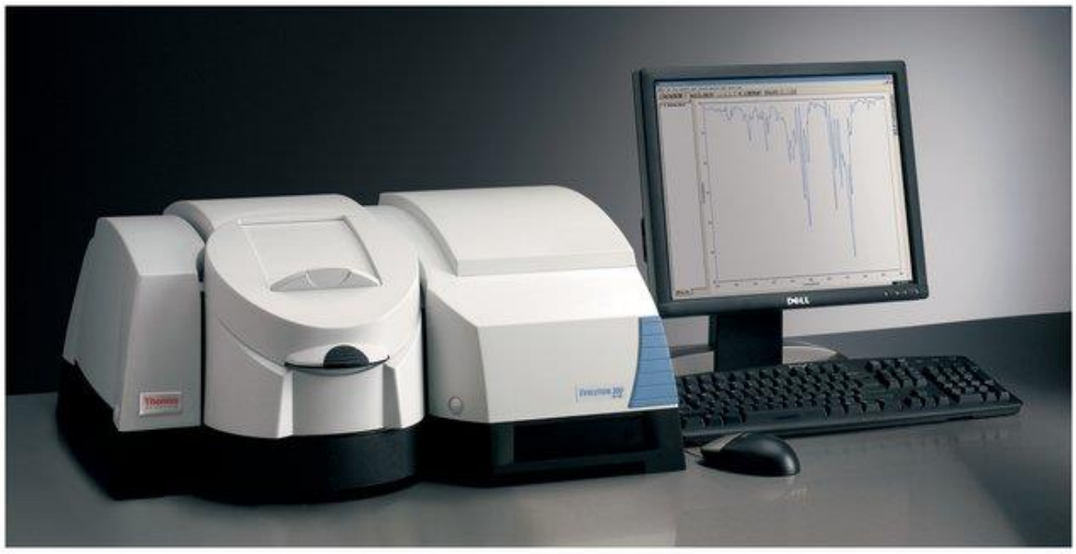
UNIVERSITY OF ILORIN
ILORIN, NIGERIA
FACULTY OF PHARMACEUTICAL SCIENCES
DEPARTMENT OF PHARMACEUTICAL &
MEDICINAL CHEMISTRY



PRACTICAL
PHARMACEUTICAL
ANALYTICAL CHEMISTRY
400 LEVEL MANUAL

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400 LEVEL MANUAL

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PART 1

PCH401 SCHEDULES

SCHEDULE I

1.1 PHOTOMETRIC CALIBRATION

INTRODUCTION

Both the wavelength and absorbance scales should be checked by calibration for good interpretation of Ultraviolet (UV) data. This is because all spectrophotometers produce information on the amount of light absorbed as function of wavelength or frequency.

Wavelength calibration of UV spectrophotometers can be done by reading spectra of solutions of samples of guaranteed purity for which reliable absorbance wavelength data are available. A spectrum of a standard Holmium glass filter which exhibits sharp absorption bands throughout the visible and ultraviolet spectral regions can also be recorded.

OBJECTIVE: To assess photometric accuracy (absorbance) in the UV region.

EXPERIMENT

1) Prepare a solution of 0.02% $^{w}/_{v}$ potassium dichromate ($K_2Cr_2O_7$) in 0.005 M sulphuric acid. From a burette, run exactly 50, 30, 15 and 5mL in to 100 mL volumetric flasks and dilute to 100 mL volume with filtered 0.005 M sulphuric acid. Label the prepared solutions A, B, C and D respectively.
2) Select a matched pair of 1cm path silica cuvettes clean and rinse them thoroughly with distilled water and then with filtered 0.005 M sulphuric acid and place them in the REFERENCE (rear) and SAMPLE (front) cuvette holders. Scan the spectrum over the range 200 – 400nm.

   NOTE: The absorbance zero must not be readjusted during this experiment.
3) Remove and drain the sample cuvette; rinse three times with the weakest dichromate solution i.e. solution D. Refill the cell and record the absorption spectrum between 400 and 200nm on the same chart.
4) Repeat step 3 with each dichromate solution (in the order C, B, A and the original solution (i.e. 0.02% $^{w}/_{v}$).

   NOTE: The order in the filling of cuvettes i.e. from weakest to the strongest solution minimizes errors associated with dilution of samples by rinsing with solvent.

EXERCISE

Locate the two absorption maxima (near 257 and 350nm) and minima (near 235 and 315nm). Measure the absorbance for each solution at all four wavelengths and enter the values in Table I. Plot graphs of absorbance (ordinate) against concentration (abscissa) at all four wavelengths. Calculate the A (1% 1cm) values and compare with the standard value given in Table 1.1 below:

Table 1.1: Spectrophotometric Data for Potassium Dichromate					
Expected wavelength maxima [λ_{max}(nm)]		235	257	313	350
Observed wavelength maxima [λ_{max}(nm)]					
Solution	Concentration (% $^{w}/_{v}$)	A	A	A	A
D	0.001				
C	0.003				
B	0.006				
A	0.01				
O	0.02				
Experimental A (1% 1cm)					
Standard A (1%, 1cm)		124.9	145.2	48.8	107.1

SCHEDULE II

1.2 THE EFFECT OF CUVETTE MATERIAL AND STRAY LIGHT ON ABSORPTION CURVES

INTRODUCTION

The type of cuvette, the sample and presence of stray lights will affect the appearance of recorded absorption curves. The effect of curette material and stray light will be observed using silica and plastic/glass cuvettes in this experiment.

Absorption cells (cuvette) of different dimension made up of different materials are available. However, the nature of the materials can affect the absorption spectra of samples due to the absorption property of cuvette materials. Based on this, quartz cells are used in the entire near UV region because of its absorption property in the visible region while glass cells are used only for the visible region.

In recoding the absorption spectra, two cells are used, one as the reference cell, the other as the sample cell. For accurate UV absorption determination, both the sample and the reference cuvettes must be made up of the same material with identical thickness.

Stray light is caused by the failure of prism to produce monochromatic radiation, diffraction effects; and imperfection, non-homogeneity coupled with unwanted surface reflection from the monochromatic radiation. Thereby permitting a small fraction of the radiation to escape through the exist slit and strike the detector as stray light.

The primary effect of stray light is a quantitative error which, may be associated with an incorrect zero energy line while the secondary effects appears when the amount of stray radiation varies markedly with frequency over the region of the band. In such case, there will be an apparent frequency shift of the band maximum which will vary slightly with the absorption band.

OBJECTIVE: To know the factors that can affect absorption of compounds.

EXPERIMENTS

A. Effect of Cuvette Material on Absorption Curve

1) Using the standard aqueous solution (0.005% $^{w}/_{v}$) phenol solution prepared by diluting 1 mL of ethanolic stock (0.5% $^{w}/_{v}$) solution of phenol to 100 mL with filtered distilled water.

a) Record the spectrum using water as the reference solution and silica cuvettes (both sample and reference) label the spectrum *as* ***A***.

b) Plastic/plastic

c) In the plastic cuvette (sample) against silica (reference), label the spectrum ***B.***

d) In glass cuvette (both sample and reference), label the spectrum **C**.

2) Comment on the differences between these spectra (***B*** and ***C***) and the standard spectrum ***A***.

B. Effect of Stray Light on Absorption Spectra

a) Record the spectrum of a VYCOR filter between the ranges of 300 - 190nm.

b) From the appearance of the curve, state whether stray light is present at short wavelength or not.

Note:VYCOR given is completely opaque to UV light below 215nm.

c) Examine all spectra previously recorded for stray light effects in the light of the results of this experiment.

EXERCISE

Define the following terms:

1) Spectrophotometer
2) A (1% 1cm)
3) Absorption maximum
4) Absorption minimum

SCHEDULE III

1.3 EFFECTS OF SOLVENTS, NATURE OF SAMPLE AND pH ON THE UV/VIS ABSORPTION SPECTRA

INTRODUCTION

Most of the spectrophotometric studies are made in solution and the most important factor in deciding the choice of solvent is that the solvent should not absorb in the same region as the solute.

Apart from solvents, the presence of colloidal and particulate matter in samples of biological origin can greatly alter the appearance of absorption spectra, not only by introducing other ultra violet absorbing components, but also due to ray light scattering of the radiation beam within the sample solution as seen in Schedule II.

Also, the pH of the medium can affect the absorption property of compounds. Acidic drugs usually show bathochromic shift (absorption at higher wavelength) in alkaline medium than in acidic or neutral medium while basic drugs like –sulphanilamide show hypsochromic shift (absorption at shorter wavelength in acidic medium than alkaline or neutral).

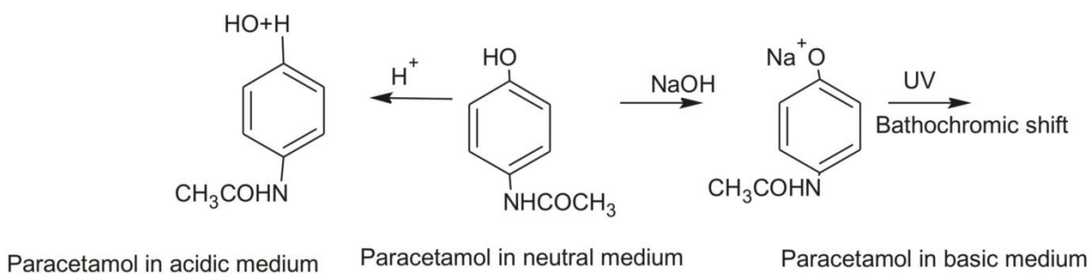


Figure 1.31: Chemical structures of paracetamol in acidic and alkaline media.

OBJECTIVE: To study factors that affect the absorption of compounds.

EXPERIMENTS

A. Effects of Solvents on Phenol Absorption Spectrum

1) Prepare solution of phenol in cyclohexane by diluting 1 mL of the ethanolic stock solution (0.5% $^{w}/_{v}$) solution of phenol to 10 mL with spectroscopic cyclohexane and further diluting 1 mL of this to 10 mL with the same cyclohexane. Record the absorption spectrum between 400 and 200 nm using 1cm path silica cuvette with cyclohexane in the reference cuvette. Label spectrum ***A***.

2) Prepare 0.005% $^{w}/_{v}$ solution of phenol in water by diluting 1 mL of ethanolic stock (0.5% $^{w}/_{v}$) solution of phenol to 100 mL with filtered distilled water. Record this on the same chart as in A, the absorption spectrum of this solution between 400 – 200 nm using 1 cm path silica corvettes with water in the REFERENCE curvette. Label spectrum ***B***.
3) Prepare solution of phenol in cyclohexane by diluting 1 mL of cyclohexane stock (0.5% $^{w}/_{v}$) solution of phenol to 10 mL, and further diluting 1 mL to 10 mL with spectroscopic cyclohexane. Record the spectrum as in step 1 and label spectrum as ***C***.
4) Prepare solution of phenol in carbon tetrachloride by diluting the phenol stock solution (see No. 3). Record the spectrum as before, using (a) cyclohexane, and (b) carbon tetrachloride as the reference solution. Label spectra ***D*** and ***E*** respectively.

EXERCISE

1) What is the concentration of each prepared solution?
2) Comment on the differences between the spectra ***A – E***.

B. Effects of Sample on Absorption Curves

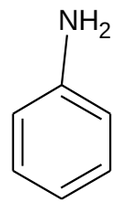
1) Record the absorption spectrum of the standard phenol solution (0.005% $^{w}/_{v}$) and the baseline absorbance. Label spectrum ***F***.
2) Prepare a dilute solution (0.5% $^{v}/_{v}$) of fresh milk (well shaken) in water by diluting 1 mL of milk to 200 mL. Record the absorption spectrum, using water as reference. Label spectrum ***G***.
3) Using the same milk solution, record the spectrum again, but use the secondary sample position (situated at the left-hand side of the cell compartment). Note the decrease in the background absorbance and label the spectrum ***H***.
4) Prepare a 0.005% $^{w}/_{v}$ solution of phenol in diluted (0.5% $^{w}/_{v}$) milk by pipetting 1m of the ethanolic stock (0.5% $^{w}/_{v}$) solution of phenol and diluting to 100 mL with the 0.5% v/v milk solution. Record the spectrum of this solution in the secondary sample position using water as reference (Spectrum ***I***). Then the 0.5% $^{v}/_{v}$ milk solution as reference (Spectrum ***J***).

EXERCISE

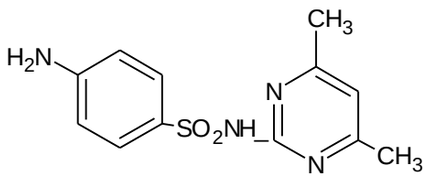
By means of comparison, discuss the five spectra obtained.

C. Effects of pH on Absorption Spectra

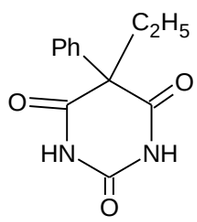
1) Using the compounds listed in Table 2 at the recommended concentration in 0.1N HCl and 0.1N NaOH, record both absorption spectra of each compounds on the chart. In addition to the measurement of λ_{max}, A_{max}. Note also the wavelength of the isosbestic point.



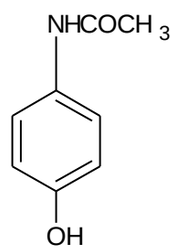
ANILINE



SULPHADIMIDINE



PHENOBARBITONE



PARACETAMOL

Figure 1.3: Chemical structures of studied drugs

Table 1.3: Effects of pH on absorption spectra								
S/No.	Compound	Conc. % $^{w}/_{v}$	solvent	λ_{max}	A_{max}	$A^{1\%}_{1cm}$	E	Isosbestic point
1	Aniline	0.02	0.1N HCl					
			0.1N NaOH					
2	Sulphadimidine	0.02	0.1N HCl					
			0.1N NaOH					
3	Phenobarbitone	0.02	0.1N HCl					
			0.1N NaOH					
4	Paracetamol	0.02	0.1N HCl					
			0.1N NaOH					

SCHEDULE IV

1.4 QUALITATIVE ULTRAVIOLET-VISIBLE SPECTROPHOTOMETRY

INTRODUCTION

Ultraviolet-visible (UV/Vis) spectra are usually recorded with samples dissolved in a non-absorbing solvent such as ethanol and sometimes a pure gas is used. The extent of absorption of ultraviolet light is directly proportional to the amount of sample through which the radiation passes. The quantitative relation is expressed by the Beer-Lambert equation:

$$A = ECL$$

Where, A = Absorbance

E = Molar absorptivity, a constant characteristic of the particular compound at a particular wavelength (λ).

C = Sample concentration in mole/L

L = Length of the sample path in cm

Absorption peaks are typically broad. They are usually recorded with wavelength increasing (energy decreasing) from left to right. The wavelength of maximum absorbance is reported as the λ_{max}, and the calculated molar absorptivity and solvent are usually indicated.

OBJECTIVE: To identify of UV spectra of some compounds.

EXPERIMENT

The absorption spectra of the various solutions should be recorded over 400 – 200 nm range using the compounds listed in Table 3 below with 1cm path silica cuvettes, using the appropriate solvent in the reference cuvette. Before recording any spectra, fill both cuvettes with the reference solvent, adjust the absorbance zero control if necessary, and record the baseline spectrum for reference purpose. For each solute, the wavelength of maximum absorption (λ_{max}) should be entered in the appropriate table, together with maximum absorbance (A_{max}), A (1%, 1cm) and E (molar absorption coefficient). For these calculations, it is assumed that, all the solutes obey the Beer-Lambert's law at the relevant concentration.

Since the path length is 1cm in this experiment, the calculation of A (1%, 1cm) and E-value is as follows

$$A\ (1\%, 1cm) = \frac{Amax}{C \times L}$$

$$E = 0.1\ [A\ (1\%, 1cm) \times \text{Molecular weight}]$$

Table 1.4: Absorption Data of some Compounds

S/No.	Compound	Molecular weight	Conc. % $^{w}/_{v}$	solvent	Observed		Calculated	
					A (1%, 1cm)	E	A (1%, 1cm)	E
1	Chloroquine	515..9	0.0015	0.01N HCl				
2	Folic Acid	441.4	0.001	0.01NNaOH				
3	Paracetamol	151.2	0.001	0.01NNaOH				
4	Pyrimethamine	248.7	0.0015	0.005N HCl				
5	Sulphadimidine	278.3	0.001	0.1N NaOH				

SCHEDULE V

1.5 COLORIMETRY DETERMINATION OF COPPER SULPHATE

INTRODUCTION

Colorimetric refers to the determination of a substance from its ability to absorb visible light. Visual colorimetric methods are based on the comparison of a coloured solution of unknown concentration with one or more coloured solution of known concentration. In spectrophotometric methods, the ratio of the intensities of the incident and the transmitted beam of light is measured at a specific wavelength by means of a detector such as a photocell.

OBJECTIVE: To determine the concentration of copper sulphate.

EXPERIMENT

Validation of Beer-Lambert Law by Colorimetric Determination of $CuSO_4$

Prepare solution of copper sulphate containing 2, 5, 7.5 and 10% $^{w}/_{v}$ $CuSO_4.5H_2O$ by dilution of the stock solution provided.

Determine the absorbance of each solution including the unknown solution provided in accordance with specification in Table 1.5A and tabulate your results by completing Table 1.5B.

Table 1.5A: Specifications for Absorptiometric Determination of $CuSO_4.5H_2O$

S/No	Maximum Transmission (nm)	Oxford Filter No.	Colour
i	470	602	Blue
ii	550	605	Yellow-green
iii	680	608	Red

Table 1.5B: Absorbance values of $CuSO_4.5H_2O$ solutions

Concentration (% $^{w}/_{v}$)	Absorbance (A) @		
	470 nm	550 nm	680 nm
2			
5			
7.5			
10			
Unknown			

EXERCISE

Plot graphs of absorbance (A) against concentration and from this graph, select the filter providing light most strongly absorbed by copper sulphate. Using the absorbance (A) at 470 nm, plot another graph of absorbance against concentration of the various solutions and determine the concentration of the unknown solution.

SCHEDULE VI

1.6 INFRA-RED ABSORPTION SPECTROPHOTOMETRY

INTRODUCTION

Infrared (IR) spectrophotometry is the branch of spectroscopy which is concerned with the measurement of absorption of electromagnetic radiation by molecules due to vibrational energy inherent in them. The vibrational energy in a molecule is within the infrared range 2.5 – 15 or 4000 to 670 cm^{-1} of the electromagnetic spectrum.

All organic compounds absorb light in this part of the spectrum. The functional group – frequency concept makes it possible to identify particular functional groups within a molecule, and makes IR spectrophotometry the simplest and often the most reliable method of assigning a compound to its class, thus contributing to the structural elucidation of organic molecules.

Interpretation of Spectra

1) Most functional groups have their characteristic absorptions (stretching frequencies) in the region 4000 – 1400 cm^{-1}. This region should be examined first when knowledge of the elemental constituents of the unknown compound has been established.
2) When classifying the possible functional groups according to group frequencies, it is important to keep in mind the physical state of the compound when the spectrum was obtained. Spectra of solids and liquids usually show a lowering in the group frequencies of polar groups due to hydrogen bonding. The lowering of group frequencies as a hydrogen bonding. The lowering of group frequencies as a result of molecular association (intermolecular bonding) is often accompanied by a broadening of the peak. Spectra obtained in dilute solution or in the vapour phase do not show these effects unless they result from intermolecular bonding.
3) Absorptions found in the region 1400 – 900 cm^{-1}(fingerprint region) are normally more difficult to interpret due to the complexity of group vibrations in this region. Many vibrational (bending and stretching) modes are possible in this region and assignments should be made with caution.

4) The region below 900 cm^{-1} is mainly useful for providing information regarding aromatic substituents patterns and for some stretching frequencies of carbon – halogen groups.

5) The following abbreviation are used for IR intensities: -

   s = strong; m = medium; w = weak; v = variable; v = stretching frequency; r, out- of-plane bending frequency; in-plane-bending frequency.

NOTE: The type of spectrometer used may have a marked effect on the IR spectrum. Instruments employing rock-salt prisms do not have the resolving power of those using diffraction gratings and consequently the IR spectra obtained in such instruments often have fewer peaks than those obtained in the latter type. For instance, a compound of the type $CH_3(CH_2)_nCH_3$ will show only two CH stretching bonds at 2944 and 2865 cm^{-1} when examined in a spectrometer equipped with rock-salt optics. However, a grating spectrometer will show four bands at 2962, 2926, 2872 and 2853cm^{-1}, i.e. the symmetric and asymmetric methyl and methylene stretching modes.

Table 1.6: Useful group frequencies in IR spectra

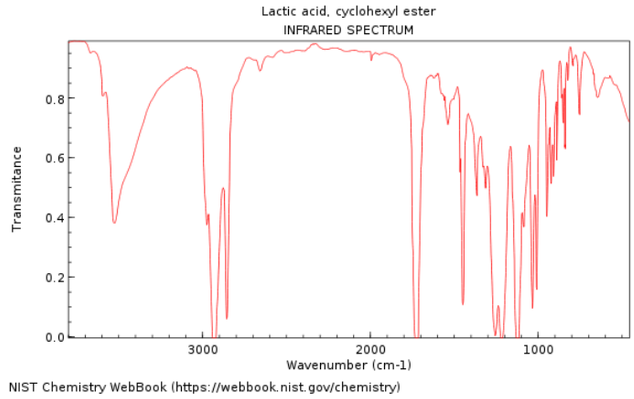
Functional group	V_{max} (cm^{-1})
O-H str	3650-3200 (depend on H-bonding)
N-H str	3500-3100 (depend on H-bonding)
N-H str	3200 and 3400 (pair of bands in amides)
=C-H str	3,300
-C-H str	3050-3010 (olefinic and aromatic; weak)
-C-H str	3000-2880
O=C-H str	2900-2700 (usually two bands, weak)
-C=N str	2250-2215
-C=N str	2260-2150
C=O str in:	
5-Ring ketones	1760-1740
6-Ring ketones and Aliphatic ketones	1720-1705
α, β-Unsaturated ketones	1690-1665
Aryl ketones	1700-1680
R-CHO	1740-1720
Ar-CHO	1715-1695
Esters	1750-1730
Unsaturated esters, Lactones and aryl esters	1730-1715
Vinyl ester	1800-1760
R, COOH	1725-1680 and 3000-2500
ArCOOH	1700-1680 and 3000-2500
RCOX	1815-1790
Ar-COX and α, β-Unsaturated	1790-1750
C=C	1680-1620
C=C (Conjugated)	(near 1600, aromatic C=C) (near 1580, present when ring is conjugated)
C=H def. (CH_3, CH_2)	1490-1430
C-H def. (CH_3)	1410-1350
C-O	1300 – 1100, (often strong)
NO_2	1500-1570, 13000 – 1370

Source: From principle of drug quality assurance and pharmaceutical analysis by A.A.

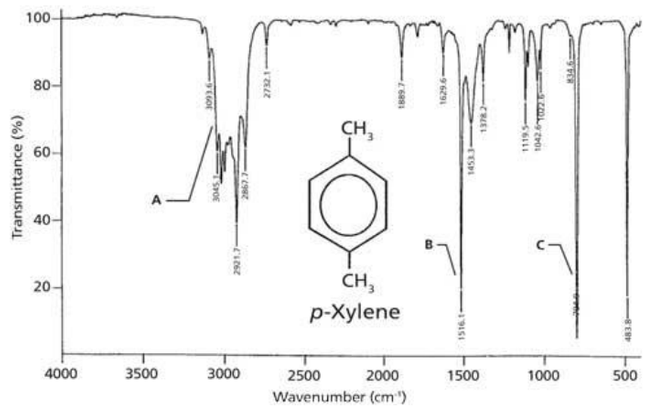
OBJECTIVE: To assess the IR spectra of some compounds.

EXERCISE

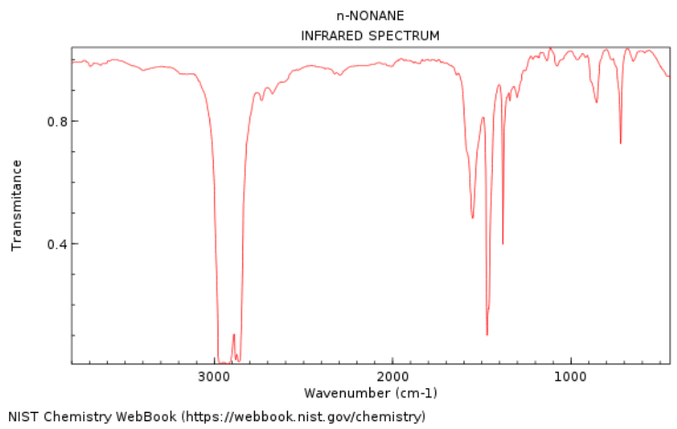
1) Identify the IR spectra a) – d) below:
2) Which groups are indicated by the peak(s) in the functional group region of the spectra?



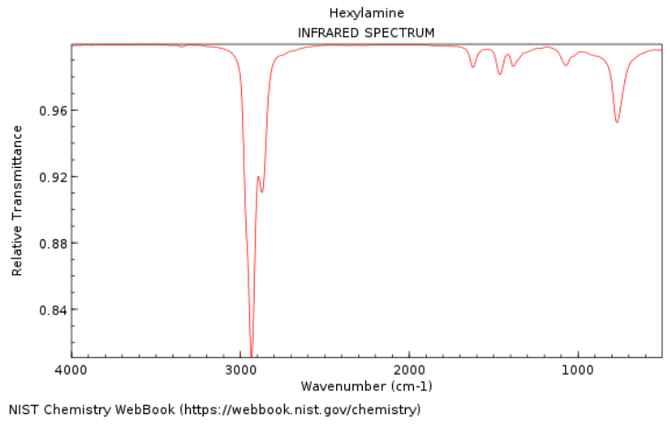
a) IR Spectrum of lactic acid



b) IR spectrum of disubstituted benzene



c) IR spectrum of nonane (C_9H_{20})



d) IR spectrum of hexylamine

Figure 1.6: IR spectra of selected compounds

SCHEDULE VII

1.7 NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY

INTRODUCTION

NMR is concerned with the magnetic properties of certain atomic nuclei, especially the nucleus of the hydrogen atom and that of the carbon – 13 isotope (13nmr). The phenomenon concerns atomic nuclei rather than electrons. The principle is based on the fact that when NMR active nuclear species are placed in a magnetic field, they distribute themselves in a number of quantized orientations corresponding to different energy levels, with respect to the field. It is the most powerful technique for structure determination of organic molecules. It explores the molecules at the level of the individual atoms. The scale of NMR is Tau(τ). It will basically give information about the type of the nuclei and their environment. Nuclei having even mass number with even atomic number (e.g. ^{16}O, ^{12}C etc.) are not active in NMR spectroscopy.

Effective field strength of each proton depends on

1) Environment of the proton
2) Electron density of the protons
3) Presence of other nearby protons.

The reference compound used when running NMR is called tetramethylsilane (TMS). It gives only one signal which comes at 101 indicating that the machine is ready.

The NMR absorption arising from shielding and deshielding are called chemical shifts. Changes in absorption will occur due to chances in subsistent attach to the atom. Attachment of electron withdrawing group e.g. Cl to a subsistent (CH_3Cl) causes a deshielding effect due to the electronegative nature of Cl atom.

Table 1.7: NMR Frequency Range

Functional Groups	τ PPM
Aromatic – OH	-0 to -2
-COOH	-2 to 0.5
-CHO	0 to 1
Aromatic ring	1.5 to 4.0
$CONH_2$	4 to 5.5
> C = C <	4.1 to 5.4
-OH	4.3 to 9.0
- C ≡ H	5.5 to 7.0
$=CH_2$	6.6
$-CH_3$	9

NOTE: Order of electronegativity –

I	<	Br	<	Cl	<	F	in CH_3X
7.83		7.35		6.90		5.20	ppm

C	<	N	<	O
9.12		7.88		6.70 ppm

OBJECTIVE: To elucidate the structure of organic molecules and to differentiate closely related compounds using NMR spectroscopy.

EXPERIMENT

There are two types of NMR spectrometers. The continuous-wave (CW) instrument and the pulsed or Fourier-Transform (FT) instruments. Most modern spectrometers are FT instruments.

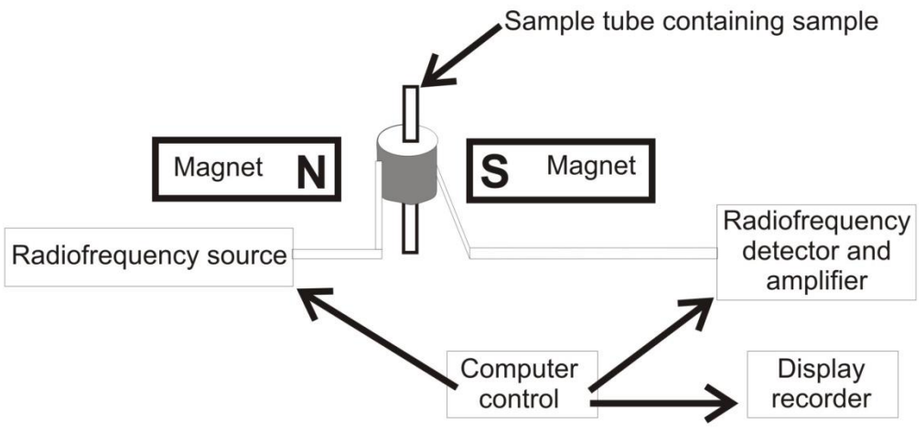


Figure 1.7: Block Diagram of NMR

The following are the basic components of a typical NMR spectrometer:

1) The *magnet* is either a permanent one or an electromagnet. Superconducting electromagnets must operate at liquid helium temperatures for high field FT spectrometers to provide a strong, stable and homogeneous magnetic field.
2) A *radio-frequency* transmitter supplies the radio-frequency signal necessary to elevate nuclei from ground to an excited state.
3) A *radio-frequency* receiver is placed around a sample to detect the energy absorbed by the sample.
4) A *sweep generator* is used to vary continuously the applied magnetic field over a relatively narrow range for a fixed frequency (field sweep – most common and preferred to frequency sweep, i.e. constant field while frequency is varied).

5) A *sample probe* holds the sample in a fixed position in the magnetic field, contains an air turbine to spin the sample (in order to increase field, homogeneity in the sample) and houses the coils that permit excitation and detection of the NMR signal. Also, it ordinarily contains two other transmitter coils – one for locking and the other for decoupling experiments. The sample holder may have a temperature-control accessory. In order to obtain high stability, the field is 'locked' to the frequency (field-frequency lock) using electronic feedback devices. In FT instruments the deuterium atom of the deuterated solvent usually provides the lock signal.
6) The *integrator* may be electronic or digital and, when connected to the spectrometer output stage on a CW instrument, determines the relative areas of resonance peaks and present these areas as a spectrum.

EXERCISE

A compound $C_3H_2O_2$ shows 2 NMR signals:

A at γ 0.3 ppm

B at γ 6.85 ppm

IR bands were at - 1707

- 2130

- 2300

- 3500

UV (λ_{max}) 253nm

Deduce the probable structure using the UV, IR and NMR Data

SHEDULE VIII

1.8 APPLICATION OF MASS SPECTROMETRY

INTRODUCTION

Mass spectrometry provides information about the qualitative and quantitative composition of both inorganic and organic analyses in a wide variety of complex molecular species. It also gives isotopic ratios of atoms in samples. The signals produced by a mass spectrometer are the direct result of chemical reactions (ionization and fragmentation).

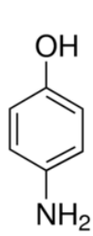
PRINCIPAL TYPE OF IONS IN MASS SPECTRUM

1) **Molecular ions:** Are ions formed by the loss of one electron from part of the molecule resulting in the formation of molecular ion peak or parent peak. The normal mass of such ion corresponds to the molecular weight.
2) **Isotopic ions:** Are other peaks of higher mass which normally accompany the parent peak and are caused by the ions which contain the heavier isotopes e.g. CH_3I has M ion at m/e = 142 derived from the highest isotopes of the constituents elements ^{12}C $^{1}H_3$ ^{127}I and there is also a peak at m/e 143 due to ^{13}C $^{1}H_3$ ^{127}I.
3) **Fragment ions:** are ions formed by the fragmentation of the molecular ion in the ion source. This can be by δ bond rupture near functional group or elimination by multiple δ bond rupture.
4) **Rearrangement ions:** Are ions formed by the rupture of a bond in the molecule. A common example is McLafferty rearrangement, a mechanism confined to compounds such as aldehydes, ketones, acids and esters.
5) **Metastable ions:** Are ions responsible for the formation of broad peaks called "Metastable peak" in a mass spectrum.
6) **Other types of ions:** in mass spectrum include the negative ions and ion by ion molecule interactions.

EXERCISE

State the possible fragmentation pattern of the following compounds:

a) $CH_3CHOHCH_3$

b) 

SCHEDULE IX

1.9 DETERMINATION OF SPECIFIC OPTICAL ROTATION OF ARABINOSE/SUCROSE

INTRODUCTION

POLARIMETRY**:** is the quantitative study of the angle of plane polarized light by various transparent substances. Optical activity is the term used to describe the ability of certain substances to rotate the plane of polarized light.

Many drugs are optically active as such they can exist in two forms possessing equal but opposite rotation and more often the biological activity resides with one of the optical isomers. However, the pharmacopoeia purpose for the measurement of optical activity is mainly to establish the identity of the substances.

It is important to note that measurement of optical rotation is presented as the angle of rotation of plane polarized light which depends on the thickness of the layer traversed by the light; wavelength of the light used and the temperature at which the measurement was taken. Hence, sodium light of wavelength 589.3 nm at the temperature of 20^{o}C is used.

DEFINITIONS

Optical rotation: of a pure liquid is the angle through which the plane of polarization is rotated by a layer of substance one decimeter in thickness.

Specific optical rotation: of a solution $[\alpha]_D^t$ in the optical rotation of a layer 1 decimeter thick containing 1 g of material per mL of liquid.

$$[\alpha]_D^t = \frac{100 \times \alpha}{L \times C}$$

Where **α** is the observed rotation

L is the length of the polarimeter tube

C is the concentration g/100 mL.

Molecular optical rotation: of a compound $[\theta]_D^t$ is the optical rotation of a one decimeter thick layer of a solution of the compound of concentration on mole per 100 mL

$$[\theta]_D^t = \frac{\alpha M}{LC}$$

where **M** is the molecular weight.

POLARIMETER

In a polarimeter, the source of light is a sodium vapour lamp (Fig. 4). The produced light is polarized by the first (fixed) Nicol prism (Polarizer). The central tube contains the solution of the sample under examination. The optical rotation is determined by rotating the second (movable) Nicol prism (Analyzer). Movement is measured on a calibrated circular scale. To facilitate the accurate determination of the balance point where the Polarizer and Analyzer are crossed, a third prism is inserted to cover half the view.

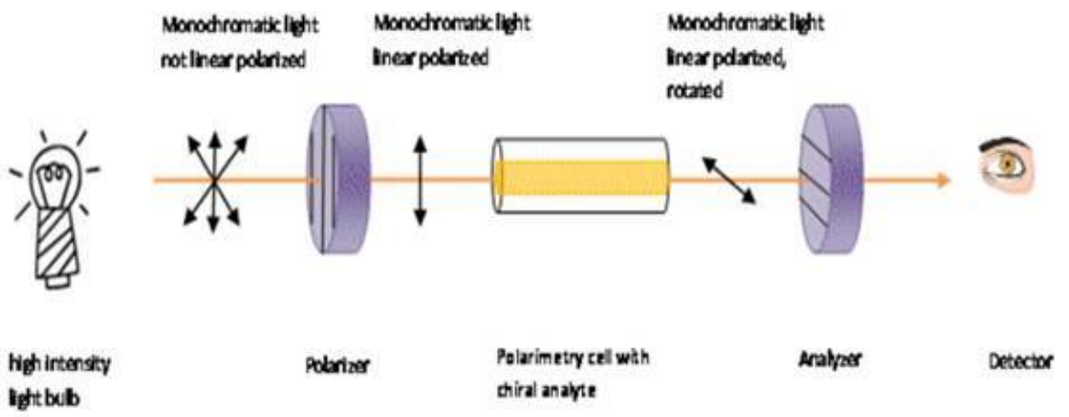


Figure 1.9: Schematic diagram of a polarimeter

OBJECTIVE: To determine the optical rotation of a compound.

EXPERIMENT

1) Determination of the specific optical rotation of Arabinose: Weigh out exactly 5, 10, and 15 g of Arabinose, dissolve in warm distilled water and dilute to 100 mL using a graduated flask. Check the polarimeter to see that the field is evenly illuminated and that the telescope is focused on the dividing line of the field of view. Clean the polarimeter tube and holding the capped end downwards; fill the tube with distilled water, making sure no bubbles are trapped. Slide the glass end plate carefully over the meniscus, then replace the end cap washer and screw up firmly. Rotate the analyzer until the two areas of the field are illuminated with equal intensity, and read both venires. Take three readings of the balance point approaching from one side, and three approaching from the other. Calculate the mean reading i.e. one mean of approximately 0^o, and one of approximately 180^o and repeat this procedure with each of three solutions of Arabinose in distilled water. Calculate $[\alpha]_D$ for each solution. Plot a graph $[\alpha]_D$ against concentration. Note and comment on the shape of the graph.

2) Determination of specific optical rotation of sucrose: Weigh out accurately 10 g dissolve in distilled water and dilute to 100 mL in a volumetric flask. Warm-slightly. Fill the polarimeter tube with distilled water and take the zero reading as above. Empty and refill the tube with the prepared sucrose solution and measure its optical rotation. Calculate the specific and molecular rotation of the sample of sucrose prepared and comment on your results. You are also supplied with sample of sucrose of unknown concentration. Measure its optical rotation and calculate the % $^{w}/_{v}$ of sucrose from the value you obtain.

SCHEDULE X

1.10 POTENTIOMETRY

INTRODUCTION

A value characteristic of an aqueous solution is its pH values, which represents conventionally its acidity or alkalinity. pH is defined by $pH = - \log_{10} [H^+]$. For a 0.1 M HCl [H] = 10 M hence pH = 1.

The potential is usually measured at zero or practically zero current.

APPARATUS

The apparatus used, (a simple potentiometer or electronic device) comprises of a voltmeter allowing readings to the nearest millivolt. The choice of indicator electrode depends on the substance being examined and may be a glass or metal electrode (for example platinum, gold, silver or mercury). The reference electrode is generally Calomel or Silver – silver chloride electrode. For indictor electrode Glass membrane electrode is commonly used.

POTENTIOMETRIC TITRATION

Plot a graph of the variation of potential difference as a function of the titrant added, continuing the addition of the titrant beyond the presumed equivalence point. The end point corresponds to a sharp variation of potential difference.

EXERCISE

Determination of pH of some mixtures using potentiometer or any other pH meter. You are expected to determine the pH of some mixture using a glass electrode, a pH meter after first standardizing the pH meter.

METHOD

1) Operate the pH meter and the electrode system according to the manufacturer's instruction.

2) Prepare the mixtures by mixing X mL of dihydrogen phosphate (0.2 M) with Y mL of citric acid (0.1 M) according to the Table 1.10 below.

X mL of Na_2HPO_4	Y mL of citric acid	Measure pH of the mixture
0.4	19.6	
7.1	12.9	
10.3	9.7	
11.6	8.4	
19.45	10.55	

3) Calibrate the apparatus using 0.05 M potassium hydrogen phthalate buffer on a primary standard (pH = 4.0) when measuring acid solutions or 0.05 M sodium borate (pH = 9.2) when measuring alkaline solutions.

4) Immerse the electrode in the solution being examined and measure the pH at the same temperature on for the standard solution at the end of the set measurement, record the pH of the solution used to standardize the meter and the electrode. If the difference between this reading and the original value is greater than 0.05 the set of measurements must be repeated.

SCHEDULE XI

1.11 POLAROGRAPHY

INTRODUCTION

Palarography is an electrochemical method of analysis. It involves the passage of gradually changing voltage into a cell containing the solution of interest. The corresponding current is measured and the result plotted graphically (either manually or automatically). The current – voltage curve (polarogram) can be used for the qualitative and quantitative analysis of electro–reducible and electro–oxidizable substances or ion.

Normally, a polarographic cell comprises of a stable reference electrode and indicator electrode often made up of dropping mercury electrode (DME) and the voltage is usually increased linearly with time.

Polarographic method of analysis is applicable to most metals and many organic compounds. Polarography is adaptable to volumes as small as 0.05 mL and can determine concentration within the range of 10^{-2} to 10^{-6} M and above.

APPARATUS

Polarograph basically consist of a potentiometer connected to two electrodes via a micrometer. The anode is normally mercury pool while the cathode is made up of dropping mercury electrode (DME).

DME consist of a glass capillary attached to a mercury reservoir. Drops of mercury fall from the orifice of this capillary at a constant rate, usually between 5 – 30 drops per minute. Each drop is the electrode while attached to the column of mercury in the capillary.

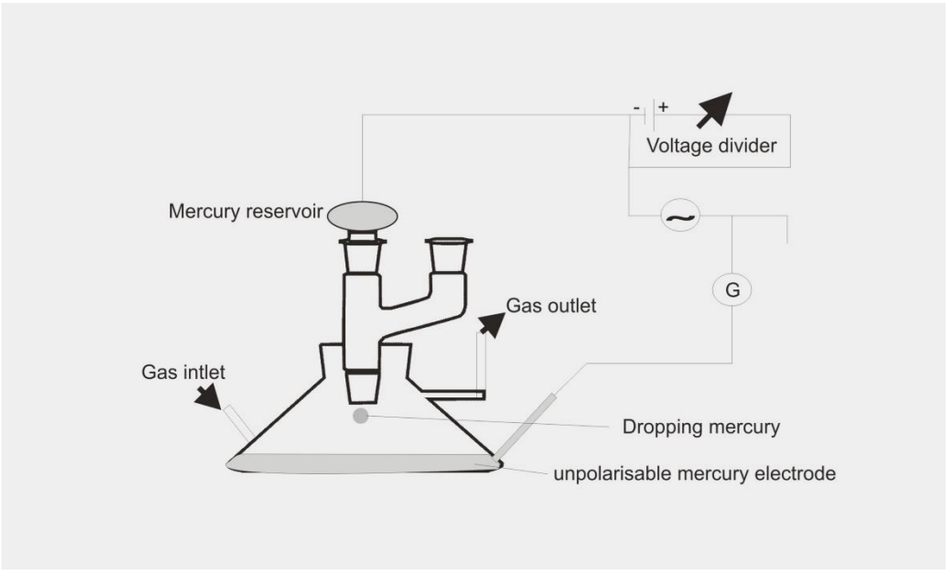


Figure 1.11A: A schematic diagram of a polarograph

The current versus voltage curve obtained with DME is very reproducible, since the surface of every new drops is fresh and practically unaffected by electrolysis at earlier drops. Supporting electrolytes are usually in Polarography to decrease resistance of the solution and also to ensure that the electro active species moved by diffusion and not by electrical migration in the voltage field across the cell. The supporting electrolytes commonly used are buffers e.g. LiOH, HCl, H_2SO_4, citrates, tartarates, tetra alkyl ammonium ions etc.

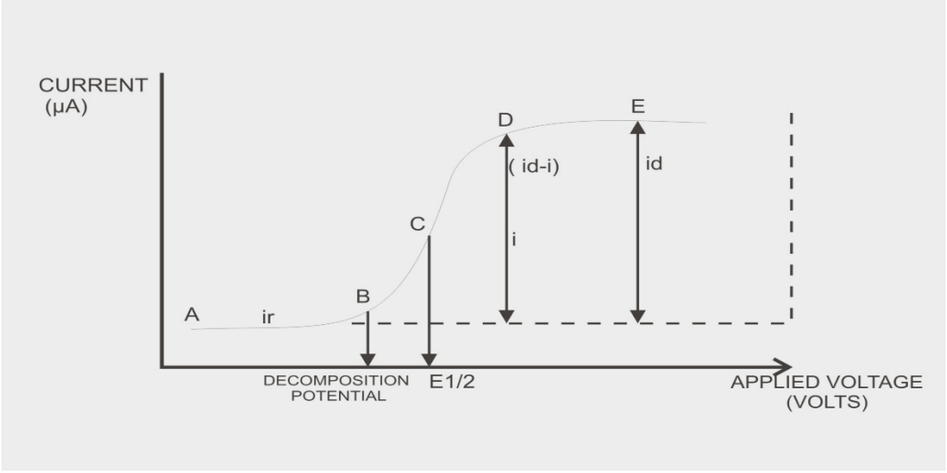


Figure 1.11B: Typical polarogram

A typical curve is shown in Figure 6 where $\mathbf{i_r}$ is the residual current, i.e. the current at any point on the wave measured with respect to the line AB produced or with respect to the residual current curve and $\mathbf{i_d}$ the diffusion current. The $E_{½}$ (half wave potential), which produced the current (at **c**) midway along the steeply rising portion of the curve, and enables qualitative analysis to be performed.

GENERAL EXPERIMENTAL METHOD

Adjust the reservoir light so as to give a drop time of about 3 to 4 seconds with the mercury tip immersed in water. Deoxygenate the test solution by passing hydrogen or nitrogen through it for 20 – 40 minutes. Rinse the electrode tip in the solution, and immerse the electrode tip in the solution. For a mercury pool anode, add 1 to 2 mL of mercury and bubble gas through the solution for a few minutes longer.

Connect the electrodes to the polarograph and adjust the galvanometer to the minimum sensitivity. Unless the correct setting of the shunt is known, the correct sensitivity is usually found by increasing the polarizing voltage to about – 1.8volts and adjusting the sensitivity control to give a large but less than full scale deflection. Plot the polarogram, either manually or automatically by the following procedure:

1) **Manual operation:** Set the potentiometer at zero and observe the meter reading. If the meter reading is negative, increase the applied potential in 0.1volt increments until a positive value is obtained. Record further average current values for gradually increasing 0.1volt increments of the applied voltage until the current changes start to increase. Now reduce the voltage increment to about 0.02volts until the current changes are small again. Finally, continue with 0.1volt increment, until the polarizing potential is about -2.0volts, plot the polarogram graphically and read the diffusion current from the curve.
2) **Automatic operation:** After standardizing the potentiometer, set the potentiometer to the described initial value and the recorder pen to the zero time. Switch on the synchronous motors used for driving the potentiometer slide wire and record chart, and assuming the sensitivity setting in the correct, the polarogram is plotted automatically. Switch the motors off when the applied potential reaches about -2.0 volts or the current readings approach full–scale deflection.

Determination of Ascorbic acid (Vitamin C) in Oranges

Anodic waves corresponding to the oxidation of ascorbic acid can be used for both qualitative and quantitative determination of vitamin C in oranges. Squeeze about 10 mL of orange juice; deoxygenate the juice by passing nitrogen gas for about 20 minutes. Adjust the pH 4 – using acetate buffer.

Using the general method above, electrolyze the orange juice. Note the fluctuations in the current at each applied potential and record the mean value in each case. Plot the anodic wave and comment on the polarogram.

SCHEDULE XII

1.12 PRACTICAL ASPECTS OF DRUG METABOLISM

INTRODUCTION

Drug metabolism is a process by which a drug taken into the body undergoes a series of biochemical reactions during its passage to the active site and it is further converted from the active site to soluble metabolites which are easily eliminated from the body. The metabolic or biochemical reactions are classified into two phases namely: phase I and phase II reactions.

Phase I reaction (biotransformation) involves oxidation, reduction and hydrolysis. These reactions convert the foreign compound to more polar metabolites by introduction of polar groups such as -OH, =NH, $-NH_2$ and -SH which then expose the metabolites to further reactions known as conjugation or phase II reaction. Phase II reactions involve the condensation of the xenobiotics with endogenous polar substances, such as glucuronic acid, sulphate radicals or amino acid to yield conjugated product and water.

OBJECTIVE: To identify the pathways by which drugs are transformed in the body and the importance of each pathway and intermediate.

EXPERIMENTS

Isolation and identification of metabolic products of aspirin following oral administration in man.

A. Paper Chromatography

Materials

a) Chromatographic tank

b) Whatman paper No. 1

c) Small capillary tube

Solvent system: Strong ammonia solution, n-butanol, water (33:100:66).

The top layer of this mixture is placed in the solvent trough and the lower layer at the bottom of the tank.

Location of spots: Freshly prepared ferric chloride solution and UV light at 254 nm.

B. Thin Layer Chromatography

Glass plate 20 x 20 cm coated with slurry of 30 g of silica gel in 60 mL of water to give a layer of 0.25 cm thick and dried at 110^{o}C for 1 hour.

Chromatographic tank: 21 x 21 x 10 cm.

Solvent System

Glacial acetic acid: Benzene: Ether: Methanol (18:120:60:1)

Location of spots:

i. UV light (254nm)

ii. Ferric chloride spray

METHODS

1) INVIVO TRIAL

a) No drug should be taken for at least 48 hours prior to the experiment.

b) On the day before the experiment, collect urine samples over a period of 6 hours, pool the urine sample and keep about 50 mL which will be used as blank.

c) After overnight fasting, take immediately that amount Aspirin preparation (2 tablets) with about 200 mL of water. You can ingest the tablet either before or after you breakfast.

d) Drink sufficient water about 100 mL, hourly or at convenient time to facilitate urination.

e) Collect all the urine voided by day, collect and tables your urine sample or follows

Sample A, 1 – 6 hours

Sample B, 6 – 12 hours

Sample C, 12 – 24 hours

Sample D, 24 – 36 hours

Store your sample in a refrigerator.

2) EXTRACTION

a) Using a clean measuring cylinder, measure about 50 mL of the blank urine sample (US) and transfer into a clear separating funnel.

b) Add approximately 2 mL of 1 M HCl to acidify the blank urine sample to about pH = 2.

c) Extract the blank urine with 3 portions each of 10 mL diethyl ether shaking gently for about similar.

   Note: Vigorous shaking should be avoided since this will result in the formation of an emulsion.

d) Allow to stand for about 2 minutes.

e) Carefully transfer the ether layer (top layer) into a 50 mL clean Erlenmeyer flask, appropriately labeled (BU). Repeat the same operation with the remaining 2 x 10 mL diethyl ether.

f) Repeat the above procedure with your 6 hour, 12 hour, 24 hour, and 36 hour urine sample. Label your sample appropriately Us6, FUs12, Us24 and Us36.

g) Evaporate each of the combined ether extract to dryness under water bath at 25^0 – 30^oC.

h) Add about 1 mL chloroform to the residue using small micropipettes, spot your sample on the paper chromatograph and TLC plate also spot your reference sample accordingly

i) Allow to dry for about 2 minutes.

j) Place your chromatographs and TLC plates into the developing chamber. Allow the solvent front to advance to the 15 cm marks.

k) Remove the chromatogram, dry it and locate spot using location method provided.

EXERCISE

1) Why is it important to study drug metabolism?
2) Predict the pathway of metabolism of the drug provided.

PART 2

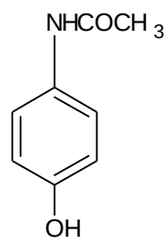
PCH402 SCHEDULES

SCHEDULE I

2.1 SPECTROPHOTOMETRIC DETERMINATION OF PARACETAMOL TABLETS

INTRODUCTION

Identification and determination of active ingredients are elements of quality control of pharmaceuticals.



PARACETAMOL

Figure 2.1: Chemical structure of paracetamol

IDENTIFICATION TESTS

1) STANDARD PARACETAMOL POWDER

Physical test

a) Comment on the appearance paracetamol powder.

b) Determine the melting point.

Chemical test

a) Dissolve 0.1g in 10 mL of water and add 1 drop of ferric chloride. Note the colour produced.

b) Boil 0.10 g of paracetamol powder in 1.0 mL of hydrochloric acid for 3 minutes, add 1 ml of water and cool, no precipitate is formed. Add 0.05 mL of 0.0167 M potassium dichromate and comment on what you observed for the next 3 minutes.

2) PARACETAMOL TABLET.

P**hysical test**

a) Comment on the appearance of the paracetamol tablet.

b) Extract the quantity of the powdered tablet equivalent to 0.5 g of paracetamol with 20 mL of acetone, filter and evaporate the filtrate to dryness. Take the melting point of the residue.

Chemical test

Boil 0.1g of the residue obtained in the test (a) with 1 mL of hydrochloric acid for 3 minutes; add 10 mL of water and cool, no precipitate is produced. Add 0.05 mL of 0.0167 M potassium dichromate. Comment on the colour which slowly develops.

ASSAY (BP 1999)

a) **Paracetamol standard powder.**

Take 0.15 g of the powder in 50 mL of 0.1M sodium hydroxide, dilute with 100 mL of distilled water, shake for 15 minutes, and add sufficient water to produce 200 mL. Mix, filter and dilute 10 mL of the filtrate to 100 mL with water. Add 10 mL of the resulting solution to 10 mL of 0.1 M sodium hydroxide, dilute to 100 mL with water and measure the absorbance of the resulting solutions to get the maximum absorption.

b) **Paracetamol tablets**

Weigh and powder 20 tablets, add a quantity of the powder equivalent to 0.15 g of the paracetamol to 50 mL of 0.1 M sodium hydroxide, dilute with 100 mL of water, shake for 15 minutes and add sufficient water to produce 200 mLs, mix, filter and dilute 10 mL of this filtrate to 100 mL with water. Add 10 mL of the resulting solution to 10 mL of 0.1 M sodium hydroxide dilute to 100 mL with water and measure the absorbance of the resulting solution to get the maximum absorption wavelength ($\leq$ or $\geq$257 nm).

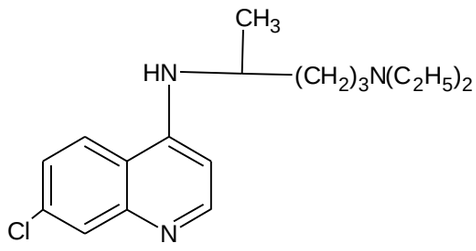
EXERCISE

Calculate the content of $C_8H_9NO_2$ in the brand of paracetamol tablet and in the standard powder taking 715 as the value of A (1%, 1cm) at the maximum wavelength determined.

Give the structure of paracetamol and predict its metabolic route

SCHEDULE II

2.2 IDENTIFICATION AND ASSAY OF CHLOROQUINE TABLETS



CHIOROQUINE

Figure 2.2: Chemical structure of chloroquine

NOTE

250 mg chloroquine phosphate ≡ 155 mg chloroquine base

200 mg chloroquine sulphate ≡ 146 mg chloroquine base

IDENTIFICATION TEST

a) Physical Test.

Examine and comment on the appearance of the tablet.

b) Chemical test.

Phosphate test: Extract a quantity of the powdered tablet containing 0.5g of chloroquine phosphate with 25 mL of water and filter. To the filtrate add 2.5 mL of 5M Sodium hydroxide and extract with three 10 mL quantities of ether.

Neutralize the aqueous layer with 2 mL nitric acid, and add 5 mL of a 4.25% solution silver nitrate. A yellow precipitate is produced, the colour of which is not changed on boiling and which is soluble in 10 M ammonia.

Sulphate test: Shake the quantity of powdered tablet containing 0.1 g of chloroquine sulphate with 10 mL of water and 1 mL of 2 M HCl and filter. To this filtrate add 2 mL of barium chloride solution, a white precipitate is produced.

ASSAY OF CHLOROQUINE PHOSPHATE TABLET

Method

Weigh and powder twenty (20) tablets. Weigh accurately a quantity of powder equivalent to 0.5 g of chloroquine phosphate. Dissolve in 20 mL of 1 M sodium hydroxide and extract with four 25 mL quantities of chloroform. Combine the chloroform extracts and then

evaporate to a volume of about 10 mL add 40 mL of anhydrous glacial acetic acid and carryout non-aqueous titration.

Calculation

$C_{18}H_{26}ClN_{3.}\ 2H_3PO_4 \equiv 2HClO_4 \equiv 2H$

515.9 g $C_{18}H_{26}ClN_{3.}\ 2H_3PO_4 \equiv 2000$ mL of $HClO_4$

0.02580 g $C_{18}H_{26}ClN_{3.}\ 2H_3PO_4 \equiv 1$ mL 0.1M $HClO_{4.}$

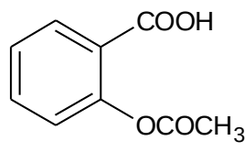
For chloroquine sulphate.

Each mL of 0.1 M perchloric acid is equivalent to 20.90 mg of $C_{18}H_{26}ClN_3H_2SO_4$

1) Calculate the percentage content of the chloroquine sample.
2) Mention the method of analysis employed.

SCHEDULE III

2.3 IDENTIFICATION AND ASSAY OF ASPIRIN TABLETS BP 1999



ASPIRIN

Figure 2.3: Chemical structure of Aspirin

IDENTIFICATION TEST

a) **Physical tes**t: Examine the two brands of aspirin tablets provided and comment on the appearance.

b) Chemical test

i. Disperse the quantity of the powdered tablet containing 50 mg of aspirin in 10 mL of warm water, boil and add 0.5 mL of iron III chloride solution. Record your observation.

ii. Boil 0.5 g of this powdered tablets for 2 to 3 minutes with 10 mL of 5 M NaOH, cool and add excess of 1 M sulphuric acids, record your observation. To a solution of precipitate in water add iron III chloride solution and record your observation.

LIMIT TEST FOR SALICYLIC ACID

Shake the quantity of a powdered tablet containing 0.20 g of aspirin with 4 mL of ethanol (96%) and dilute to 100 mL with water at a temperature not exceeding 10^{o}C filter immediately, transfer some of the filtrate to a Nessler cylinders, add 1 mL of freshly prepared ammonium iron III sulphate [$FeNH_4(SO_4)_2$] solution, mix and allow to stand for 1 minute. Any violet colour produced is not more intense than that obtained by adding 1 mL of freshly prepared ammonium iron III sulphate solution to a mixture of 3 mL of a freshly prepared 0.01% $^{w}/_{v}$ of salicylic acid.2 mL of ethanol 96% and sufficient water to produce 50 mL containing in the second Nessler cylinder (0.3%).

ASSAY

Weigh and powder 20 aspirin tablets. To a quantity of the powder containing 0.5 g of aspirin add 30 mL of 0.5M sodium hydroxide. Boil gently for 10 minutes and titrate the excess alkali with 0.5 M hydrochloric acid using phenol red as indicator. Repeat the operation without the

substance being examined. The difference between the titration represent the amount of sodium hydroxide required.

Each mL of 0.5 M sodium hydroxide is equivalent to 45.04 mg of $C_9H_8O_4$.

EXERCISE

1) Calculate the percentage content of aspirin.
2) Give the structure of aspirin and predict its metabolic route.

SCHEDULE IV

2.4 DISSOLUTION RATE TEST

INTRODUCTION

Dissolution rate test is the process by which a drug becomes dissolved in a solvent. It provides an estimate of the extent to which to which the drug substance is released from the dosage unit into a dissolution medium under standardized experimental condition.

OBJECTIVE: To determine the time course of the dissolution process and to study the influence of excipients or other processing variable on the dissolution rate.

EXPERIMENT

Method described in B.P. 1993 i.e. Rotating basket.

In Vitro Experimental (Dissolution Test)

The USP XIX basket method is described for the dissolution rate tests. The apparatus consists of cylindrical, flat bottom glass vessel with an internal diameter of 100 mm, a flanged upper rim, and a capacity of 1000 mL; a variable speed motor; a cylindrical stainless steel basket assembly made of woven wire cloth, with a nominal aperture size of 425 micrometer; joined down one side with a narrow welded seam and with a flanged rim at each end. The variable speed motor causes the basket to rotate in the vessel. A speed of 100 revolutions per minute is used. The motor drives the basket by means of a shaft which is approximately 5 mm in diameter and about 300 mm long in such a way that the basket revolves smoothly and without perceptible wobble.

The shaft passes through the vessel and opening in the lid of the vessel and is positioned so that its axis is not more than 2 mm from the vertical axis of the vessel. It carries at its lower end a stainless-steel disc of 25 mm in diameter in which there is a 2 mm vent.

The vessel is securely clamped in a water bath in such a way that displacement vibration from other equipment including water circulating device is minimized. The water bath is set to maintain the temperature of the dissolution medium in the glass vessel at 37 ± 0.5^{o}C. See the next figure for the assembled apparatus.

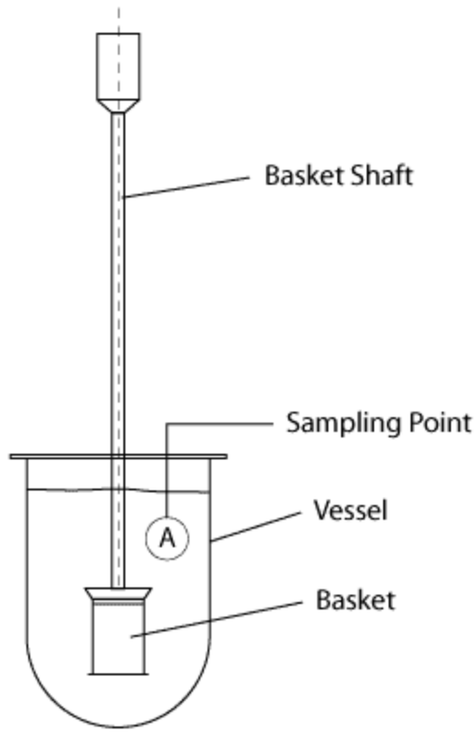


Figure 2.4: Schematic Diagram of the Rotating Basket Dissolution Apparatus

METHOD

One liter of 0.1M hydrochloric acid, as the dissolution medium, was introduced into the vessel and immersed in a constant temperature water bath, maintained at 37 ± 0.5^{o}C. For each test, one tablet was placed in the basket. When the required temperature was attained, the apparatus was assembled and the distance between the bottom of the basket and the interval surface of the vessel adjusted to between 18 and 22mm. The motor was started at a rotational speed of 100 revolutions per minute. A sample of 5mL of the dissolution medium was withdrawn at 45 minutes. Samples were withdrawn from a point half way between the basket wall and the wall of the vessel, level with the mid-point of the side of the basket, to ensure that the normal pattern of flow of the dissolution medium was not disturbed. Samples of the dissolved drug were filtered and analyzed for drug content. The procedure is repeated four times.

EXERCISE

1) What is the importance of dissolution test?
2) Give reasons for the use of 0.1 M HCl as the dissolution medium.
3) Comment on the dissociation of your sample in stomach and in the intestine.
4) Predict the metabolic route of your sample.

SCHEDULE V

2.5 QUALITY CONTROL OF ORAL LIQUIDS (PARACETAMOL SYRUP)

INTRODUCTION

Oral liquids usually consist of solutions or emulsions of one or more active ingredients in a suitable vehicle; they consist of liquid active ingredients and be intended to be swallowed either undiluted or after dilution. Oral liquids may be prepared before use from concentrated liquid preparations or from granules or powder. Suspension may show sediment that is readily dispersible on shaking. Emulsions may show evidence of phase separation but are easily reformed on shaking.

OBJECTIVE: To assess the quality of oral liquid formulations.

IDENTIFICATION TEST (BP 1993)

1) Carry out the method for thin layer chromatography, using silica gel GF_{252} as the coating substance and a mixture of 65 mL of chloroform, 25 mL of acetone, 10 mL of toluene and 0.5mL of glacial acetic acid as the mobile phase. Apply 10 µL each of the following solutions separately to the plate and develop to 15 cm.
    a) Dilute 1 mL of the paracetamol syrup to 10 mL with methanol, filter if necessary.
    b) 0.24% $^{w}/_{v}$ of paracetamol reference solution in methanol.

    After developing the plate, dry in a current of warm air, examine under ultraviolet light (254nm).

    Observation: The principal spot in the chromatogram obtained with solution (a) correspond to that in the chromatogram obtained with solution (b).
2) The ultraviolet absorption spectrum of paracetamol sample in 0.1 M sodium hydroxide solution exhibits maximum at the same wavelength as that of the reference standard.
3) The infra-red absorption of paracetamol will produce the same pattern as that of the reference standard.
4) Add one drop ferric chloride solution to 10 mL of 1%$^{v}/_{v}$ solutions of the sample. A violet – blue colour is produced.

QUANTITATIVE TEST

Preparation of Chromatographic Column

Pack a plug of fine glass wool in the base of a chromatographic tube (25 mm x 250 mm tube which is fused to a 5 cm length of 7 mm tubing) with the aid of a tamping rod about 1 mm less than that of the tube. To 2 g of purified siliceous earth in a 100 mL beaker add 2 mL of a solution containing 1.0 g of sodium bicarbonate and 4.5 g of sodium carbonate in each 100 mL, and mix until a fluffy mixture is obtained. Transfer the mixture to a chromatographic tube, and gently to compress the material to uniform mass.

Calibration Curve

Accurately weigh 50mg of paracetamol powder and dissolve in 100 mL distill water in 100 mL volumetric flask. Transfer 1, 2, 3, 4, 5 and 6.0 mL of the standard solutions to series of 100 mL volumetric flasks. Add sufficient solvent mixture of 1 mL 0.1 M hydrochloric acid per 100 mL of methanol to volume. Determine the absorbance of the preparations in 1 cm cells at the wavelength of maximum absorbance at about 249 nm. Use a solvent mixture consisting of 1 mL of 0.1 M hydrochloric acid per 100 mL of methanol as blank.

Assay

Transfer an accurately measured volume of paracetamol syrup, equivalent to 250 mg of paracetamol to a 250 mL volumetric flask, add 2 mL of 1 M sodium hydroxide dilute with water to volume, and mix. Transfer 2.0 mL of this solution to a 100 mL beaker, add 1 drop of hydrochloric acid, swirl to mix, then add 3.0 g of purified siliceous earth. Mix, and transfer to the chromatographic column. Scrub the beaker with 1 g of purified siliceous earth mixed with two drops of water, transfer the washing to the column, and tap gently. Place a plug of fine glass wool on top of the column. Wash the column with 100 mL of the water-saturated chloroform, and discard the eluate. Eluate the acetaminophen with 150 mL of water - saturated ether, collecting the eluate in a 400 mL beaker. Evaporate the ether on a steam bath with the aid of a current of air just to the dryness. (NOTE – Avoid prolonged drying, to prevent loss of acetaminophen). Dissolve the residue in 1 mL of dilute hydrochloric acid per 100 mL of methanol (1 in 100), and transfer to a 50-mL volumetric flask. Rinse the beaker with the solvent mixture, adding the rinsing to the flask, dilute with the solvent mixture to volume, and mix. Transfer 10 mL of this solution to a 100 mL volumetric flask, dilute with the solvent mixture to volume and mix.

METHOD

Determine the absorbencies of the standard preparation and assay preparation in 1-cm cells at the wavelength of maximum absorbance at about 249 nm. Use a solvent mixture consisting of 1 mL of 0.1M hydrochloric acid per 100 mL of methanol as the blank. Calculate the % content of paracetamol by the formula:

$$\frac{\text{Concentration of sample (mg) x dilution factor x 100}}{\text{250 mg}}$$

Paracetamol syrup contains not less than 95.0% and not more than 105.0% of the labeled amount of $C_8H_9NO_2$ (U.S.P. 1980).

EXERCISE

1) State the methods of analysis applied in the assay.
2) What is purified siliceous earth?

SHEDULE VI

2.6 ASSAY OF PARACETAMOL CAPSULES

INTRODUCTION

Capsules are solid preparations with hard or soft shells of various shapes and capacities, usually containing a single dose of active ingredient. They are intended for oral administration.

IDENTIFICATION TESTS

Carry out the identification test as described under paracetamol tablets.

ASSAY

Weigh the content of not fewer than 20 paracetamol capsules. Mix the contents, and transfer an accurately weighed portion of the powder containing 0.15 g of paracetamol to 50 mL of 0.1 M sodium hydroxide. Proceed as directed for paracetamol tablets.

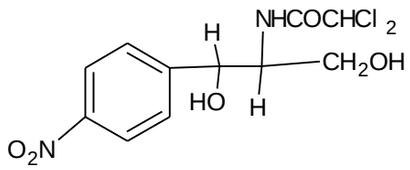
Acetaminophen capsules contain not less than 95% and not more than 105% of labeled amount of $C_8H_9NO_2$

EXERCISE

What advantage has the capsule over the tablet?

SCHEDULE VII

2.7 SPECTROPHOTOMETRIC DETERMINATION OF CHLORAMPHENICOL CAPSULES



CHLORAMPHENICOL (molecular weight = 323.1)

Figure 2.7: Chemical structure of chloramphenicol

IDENTIFICATION TEST

A. Physical test

Comment on the appearance of the brands of chloramphenicol capsules. Empty the content of the capsules on a clean dry glass and briefly describe the appearance of powder. Determine and comment on the melting point of the powder.

B. Chemical test

a) Paper chromatography

Prepare a standard solution of about 100g/mL of the test sample in ethanol. Using a clean micropipette spot the test sample on a chromatographic paper, allow to dry and develop in solvent system containing ethyl acetate: methanol: acetic acid (50: 30: 20) for about 5 hours. Remove the paper and allow it to dry. Examine the spot under the UV light at 254 nm and record the R_f value.

b) Colour test - Fujiwara's test

Dissolve 0.2 g of content of the capsule in 2 mL of pyridine. Add about 2 mL of 20% NaOH and heat the mixture on a water-bath for 3 – 5 minutes with vigorous periodic shaking. Comment on your observation. Carry a similar test using standard chloramphenicol samples provided.

ASSAY BP (1999)

Dissolve the quantity of the mixed content of 20 capsules containing 0.2 g of chloramphenicol in 800 mL of water, warming if necessary, to effect a solution and add sufficient water to produce 1000 mL. Dilute 10mL to 100 mL with water and measure the absorbance of the resulting solution at the maximum at 278 nm.

Calculate the content of $C_{11}H_{12}Cl_2N_2O_5$ taking 297 as the value of A (1%, 1cm) at the maximum at 278 nm.

EXERCISE

1) Comment on your findings
2) Suggest way by which fake chloramphenicol capsule can easily be identified.
3) Give the structure of chloramphenicol.

SCHEDULE VIII

2.8 ASSAY OF CHLORPHENIRAMINE PARENTERAL PREPARATIONS

INTRODUCTION

Parenteral preparations are sterile preparations intended for administration by injection, infusion or implantation into the body.

OBJECTIVE: To know whether the chlorpheniramine injection which is a sterile solution of chlorpheniramine maleate, $C_{16}H_{19}ClN_2$, $C_4H_4O_4$ in water for injection complies with the requirement stated under parenteral preparations.

NOTE: Protect the injection from light.

UNIFORMITY OF CONTENT

Determine the content of active ingredient of each of 10 injections taken at random using a suitable analytical method. The preparation being examined complies with the test if the individual value thus obtained is all between 85 and 115% of the average value.

The preparation being examined fails to comply with the test if more than one individual value is outside the limits 85 to 115% of the average value. (BP 1993).

IDENTIFICATION TEST

A. Apply separately to TLC plate 2 mL of each of following two (2) solutions:

1) Evaporate an appropriate volume to dryness in a current of nitrogen or water bath using minimum amount of heat, dissolve the residue as completely as possible in sufficient chloroform to produce a solution containing 0.5% $^{w}/_{v}$ of chlorpheniramine maleate and centrifuge.

2) 0.5% $^{w}/_{v}$ of chlorpheniramine maleate standard in chloroform.

Use a mixture of 50 volumes of ethyl acetate, 30 volumes of methanol and 20 volumes of 1M acetic acid as the mobile phase. Heat the plate at 105^{o}C for 30 minutes before use. After removal of plate, allow to dry in air and examine under ultraviolet light (254 nm). The two principal spots in the chromatogram obtained with solution (1) correspond to those in the chromatogram obtained with solution (2).

B. Spray the plate with dilute potassium iodobismuthate solution. (1) Correspond with that in the chromatogram obtained with solution (2).

C. Evaporate a volume of injection, equivalent to about 25mg of chlorpheniramine maleate, on a steam bath to dryness, and dry the residue at 105ºC for 1 hour. It melts between 128ºC and 135ºC.

D. pH is between 4.0 and 5.2.

ASSAY

Dilute a volume containing 10 mg of chlorpheniramine maleate to 500 mL with 0.25 M sulphuric acid and measure the absorbance of the resulting solution at the maximum at about 265 nm. Calculate the content of $C_{10}H_{19}ClN_2$, $C_4H_4O_4$ taking 212 as the value of A (1%, 1cm) at the maximum at 265 nm.

EXERCISE

1) Give the structure of chlorpheniramine
2) Give the class of chlorpheniramine

SHEDULE IX

2.9 ANALYSIS OF PACKAGE WATER

INTRODUCTION

Water is the most important compound on the surface of the earth. About three quarter of the earth's surface is covered with liquid water and it is the principal constituent of all living organisms. Water is a clear colourless liquid, practically odorless and tasteless. Water ordinarily refers to portable water freshly drawn from public supply, naturally occurring water frequently contain dissolved mineral substances indigenous to the region in which they are found. Since water dissolves a part of nearby things which it comes in contact with, absolutely pure water does not occur.

SAMPLE COLLECTION

Collect the samples directly from tap water into a polythene bag and treat with nitric acid use also the loose sample of "pure water" provided and teat in a similar manner. Take the pH of the two samples. Carry out qualitative analysis for iron, lead and any other two metals.

QUALITATIVE ANALYSIS

Iron

Take about 2 mL of your sample and add 1 mL of ammonium sulphide. A precipitate will be produced which can be dissolved by the addition of cold 3 M HCl with the evolution of hydrogen sulphide. Ferric salt will yield a reddish-brown precipitate with an excess of 1 M sodium hydroxide but Ferrous salt will yield a green white precipitate with 1 M sodium hydroxide colour will rapidly change to brown when shaken.

Lead

Add 1 mL of 2 M sulphuric acid to 2 mL of the sample. White precipitate which is insoluble in 3 M hydrochloric acid or 2 M nitric acid is produced. The precipitate is soluble in warm 1 M sodium hydroxide and in ammonium acetate.

Copper

Add excess 6 M ammonium hydroxide to small part of the sample. A bluish precipitate and then a deep blue coloured solution is produced.

Silver

Add hydrochloric acid to part of the sample. White curdy precipitate which is insoluble in nitric acid but readily soluble in 6 M ammonium hydroxide is produced.

Mercury

To part of the sample add 1 M sodium hydroxide. Yellow precipitate is produced. Mercuric salt responds to this test. For mercurous compound addition of 1M sodium hydroxide to part of the sample will produce a black colour.

QUANTITATIVE DETERMINATION OF IDENTIFIED HEAVY METALS USING TITRIMETRY

A. Determination of Ferrous ion (Fe^{2+})

Add 200 mL of N^- sulphuric acid (195 mL of H_2O + 5 mL of conc. H_2SO_4) to 25 mL of ferrous iron solution in a 25 mL volumetric flask and make up with distilled water. After vigorous shaking, remove 25 mL of the solution with a pipette into a 250 mL conical flask and add 25 mL of N – sulphuric acid, titrate with standard 0.1 M potassium permanganate solution to the first permanent faint pink colour. This titration would be repeated with two other 25 mL portions of the ferrous solution.

$MnO_4^- + 5\ Fe^{2+} + 8H^+ = Mn^{2+} + 5Fe^{3+} + 4H_2O$

1 mL – N – $KmnO_4$ = 0.05585 g Fe.

B. Determination of Lead (Pb) Using Xylenol Indicator

Take 25 mL of lead ion solution into 250mL volumetric flask using a pipette, dilute this solution with 25 mL of distilled water and add 3 drops of the indicator solution. When the colour of the solution is red, add dilute nitric acid cautiously with stirring until the solution acquired a yellow colour. Add powdered hexamine (hexamethylene tetra mine) until the colour is intensely red. This step is to ensure that the solution maintained a correct pH (about 6) for the subsequent titration. Titrate the solution with standard EDTA until the colour change to lemon yellow.

In each titration carried out, the final burette reading would be taken after 2 minutes.

1mL EDTA = 10.3605 mg Pb.

SCHEDULE X

2.10 DETERMINATION OF CHLORINE IN TAP AND PACKAGE WATER

INTRODUCTION

Chlorine belongs to the group of elements called the halogens. Although halogens compounds are known and used, their great affinity for other compounds render their displacement from their compounds very difficult. Chlorination of water supplies and their polluted waters serves primarily to destroys or deactivates diseases producing microorganisms.

OBJECTIVE: To determine the presence and amount of chlorine in samples of water provided.

EXPERIMENT

NOTE: - start determination immediately after sampling do not store the sample

a) Take pH of the samples

b) Take about 10 mL of water sample in a test tube. Add acidified silver nitrate solution allow the solution to stand for five (5) minutes. Observe the presence of white precipitate.

EXERCISE

1) Define water?
2) Give the pH of your water samples?
3) Why do we chlorinate water?
4) What are the factors affecting the bactericidal efficiency of chlorine?
5) What are the effects of excess chlorine in water?

SCHEDULE XI

2.11 ASSAY OF DICLOFENAC TABLETS

INTRODUCTION

Diclofenac [2-(2,6-dichloroamino) phenyl] acetic acid is a non-steroidal anti-inflammatory drug. It is usually found as a sodium or potassium. It is easily available and effective and thus are extensively used. It is an effective anti-inflammatory, analgesic and antipyretic agent which is commonly used in the treatment of acute and chronic pain, rheumatoid and osteo arthritis.

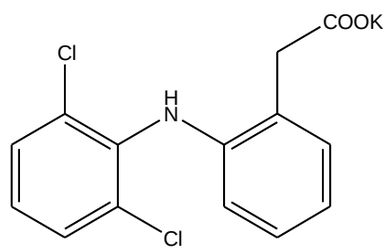


Figure 2.11: Chemical Structure of Diclofenac Potassium

OBJECTIVE: To determine the % content of active diclofenac in diclofenac tablets

EXPERIMENT

Weigh and crush twenty (20) tablets of the given brand of diclofenac tablet. From the powder obtained, accurately weigh a quantity of the powder equivalent to 100 mg and dissolve in a volumetric flask with sufficient distilled water to produce 100 mL. Prepare in triplicate and allow the resulting solutions to stand for about 10 minutes after which it can be filtered. Dilute 10 mL of these solutions to 50 mL with distilled water. Further dilute 5 mL of the second solutions to 50.0 mL with distilled water. Measure the absorbances of the final solutions with the UV spectrophotometer using a 1 cm layer quartz cuvette.

EXERCISE

What is the concentration and λ_{mas} of the final solution?

From the regression equation: y = 0.0264x + 0.0058 of the standard calibration curves, calculate the % composition of the active diclofenac in the studied tablet and compare to pharmacopoeia specifications.

SCHEDULE XII

2.12 ASSAY OF MORPHINE INJECTIONS

INTRODUCTION

Morphine is used for analgesia (pain relief), in severe pains resistant to non-narcotics. Specifically, the basic structure of morphine is that of a phenanthrene nucleus, with the methylated nitrogen in a heterocyclic ring structure (Figure 2.12). Morphine is a benzylisoquinoline alkaloid with two additional ring closures. It has a rigid pentacyclic structure consisting of a benzene ring, two partially unsaturated cyclohexane rings, a piperidine ring and a tetrahydrofuran ring.

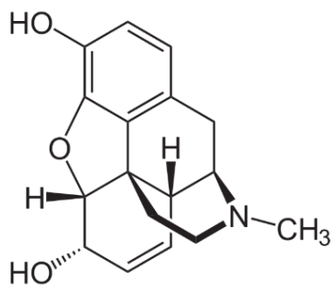


Figure 2.12: The Chemical Structure of Morphine

OBJECTIVE: To determine the % content of active morphine in morphine parenteral preparations

EXPERIMENT

Dissolve a quantity of morphine hydrochloride or sulphate injection equivalent to 10 mg or 15mg in a 100 mL volumetric flask containing about 20 mL of distilled water or 0.1N NaOH. Make up the solution to 100 mL with distilled water or 0.1N NaOH. Prepare the sample solutions in triplicate and measure their absorbances. Calculate the percentage contents of active morphine in the parenteral preparations using the British Pharmaceutical Codex (1979) A (1%, 1 cm) specifications of 41 for water and 70 for 0.1N NaOH.

EXERCISE

1) Compare the % content of the sampled morphine injection using water with that obtained using 0.1N NaOH as assay solvent.
2) Compare the obtained % content of the sampled morphine injection to the USP pharmacopeia specifications.