SREE DATTHA INSTITUTE OF PHARMACY

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BIOPHARMACEUTICS AND PHARMACOKINETICS

Lab Manual

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STANDARD CURVE OF SALICYLIC ACID

Aim: To plot the standard curve of salicylic acid by colorimetric method.

Requirement: Test tube, volumetric flask.

Chemicals: Ferric nitrate reagent, salicylic acid

Principle:
Colorimetric estimation of salicylic acid involves formation of light violet colored complex with ferric nitrate reagent which shows maximum absorbance at 520 nm in visible region. Qualitative analysis by visible spectroscopy depends on beer lamberts law,which states that absorbance of substance depends on concentration and path length. Absorbance changes in directly proportionate with concentration and inversely proportionate with pathlength.

A = log Io/P =abc
A = absorbance
b =path length
Io = Intensity of incident light
P = intensity of transmitted light
a = absorptivity
c = concentration of a given substance.

Procedure:

1. Dissolve 100mg of drug in 100ml of distilled water to prepare 1mg/ml concentration of stock solution.
2. From the above stock solution prepare 10, 20, 30, 40, 50, 60µg/ml dilutions and check the absorbances of the dilutions at λ_{max} (520 nm).
3. Then plot the graph between concentration (X-axis) and absorbance (Y-axis), find out the slope of the obtained straight line.

Report:

EFFECT OF CO- SOLVENT ON SOLUBILITY OF SALICYLIC ACID

Aim: To study the effect of co- solvent on solubility of salicylic acid.

Reagents: 1% ferric nitrate, 1% propylene glycol, 5% glycerin.

Requirements: Test tube, beaker, pipette, volumetric flask.

Principle:

The solubility of weak electrolyte (or) non polar compound in water can be improved. Use of water miscible solvent in combination with water to improve the solubility of compound is called co-solvent. If we add co solvent to the actual solvent then the solubility in this system is greater than that can be predicted from the material solubility in each individual solvent. The principle behind using co-solvent or combinations of solvents is to bring dielectric constant of solvent system to a value at which drugsolubility is maximum. Ideally suitable blend should posses value of dielectric constant between 25 to 80. The choice of suitable co-solvent is some what limited for pharmaceutical use, because of possible toxicities and irritancy. The most widely used system which will cover this range is water ethanol blend & other suitable solvents are sorbitol, glycerol, propylene glycol and syrup.

Procedure:

Prepare saturated solutions of salicylic acid in 10 ml of given co-solvent system. From the above saturated solution prepare 1/10 and 1/50 dilutions. From the abovedilutions take 1 ml, add 4 ml of ferric nitrate reagent ,set aside for 15 min.
Measure the absorbance of above solution at 520nm.Using standard plot of salicylic acid calculate the amount of drug soluble in given solvent systems.

Preparation of blank:

Take 1 ml of solvent and add 4 ml of $FeNO_3$ reagent. Use this solution as blank.

Observations and calculations:

Co-solvent	Dilutions	Absorbance	conc(µg/ml)	Amount of drug(mg/ml)

Concentration = absorbance/slope (from standard plot of salicylic acid)
Amount of drug soluble in 10ml = concentration x10

Report:

EFFECT OF pH OF SOLVENT ON SOLUBILITY OF SALICYCLIC ACID

Aim: To study the effect of pH of solvent on solubility of salicylic acid

Reagents: $FeNO_3$, regent, 0.1N HCL, 0.1N NaOH.

Requirements: Test tubes, beaker, pipette, volumetric flask.

Principle:
The choice for solvent is water, however although the drug is freely soluble in water it is unstable in aqueous solution. Hence water miscible solvents are used as co-solvents in the formulation to improve the solubility & Stability of the formulation. Co-solvents are used in analysis, extraction and separation. Suppose in aqueous pH, co solvent solubility and stability is unattainable then break down occurs. This is termed as solvlysis. The recommended solvents are

Solvent	*Applications*
Water	for all purpose
Methanol	extraction & separation
0.1N HCL	dissolution acid base extraction
0.1N NaOH	dissolution
Ethanol	formulation and extraction
Propylene glycol	formulation
PEG300	formulation

Procedure:
1. Prepare saturated solution of salicylic acid using the given solvent.
2. From the above stock solution prepare 1/10 and1/ 50 dilutions using the respective solvent
3. To the 1ml of above solution add 4 ml of $FeNO_3$ reagent set aside for 15 min.
4. Measure the absorbance of solution at 520 nm.

Preparation of blank:

Take 1 ml of solvent add 4 ml of FeNo3 reagent. Use this solution as blank.

Observations and calculations:

Solvent	Dilutions	Absorbance	conc(µg/ml)	Amount of drug(mg/ml)

Report:

EFFECT OF SUREACTANT ON SOLUBILITY OF SALICYLIC ACID

Aim: To study the effect of surfactants on solubility of salicylic acid.

Reagents: 1%$FeNO_3$, 2% tween80, 1%SLS,

Requirement: Test tubes, beakers, pipette, volumetric flask.

Principle:
The solubility of poorly soluble drug can be improved by addition of surfactant. This phenomenon of the micellar solubility and solubilization has been widely used for the formulation of poorly soluble drug. The amount of surfactant used for this purpose must be carefully controlled. Large excess is undesirable because of cost and possible toxic effects and 1/3 effect on product aeration during manufacturing. Excessive amounts of surfactant may also reduce bio availability of drug due to strong absorption with in the micelles and it may also lead to precipitate on storage (or) dilution of product.

Procedure:
Prepare saturated solution of salicylic acid (10ml) in water with the help of surfactants like 2%tween80, 1%SLS. From the above solutions prepare 1 in 10 and 1 in 50 dilutions. Take 1ml from the dilutions and add 4ml of $FeNO_3$ agent to it and set aside for 15 min. Measure the absorbance of the above solution at 520nm.

Preparation of blank:
Take 1ml surfactant solution and add 4ml of $FeNo_3$ reagent.

Observations and calculations:

Surfactant	Dilutions	Absorbance	Concentration(µg/ml)	Amount of drug

Report:

EFFECT OF PARTICLE SIZE ON DISSOLUTION RATE

Aim: To study the effect of particle size on dissolution rate.

Requirement: Beakers, test tubes, standard sieves, dissolution apparatus.

Principle:
Surface area of drug particle is a parameter which influence drug dissolution and as particle size decreases, surface area increases and rate of dissolution is directly proportional to surface area. Dissolution is a process in which solid substance solubilizein a given solvent i.e mass transfer from solid surface to liquid phase. Noyes Whitney equation is given to show proportionality between surface area & dissolution.

$$\frac{dc}{dt} = \frac{DAK\,w/o\,(C_s - C_b)}{vh}$$

$\frac{dc}{dt}$ = rate of dissolution

D = Diffusion co-efficient of drug
A = surface of dissolving solid
K = partition co- efficient
h = thickness of stagnant layer
C_s = concentration of drug in stagnant layer
C_b = concentration of drug in bulk

Procedure:

- Take 10g of salicylic acid and prepare a damp mass by using 2% acacia as granulating agent.
- Pass the damped mass through sieve no 10 to obtain granules.
- Dry the obtained granules at 70 C for 30 min, pass the dried granules through sieve no 10,22,85,30,60,
- Take 500mg of powder retained on each sieve and subject to dissolution (dissolution media=water,900ml).
- Take out the sample at 0,10,20,30,40,50,60 time intervals
- Then add 4ml of 4% FeNo3 reagent to the collected samples, then measure the absorbance of above complex at 520 nm.
- Then calculate the % of drug release.

Observations and calculations

Time	Absorbance	conc(µg/ml)	Concxd.f	Amount of drug	% drug release

d.f = dilution factor

Report:

DIFFUSION RATE OF SALICYLIC ACID THROUGH A SEMI PERMEABLE MEMBRANE

Aim: To study the diffusion rate of salicylic acid through a semipermeable membrane.

Requirement: Onion membrane (thin), salicylic acid, water, ferrous reagent or $FeNo_3$ reagent

Principle:

Diffusion means the passage of molecules (drug) from high concentration to low concentration through semipermeable membrane. It is necessary for a drug absorption, distribution. A drug molecule must pass through several cell membranes, intracellular fluid and extra cellular fluid to reach the general circulation. In body different semipermeable membranes like BBB. Placental barrier etc..are there through which drug has to pass to show its effect.

Rate of diffusion expressed by Ficks law of diffusion rate of diffusion =

$$\frac{dq}{dt} = \frac{-DKA\,(cp-ct)}{h}$$

- cp = drug conc in plasma
- ct = drug conc in tissue
- A = surface area of membrane
- h = thickness of membrane
- k = lipid/water partition co- efficient
- D = diffusion co- efficient of drug

Procedure:

1. Take 50 mg of salicylic acid in a beaker containing 100 ml of water.
2. Then collect membrane of onion tie to the open end of the test tube and the other end is kept open.
3. Fit that membrane test tube with the help of stand, and arrange it in a manner such that the open end face towards opposite upper side.
4. Take 10 ml of drug solution and pour in to the open end of test tube and place lower point of test tube in a beaker containing 50 ml of distilled water.
5. Collect samples at different time intervals like 0,10,20,30,40,50,60,90,and 120, min and simultaneously replace with water (pure water)
6. Take 1 ml from the samples collected at different time intervals and add 4 ml of 4% FeNo3 reagent
7. Then measure the absorbance value using Uv-visible spectroscopy.

Report:

DISSOLUTION STUDIES OF ENALAPRIL MALEATE COMMERCIAL TABLETS

Aim: To perform the dissolution studies of enalapril maleate commercial tablets

Reagent: pH 7.4 phosphate buffer, 0.1N Hcl, 0.1N NaOH, distilled water

Preparation of PH 7.4 phosphate buffer:

Dissolve 6.8 gm of potassium hydrogen phthalate and 1.56 g of sodium hydroxide in distilled water and make up the volume 1000ml.

Principle:

Dissolution is defined as the process of which solid substance enters the solvent. It plays important role in bio availability of drug. It provides data to distinguish between good and bad formulations. Changes in production process that might influence bio availability of drug dissolution depends on drug related factors, formulation factor and instrumental factors. The dissolution rate (time required by solute to dissolve in solvent) change from one manufacture to another. In this experiment take different brands of enalapril maleate to determine dissolution rate & percent drug release.

Procedure:

Plotting of standard graph of enalapril maleate:

1. Take 100mg of drug and dissolve in 100ml of water. From this pipette out 1ml and make the volume to 50ml with distilled water to prepare stock solution of 20µg/ml
2. Prepare different concentrations like 2,4,6,8 and10 µg/ml from the above stock solutions.
3. Measure the absorbance of dilutions using uv/visible spectrophotometer at λ_{max} 215 nm.
4. Then plot the standard graph of enalapril by taking concentrations on X-axis and absorbance on Y-axis.

Dissolution study:

1. Take three different commercially available tablets as mentioned above.
2. Prepare the dissolution media of pH 7.4 phosphate buffer and set the pH.
3. Set up rpm as per monograph i.e 50 rpm and keep temperature at 37±0.5^{o}c
4. Collect the samples at different time intervals like 0,10,20,30,40,50,60 min and simultaneously replace with same amount of solvent (media).
5. Then measure the absorbance value at 215 nm.
6. Plot the graph between % drug release & time interval.
7.

Observations and calculations:

Time	Absorbance	conc(µg/ml)	Concxd.f	Amount of drug(mg/ml)	% drug release

Report:

DIFFUSION STUDIES OF SALICYLIC ACID OF DIFFERENT PREPARATIONS

Aim: To determine the rate of salicylic acid diffusion from different ointment bases.

Apparatus: Petriplates, glass rod, beakers, measuring cylinder.

Reagents: Agar medium, different ointment bases, 4% ferric nitrate.

Principle:
The release of medicament from a semi solid is prerequisite to produce either a local effect at the site of application or for cutaneous absorption to produce systemic effect. Release of medicament from semisolid dosage form depends upon factors like nature and composition of semisolid vehicle or physicochemical properties of medicament like partition coefficient, solubility, particle size etc. In this experiment the release of salicylicacid from various semisolid bases is evaluated by using agar cup plate method .In this method salicylic acid is incorporated into differentiated bases like simple ointment, vanishing cream and gel. In an agar plate cup bases diffusion of salicylic acid was measured for 30, 60, 90 min and latter with interval of one hour.

Procedure:
Prepare 10 g of simple ointment, vanishing cream and gel according to the formula of I.P. and incorporate 500 mg of salicylic acid into each formulation. Prepare 100 ml of agar medium and add 10 ml of ferric nitrate to agar plate before solidification and label it.
Create 3 cups in each petriplate, then put the prepared ointment, gel and cream into cups without touching the surroundings. Observe it for every 20 min for 1 hour and every 60 min for 3 hours. Every time observe the diameter of the zone ,measure with scale .In this salicylic acid slowly diffuses into the surrounding medium from the preparation and reacts with ferric nitrate to give a purple color. The size of the zone indicates the measure of salicylic acid diffusion observed.

Observations and calculations:

Time(min)	Simple ointment	Vanishing cream	Gel

Report:

DISSOLUTION STUDIES OF LEVOFLOXACIN

Aim: To perform the dissolution studies of levofloxacin

Reagent: pH 7.4 phosphate buffer, 0.1N HCl, distilled water

Principle:

Dissolution is a process of which solid substance enters the solvent. It plays an important role in bio availability of drug. It provides data to distinguish between good and bad formulations. Changes in production process that might influence bio availability of drug dissolution depend on drug related factors like formulation factors, instrumental factors. The dissolution rate (time required by solid amount of solute to dissolve in solvent) change from one medium to another medium. In this experiment levoflaxacin is used to estimate it dissolution rate in different media i.e. 0.1N HCl & pH 7.4 phosphate buffer.

Procedure:

***Preparation of PH 7.4 phosphate buffer*:**

Dissolve 6.8 gm of potassium hydrogen phthalate and 1.56 g of sodium hydroxide in distilled water and make up the volume 1000ml

***Preparation of 0.1N Hcl**:*

Dilute about 0.85 ml of HCl up to 100ml with distilled water.

Procedure

Plotting of standard graph of levofloxacin:

1. Take 100mg of drug and dissolve in 100ml of water, from this pipette out 1ml and make the volume to 50ml with distilled water (20µg/ml)
2. Prepare different concentrations like 2,4,6,8,10 µg/ml from the above stock solution.
3. Measure the absorbance at 281 nm using uv/visible spectrophotometer.
4. Then draw the graph between concentration (X-axis) and absorbance (Y-axis)
5. Determine the slope from the obtained straight line.

Dissolution studies:

1. Prepare dissolution media of P^H7.4 phosphate buffer &0.1N HCl.
2. Carry out Dissolution of levofloxacin in this two media.
3. Set the rpm as per monograph- 50rpm at a temperature of 37±0.5^{o}c.
4. Then carry out the process of dissolution using type-1 apparatus.
5. Collect the samples at 0,10,20,30,40,50,60 min time interval.
6. Measure the absorbance values of collected samples at 281 nm .
7. Plot the graph between % drug release & time.

Observations and calculations:

Time	Absorbance	conc(µg/ml)	Concxd.f	Amount of drug(mg/ml)	% drug release

Report:

DETERMINATION OF ABSORPTION RATE CONSTANT (K_a) BY WAGNER NELSON METHOD

Aim: To determine the absorption rate constant by Wagner nelson method.

Principle:
The method involves the determination of K_a from percent unabsorbed and time plot. This does not require the assumption of zero or first order absorption.

After oral administration of a single dose of a drug at any given time the amount of drug absorbed in to systemic circulation (X_a) is the sum of drug in the body (X) and amount of drug eliminated from the body (X_e) through renal route.

thus ,

$$X_a = X + X_e \qquad \square(1)$$

The amount of drug eliminated at any time "t"

$$X_e = K_E \,.Vd.\, (AUC)_0^t \qquad \square(2)$$

AUC (area under curve) is the total amount of drug absorbed in to the system,
Vd=volume of distribution.

$$X = Vd.C \qquad \square(3)$$

C=concentration at a particular time't'
Substitute eq(2) & (3) in eq(1) then we get

$$X_a^t = Vd.C + K_E \,.Vd.\, (AUC)_0^t \quad (X_a \text{ at time t}) \qquad \square(4)$$

At ∞ time i.e. at t=∞

$$X_a^\infty = Vd.C^\infty + K_E.Vd.\, (AUC)_{0^\infty}$$

But at t=∞; C^∞ will be zero, so $Vd.C^\infty = 0$

$$X_a^\infty = K_E \,.Vd.\, (AUC)_0^\infty \qquad \square(5)$$

fraction of drug absorbed $= X_a^t / X_a^\infty$, by substituting eqs (4) & (5) here, we get

$$X_a^t / X_a^\infty = [Vd.C + K_E \,.Vd.\, (AUC)_0^t \,] \,/\, [K_E \,.Vd.\, (AUC)_0^\infty \,]$$

Vd common term will get cancelled, then above equation will become

$$X_a^t / X_a^\infty = [C + K_E . (AUC)_0^t] / [K_E . (AUC)_0^\infty] \quad \square(6)$$

Thus % amount of drug remaining to be absorbed is $(1 - X_a^t / X_a^\infty) * 100$

A semi log plot of % unabsorbed (% ARA) Vs 't' yields a straight line with slope $-K_a / 2.303$, log concentration Vs time plot gives a straight line with slope of $-K_E / 2.303$, AUC can be obtained from graph of concentration Vs time plot.

Report:

DETERMINATION OF ABSORPTION RATE CONSTANT BY METHOD OF RESIDUALS

Aim: To determine the absorption rate constant (K_a) for the given data by method of residuals

Principle:

If a subject receives a single dose of 100mg is in tablet dosage, from the data obtained after oral administration of the drug the K_a value is calculated by using "method of residuals" for kinetics by administering intravenously. The concentrate of drug of plasma is expressed by exponential equation.

$$C=\left[\frac{K_a FX_0}{Vd\,(Ka-KE)}\right] * (e^{-K_E t} - e^{-K_a t}) \quad \square\ (1)$$

Say $\left[\frac{K_a FX_0}{Vd\,(Ka-KE)}\right] = A$ (hybrid constant)

Then eq 1 becomes

$$C=(Ae^{-K_E t} - Ae^{-K_a t}) \quad \square\ (2)$$

Drug elimination $K_a >> K_E$ so Ka reaches ∞ value, thus $e^{-K_a t}$ becomes '0'

Now eq 2 becomes $C= Ae^{-K_E t}$ □ (3)

By taking natural logarathims on both sides

$$\log C=\log A - \frac{K_E.t}{2.303} \quad \square(4)$$

So graph between log C Vs time plot gives a straight line with slope of $-K_E/2.303$
Then back extrapolate the line, so that we will get a interecept of 'log A'on Y-axis
Now take the difference between this back extrapolated concentrations and actually (experimentally) obtained concentrations, which is nothing but the residual concentration.
So C_r = extrapolated concentration – actual concentration

Now $C_r= Ae^{-K_a t}$

By taking natural logarthms on both sides

$$\log C_r = \log A - \frac{K_a t}{2.303} \quad \square \text{eq } 5$$

Now the semi log graph between log C_r Vs time gives a straight line with a slope of $-K_a/2.303$ from which we can calculate the absorption constant.

Report:

DETERMINATION OF ELIMINATION RATE CONSTANT AND BIOLOGICAL HALF LIFE FROM URINARY EXCRETION DATA

Aim: To determine elimination rate constant and biological half life from urinary excretion data.

Principle : The half-life of a drug is a time required to reduce the amount of drug in the body (or) plasma by 50% (or a first order process). The half-life is the constant and is independent of starting value of amount of drug in the body. The plasma half-life can be determined from the graph (or) from K_E

$$t_{½} = 0.693/k$$

K_E represents overall elimination by compiling parallel pathways and is equal to sum of rate constant that define the various simultaneous contributory process such as metabolism, renal excretion and biliary excretion

Half-life is clinically useful parameter of pharmacokinetics. It is useful in determining.

1. The fluctuation of plasma concentration between doses.
2. The time required to reach steady state equilibrium after continuous administration
3. The existence of drug in the system once the drug administered has ceased

Determination of K_E from urinary excretion data by 2 methods.

1. Rate of excretion method
2. Sigma minus method

Rate of excretion method : The rate of urinary drug excretion dX_u/dt is proportional to amount of drug in the body (X) written as

$$dX_u/dt = K_e X \qquad (1)$$

Where K_E = first order urinary excretion rate constant according to first order kinetics

$$X = X_0 e^{-k_E t}$$

Substituting in above equation in eq1 yields [X_0= dose administration (I. V.bolus)]

$$dX_u/dt = K_e . X_0 e^{-K_E t} \qquad (2)$$

taking logarithms eq 2 becomes

$$\log dX_u/dt = \log K_e X_0 - K_E t/2.303 \qquad (3)$$

The above equation states that semi log plot of rate of excretion Vs time yields a straight line with slope $-K_E/2.303$. It must be remembered that slope is related to elimination rate constant(K_E) and not to excretion rate constant(K_e).The excretion rate constant can be obtained from Y-intercept ($\log K_e.X_0$)

Report:

DETERMINATION OF BIOLOGICAL HALF-LIFE OF RIFAMPICIN BY URINARY EXCRETION

Aim: To estimate the biological half-life ($t_{1/2}$) of rifampicin by urinary excretion data.

Principle:

In the absence of plasma level concentration Vs time data useful information can still be obtained from urine data regarding elimination kinetics of drug .The urinary excretion method is useful where there is a lack of sufficient substitute analytical method tomeasure the concentration of drug in plasma with accuracy. Often or less sensitiveanalytical method can be used for determining urine drug concentration. The method is non-invasive and therefore better subject compliance is assumed. Collecting urine sampleis convenient when compare to drawing of blood periodically .In urinary excretion data, the urine excreted at defined time intervals following the administration of a drug is collected and analyzed for drug content .Amount increases in various time intervals and excretion rates are then calculated based on excretion data.

K_E can be determined from urinary excretion data by 2 methods.

1. Rate of excretion method
2. Sigma minus method

sigma minus plot method:

The rate of amount of drug excreted in urine unchanged is

$$dX_u/dt = Ke.X_0 e^{-K_E t}$$

Integration of the above eq yields

$$X_u = Ke\ X_0/K_E(1- e^{-K_E t}) \quad \square\ (1)$$

X_u is cumulative amount excreted as unchanged from urine at time t

At infinity time $t=\infty$, $e^{-K_E t}$ becomes 0

$$X_u^{\infty} = K_e X_0 /K_E \quad \square\ (2)$$

Substitute eq2 in eq1 then,

$$X_u = X_u^{\infty}(1- e^{-K_E t}) \quad \square\ (3)$$

$$X_u^{\infty} - X_u = X_u^{\infty}. e^{-K_E t}$$

By taking natural logarithms on both sides

$$\log X_u^{\infty} - X_u = \log X_u^{\infty} - \frac{K_E t}{2.303} \quad \square \text{eq } 4$$

Eq 4 indicates that semilog plot between amount remaining to be eliminated (log X_u^∞ - X_u) Vs time gives a straight line with slope of $-K_E/2.303$

Report:

DETERMINATION OF BIOAVAILABILITY OF FOUR BRANDS OF A DRUG

Aim : To estimate the bioavailability parameter from the plasma concentration data of four brands of drug .

Principle: A drug is available in different dosage forms. The therapeutic efficacy of a drug from a dosage form depends on rate and extent at which it reaches the blood. This property of dosage form is known as bioavailability. It is an absolute term that indicates measurement of both the true rate and total amount of drug that reaches the general circulation from an administered dosage form. Equivalence is more a relative term that compares one drug with another (or) with a set of established standards. Bioavailability is the most important intensity and duration of therapeutic effect of the drug. The essential process involved in the bioavailability concept is the absorption process. Absorption refers to transfer of drug from site of administration to blood.

When the bioavailability of a drug administered orally is compared to its IV administration is called absolute bioavailability. When the bioavailability of a drug after oral administration is compared with that of an oral standard of the same drug is called relative bioavailability.

A selection of a drug (or) standard plot of a drug administered orally is taken as reference standard for determining relative bioavailability. An analysis of all characteristics is required before one can implicate only one factor (or) parameter as indicates bioequivalence (or) lack of it. The blood concentration time curve is the focal point of bioavailability assessment and is obtained when serial blood samples taken after drug administration are analyzed for drug concentration.

Relative bioavailability is used to characterize absorption of a drug from its formulation. Both pharmacokinetic and pharmacodynamic methods are used to measure the bioavailability. The kinetic methods used are plasma level time studies and urinary excretion studies. The plasma time studies method is most relative method and method of choice in comparison to urine data.

Bioavailability and bioequivalent concepts are important in clinical practice when substitution of one brand for another is to be made. When the products are equivalent such substitution leads to failure of treatment. The bioavailability of concept is useful in

1. Primary stage of development of a suitable dosage form for a new drug entity
2. Determination of influence of excipients, patients related factors
3. Development of new formulation of existing drugs
4. Control of quantity of a drug.

Several parameters are used to provide a general evaluation of overall rate and extent of absorption of a drug. An analysis of all characteristics is required before one can implicate only one factor (or) parameter as indicative bioequivalence (or) lack of it.

As the drug is absorbed, increased concentration of drug are observed in successive samples until maximum concentration is achieved. This point of maximum concentration is called peak of concentration. In the concentration-time curve, the section of curve to left of the peak represents the absorption phase during which the rate of

absorption exceeds rate of elimination. The section of curve to right of peak of blood concentration time curve is elimination phase. Height of the curve represents the highest drug concentration after oral administraton. The second parameter is the measurement of length of time necessary to achieve maximum concentration after drug administration called as time of peak plasma concentration. This is related closely to rate of absorption of drug from a formulation and used as a single measurement of rate of absorption.
The third important parameter for evaluation is under the serum is reported in µg/ml and can be considered in representative of a single dose of a drug.

Report:

DETERMINATION OF PHARMACOKINETIC PARAMETRS BY ONE COMPARTMENT MODEL.

Aim: To evaluate and calculate some of the pharmacokinetic parameters as per one compartment model

Theory and Principle:

The following data refers to the plasma concentration vs time offers i.e. bolus dosing of 300mg (X_0) of a drug.

(a) half life drug
(b) concentration of drug in plasma at '0' time
(c) volume of distribution (Vd)
(d) renal clearance

One compartment open model:

One compartment model is the simplest model which depicts the body as single kinetically homogenous unit that as no barriers to the movement of drug. Final distribution equilibrium between the drug in plasma and other body fluids is obtained instantaneously and maintained at all times.

The term open indicate that the input (bioavailability) and output (elimination) are Unidirectional and that the drug can be eliminated from the body.

Elimination rate constant(K_E) : It is overall elimination rate constant of urinary excretion, metabolism, biliary excretion, pulmonary excretion and other mechanism involved there in.

Biological half life/elimination halflife: It is defined as time taken for the amount of drug in the body as well as plasma concentration to decline by one half or 50% its initial value.

$t_{1/2}$=0.693/K

Apparent volume of distribution. It is the hypothetical volume of body fluid into which a drug is dissolved or distributed. Volume of distribution is a proportionality constant pharmacokinetic parameter that permits the use of plasma drug concentration in place amount of drug in body

$$\mathbf{Vd = \frac{Amount\ of\ drug\ in\ the\ body}{Plasma\ drug\ concentration}}$$

***Clearance*:** It is the theoretical body fluid containing the drug [i.e., fraction of Vd] from which the drug is completely removed in a given period of time

Clearance = rate of elimination / plasma drug concentration

Cl = dX/dt/C or K_E.Vd

C_0 : Plasma drug concentration immediately after injection i.e. zero time.

In IV bolus administration the elimination process follows first order kinetics. The graph between log C Vs time gives a straight line with slope of -K_E/2.303.

Using K_E and above formulas we can determine biological half-life.

Report:

DETERMINATION OF BIOLOGICAL HALF LIFE OF METRONIDAZOLE TABLETS FROM URINARY EXCRETION DATA

Aim : To determine the biological t1/2 of metronidazole tablets from urinary excretion data.

Requirements : Beakers, U.V.Spectrophotometer, measuring cylinder.

Theory :

In the absence of plasma level concentration Vs time data useful information can still be obtained from the urine data regarding elimination kinetics of drug. The urinary excretion method is useful where there is lack of sufficient substitute analytical method to measure the concentration of drug in plasma accuracy. Often or less sensitive analytical method can be used for determining urine drug concentration as compared to plasma concentrations.

If the urinary drug concentrations are low assigning a large volume is relatively easy and serve the purpose. The method is non invasive and therefore better subject compliance is assumed. Collecting urine sample is convenient when compared to drawingof blood periodically. In urinary excretion data the urine excreted during different time intervals following the administration of a drug is collected and analysed for the drug content. Amount increases in various time intervals and excretion rates are then calculated based on excretion data.

Procedure :

Plotting of standard graph of metranidazole:

1) Prepare the artificial urine, from the artificial urine prepare different concentrations like 1, 2, 3, 4, 5, 6, 7, 8, 9, 10.(µg/ml).
2) Measure the absorbances of the prepared standard solution using U.V.Spetroscopy at 277nm.
3) Draw the plot between concentration (X-axis) and absorbance (Y-axis), find out the slope of the line.

Finding out amount of drug excreted:

4) Initially select a healthy subject.
5) According to the formula prepare artificial urine and take as the reference.
6) Then administer the subject with drug of metronidazole 400mg tablet. After the drug administration, collect the urine samples at regular intervals of 0, 1, 2, 3, 4, 5, 6(hrs)
7) Then analyse samples using U.V.Spectroscopy at 277nm.
8) Determine the amount excreted from the collected data using standard graph.

Standard concentration(µg/ml)	Absorbance

Time(hrs)	Different time	Midpoint	Absorbance	Volume of (V)urine(ml)	Concentration(C)	Amount A=VxC	Rate of excrete	Log rate of excretion

Report :

SREE DATTHA INSTITUTE OF PHARMACY

Nagarjuna Sagar Road, Sheriguda (V), Ibrahimpatnam,

Hyderabad – 501510

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PHARMACEUTICAL ENGINEERING

Lab Manual

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INDEX

DETERMINATION OF ABSOLUTE HUMIDITY & RELATIVE HUMIDITY BY PSYCHOMETRIC METHOD

Aim: To determine the absolute humidity and relative (percentage) humidity of the air by Psychometry

Requirements: Psychometer and humidity chart.

Principle:

A Psychometer contains both dry bulb & wet bulb thermometers. In the Psychometric method, the dry bulb and wet bulb temperatures are simultaneously noted. By knowing the dry bulb and wet bulb temperatures, the absolute humidity and the percentage humidity can be calculated from the humidity chart or psychometric chart. Dry bulb temperature was always greater than the wet bulb temperature. Absolute humidity can be calculated by the following equation

$$W_W - W_G = (h_G/29\ K_G\ \lambda_W)\ (t_G - t_W)$$

W_W = Absolute humidity of the specific wet bulb temperature or Saturation humidity at Specific wet bulb temperature.

W_G = Absolute humidity at air to be estimated.

h_G = Heat transfer coefficient from the air to the wet surface by convection process.

K_G = Mass transfer coefficient from the wet surface into air.

λ_W = Latent heat of vaporization of water at the specific wet bulb temperature.

t_G = Dry bulb temperature. t_W = Wet bulb temperature.

In the above equation, $h_G/29\ K_G = 0.26$ **(constant)**

It is taken as constant for the air-water vapour system. 29 refers to the mol.wt. Of air.

The value of λ_W can be obtained from the theoretical table (Appendix 9 in Badger, p.no.733)

Saturation humidity (W_W) was calculated from the humidity chart at the specific wet bulb temperature. Therefore, knowing the dry bulb and wet bulb temperatures and above factors, the absolute humidity can be calculated by the above equation.

$$\text{Relative Humidity} = \frac{\text{Absolute humidity of atmospheric air}}{\text{Absolute Humidity at dry bulb temp. from 100\% saturated air curve}} \times 100$$

Or

$$\text{Relative Humidity} = \frac{\text{Absolute humidity of air}}{\text{Saturation humidity at Dry bulb temperature}} \times 100$$

Procedure:

1. Take the Psychometer and saturate one of the bulb of the thermometer with water using a wet wig (cloth). Wig should be continuously wet with water.
2. Note the temperatures of both dry and wet bulb thermometers till the concurrent readings are obtained.
3. Estimate the absolute & relative humidity by the equations included in the principle.
4. Also, record the values of absolute & relative humidities from the humidity chart.

Note down the values of wet bulb temp & dry bulb temp on the humidity chart (Psychometric chart). From the wet bulb temperature draw a straight vertical line to the 100% saturated air curve. Then

descend along the nearest adiabatic cooling line till the adiabatic cooling line intersects vertical line from the dry bulb temperature. Draw a straight horizontal line from the point of intersection to the Y-axis, this gives the value of absolute humidity in lb (pounds).
At the point of intersection of the adiabatic cooling line and the dry bulb temp, the nearest percentage curve, indicates the values of relative (percentage) humidity.

Observations & Calculations:

Time (mins)	**Dry bulb temp (^{0}C)**	**Wet bulb temp (^{0}C)**
0		
5		
10		
15		

Calculation of relative (percentage) humidity by equation:

$$\textbf{Relative Humidity} = \frac{\text{Absolute humidity of air}}{\text{Saturation humidity at Dry bulb temperature}} \times 100$$

Applications: The control of temperature & humidity was very important in pharmaceutical industry. The processing of tablets & capsules was done under air conditioned atmosphere (24^{0}C and RH 40-45%).
The Aspirin tablets and Effervescent tablets are manufactured by dry granulation method, in a dehumidified area, with RH < 30% and temp 21^{0}C.

Report: The absolute humidity was found to be
The relative humidity was found to be

OVERALL HEAT TRANSFER COEFFICIENT OF GLASS

Aim : To determine the overall heat transfer coefficient (OHTC) of glass using a glass condenser.

Requirements : Steam generator, safety tube, glass condenser, stand, beakers etc.

Reference : Introduction to Chemical Engineering by Badger & Banchero, Page no. 143.

Principle : The overall heat transfer coefficient is estimated by dividing the total amount of heat transferred by the product of log mean temperature drop and area of heat transfer.

$$U = Q/A \Delta t_m$$

U = Overall heat transfer coefficient. **Q** = quantity of heat transferred through the condenser.
Δt_m = log mean temperature drop
A = Area of condenser through which heat is transferred = $2 \pi r l$, where
r = inner radius of given condenser. **l** = length of given condenser.

The total heat transferred through condenser is equal to either heat lost by steam or heat gained by the circulating water. Heat lost by steam is expressed as ;

$$Q_1 = m_1 S_1 \Delta t_1 + m_1 L$$

Heat gained by circulating water is given as;

$$Q_2 = m_2 S_2 \Delta t_2$$

$$Q = (Q_1 + Q_2) / 2 \qquad \Delta t_m = (\Delta t_1 - \Delta t_2) / 2.303 \log (\Delta t_1 / \Delta t_2)$$

Where, T_1 = temperature of steam; T_2 = temperature of condensate.
T_3 = temperature of inlet tap water; T_4 = temperature of water at condenser outlet.
Δt_1 = temperature drop of steam = $T_1 - T_2$, Δt_2 = temperature rise of water = $T_4 - T_3$
Δt_m = log mean temperature drop
m_1 = mass of condensate obtained within some time interval say 5 minutes.
m_2 = mass of hot water obtained from outlet of condenser within 5 minutes.
S_1 = specific heat of steam = 1Kcal S_2 = specific heat of water = 1 Kcal
L = Latent heat of vapourisation of water = 540 Kcal.
r = inner radius of the condenser in meter:
l = length of condenser in meter. **A** = area of condenser in m^2 = $2 \pi r l$,
Q_1 = Quantity of heat lost by steam Q_2 = Quantity of heat gained by water.
Q = Quantity of heat transferred through the condenser.
U = Overall heat transfer coefficient in Kcal/m^2 ^{0}C

Procedure :

1. Place the steam generator on the tripod stand and fill it with water upto 3/4th of its volume. Attach a safety tube to steam generator to prevent explosion.
2. Fix the condenser to a stand. Attach the steam outlet of steam generator to the glass condenser through a rubber tube and a glass tube. Use Teflon tape for tight attachment and to prevent leakage of steam.
3. Heat the steam generator. Pass the generated steam through a water jacketted glass condenser.
4. Steam is condensed by circulating water in the counter current fashion.

5. Estimate the temp.of steam (T_1) and temperature at the inlet of circulating water (T_3). T_1 & T_3 remain constant throughout the experiment.
6. Collect the condensate in a beaker, which is previously weighed.
7. Weigh the condensate collected in 5 min. measure the temperature of the condensate (T_2).
8. Collect the circulating water in a 2000 ml measuring cylinder by employing a funnel and rubber tube. Note the temperature of water obtained at the outlet of condenser (T_4). Also, observe the mass of circulating water obtained in 5 minutes (m_2).
9. Estimate the length and radius of condenser. calculate the overall heat transfer coefficient by the equation discussed in Principle.
   Repeat the experiment 3 times.

Calculations :

Length of condenser (l) =---------- m
Inner radius of condenser (r) =---------- m
Area of condenser (2π r l) =---------- m^2
Weight of empty dry beaker (W_1) =---------- Kg.
Weight of beaker + Condensate =---------- Kg
Weight of condensate (m_1) =---------- Kg

Tabulation :

Temperature		**Temp of circulating water**		**Weight of water collected in 5 min.**	
Steam T_1 (^{0}C)	**Condensate T_2 (^{0}C)**	**Inlet T_3 (^{0} C)**	**Outlet T_4 (^{0}C)**	**Condensate (m_1) kg**	**Circulated water (m_2) kg**
I 100^0 C					
II 100^0 C					
III 100^0 C					

Calculation of overall heat transfer coefficient :

Trial 1 : $\Delta t_1 = T_1 - T_2$ = ----------

$\Delta t_2 = T_4 - T_3$ = ----------

$\Delta t_m = (\Delta t_1 - \Delta t_2) / 2.303 \log (\Delta t_1 / \Delta t_2)$ = ----------

$Q_1 = m_1 S_1 \Delta t_1 + m_1 L$ = ----------

$Q_2 = m_2 S_2 \Delta t_2$ = ----------

$Q = (Q_1 + Q_2) / 2$ = ----------

$U = Q / A \Delta t_m$ = ----------K cal /m^2 ^{0}C

Repeat the Trial 2 & Trial 3

Result : Overall heat transfer coefficient of glass ;

Trial 1= ----------K cal /m^2 ^{0}C
Trial 2 =----------K cal /m^2 ^{0}C
Trial 3 =----------K cal /m^2 ^{0}C

EFFICIENCY OF STEAM DISTILLATION

Aim: To calculate the efficiency of steam distillation of Aniline.

Requirements : Steam generator, condenser, round bottom flask (RBF) with long wide neck, 3 holed cork, thermometer (0-110^{0}C), beakers, separating funnel, Aniline, sodium chloride.

Principle: Steam distillation was a technique employed to separate the high boiling point substances from the non volatile impurities or it can be employed for the removal of volatile impurities from substances having extremely high boiling point. An important requirement for steam distillation was that the desired product must be immiscible with water. In steam distillation, the distillation temp was less than 100^{0}C, because distillation occurs when the sum of the partial pressures of the two immiscible liquids just exceeds the atmospheric pressure.

% efficiency of steam distillation = (Practical yield) x100 / (Theoretical yield)

Practical yield = Weight of substance (Aniline) / weight of water = W_a/W_b

Theoretical yield = $(M_a P_a)/ (M_b P_b)$ where,

M_a = Mol.wt.of Aniline = 93.13; **P_a =** Partial pressure of Aniline.
M_b = Mol.wt.of water = 18; **P_b =** Partial pressure of Water.

As per Avogadro's hypothesis, number of molecules of each component in vapour phase was proportional to its vapour pressure. Hence,

$n \propto P$; $n_a \propto P_a$ & $n_b \propto P_b$
$n_a/n_b = P_a/P_b$ → equation (1)

Number of moles = Weight of substance / Mol.wt.of substance
$n_a = W_a/M_a$; $n_b = W_b/M_b$

Therefore taking the values of **n_a** & **n_b** in equation (1)

$(W_a/M_a)/ (W_b/M_b) = P_a/P_b$ or $W_a/W_b = P_a M_a/P_b M_b$

Procedure:

1. Place the steam generator on a tripod stand, fill about 3/4th of its volume with water. Attach a safety tube to the steam generator to prevent explosion.
2. Take 10 ml of Aniline in RBF. Fit three holed cork, one for steam inlet tube, second for thermometer, third for glass tube for vapour outlet.
3. Pass the steam generated into RBF, by employing a rubber tube and a bent glass tube. Use Teflon tape and attach the rubber tube tightly to prevent the leakage of steam.
4. After some time, the liquid starts boiling. Observe the constant temperature at which distillation occurs, (boiling point of the liquid being distilled), note it as the boiling point of aniline plus water.
5. Knowing the boiling point, observe the partial pressure of water, P_b (at this temperature) from the vapour pressure table. Estimate the partial pressure of aniline (P_a) by the formula ($Pa = 760$ mm Hg $– P_b$).
6. The hot vapour obtained through distillation, was condensed by circulating the water in the counter current fashion.

7. Collect sufficient quantity of condensate into a beaker, transfer into a separating funnel, add few grams of sodium chloride and shake the liquid mixture thoroughly, then allow it to rest. This was done because NaCl dissolves in water, if any traces of aniline was dissolved in water, the aniline droplets will be separated out into the aniline layer. Consequently, separate the aniline layer and the aqueous layer.
8. Estimate the weight of condensate, aniline and water. Repeat the experiment in three trials and calculate the efficiency of steam distillation.

Precautions:

1. Connect the steam tube between steam generator and distillation flask such that the steam tube will be completely immersed in aniline.
2. Insert the thermometer upto the neck of the distillation flask.
3. Cover the distillation flask and the condenser with asbestos rope (insulator) so as to prevent the heat loss due to radiation.

Calculations: **Trial I**

Weight of empty beaker = ----------

Weight of beaker + condensate = ----------

Weight of condensate = ----------

Weight of aniline (W_a) = ----------

Weight of water (W_b) = Weight of condensate – weight of aniline (W_a) = ----------

Distillation temperature (boiling point) = ----------

Partial pressure of water (P_b) at distillation temperature (from vapour pressure table) = ----------

Partial pressure of aniline (P_a) = 760 mm Hg – P_b = ----------

M_a = Mol.wt.of aniline = 93.13

M_b = Mol.wt.of water = 18

Practical yield = W_a/W_b = ----------

Theoretical yield = $P_a M_a/P_b M_b$ =

Efficiency = (Practical yield) / (Theoretical yield) x 100 =

Result: Efficiency of steam distillation of aniline =

Trial I:

Applications: Steam distillation was used for :

1. Purification of organic compounds with high boiling point, e.g. Aniline, Nitrobenzene.
2. Extraction of Volatile oils, e.g. Clove oil, Eucalyptus oil.

RATE OF DRYING

Aim: To determine the rate of drying for the given sample and to determine the values of

a) Critical Moisture Content – CMC

b) Equilibrium Moisture Content – EMC

c) Drying Time.

Requirements: Tray dryer, trays, digital balance, test sample.

Theory: When a substance, having certain moisture content, was exposed to air at a certain temperature and humidity, it looses/gains moisture until equilibrium moisture content (EMC) was attained.

The value of EMC depends on 1) the nature of the substance, 2) temperature and humidity of air to which the substance was exposed.

If the substance contains more moisture than the equilibrium value, it was known as free moisture content (FMC). It was the free moisture content (FMC) which it lost by the substance during drying.

Drying rate curve was obtained by plotting the average FMC on X-axis and drying rate on Y-axis.

Drying time curve was plotted between drying time (X-axis) and average moisture content (Y-axis).

Procedure:

1. Take a rectangular metallic tray and note its length (l), breadth (b) and empty weight (W_1).
2. Calculate the area of the tray by the formula, A = l x b.
3. Fill the tray with sample (sand) upto $3/4^{th}$ the height of the tray and observe its weight (W_2).
4. Add water to the tray containing sand, so that slurry was obtained; observe the weight of tray + slurry of sample (W_3).
5. Place the tray in the tray dryer/hot air oven and maintain the temperature at 70^0C.
6. At the interval of 15 min (0.25 hr), remove the tray from the dryer and observe its weight (W_T).
7. Continue the drying of tray until the weight of tray undergoes no change and until its weight (W_T) was only slightly higher than (W_2).
8. Plot the following graphs:
   a) Drying rate curve: Average MC (X-axis) and Drying rate (Y-axis).
   b) Drying time curve: Drying time (X-axis) and average moisture content (Y-axis).
9. Drying rate and moisture content (M.C.) are calculated as:
   Drying rate = (Moisture lost in each interval) / (time interval x area of tray)
   Moisture content (M.C.) = Weight of moisture in sample/ dry weight of sample.

$(M.C)_0 = (W_3 - W_2) / (W_2 - W_1)$ at time = 0 hrs

$(M.C)_{0.25} = (W_T - W_2) / (W_2 - W_1)$ at time = 0.25 hrs

Average M.C. = $[(M.C)_0 + (M.C)_{0.25}] / 2$

Observations & Calculations:

Area of petridish ($A = \pi r^2$) =

Weight of empty petridish = w_1 =

Weight of empty petridish + sample = w_2 =

Weight of empty petridish + sample + water = w_3 =

Weight of sample ($CaCO_3$) =

Weight of water added =

Weight of sample after 15min drying =

Moisture content (M_c) = $W_3 - W_2 / W_2 - W_1$

Average moisture content = $(Mc_1 + Mc_2) / 2$

Rate of drying = $(W_n - W_{n+1}) / \Delta tA$

Time in hr.	**Wt.of Petri dish water (gm)**	**Total moisture (W_T-W_2) (gm)**	**Moisture content $\frac{(W_T-W_2)}{(W_2-W_1)}$**	**Avg.M.C (gm/gm)**	**Drying rate (gm/hr cm^2)**

Inference :

1. CMC of the given sample = ---------- gm.
2. EMC of the given sample = ----------
3. Time required for drying (to reach EMC) = ---------- hrs

SIEVE ANALYSIS

Aim: To determine the particle size distribution of the given sample of granules by sieve analysis.

Requirements: Granules, standard sieves of different aperture sizes, Sieve shaker, brush.

Principle:

Sieve number was defined as the number of openings present per linear inch in a given sieve.

A set of standard sieves was taken, with the coarsest sieve at the top and the finest sieve at the bottom. The test sample (granules) was taken on the coarsest (uppermost) sieve. Size separation equipment, crushing and grinding equipment will have the required sieve/set of sieves to separate the different particle sizes of the powder or granular materials present in the given sample. In the sieve analysis, a certain amount of sample will pass through a screen of a given aperture (d) and will be retained on the next subsequent sieve placed below the upper sieve.

The average size of the particles retained on a specific sieve = Average aperture or sieve opening (d) of upper sieve and the sieve on which the sample is retained.

Procedure:

1. Take the stack of sieves in the following order.

Uppermost		Lowermost
Coarsest	Sieve no. 8, 36, 52, 72, 100, 150, 200, pan	Finest
Top		Bottom

2. Weigh 100 gm of granules and place it on the coarsest sieve. Operate the sieve shaker for 20mts.
3. Weigh the sample retained on each sieve (W).
4. Record the weight of sample (W) retained on each sieve. Calculate the following from the date recorded.
   a) % wt retained b) Cumulative wt% under size c) Cumulative wt% oversize
   d) Wd^2, Wd^3, Wd^4
5. Draw the following plots:
   a) Frequency size distribution plot: % weight retained (Y-axis) and average aperture (particle) size (X-axis).
   b) Cumulative size distribution plot: Cumulative wt% undersize and cumulative wt % oversize (Y-axis) and average aperture (particle) size (X-axis).
   c) Log probability plot: Cumulative wt% undersize (X-axis) on probability scale and average aperture (particle) size (Y-axis) on log scale. Record the values of mean, median and mode from plots.

d) Calculate the following statical diameters: d_{avg}, d_s, by both the methods:

Method 1: By formula.

Method 2 : By Hatch Choate equations.

Sieve No.	Aperture size (mm)	Sieve No. Passed/Retained	Average aperture size (mm) (d)	Weight retained (gm) (W)	% W	% W x d	Cumulative wt% over size	Cumulative wt % Under size

$\sum W =$ $\sum \%W =$ $\sum \%Wx\ d =$

Log d	W log d	Wd	Wd^2

$\sum Wd =$

Average particle size in mm = $(\sum \%W \times d) / \sum \% W$ or $(\sum W \times d) / \sum W$

d_{avg} Arithmetic mean = $(\sum \% W \times d) / 100$,

d_s Mean surface diameter = $\sqrt{(\sum W d^2)} / \sum W$

Result: From graph:

d mean = ---------- mm, d mode = ---------- mm,

d median = ---------- mm

Statical diameters (in mm)	By formula	By Hatch Choate equation
d_{avg} d_s		

BALL MILL

Aim: To study the operation of Ball mill and to study the particle size distribution of the milled product by sieve analysis.

Requirements: Ball Mill, chalk pieces, Sieves, Sieve shaker.

Theory: Ball mill works on the principle of impact and attrition. The grinding media consists of balls, which are made of stainless steel or porcelain. Ball size varies from 75 mm – 150 mm. Ball mill should be operated at optimum speed for efficient milling. At low speed, balls only slide over each other, at very high speed, balls undergo centrifugation. At optimum speed of the mill, the balls cascade over each other, this leads to efficient milling due to both impact and attrition. Diameter of aball mill was less than the length of the mill. Diameter varies from 1-3 m. If the density of ball was high it forms a fine product. Ball mill can be used for sterile milling.

Sieve analysis of the milled product: The milled product obtained from Ball mill was subjected to sieve analysis. Sieve number was defined as the number of apertures or openings in a linear inch of the given sieve. The milled product (100 gm) was placed on a set of a sieves, the coarsest sieve was at the top and the finest sieve was at the bottom. The sample on the stack of sieves was shaken in a sieve shaker for 5 minutes. The average particle size of the sample retained on each sieve was equal to the average aperture size of the upper sieve and the lower sieve on which the sample was retained.

Procedure:

1. Take the sample to be milled in a Ball Mill upto 2/3rd the volume of the mill.
2. Mill the sample (chalk pieces) for 2 1/2hr. At the interval of every 15 minutes stop the rotation and observe the milled powder for the required degree of fineness.
3. After the completion of milling, note down the weight of the milled weight.
4. Place 100 gm of milled product on a set of standard sieves. Place the stack of sieves on a sieve shaker. Operate the sieve shaker for 5 minutes.
5. Weigh the sample retained on each sieve.
6. Plot the following graphs:
   (a) Frequency size distribution plot: Percentage weight retained (Y-axis) and average aperture size (X-axis).
   (b) Cumulative size distribution plot: Cumulative wt.% undersize & cumulative wt.% oversize (Y-axis) & average aperture size (X-axis).
   (c) Log-probability plot.

7. Note the values of mean, median and mode from the graphs.
8. Calculate the statistical diameters such as arithmetic mean, mean surface, mean volume, mean volume surface, weight mean, geometric mean, by two methods,
   (a) by Formula (b) by Hatch Choate equations.

Average particle size in mm = $(\sum \%W \times d) / \sum \% W$ or $(\sum W \times d) / \sum W$

d_{avg} Arithmetic mean = $(\sum \% W \times d) / 100$,

d_s Mean surface diameter = $\sqrt{(\sum W d^2) / \sum W}$

Sieve No.	Aperture size (mm)	Sieve No. Passed/Retained	Average aperture size (mm) (d)	Weight retained (gm) (W)	% W	% W x d	Cumulative wt% over size	Cumulative wt % Under size

$\sum W =$ $\sum \%W =$ $\sum \%Wx\ d=$

Log d	W log d	Wd	Wd^2

$\sum Wd =$

Report: From graph

Mean =----------mm

Median =.....mm

Mode =.....mm

Diameters	From formula	From equations
d_{avg} d_s		

OPEN PAN EVAPORATOR

Aim: To determine the rate of evaporation and overall heat transfer co-efficient and steam economy of open pan evaporator when evaporating saturated sodium chloride solution.

Theory: The objective of an evaporator is used to increase the concentration of the solution. Evaporation is an heat transfer operation which converts a liquid into vapour leaving behind a concentrated solution.

The capacity of an evaporated is defined as number of kgs of water evaporated per hour.

The steam economy of an evaporator is defined as number of kgs of water evaporated per number of kgs of steam fed into the unit.

The steam economy is always less than 1 for single effect evaporators and more than 1 for multiple effect evaporators.

Requirements: Open pan evaporator, NaCl, Water.

Procedure:
Prepare approximately 20kgs of saturated NaCl dried (20% W/W)

- Charge this material into an open pan evaporator with bottom drain value in closed position.
- Open the steam supply value and also the steam to flow into the steam jacket collect the condensate in a separate vessel. Also note down the pressure indicated by the pressure gauge.
- After an hour stop the steam supply and measure the volume of condensate collected as well as temperature of the boiling liquor.
- Open the bottom drain valve of an evaporator and weigh the concentrate of NaCl.
- Wash the open pan evaporator to remove any trace of NaCl.

Observations and Calculations:

- Initial weight of saturated NaCl or brine taken =
- Final weight of saturated NaCl drained =
- Time of operation =
- Pressure of steam supplied to the steam jacket =
- Amount of condensate collected (W) =
- Latent heat of vaporization of steam at the Given pressure from steam table (λ) =
- Temperature of steam =
- Temperature of boiling solution =
- Amount of water evaporated (M) =
- Initial weight of NaCl – Final weight of saturated Drained (20% W/W) NaCl drained =
- Total energy given up by the steam to the brine solution (Q) = λW =
- Evaporator capacity / rate of evaporation =

= Amount of water evaporated / hour =

- Steam economy = $\frac{\text{Amount of } H_2O \text{ evaporated}}{\text{Amount of steam consumed}}$ =

- Overall heat transfer coefficient $U = \frac{Q/\theta}{A.\Delta T}$

$Q = W \times \lambda$

$A = 2\pi r^2$

Report:

1. Rate of evaporation was found to be_____.
2. Steam Economy was_____.
3. Overall heat transfer coefficient was_____.

EFFECT OF FILTER AID ON RATE OF FILTRATION

Aim: To study the effect of filter aid on rate of filtration and to determine the optimum concentration of filter aids,

Requirements: Beakers, funnel, filter paper.

Chemicals: $CaCo_3$, Bentonite, distilled water.

Principle: A granular or fibrous material capable of forming a highly permeable cake is called as filter aid. Eg: Diatomaceous earth, bentonite, Calcium Carbonate, talc, paper pulp, cellulose and asbestos.

IDEAL PROPERTIES OF FILTER AID:

- Filter aids should be rigid and porous in nature.
- It should have low bulk density.
- It is chemically inert to filter aid.
- It should be perfectly recoverable.

Procedure:

- Take 5gm of calcium carbonate in beaker add bentonite in concentrations of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%.
- To it, add 100ml of distilled water and dissolve calcium carbonate.
- Filter the samples through a filter paper and note down the time taken for filtration.

Determination of Optimum Concentration:

To know the optimum concentration of filter aid, graph was plotted by taking concentration of filter aid on X-axis and rate of filtration on Y-axis sudden change in the graph gives the optimum concentration of filter aid.

Observations and Calculations:

S.No.	Concentration of filter aid	Volume of filtrate (ml)	Time (mins)	Rate of filtration (ml/min)

Report:

As the concentration of filter aid was increased, the rate of filtration also increases. Optimum concentration of filter aid was_____.

FACTORS AFFECTING RATE OF FILTRATION

Aim: To study the effect of various factors on rate of filtration.

Apparatus: Filter papers, glass rod, beakers.

Chemicals: Calcium Carbonate, glycerine.

Principle: The rate of filtration was rate of drying force and resistance. Driving the upstream and downstream of the filter, the distance is not constant.

$$V = \frac{\pi \Delta P\text{-}r^4}{8l\eta}$$

Where, V = Rate of flow

ΔP = Pressure difference

r = Radius of capillary in filter belt

l = thickness of filter cake

η = Viscosity of filtrate

$$V = \frac{kA\Delta P}{\eta l}$$

k = Permeability coefficient

A = Surface area

FACTORS AFFECTING:

1. Area of the filter
2. Resistance of cake
3. Viscosity of filtrate
4. Pressure difference across the filtrate

Procedure:

Effect of thickness of cake: Two solutions of $CaCo_3$ were prepared using water as solvent in the concentration of 5% & 10%, in the concentration of 5%, time taken for the filtration was noted calculate the rate and compare.

Effect of Viscosity: Two different solutions of 5% $CaCo_3$ were prepared once using water as a solvent and the other using 10% glycerin as a solvent. Both the solutions were filtered. Time takenfor filtration was noted down. Rate of filtration was calculated and compared.

Effect of area of filter medium: Two solutions of $CaCo_3$ were prepared and filtered one using average (large) funnel and other using small funnel. Time taken for filtration was noted down. Rate of filtration was noted and compared.

Plot a graph between rate of filtration and time taken.

Rate of filtration on Y-axis and time taken on X-axis for all the three parameters.

Observations and Calculations:

Weight of empty specific gravity bottle =
Weight of empty bottle + Water =
Weight of water =
Weight of empty bottle + $CaCO_3$ solution (water) =
Weight of $CaCo_3$ solution (water) =
Empty bottle + $CaCO_3$ solution (glycerin) =
Weight of $CaCO_3$ solution =
Density of water =
Density of $CaCO_3$ (Water) =
Density of $CaCO_3$ (Glycerin) =

Viscosity
5% $CaCO_3$ → using water as solvent time (T)
5% $CaCO_3$ → using glycerin as solvent time (T)

$\eta_2 = (t_2\rho_2 / t_1\rho_1)\ \eta_1$ ($CaCO_3$ – Water)
η_1 = 1.009cps
$\eta_2 = (t_2\rho_2 / t_1\rho_1)\ \eta_1$ ($CaCO_3$ – Glycerin)

Area of funnel = πr^2
Area of small funnel =
Area of large funnel =

Effect of area on filter medium:

	Time (min)	**Volume of filtrate (ml)**	**Area (m^2)**	**Rate of filtration (ml/min)**
Large Funnel Small Funnel				

Effect of Viscosity:

	Time (min)	**Volume of filtrate (ml)**	**Viscosity**	**Rate of filtration (ml/min)**
5% $CaCO_3$ (10% glycerin) 5% $CaCO_3$ (Water)				

Result:

1. With the increase in area of filter medium, rate of filtration also increases.
2. With the increase in viscosity of solvent, rate of filtration decreases.
3. With the increases in concentration, rate of filtration decreases.

VENTURIMETER

Aim: To determine the coefficient of discharge of venturimeter and orifice meter.

Apparatus: Venturimeter, Orifice meter, sump tank, measuring tank, globe valve, stop watch.

Theory: These flow meters are based on the general energy conservation equation. Because pressure drop is measured after converted into down stream velocity (measured at minimum cross sectional area) which in then converted into volumetric flow rate by multiplying it by volumetric flow rate by that cross sectional area. For unit mass, general conservation equation is,

$P_1/\rho + V_1^2/2 + g Z_1 + U_1 + q = P_2/\rho + V_2^2/2 + g Z_2 + U_2 + W_s$ ----------(1)

In the absence of any pump, any heat interaction any change in internal energy and any change in absence of friction (ideal flow) eq. 1 reduces l incompressible fluid i.e.

$\rho_1 = \rho_2 = \rho$

$P_1/\rho + V_1^2/2 + g Z_1 = P_2/\rho + V_2^2/2 + g Z_2$

This is Bernoulli's equation which represents energy 1 mars. So if there is no change in elevations. (i.e. $Z_1 = Z_2$)

$P_1/\rho + V_1^2/2 = P_2/\rho + V_2^2/2$

$P_1/\rho - P_2/\rho = V_2^2/2 - V_1^2/2$ ---------- (2)

From continuity equation

$A_1V_1 = A_2V_2$

$\rightarrow V_1 = (A_2 / A_1) V_2$ ----------(3)

Where A_1 = Cross sectional area of pipe
A_2 = Cross sectional area of throat
V_1 = Velocity of fluid in pipe
V_2 = Velocity of fluid in throat

Putting the value of V_1 in eq-3 we get

$$V_2 = \frac{1}{\sqrt{1-\left[\frac{A_2}{A_1}\right]^2}}\sqrt{\frac{2\Delta P}{\rho}} \quad m/s$$

Since $\Delta P = \rho g H$,

$$V_2 = \frac{A_1}{\sqrt{A_1^2 - A_2^2}}\sqrt{2gH} \quad \text{m/s}$$

At venture throat, theoretical volumetric flow rate

$Q_{th} = A_2V_2$ m^3/s

$$= \frac{A_1 \times A_2}{\sqrt{A_1^2 - A_2^2}}\sqrt{2gH} \ m^3/s$$

Actual discharge $Q_a = \frac{\text{Volume}}{\text{Time}}$ m^3/s

The velocity V_2 has been derived without considering any losses so it is ideal velocity at the minimum cross sectional area or throat. The actual velocity can be obtained by multiplying the theoretical value by a factor Cd.

Again the losses in velocity may be because of two reasons:

- Due to the formation of Rena contract due to the contraction of cross sectional area of flow respectively it is represented by C_C.
- Due to frictional losses so actual mean velocity will be less than the ideal mean velocity C_V.

Procedure:

- Check all the clamps for tightness.
- Open the gate valve and start the flow.
- Open the outlet valve of the venturi meter and close the valve of orifice meter.
- First open air cocks, then the venturi meter and close the air cocks slowly and simultaneously so that mercury doesn't go away into water.
- Repeat the procedure by changing the discharge is also for orifice meter.

a) For Venturi meter:

1. Actual discharge, $Q_a = \frac{\text{Volume}}{\text{Time}}$ m^3/s

Let 'H' be the pressure lead across manometer.
H= Manometer diff (specific gravity of mercury – specific gravity of water)

H = manometer difference x (13.6-1)
= h x 12.6 =----------m

2. Theoretical discharge Q_{th} :

$$Q_{th} = \frac{A_1 \times A_2 \times 2gH}{A_1 - A_2}$$

Where A_1 = Cross sectional area of pipe =
A_2 = Cross sectional area of throat =
H = pressure head across manometer

3. Coefficient of discharge, $C_d = Q_a / Q_{th}$

Precautions:

1. Operate the manometer valve gently. Air bubbles are to be removed so that mercury does not run away into water.
2. Don't lose the outlet valve.
3. There should be no air bubbles entrapped while taking reading of liquid level in tubes.

Graphs:

Draw a graph between C_d on Y-axis and Q_{th} on X-axis for venturi meter.

Observations and Calculation:

Manometer difference =
Time, T_1 =
Q_a = Volume / Time
H = h x 12.6 =

$C_d = Q_a / Q_{th}$

S.No.	Manometer difference (h)			Time for 10 lit	$Q_a = \frac{\text{Volume}}{\text{Time}}$ (m^3/sec)	H = h x 12.6		$C_d = Q_a/Q_{th}$
	1	2	diff					
1.								
2.								
3.								
4.								
5.								
6.								
7.								
8.								

Report:

The coefficient of discharge of venturi meter or venture coefficient was found to be =-----

REYNOLD'S APPARATUS

Aim: To determine whether the flow is laminar, turbulent or transition.

Apparatus: Dye tank, transparent tube, Supply tank, measuring tank, sump tank, flow control valve.

Theory: When a liquid was flowing through a pipe, the flow was either viscous, laminar or turbulent when fluid was flowing in parallel layers or laminar sliding part adjacent laminar it was called laminar flow. When the fluid does not flow in parallel layers or laminar sliding part, adjacent laminar, it was called laminar and there was intermixing of fluid particles then the flow was said to be turbulent existence of there types was first demonstrated by Reynolds in 1883. The apparatus consistsof a constant head supply tank with water. This tank is provided with a bell mouth outlet to which a transparent tube is fitted. At the outlet of the tube, a regulating valve was provided a dye tank containing colored dye was fitted above the supply tank. The water flows through the pipe and dye was injected at the center of the pipe when the velocity of flow was low.

The occurrence of laminar and turbulent flow was governed by relative magnitudes of inertia and viscous forces. Reynolds's related the inertial force to viscous and arrived at a dimension less parameter now called Reynolds's number.

Procedure:

- Put a small amount of potassium permanganate in the dye tank.
- Check whether the water lend in the tank remains constant such that the water entering the tank was equal to the water leaving the tank.
- Now check the flow of the dye while it moves in a parallel layer it would be laminar and solution it is not in parallel layer it may be turbulent as transition.
- Now note the time required for collecting lot of water.
- Repeat the experiment for various flow rates and find the type of flow.

Observations and Calculation:

Time, t =

u =

Diameter of pipe =

Area = $(\pi / 4) \times d^2$

q =

Volume, v = q / a Reynolds

number = $du\rho / \mu$

Time taken for collecting 10 lit	q = volume / time	v = q /a	Re = duρ / μ	Type of flow

Report:

From the experiment it was observed that the flow was laminar for N_{RE} upto 2000, transition for <2000, turbulent for N>4000

The critical Reynolds's number was found to be =

FRICTIONAL LOSSES IN PIPE

Aim: To determine the coefficient of friction or friction factor for the given pipe.

Apparatus: 17gmm galvanic iron pipe, 14mm cu pipe, 14mm aluminum pipe, globe valve tappings, stop watch, sump tank, measuring tank and centrifugal pump.

Theory: The liquid flowing under pressure in a pipe line will be subjected to head loss which may be major or minor. This was also called head loss due to friction. Minor losses are caused due to losses at entry or exit and major losses are due to change in cross section of the pipe line, suddencontraction in pipe lined due to elbow or bends which change direction of the fluid and loss due to obstruction in path of flow. In long pipe minor losses are very less compared to major losses due to friction. However, minor losses will be appreciable when a fluid is flowing through pipes, it is subjected to resistance to the flow due to shear forces between pipe value and fluid particles and in between the fluid particles also.

Procedure:

- Fill up the water on the sump tank
- Open the inlet valve fully
- Open the outlet valve and start the pump
- Check the leakage by closing outlet valves for each pipe and correct the leaks if any.
- Open the outlet valves of the pipe to be tested.
- Remove all the air bubbles from manometer and connect to pipes.
- Reduce the flow to adjust the outlet valve so that water heads in manometer are to be nearable height.
- Note down the heads and flow rate.
- Now in the flow and accordingly adjust the outlet valve so that liquid will not over flow note down heads and flow rate.
- Repeat the procedure for other pipes.
- Graph was drawn by f on Y-axis and Re on X-axis on log graph or logf on Y-axis and log Fe on X-axis.

Observations and Calculation:

Area of galvanic iron pipe, $A = \pi d^2/ 4$

Area of galvanic copper pipe, $A = \pi d^2/ 4$

Length of pipe = 1m

$g_c = 1$
$\rho =$
$\mu =$

S.No.	Manometer difference (h)		Time for 10 lit	$q_a = \frac{0.01}{t}$ (m^3/sec)	V = q/a m/sec	$f = hDg_c / 2lV^2$	$N_{Re} = DV\rho/\mu$
	Galvanic iron pipe	diff					
1.							
2.							
3.							
4.							
5.							
6.							
7.							
8.							

Result:

Coefficient of friction for galvanic iron pipe was found to be

Coefficient of friction for iron pipe was found to be

SEDIMENTATION BY CENTRIFUGATION

Aim: To estimate the sedimentation time for the formulation having different concentrations of suspending agents by using centrifugation.

Requirements: Calamine lotion prepared with different concentration of suspending agents of centrifuge, scale, stop clock etc.,

Procedure: Centrifugal force was used to provide the derived force to replace the gravitational force in sedimentation process. In this experiment, the relative sedimentation of fore aggregate material (Bentonite) concentration (3%, 2%, 1% & 0% control respectively calamine lotion). Using a centrifuge was compared. sedimentation by centrifugation involves utilization of centrifugal force for sedimentation of solids. During the process of centrifugation, coarse material falls to bottom and above which layer of settled solid with transition zone of partly thickened material above it. The boundary between transition zone and settle solid region was usually observed and through this waterescapes from lower layer which are under compression. Above transition zone, pulp at original concentration and layer of clear water are found. As thickening progress due to sedimentation the tip two layers disappear layer of settle solids shrink, because of compression. Thus, as the time of sedimentation increases the weight of sediments of prepared concentration of suspension decreases tosome extent after with the weight remains constants.
As the time count sediments are formed.

Theory: Centrifuging (also called centrifugation) was a common unit operated in pharmacy. Centrifugal force commonly expressed in multiple of the force of gravity, varies with the rotational speed and with the radial distance from the center of rotation.

A centrifuge was an apparatus utilizing centrifuged force for the separation of lipids form solids. It was essentially a development of gravity filter where in the force acting on the liquid, instead ofbeing restricted to gravity is enormously increased by utilizing centrifuge force. This increased force can also be applied to the separation of immiscible liquids.

Sedimentation Centrifuge: A centrifuge that produces sedimentation of solids based on the difference in the densities of two or more phases of the mixture.
Eg: Top-suspended centrifuges are used extensively in sugar refining automatic batch centrifuges and continuous filtering centrifuges etc.,

Procedure:

- Prepare four 10 ml calamine lotions by using 0%, 1%, 2% and 3% suspending agent (Bentonite) respectively.
- Transfer into the centrifuge tube and measure the height of sediment in each tube.
- Operate centrifuge and determine height of sediment of the every 5 minutes.
- Take 6 readings or until constant heights obtained.
- Plot a graph between height of sediment on Y-axis and time on X-axis

Observations and Calculations:

Ingredients	Official Formula (100ml)	Working formula (10ml)
Calamine Zinc Oxide Bentonite Sodium Citrate Liquefied Phenol Glycerin Water		

Report:

Sedimentation time for 0% concentration calamine lotion was
Sedimentation time for 1% concentration calamine lotion was
Sedimentation time for 2% concentration calamine lotion was
Sedimentation time for 3% concentration calamine lotion was

MIXING

Aim: To determine the percentage of mixing present in the give sample.

Requirements: Mortar, Pistle, Volumetric flask, Colorimeter.

Chemicals: $CaCO_3$, Amaranth dye.

Principle: Mixing was defined as a process that tends to result in a randomization of dissimilar particles within a system. The principle mechanism involved in solid-solid mixing are:

- Converting Mixing – It was achieved by inversion of powder bed using blades. It is referred as macro mixing.
- Shear Mixing: In this type, the forces of attraction are broken down so that each particle moves on its own i.e., shear forces reduces the attraction forces and reduce scale of segregation.
- Diffusion mixing: It involves random motion of particles within powder bed, it occurs at the interfaces of dissimilar regions. It is sometimes refereed as micro mixing.

Procedure:

- 100mg of $CaCO_3$ was taken in a mortar.
- To this 25mg of Amaranth dye indicator was added.
- The above mixture was mixed in mortar for about 20 mints in the same direction.
- Later the sample was collected in such a way that 25mg from the middle of the mortar and another 25mg of sample from the side walls of mortar.
- One of the collected sample was taken in a 100ml volumetric flask and volume was made up to 100ml.
- Then filter the above solutions through normal filter paper.
- A required quantity of sample was taken in a colorimeter tube.
- The absorbance dissolution point was observed and the value should be less than 0.2.
- The dilution will be repeated again. By taking 10ml of previously diluted solution in a volumetric flask and make the volume up to 100ml.
- The same procedure was repeated for another sample collected.

Report: