_{NO^-} = \lambda_{AB\text{-}NO^-} + \lambda_{EM\text{-}NO^-} \text{ and } \nu_{NO^-} = c/\lambda_{NO^-} \quad \text{Equation (4)}$$

In calculating the frequencies, be sure that the speed of light and the wavelengths are in meters per second and meters, respectively.

Calculations for Part I

A. Determination of Ground State Ionization Constant

1. In solution 3 (acid solution) the 2-naphthol is all in its free acid form (NOH). In solution 1 (basic solution) the 2-naphthol is in its conjugate base form (NO^-). From the absorption spectra, determine the molar absorptivities of these two forms of 2-naphthol at the wavelength of maximum absorbance for the **conjugate base** form (around 345 nm). To do this use the Beer's law relationship, $A = \varepsilon b c_o$, where A is the absorbance, ε is the molar absorptivity, b is the path length (1.00 cm) and c_o is the concentration of the 2-naphthol species.

2. The concentrations of NOH and NO^- in the buffered solution (solution 2), can be determined from the absorbance at the wavelength used to get the molar absorptivity. The following equations illustrate how this is done:

For each solution

$$c_o = [NOH] + [NO^-]$$

$$A = \varepsilon_{[NOH]}[NOH] + \varepsilon_{NO-}[NO-]$$
$$A = \varepsilon_{NOH}[NOH] + \varepsilon_{NO-}(c_o\text{-}NOH])$$

$$A = (\varepsilon_{NOH} - \varepsilon_{NO-})[NOH] + (\varepsilon_{NO-})c_o$$

Use this last equation to get [NOH] and $[NO^-]$ for the buffered solution.

3. Starting with $K_a = \dfrac{[H_3O^+][NO^-]}{[NOH]}$

You can show that

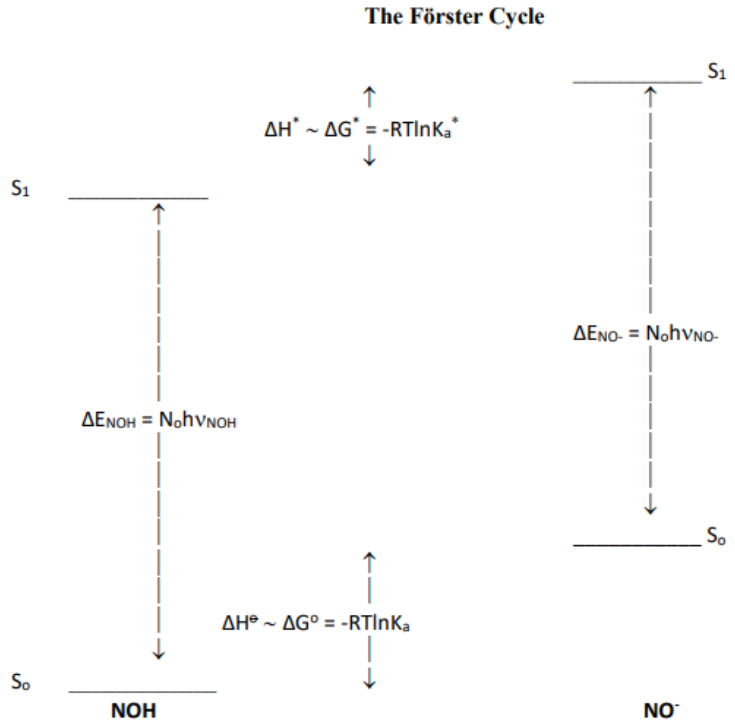
$$-\log K_a = -\log[H_3O^-] + \log\frac{[NOH]}{[NO^-]}$$

$$pK_a = pH + \log\frac{[NOH]}{[NO^-]}$$

Use this equation to determine the pK_a for the buffered solution. Compare your value to the literature value which can be found in the Handbook of Chemistry and Physics.

B Determination of the Excited State Ionization Constant

In order to get the excited-state ionization, K_a*, the Förster Cycle method will be utilized. In this method, the excited-state ionization constant is determined from the ground-state ionization constant and the spectroscopic transition energies of 2-naphthol and its conjugate base. The transition energy for each species is the energy needed to excite each from its ground state to its lowest excited singlet state. The diagram on the following page illustrates the Förster Cycle method. In this diagram, S_o and S_1 represent the ground and excited state electronic energy levels of the NOH and NO^- species.



In this diagram, R is the gas constant, No is Avagadro's number, c is the speed of light and h is Planck's constant. From the diagram, it should be clear that

$$\Delta E_{NOH} + \Delta G^* = \Delta G^o + \Delta E_{NO^-}$$

Therefore, $\Delta G^* = \Delta G^o + \Delta E_{NO^-} - \Delta E_{NOH}$

$$-RT \ln K_a^* = -RT \ln K_a + N_o h (\nu_{NO^-} - \nu_{NOH})$$

or $$-\log K_a^* = -\log K_a + (N_o h/2.303RT) (\nu_{NO^-} - \nu_{NOH})$$

Therefore $$pK_a^* = pK_a + (N_o h/2.303RT)(\nu_{NO^-} - \nu_{NOH})$$

In the above equation $\nu = c/\lambda$ where λ is the transition wavelength in meters. The transition wavelengths for NOH and NO^- will be determined from the wavelengths of maximum absorbance and emission of their respective spectra.

Preparation of Solutions for Part I and Part II:

The following aqueous stock solutions will be provided:

A. 1.0×10^{-3} M 2-naphthol
B. 0.10 M H_2SO_4
C. 0.10 M NaOH

Obtain 2–25 mL volumetric flasks and prepare the following solutions:

	2-naphthol	H_2SO_4	NaOH
1.	5.0 mL	0.0 mL	10.0 mL
2.	5.0 mL	10.0 mL	0.0 mL

Fill each of the flasks to the mark with distilled water.

B. Determination of The Excited State Ionization Constant

Determine the excited state pKa for 2-naphthol by application of the Förster Cycle equation (Equation 2). In order to obtain the transition frequencies of the 2-NOH and 2-NO^-, the transition frequencies that you have determined (Equation (3) and Equation (4)) will be used. Apply these frequencies along with the ground state pKa to Equation (2) to calculate pKa^*.

EXPERIMENT 7

Verification of Ostwald's Dilution Law by Conductometric Method

Objective : Verification of Ostwald's Dilution Law using acetic acid as the weak acid

Discussion : Equivalent conductance (Λ) of an eletrolyte solution is related to the specific conductance (κ) according to the relation: $\Lambda = 1000\,\kappa / c$

Where *c* is the concentration of the electrolyte in *g equivalent/L*

Thus, if the specific conductance and the concentration of a solution are known, the equivalent conductance of the solution can easily be calculated.

The dissociation of a weak binary electrolyte (AB) with degree of dissociation α at a concentration *c* mol L^{-1} may be represented as,

$$AB \rightleftharpoons A^+ + B^- \quad \ldots\ldots(1)$$

$$c(1-\alpha) \qquad c\alpha \quad c\alpha$$

The dissociation constant K_a is given by,

$$K_a = (a_{A^+}.a_{B^-})/a_{AB} \quad \ldots\ldots(2)$$

Where a_i represents activity of the ith species. For the dissociation of a weak acid (HA) equation (1) can be written as follows.

$$HA + \text{water} \rightarrow H^+(aq) + A^-(aq) \quad \ldots\ldots(3)$$

Ionisation constant (K_a) of the weak acid, HA, may be defined according to,

$$K_a = [H^+][A^-]/[HA].f_{H^+}.f_{A^-}/f_{HA} \quad \ldots\ldots (4)$$

here, terms in the square bracket represent concentrations ***c*** in $gmolL^{-1}$ or $gionL^{-1}$. For a dilute solution of weak acid, the ionic strength of the medium will be very low, and the numerical value of the activity coefficients f's are very close to unity (*Debye-Huckel limiting law*). Under this condition, equation (4) may be written as:

$$K_a = [H^+][A^-]/[HA] = c\alpha^2/(1-\alpha) \quad \ldots\ldots(5)$$

The degree of ionization (α), at a particular concentration (c) of the week electrolyte, HA, may be well approximated by the ratio, Λ/Λ_o, where Λ is the equivalent conductance of the weak acid HA at concentration c and Λ_o is its equivalent conductance at *infinite dilution*.

Substituting, $\alpha = \Lambda/\Lambda_o$, one obtains from equation

$K_a = (\Lambda^2 c)/[(\Lambda_o - \Lambda)\Lambda_o]$……(7)

Which on rearrangement yields,

$1/\Lambda = 1/\Lambda_o + 1/(K_a . \Lambda_o^2)\ \Lambda c$……(8)

Thus, a plot of $1/\Lambda$ against Λc at different concentration should give a straight line, values of K_a and Λ_o can be obtained from the slope and intercept.

Equipments/Glass Apparatus: Conductivity bridge, burette, pipette, conical flask, beaker.

Reagents: oxalic acid ($C_2H_2O_4$, $2H_2O$), sodium hydroxide, acetic acid (CH_3COOH), potassium chloride.

Procedure :

1. Prepare 100 mL standard 0.1N oxalic acid solution and 0.1 N (exact) KCl solution (100 mL). Prepare solutions of $\sim$ 0.1N NaOH (100 mL) and $\sim$ 0.1N acetic acid (100 mL).

2. Prepare 100 mL 0.01N standard KCl solution by proper dilution of 0.1N solution.

3. Rinse a 100 mL beaker and the conductivity cell with the exact 0.01N KCl solution thoroughly and then pour sufficient volume of this solution into the beaker so that the electrodes of the cell are completely immersed in the solution. Record the conductance. Repeat this procedure with the exact 0.1N KCl solution. Calculate the *mean cell constant* from the measured conductance values of these two solutions, using the literature values of the specific conductance of KCl solutions at these concentrations at the same temperature. Find the mean cell constant.

4. Take 60 mL of the water in a 100 mL beaker. Dip the clean, dry conductivity cell into it. Add 0.5 mL acetic acid, to water (in the conductivity cell), stir well and record the conductance. Repeat the procedure of adding 0.5 mL acetic acid for at least eight times, and measure the conductivity of the solution after each addition.

5. Calculate the concentration and equivalent conductance values of all the acetic acid solutions.

Results:

Table 1. Preparation of 100 mL standard 0.1N oxalic acid solution

Weight taken (g)	Weight to be taken (g)	Strength of oxalic acid solution

Table 2. Standardization of NaOH solution using standard oxalic solution

Sl. No.	Vol. of oxalic acid solution/ mL	Burette Reading /mL			Average volume/mL	Strength of NaOH solution
		Initial	Final	Difference		

Table 3. **Standardization of Acetic acid solution against NaOH solution**

Sl. No.	Volume of acetic acid solution/ mL	Burette reading/ mL			Average volume/mL	Strength of Acetic acid solution
		Initial	Final	Difference		

Table 4. Determination of specific conductance for KCl solution

Sl. No.	Strength of KCl	Conductance (S)	Specific conductance

Table 5. Cell constant determination

Sl. No.	Concentration of KCl	Specific conductance	Resistance	Cell constant	Mean cell constant

Table 6. Conductance of acetic acid solution of different concentration

Volume of water = …….mL

Sl. No.	Volume of acetic acid solution added (mL)	Concentration of acetic acid solution	Conductance (S)	Equivalent conductance

Calculation : Calculate K_a and Λ_o from the slope and the intercept.

EXPERIMENT 8

Measurement of Rate-Constant of Fluorescence Quenching: Stern Volmer Method

Aim: To determine the rate constant of fluorescence quenching of a fluorophore by a quencher in a solvent.

Introduction: The relative fluorescence intensity of a fluorophore depends on the number of fluorophores, natural lifetime of its first excited singlet state and on the rate that the first excited state is deactivated by nonradiative processes. Fluorescence quenching refers to any process that decreases the fluorescence intensity of a sample. A variety of molecular interactions can result in quenching. These include ground-state complex formation, excited-state reactions, molecular rearrangements, energy transfer, and collisional quenching.

Much of these nonradiative deactivations come about because of collisions of the excited molecule with solvent molecules. However, some substances are particularly efficient at deactivating, or quenching, excited states. These tend to be substances with heavy atoms (for example halogens) or paramagnetic compounds. Oxygen is a particularly good quencher; therefore it is often necessary to remove the oxygen from solution before measuring fluorescence spectra. Fluorescent probes, such as dansyl chloride, are used in biochemistry to study the various binding sites in large macromolecules through the difference of the quenching rates of the bound verses free probe.

Theory: The emission intensity of the fluorophore can be quenched by a quencher (Q) by complex formation in the ground state (static quenching) or in excited-state fluorophore quencher reaction (dynamic quenching). The following general scheme illustrates the nature of processes that deactivate an electronically excited state of a molecule.

$$A + h\nu \xrightarrow{k_{ex}} A^* \quad \text{(excitation process)}$$

$$A^* \xrightarrow{k_r} A + h\nu \quad \text{(radiative)}$$

$$A^* \xrightarrow{k_{nr}} A + heat \quad \text{(non-radiative)}$$

$$A^* + Q \xrightarrow{k_q} A + Q^* \quad \text{(energy transfer)}$$

$$A^* + Q \xrightarrow{k_{ET}} A^- + Q^+ \quad \text{(electron transfer)}$$

If quenching happens in ground state then the equation for quenching reads as:

$$\frac{F_0}{F} = 1 + K_S[Q]$$

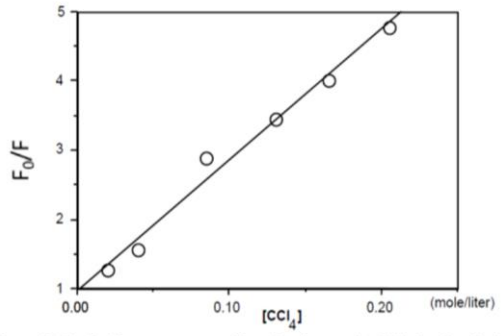
If quenching happens in excited state then the equation for quenching reads as:

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_D[Q]$$

In these equations F_0 and F are the integrated fluorescence intensities in the absence and presence of quencher, respectively; K_S and K_D are static and dynamic quenching constant. For both static and dynamic quenching a linear plot is obtained. k_q is the bimolecular quenching constant; τ_0 is the lifetime of the fluorophore in the absence of quencher, and [Q] is the concentration of quencher. The Stern-Volmer quenching constant is given by $K_D = k_q\tau_0$. If the quenching is known to be dynamic, the Stern-Volmer constant will be represented by K_D. Otherwise this constant will be described as K_S *or* K_{SV}. Quenching data are usually presented as plots of F_0/F versus [Q]. This is because F_0/F is expected to be linearly dependent upon the concentration of quencher. A plot of F_0/F versus [Q] yields an intercept of one on the *y*-axis and a slope equal to K_S or K_D. From the linear plot of F_0/F versus [Q], it is not possible to say whether the quenching mechanism is static and dynamic. Time resolved fluorescence studies are necessary to identify whether the quenching mechanism is static or dynamic. For static quenching excited state fluorescence lifetime does not change but for dynamic quenching fluorescence lifetime decreases with increase in [Q]. Therefore, for static quenching $\tau/\tau_0 = 1$, whereas, for dynamic quenching, $F_0/F = \tau_0/\tau$.

Examples of Quencher: One of the best-known collisional quenchers is molecular oxygen, which quenches almost all known fluorophores. It is frequently necessary to remove dissolved oxygen to obtain reliable measurements of the fluorescence yields or lifetimes. Known quenchers include halides, heavy metals, different amines etc.

Generally linear Stern-Volmer plot is observed however, there are several reasons which make the Stern – Volmer plot non-linear.



Stern-Volmer plot for the fluorescence quenching of anthracene by CCl_4 in ethanol solution.

Reference: *Principles of Fluorescence Spectroscopy, 3rd Edition, J. R. Lakowicz.*

Chemicals Required: Anthracene, CCl_4, ethanol (95%).

Equipments Required: Fluorescence cuvette, Volumetric flasks, Pipettes; Spectrophotometer (to check absorbance of the stock anthracene solution); Spectrofluorimeter (to measure fluorescence of different solutions).

Procedure:

1. Make a ~5.0 x 10^{-4} M solution of anthracene by dissolving about 1 mg of anthracene in 10mL of ethanol in volumetric flask.
2. Dilute this solution by 50 times by transferring 1 mL of this solution to a 50 mL volumetric flask thus making a 1.0 x 10^{-5} M stock solution.
3. Calculate how much amount of CCl_4 is required to make the final concentration as 0.02, 0.04, 0.12, 0.16, 0.20 moles/litre.
4. There will be total six solutions, one without CCl_4 and other five solutions will be with different concentrations of CCl_4. Keep it in mind concentration of anthracene should be exactly same in all solutions.
5. Measure the fluorescence of the stock solution of anthracene (i.e. without CCl_4) and note down the integrated intensity (F_0) (area under the fluorescence curve).

6. Measure the fluorescence of all other solutions with equal concentration of anthracene but with different concentration of CCl_4 and note down the integrated intensity (F) for each five solutions.
7. Make a table of F_0/F against the quencher concentration [Q].
8. Plot F_0/F against the six different quencher concentrations.
9. Fit the plot with fixed intercept at 1.
10. Calculate the slope of the linear plot.
11. Comment on the goodness of the linear fit.
12. Write down the sources of error that could creep into the result.
13. If fluorescence lifetime of anthracene at 25^0C is 5 nanosecond then calculate the value of k_q. *(Assume quenching to be dynamic)*
14. Write the experiment report and submit the notebook to the instructor.

Cautions:

1. It is important that the absorbance (optical density) of the solution is not more than about 0.2 so that the fluorescence intensity is uniform throughout the solution.
2. Rinse the fluorescence cuvette several times with the new solution before taking its fluorescence spectrum.
3. Experimental conditions for fluorescence measurements, i.e. excitation wavelength, slits etc. should be same for all measurements.