Lab No. 4

Kinetics of P-Nitrophenyl Acetate Hydrolysis

I.Objectives:

To determine the kinetic parameters of the alkaline hydrolysis of p-nitrophenyl acetate in the presence of Tris buffer (PH 8.5 and 0.05M),mainly:

1. The hydrolysis Rate constant.
2. The half-life of the reaction at three different temperatures (30°C,40°C and 50°C).
3. The heat of activation for the overall reaction.

II.Keywords : *Rates & orders of reactions, influence of temperature & other factors on reaction rates, Tris buffer.*

III.Introduction:

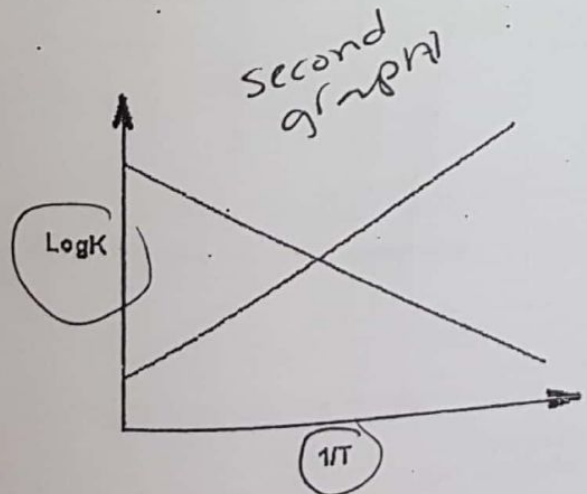
A number of factors other than concentration may affect the reaction velocity among these are temperature ,solvents,catalysts,and light.
The speed of many reactions increases about two to three times with each 10° rise in temperature.
The effect of temperature on reaction rate is given by the equation ,first suggested by Arrhenius,

$$K = Ae^{(-Ea/RT)}$$

$$\text{Log } K = \text{Log } A - Ea/2.3*(1/RT) \quad \ldots\ldots(1)$$

K is the specific reaction rate.
A is a constant known as the *Arrhenius factor* or the frequency factor.
Ea is the *energy of activation* .
R is the gas constant (1.987 cal/deg mole).
T is the absolute temperature.
The constants ,A and Ea , may be calculated by determining K at several temperatures and plotting 1/T against Log K; i.e. a plot of *Log K* Vs *1/T* yield a straight line.

$$\text{slope} = \frac{-Ea}{2.3R}$$



The slope of the line so obtained is *–Ea/2.3R* and the intercept on the vertical axis is *LogA*, from which Ea & A may be obtained .

It should be observed that since the reciprocal of the absolute temperature is plotted along the horizontal axis, the temperature is actually decreasing from left to right across the graph.

Ea may also be obtained by writing equation (1) for a temperature T_2 as

$$\text{Log } K_2 = \text{Log } A - Ea/2.303R*(1/T_2)$$

And for another temperature T_1 as

$$\text{Log } K_1 = \text{Log } A - Ea/2.303R*(1/T_2)$$

Subtracting these two expressions yields

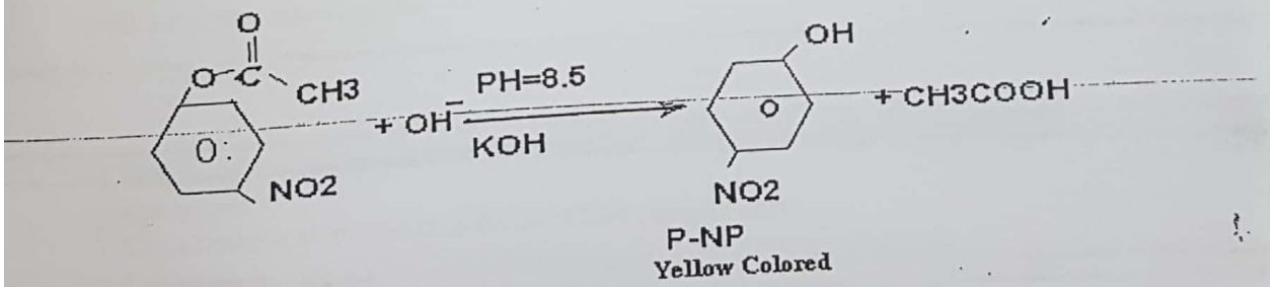
$$\text{Log } K_2/K_1 = Ea/2.303R*(T_2-T_1/T_2T_1)$$

IV.Experimental Work:

1.Allow the spectophotometer to warm up for 15minutes.
2.Place a 100ml volumetric flask containing 98ml of tris buffer solution (PH=8.5) ,0.05M) in the designated water bath adjusted at the proper temperature (30°C,40°C or 50°C) until equilibrium is reached.
3.Pipette 2.0ml of the P-NPA stock solution (0.5mg/ml in acetonitrile) into the flask ,shake well and note the time.
4.Withdraw 5ml from the flask at the scheduled times.
5.Immediately ,measure the absorbance at λ_{max}=398.5nm using the buffer as a blank. If there is a delay for any reason ,place the sample in ice until you are ready to do so.
6.Record your A_t values and other data on the attached table and on the board.

V.Data Analysis:

In this experiment you will study the *hydrolysis of P-NPA in alkaline PH.*



P-NP
Yellow Colored

$$\text{Rate} = -d[PNPA]/dt = d[PNP]/dt = d[AA]/dt$$

$$-d[PNPA]/dt = K_{OH}[E][OH]$$

Where the minus sign signifies that the ester concentration is decreasing

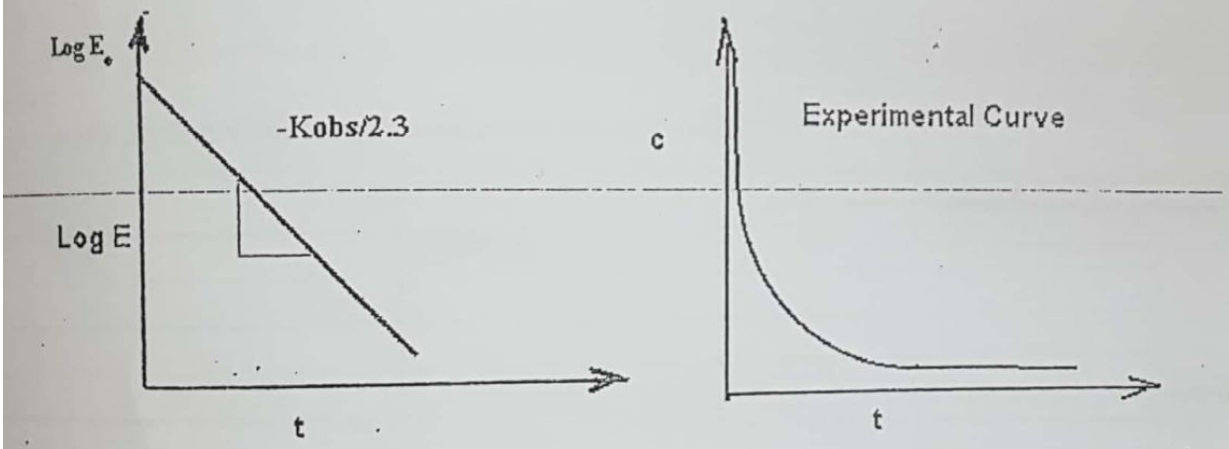
[OH-] is in excess & constant because we have a buffer.
Thus $K_{OH}[OH^-] = K_{obs}$ i.e. *pseudo first order reaction.*

$$-d[PNPA]/dt = K_{obs}[E]$$

integrate this equation after arrangement

$$\int -d[E]/E = \int K_{obs}[E]/dt$$

$$\text{Log } E = \text{Log } E_o - K_{obs}\, t/2.303$$



Where LogE is the amount remaining *unhydrolysed* at time t
Slope = $-K_{obs}/2.3$, i.e.K=slope*2.3
$t_{1/2} = 0.693/K$ constant & not depending on concentration.

Experimentally
Amount remaining = $A_\infty - A_t$
A_t is the amount of PNP a colored compound and absorbance of it is measured at 398.5nm λmax.
At infinite time all E is hydrolysed → PNP thus at A_∞ amount of PNP = amount of E and amount remaining=$A_\infty - A_t$.

1.Plot Log($A_\infty - A_t$) versus t for the three temperatures on a single piece of semilog graph paper.
2.Calculate the slope and then Kobs at three temperatures.
3.Calculate the $t_{0.5}$ for the P-NPA hydrolysis at the three temperatures.
4.Plot **Log K_{obs}** versus **1/T** and calculate the heat of activation for the overall reaction.

Lab No. 4

Kinetics of alkaline hydrolysis of p-Nitrophenyl acetate

Report Sheet

Name: Number: Section:

*Objectives

* Write the equation describing the alkaline hydrolysis of p-Nitrophenyl acetate.

* Write the integrated rate equation for this reaction and show the order.

*Data and calculations

Time	30 C°		40 C°		50 C°	
(min.)	A_t	$A_\infty - A_t$	A_t	$A_\infty - A_t$	A_t	$A_\infty - A_t$
5						
10						
15						
20						
25						
30						
45						
60						
∞						

* Calculate the reaction rate constant, the half life and shelf life at 30, 40 and 50C°.

* Calculate the activation energy (cal./mole) for this reaction.

* Summarize your data in the following table.

	30 C°	40 C°	50 C°
Reaction rate constant (min^{-1})			
Half life (min)			
Shelf life (min).			
Activation energy (cal./mole)			

*Conclusion.

Lab No. 6
Dissolution

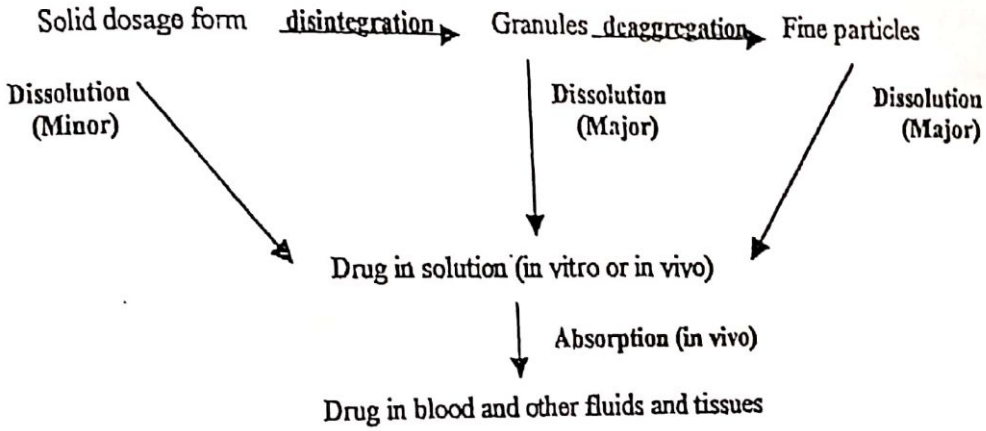
Objectives

1. Determination of the intrinsic dissolution rate for salicylic acid.
2. Studying the effect of pH on the dissolution rate of salicylic acid.

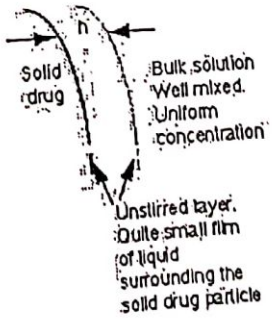
Introduction

Dissolution is a process by which two components or phases interact to form a homogeneous one-phase system. It is important, however, to distinguish between dissolution as a general term, and the dissolution rate, which is a quantitative term used to describe the velocity of the process i.e. how fast a substance would dissolve in a solvent.

Dissolution importance can be understood by looking at the steps involved in the release of drugs from solid dosage forms.



Obviously, for a drug to be absorbed it has to be in solution form (figure 1). In case of drugs of low solubility (below 1 mg/ml), the dissolution step is the rate limiting step, since it is the slowest among the steps involved. It is therefore, important to study the rate at which sparingly soluble drugs go into solution, since their bioavailability is ***dissolution rate limited.***



The need to quantitate the dissolution process, lead to the introduction of the ***diffusion-layer model*** or ***film theory***. When a solid comes into contact with the surrounding liquid medium, it immediately forms a saturated solution within a thin film of liquid of thickness "h". For the dissolution process to continue, molecules must diffuse across this ***"stagnant diffusion layer"*** to the bulk medium (figure 2).

The thickness "h" represents a stationary layer of solvent, in which the solute molecules exist in concentration from C_s to C_b. At X = 0 (figure 3), the drug in the solid is in equilibrium with the drug in the diffusion layer. At X greater than h, mixing occurs in the solution , and the drug is found at a uniform concentration, C_b, within the bulk medium. The change in concentration with distance is constant, as shown by the straight line, downward sloping across the diffusion layer. Based on this theory, the dissolution process is described to be *diffusion-controlled*.

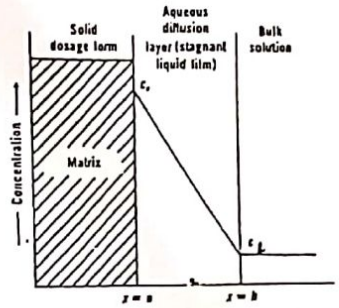


Fig. 3 :. Dissolution of a drug from a solid matrix, showing the stagnant diffusion layer between the dosage form surface and bulk solution.

Noyes and Whitney introduced an equation to quantify a diffusion-controlled dissolution process in the absence of any chemical interaction

$$\frac{dM}{dt} = \frac{DS}{h}(C_s - C_b) \qquad \text{——} \quad (1)$$

in which:
dM/dt is the rate of dissolution (mass M of solute dissolved in time t).
D is the diffusion coefficient of the solute in solution.
S is the surface area of the exposed solid.
h is the thickness of the diffusion layer.
C_s is the solubility of the solid in the dissolution solvent (concentration of a saturated solution of the solute at a fixed temperature).
C_b is the concentration of the solute in the bulk solution at time t.

Under ***sink conditions***, achieved by using a large volume of dissolution medium or by continuous renewal of the solvent, $C_b \ll C_s$, and the equation becomes:

$$\frac{dM}{dt} = \frac{DS}{h}C_s$$

the quantity D/h can be referred to as K or ***the intrinsic dissolution rate.***

In order to quantify the dissolution rate experimentally, the number of variables must be limited to the one under investigation. Working with conventional tablets, the dissolution rate is determined under constant pH, temperature, volume and rotation speed, and is known as the total dissolution (mg min^{-1}), since the surface area is not controlled. However, a quantity more often determined during the early stages of formulation of a new drug dosage form, is the intrinsic dissolution rate *(IDR)*.This term requires the surface area to be constant and not decreasing with time. The IDR can be determined by compressing the pure powdered drug under extremely high

pressure in the absence of any additives. The resultant *non-disintegrating disc* has a constant surface area that can be determined easily.

Equation (1) can be reduced by using a non-disintegrating disc, a constant rotation speed and a large dissolution volume, becoming

$$\frac{dM}{dt} = K_1 C_s$$

Working under constant pH and temperature, the equation can be written as

$$\frac{dM}{dt} = K_2$$

which is integrated to become

$$M = K_2 t$$

A plot of the amount dissolved (M) as a function of time yields a straight line. Knowing the disc surface area and the saturation solubility, the slope of the straight line can be used to determine the IDR (k = D/h).

Procedure

1. Calculate the geometric surface area of the provided salicylic acid disc.
2. Place the disc in the center of a 100 ml beaker, and carefully add ***100 ml*** of water.
3. Adjust the stirrer to the required speed.
4. Using a pipette take ***2 ml*** samples at *5* minute intervals, add ***2 ml*** of $FeCl_3$ to each sample, and measure the absorbance at 528 nm.
5. From the provided calibration curve, calculate the total amount of salicylic acid present in solution.
6. To study the effect of pH, repeat the above steps using ***0.01 M HCl*** as the dissolution medium instead of distilled water.

Lab No. 6
Dissolution
Report Sheet

Name: Number: Section:

*Objectives:

*Data and Calculations

Time (min)	Distilled water			0.01 M HCl		
	A	Concentration (mg/ml)	Total Amount (mg)	A	Concentration (mg/ml)	Total Amount (mg)
5						
10						
15						
20						
25						
30						

1. On an ordinary graph paper, plot total amount dissolved versus time.
2. Calculate the slope for each line, then calculate the intrinsic dissolution rate, knowing that the saturation solubility of salicylic acid at room temperature is 2 gm/l.
3. Comment on your results, then discuss the various factors affecting dissolution rate based on Noyes and Whitney equation.

Lab No. 7
Rheology of Fluids

Objectives:

1. To study the rheological properties of some fluids.
2. To learn how to use different types of viscometers.

Introduction:

Rheology is the study of flow behavior of fluids. This is of particular importance in pharmacy, since many pharmaceutical formulations are liquids and semisolids. Flow properties are involved in pouring a product in and out of the container, spreading the product on the skin, mixing and flow into containers during preparation and stability of emulsions and suspensions.

Fluids are classified depending on their flow properties into Newtonian and non-Newtonian. Most liquids like water, alcohol, glycerin and syrup fall under the category of Newtonian flow. Their viscosity (η) is constant with increasing shearing stress and is given in poise. Other pharmaceutical preparations like ointments, suspensions and polymer solutions, show flow properties that depend on the shearing stress applied i.e. viscosity is not constant, and are said to be non-Newtonian. A complete rheogram or flow curve should be used to fully describe their flow behavior, whether it is plastic, pseudoplastic or dilatant.

Measurement of viscosity:

The type of instrument used for measurement of viscosity is dependent on the flow pattern of the preparation. Based on this, instruments called viscometers are divided into:

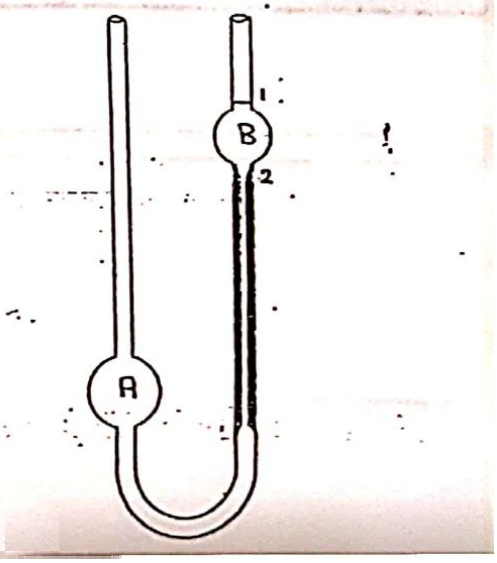
a. *One-point viscometers*: These operate at a single rate of shear, which provides a single point on the rheogram. Extrapolation of a line through this point to the origin will result in a complete rheogram. Such viscometers are suitable only for Newtonian fluids.

b. *Multipoint instruments*: These operate at a variety of rates of shear, which makes it possible to obtain a complete rheogram. Such instruments are suitable for the determination of non-Newtonian flow properties.

Types of viscometers:

a. Capillary (Ostwald) viscometer:

This is a U-shaped glass tube containing a very thin capillary tube and two bulbs located at different levels. Its use is based on measuring the time required for a liquid to flow by gravity between two marks . and :. This time is related to viscosity according to the equation based on *Poiseuille's law*:



$$\eta = k \rho t$$

where:

η is the absolute viscosity of the test liquid in centipoise.
ρ is the liquid's density.
t is the flow time of the test liquid.
k is an instrumental constant related to the length and inner radius of the capillary tube, bulb's volume and difference in height.

To eliminate the instrumental constant, the flow time of the test liquid is related to that of a standard liquid (usually water) and the equation becomes:

$$\frac{\eta_1}{\eta_2} = \frac{\rho_1 t_1}{\rho_2 t_2}$$

where η_1 and η_2 are the absolute viscosities for the test and standard liquids, respectively. The absolute viscosity of the test liquid is then calculated using the equation

$$\eta_1 = \eta_2 \left(\frac{\rho_1 t_1}{\rho_2 t_2}\right)$$

b. *Falling sphere viscometer:*

In this type of viscometers a glass or steel ball rolls down an almost vertical glass tube containing the test liquid at a given temperature. The rate at which a ball of a particular density and diameter falls is an inverse function of the viscosity of the sample. To calculate the viscosity, the time for the ball to fall between two marks is accurately measured and substituted in the following equation

$$\eta = t\,(S_b - S_f)\,B$$

where:

η is the absolute viscosity of the test liquid in centipoise.
t is the time interval in seconds.
S_b is the specific gravity of the ball.
S_f is the specific gravity of the test liquid.
B constant for a particular ball.

To eliminate B, the viscosity of the test liquid is compared to that of a known liquid, usually water and the equation becomes

$$\frac{\eta_1}{\eta_2} = \frac{t_1\,(S_b - S_{f1})}{t_2\,(S_b - S_{f2})}$$

where η_1 and η_2 are the absolute viscosities for the test and standard liquids, respectively. The absolute viscosity of the test liquid is then calculated using the equation

$$\eta_1 = \eta_2 \left(\frac{t_1 (S_b - S_{f1})}{t_2 (S_b - S_{f2})} \right)$$

c. Rotational Viscometers:

These instruments depend on the fact that a solid rotating body immersed in a liquid is subjected to a retarding force due to the viscous drag, which is proportional to the viscosity of the preparation. The advantages of rotational viscometers are that the shear rate can be varied over a wide range of values, and that continuous measurement at a given shear rate or shear stress can be made for extended periods of time, affording measurements of the time dependency as well as the shear dependency of the viscosity. Common examples are the Brookfield viscometer and the cone and plate viscometer.

Procedure:

a. Capillary (Ostwald) viscometer:

1. Clean and dry the viscometer.
2. Set the viscometer upright in a thermostated water bath adjusted at 25 C°.
3. Fill the viscometer with the liquid to the mark located on top of the lower bulb.
4. Using a pipette filler, suck up the liquid until it rises beyond the mark located on top of the upper bulb.
5. Determine the flow time of the liquid between the upper and lower marks of the upper bulb.

b. Falling sphere viscometer:

1. Fill the burette with the liquid to the top.
2. Slowly let the sphere fall through the liquid according to its own gravity.
3. Record the time taken by the sphere to pass a certain distance.
4. Repeat three times and take the average.

Lab No. 7
Determination of Viscosity
Report Sheet

Name: Number: Section:

*Objectives:

*Data and Calculations

1. Capillary Viscometer:

Liquid	*Density (g/ml)*	*Time (sec)*	η_1 / η_2	η_1 *(cp)*

2. Falling Sphere Viscometer:

Liquid	*Specific Gravity*	*Time (sec)*	η_1 / η_2	η_1 *(cp)*

3. Rotational Viscometer:

Conclusion:

Lab No. 8
Incompatibility

Objectives

Introduction

Incompatibility may be defined as an interaction of two or more components to produce changes in the physical, chemical, microbiological or therapeutic properties of a preparation. It may affect safety, efficacy and appearance of a preparation. Although incompatibility may be found in all dosage forms, it is more likely to occur in liquid than in solid preparations.

Physical incompatibility is visually detected in the form of immiscibility, insolubility, color change or liquefaction. This produces unsightly, non-uniform product from which it is difficult to remove an accurate dose.

Chemical incompatibility is due to chemical reactions that produce inactive or toxic products. Most chemical changes result from hydrolysis, oxidation-reduction or complexation and may or may not be detected visually. It may be described as a physicochemical process, since physical incompatibilities may also result from chemical changes.

Therapeutic incompatibility results in undesirable antagonistic or synergistic pharmacological activity.

Microbiological incompatibility may be due to any interaction that diminishes the activity of the preservative used.

The following sections include examples of incompatibilities that may be found in some pharmaceutical preparations.

1. *Cationic and anionic compounds of high molecular weight*

The valuable pharmaceutical and pharmacological properties of many organic compounds are associated with a large ***cation*** or a large ***anion***. Interaction of ions of opposing types yields ***salts*** of very high molecular weight, which, usually, are insoluble or slightly soluble in water and lack the useful properties of the ions. Interacting ions may be the drug itself or the adjuvants used like dyes, emulsifying agents, thickening agents and preservatives.

2. *PH effects*

Most drugs are often salts of weak acids and bases. These salts are soluble in water, whereas their corresponding unionized forms are practically insoluble. Therefore, if a solution of a salt of a weakly basic drug is made alkaline, the free base may be precipitated, while precipitation of the free acid may occur if a solution of a weakly acidic drug is acidified. Whether precipitation occurs or not depends on:

- The solubility of the unionized acid or base.
- The pH of the solution.
- The pKa of the acid or base.

According to the Henderson-Hasselbalch equation, the relationship between the pH, pKa and the relative concentrations of the unionized acid or base and its salt form is as follows:

$$pH = pKa + \log(salt/acid) \qquad \text{(for a weak acid)}$$

$$pH = pKw - pKb + \log(base/salt) \qquad \text{(for a weak base)}$$

Changes in the solubility brought about by alterations in solvent pH can be predicted using the pHp equation. The pHp for a weak acid is the pH *below* which the free acid starts to precipitate

$$pHp = pKa + \log(S - S_o / S_o)$$

The pHp for a weak base is the pH *above* which the free base starts to precipitate

$$pHp = pKw - pKb + \log(S_o / S - S_o)$$

where:
S_o is the molar solubility of the unionized acid or base.
S is the molar concentration of the salt form of the drug initially added.

Procedure

1. ***Cationic and anionic compounds of high molecular weight***

Using test tubes, mix 2 ml quantities of each of the following:
a. S.L.S. solution + cetrimide solution.
b. S.L.S. solution + Tween 80 solution.
c. S.L.S. solution + C.M.C. Na solution.
d. Cetrimide solution + C.M.C.Na solution.
e. Cetrimide solution + Tween 80 solution.

Record any incompatibility observed in the form of precipitation or cloudiness.

2. ***PH effects***

a. Soluble salicylates and acids.

Starting with *3% w/v* sodium salicylate solution, prepare 6 ml of *1.5% w/v* sodium salicylate syrup as follows:
1. Using Lemon syrup B.P. containing citric acid.
2. Using Simple syrup B.P flavoured with lemon tincture.

Record your observations.

b. PHp of weak acids:

1. Transfer 20 ml of 0.1 M sodium benzoate solution to an Erlenmeyer flask.
2. Determine the pH of the solution.
3. Add 0.5 M HCl dropwise until a white precipitate starts to form.
4. Record the pH of the solution which is pHp of 0.1 M sodium benzoate solution.
5. Continue adding 0.5 M HCl until complete precipitation is observed, record the pH.
6. To 20 ml of 0.1 M sodium benzoate in 50% glycerol, add the same volume of 0.5 M HCl used, record your observations.

3. *Aspirin suspension*

a. Prepare 100 ml of a suspension containing 300 mg/5 ml or 6.0 gm per 100 ml aspirin (it should be a suspension since the solubility of aspirin is 1gm /300 ml water). Transfer to a suitable glass bottle.
b. Take 10 ml and filter. To the filtrate add few drops of $Fecl_3$. Record your observations and keep the preparation for your next lab session.
c. After one week, take another 10 ml and filter. Add few drops of $Fecl_3$.
d. Sniff the contents for any vinegar smell. Record your observations.

Lab No. 8
Incompatibility
Report Sheet

Name: Number: Section:

*Objectives:

*Results:

1. Incompatibility between large anionic and cationic molecules

			Result
1.	SLS	CMC Na	
2.			
3.			
4.			

Comment:

2. PH effects:

a. Sodium Salicylate solution.

** Calculations*

** Results*

		Result
1.	*Sodium salicylate flavored with lemon syrup*	
2.	*Sodium salicylate flavored with neutral lemon syrup*	

Comment:

b.Sodium benzoate solution

1.	PH of sodium benzoate solution before addition of HCl.	
2.	PHp of sodium benzoate solution.	
3.	PH after complete precipitation.	

Comment on the results obtained for sodium benzoate solutions in water and in 50% glycerol.

4. **Aspirin Suspension**
   - *Write the equation of the chemical change occurring for aspirin.*

   - ***Results:***

   - ***Comment:***

Questions:

1. *Calculate the pHp for a 0.10 M sodium benzoate solution, then compare it with the value obtained in your experiment.*

2. *Check the different dosage forms of aspirin available in the market.*

3. *Write the formula for lemon syrup and neutral lemon syrup. (Refer to the B.p.).*

Lab No. 9
Surface Tension

Objectives
Determination of surface tension for some liquids and solutions.

Introduction

Molecules at the surface of a liquid are subjected to an unbalanced force of molecular attraction. Because of this, they experience an inward force towards the bulk, which pulls the molecules together and contracts the surface. The force per unit length that must be applied parallel to the surface to counterbalance the inward pull is termed ***surface tension***. The value of this physical property can serve as an index for the strength of intermolecular attractive forces of liquids. Thus, the surface tension of polar liquids (dyne/cm) is higher than that of non-polar liquids.

Methods used to measure surface tension are numerous and include:

1. Capillary rise method

A liquid rises spontaneously into a capillary tube due to surface tension. If it rises to a height, h, above the bulk liquid in a capillary having a radius, r, a force, F, will act upward and vertically along the circle of liquid-glass contact. Based upon the definition of surface tension, this force will be equal to the surface tension, γ, multiplied by the circumference of the circle, $2\pi r$. This force upward must support the column of liquid, and since the mass, m, of the liquid is equal to the density, ρ, multiplied by the volume of the column, $\pi r^2 h$, the force W opposing the movement upward will be $W = mg = \pi r^2 \rho g h$

Equating the two forces at equilibrium gives

$$\gamma 2\pi r = \pi r^2 \rho g h$$

so that

$$\gamma = \frac{1}{2} \rho g h r$$

Thus, the greater the surface tension, and the finer the capillary radius, the higher the rise of liquid in the capillary.

If the surface tension of a liquid, γ_1, is related to that of water, γ_1, the equation becomes

$$\frac{\gamma_1}{\gamma_2} = \frac{\rho_1 h_1}{\rho_2 h_2}$$

2. Number of drops method

The weight of a drop of liquid detached from a tube of known radius r depends on surface tension. At equilibrium, the weight of the drop is equal to surface tension acting on the circumference of the tube

$$\therefore \quad m\,g = \gamma\, 2\pi r$$

so that

$$\gamma = \frac{m\,g}{2\pi r}$$

Since mass of a drop $m = v_d\,\rho$, the equation becomes

$$\gamma = \frac{v_d\,\rho\,g}{2\pi r}$$

And for a given volume of liquid v_T, $v_d = v_T / n$

$$\frac{\gamma_1}{\gamma_2} = \frac{\rho_1\, n_2}{\rho_2\, n_1}$$

where γ_1 is the liquid's surface tension, and γ_2 is the surface tension of water. Thus, as surface tension is reduced, the volume of a drop is decreased, and the number of drops per certain volume is increased. However, the viscosity factor should be considered.

3. Du Nouy ring method

The method depends on measurement of the force required to detach a platinum ring immersed at the surface of a liquid. This force is proportional to surface tension.

Procedure

1. Capillary rise method

Immerse a capillary tube in the test liquid. Record the height of the liquid's column which rises above the liquid's surface. Repeat 2-3 times and take the average. Be sure that the capillary tube is clean and that it is immersed in a vertical position.

2. Number of drops method

Count the number of drops per 2 ml of liquid sample using a burette. The drop must form slowly to minimize variation. Repeat twice for each sample and take the average.