**King Saud University**

***College of Pharmacy***

***Pharmaceutical Chemistry***

**PHARMACEUTICAL CHEMISTRY 4 (428)**

***Laboratory Manual***

**Dr. Motiur Rahman**

**2017-2018**

**Course syllabus**

**Course title:** Pharmaceutical chemistry

**Course number:** 428

**Course credit:** 1.5 credits

**Number of hours:** 3 hrs /week

***Course description***

This course intended to provide students with the experimental tools necessary for drug analysis. Experiments are designed to encourage students to think in a systematic way by conducting analytical chemistry experiments in relation with their everyday life, analyzing the results and evaluating the answers via a comparison of the data to other reported literature values. Also, the course will teach students to present their findings in a scientific recognized format by writing a report detailing the experimental procedures, the results, the discussion and their conclusions.

***Course objectives***

At the end of this course the students will be able to:

 Think about a suitable identification chemical test for each drug according to its structural functional group/s (-OH, >C=O, -COOH, -CHO, -NH2, etc.).

 Choose the suitable analytical basis (Volumetric or UV-spectrophotometric assay) for quantitative analysis (purity in crude form or % of active ingredient in the marketed forms) of any drugs.

 Design acceptable mathematical forms for calculation of the purity or % active ingredient in any drug form in case of volumetric or UV-spectrophotometry.

 Use different types of titration (Direct or back Acid-base, Redox and

Complexometric) for drug analysis.

 Use of UV-spectrophotometer for reading of absorption (A) of different

solutions at certain λmax.

 Understand and use the theoretical basis of Beer-Lambert's law and its application for determination of A1% and/or ξ (mathematically or graphically as physical constants) and % of the active ingredient in any marketed drug.

***Laboratory Assessment***

First Practical Exam ………………………………………….………..……………….…… (10 pts). Lab. Activity (participation, attendance and evaluation) ………………...………….....…… (3 pts). Final Practical Exam …………………………………………………………………...…… (12 pts).

***Course instructors***

**Name:** Dr. Motiur Rahman

**Office No.:** 2A117

**Phone No.:** 4679096

**Lab. Location:** 1B23

**Lab. Time:** 13 – 16 (Thursday)

**Lab. 1** (page 5)

**Content**

***Introduction*:** Safety rules – General lab instructions.

**Lab. 2** (page 11)

***Analysis of paracetamol tablets***

Identification (FeCl3 soln.)

UV-spectrophotometry: Preparation of serial dilutions – Detn. A1% for paracetamol**. Lab. 3** (page 13)

***Analysis of aspirin tablets***

Identification (based on alkali hydrolysis and FeCl3 soln.)

% of acetylsalicylic acid in aspirin tablet by Back acid-base titration**.**

Limit test: definition – Why and How????

**Lab. 4** (page 16)

***Analysis of aminophylline tablets***

Identification (CuSO4 soln.)

% of theophylline in tablet by UV-spectrophotometry**.**

% of ethylene diamine in tablet by Direct acid-base titration**. Lab. 5** (page 19)

***Analysis of Pyridostigmine bromide tablets or ampoules***

Detn. of A1% mathematically and graphically.

Detn. of % purity for the active ingredient by UV-spectrophotometry**. Lab. 6** (page 21)

***Analysis of ascorbic acid tablets***

Identification (2,6-DCPIP and AgNO3 soln.)

Detn. of purity and % of Vit-C in tablet by Complexometric titration**. Lab. 7** (page 23)

***First practical exam. ……………………………………………..……………….. (****10 pts****)***

**Lab. 8** (page 24)

***Analysis of Chloramphenicol capsules***

Identification (Azo-dye formation by diazotization reaction with 2-naphthol)

Detn. of A1% and/or ξ (mathematically or graphically) and purity by UV-spectrophotometry**. Lab. 9** (page 27)

***Analysis of Calcium lactate tablets***

Identification (KMnO4 soln. and Moist powder with Conc. HCl/Pt-wire – Bunsen burner flame) Detn. of % purity by Complexometric titration**.**

**Lab. 10** (page 30)

***Analysis of Inderal (propranolol hydrochloride) tablets*** Detn. of purity and % in tablet by UV-spectrophotometry**. Lab. 11** (page 32)

***Analysis of Penicillamine capsules***

Identification (Phosphotungestic acid)

Detn. of purity of penicillamine by Complexometric titration**. Lab. 12** (page 34)

***Analysis of Warfarin (as Sodium salt) tablets***

Detn. of purity and % in tablet by UV-spectrophotometry using liquid-liquid extraction technique for purification**.**

**Lab. 13**

***Final practical exam. ……………………………………………..……………….. (****15 pts****)***

**Lab. 14** (page 37)

***Further Experiments and Readings***

***Analysis of Kemistine (2%chloramphenicol) or Riachol (1%) ointment***

Detn. of purity and % in ointment by UV-spectrophotometry by using liquid-liquid extraction technique for purification**.**

**Lab. 15** (page 39)

***Analysis of Methyldopa tablets***

Identification (FeCl3 soln. with Ammonia soln. or NaOH soln.) Detn. of A1% graphically and % purity by UV-spectrophotometry**. *Further useful readings*** (page 39)

**Lab. 1**

***Introduction: Safety rules – General lab instructions***

It is essential to develop a high standard of professional practice during training. Arrive for your class well prepared and try to work independently. Confidence in your own ability will grow with self-reliance. Remember that teaching staff is usually a more reliable source of help than fellow students. Mistakes are an inevitable and useful part of learning process so long as they are recognized and corrected.

1. Wear a freshly laundered lab coat to protect your clothes and the preparation you are dealing with. Class participation in laboratory means being in class ON TIME and ready to go.

2. Provide yourself with a clean towel and a sponge, so that spilled chemicals can be cleaned immediately.

3. Work in a clean tidy manner, so as to reduce the risk of errors and contamination. The working space should be kept clear of books and paper. Use clean equipment (spatula, pipets, …etc) to avoid contamination of stock reagents.

4. Each student is responsible for the appearance of his desk and balance at all the time, and should spend about 10 minutes at the end of each period cleaning his equipment, working area, and sink.

5. Be sure to use the balance properly, since it is a sensitive and delicate apparatus. Use weighing paper to protect the pan, and immediately clean any spilled chemicals on the balance to avoid corrosion. The balance should be in the stand by position **WHEN NOT IN USE**.

6. Don't discard solid waste materials in the sink; it should be discarded in special garbage.

7. You should also bring a **CALCULATOR**.

8. In order to prevent confusion, students are not to leave the laboratory without first obtaining permission from an instructor. Receiving said permission, the student is to minimize his or her absence.

9. The laboratory manual about each experiment should be read before lab. A quiz may be asked to at the end of each laboratory period.

10. Exams will not be strictly cumulative but it is difficult to cover material without reference to material coverd earlier, even last semester. Also eace regular exam will contain some questions which will cover pharmaceutical calculations which are always cumulative.

**Safety Regulations and Practices**

**LABORATORY SAFETY IS AN IMPORTANT COMPONENT OF LABORATORY WORK. STUDENTS ARE EXPECTED TO OBSERVE ALL SAFETY REGULATIONS AT ALL TIMES IN THE LABORATORY.**

**DESREGARD FOR THE LABORATORY SAFETY REGULATIONS WILL RESULT IN THE DEDUCTION OF POINTS FROM THE LABORATORY GRADE. REPEATED DISREGARD FOT THE LABORATORY SAFETY REGULATIONS WILL LEAD TO EXPULSION FROM THE LABORATORY.**

1. **Safety goggles must be worn over the eyes at all times in the laboratory!** Contact lenses are  **not** to be worn in the laboratory. They offer no protection in themselves; they are unsafe even under safety goggles. Various fumes may be concentrated under the lenses and against the eye. **You may not work in the lab wearing contact lenses.**

2. Smoking, drinking, and eating are forbidden in the laboratory because of the possibility of the chemicals getting into the mouth or lungs.

3. Clothing worn in the laboratory should protect arms, legs, and feet. Shorts or sandals should not be worn into the laboratory. Plastic gloves are available in the laboratory and are to be used when handling corrosive or caustic chemical irritants.

4. Confine long hair and loose clothing when in the laboratory. Avoid wearing jewelry that catch on objects, or under which chemicals can be trapped.

5. Do not run in the laboratory. Do not wash. Give adequate room for people to move behind or around your work area.

6. Keep drawers and cabinets closed while working. Keep aisles free of obstructions such as chairs, stools, boxes, and receptacles.

7. Avoid slipping hazards. Pick up ice, stoppers, glass beads, broking glass, and other small objects from the floor. **Clean up all spills** – both liquids and solids  **immediately**.

8. Keep work space uncluttered. Work areas should be kept clear of chemicals and scraps of paper. Keep measuring equipments such as graduated cylinders and volumetric flasks where they will not be knocked over easily. Support all small and top heavy containers.

9. Never point an open container of hot liquid at anyone, including yourself. Likewise, never vent a separatory funnel toward anyone, including yourself.

10. Unauthorized experiments are prohibited. Never perform work in the laboratory alone.

Laboratory work is not to be conducted unless there are at least two people present in the laboratory and the instructor authorized the work. **Never leave your experiment unattended**.

11. Learn the location of, and how and when to use eye wash fountains safety showers, fires extinguishers, fire blanket, first aid kit, and spill-cleanup chemicals. Ask your instructor to familiarize you with the proper uses of these facilities.

12. Learn what to do and where to go when an alarm sounds.

13. Most chemicals are harmful to some degree. Avoid direct contact with any chemical. It is especially important to keep chemicals from the hands, face, arms, legs, clothes, and shoes.

Wash thoroughly with soap and warm water whenever a chemical contacts your skin.  **Never taste a chemical**. **Never smell a chemical directly**. When instructed to smell something, bring a small sample of the vapor to your nose by means of a cupped hand.

14. Never distill a liquid to dryness. Peroxides may form in solvents (especially ethers) upon prolonged storage (exposure to O2 in air) and may explode if concentrated and heated in a distillation flask.

15. Operations involving flammable gases, toxic vapors, or noxious odors should be performed in the hood.

16. Before using an open flame or spark-producing equipment such as motors or open heaters, be sure there are no flammable liquids, or vapors nearby. Use **no open flames** without permission or direction of the laboratory instructor. Never reach across a lighted burner.

17. Apparatus attached to a ring stand should be positioned so that the center of gravity of the system is over the base and not to one side or behind the base.

18. Always set up your apparatus so that the heat source can be quickly withdrawn if necessary.

19. Mouth suction should never be used to fill by pipettes. Use a pipette pump.

20. Carefully read the label before removing a reagent from its container. Names of distinctly different substances are sometimes nearly alike and using the wrong substances can lead to accidents.

21. Always add a reagent slowly; never "dump" it in. Beware of exothermic reactions.

22. When adding liquids or powders to a vessel, do not pour towards you. Use a funnel when practical, especially if the opening being poured into is small. Before pouring a liquid into burette, dropping funnel, or separatory funnel, make sure the stopcock is closed. Use a stirring rod to direct the flow of liquids being poured.

23. To avoid splattering always pour more concentrated solutions carefully -true of concentrated sulphuric acid where tremendous quantities or heat are generated. Always add acids to water. Do not mix strong acids with strong basis directly.

24. First-aid material must be provided, and staff instructed in first-aid technique and emergencies.

25. Broken glass causes most of the injuries associated with the laboratory.

26. Special caution is advised when inserting (or removing) glass tubing or thermometers through rubber stoppers, tubing corks, rubber hoses, … etc. when inserting glass tubing or rods into corks, rubber stoppers, or rubber hoses, follow these procedures:

a) Fire polish the end of the glass to be inserted.

b) Lubricate the glass with either water or glycerol if it will not contaminate the experiment. c) Wrap a cloth around the glass and protect your hand with a cloth or protective gloves.

d) Hold the wrapped piece of glass not more than 5 cm from the nearest end to be inserted.

e) Insert the glass into the cork, stopper or hose with a twisting motion, avoiding too much pressure and torque.

f) Consult your instructor if in doubt.

27. Small quantities of water soluble neutral substances may be flushed down the drain of the sink with large quantities of running water. Waste solids, matches, labels, paper towels, … etc. are disposed of in the waste

28. All accidents and personal injury, no matter how minor, must be reported to the instructor immediately.

29. Before leaving the laboratory each day, be sure to wipe off your laboratory bench with a wet sponge. Be sure that the water and gas at your laboratory station are turned off before you leave.

30. If equipment is used, clean and put it away when finished. Also equipment removed from laboratories and workshops must be signed out in a register.

31. When using hazardous goods, ensure that the MSDS is understood and advice adhered to.

32. All wastes must be disposed of in the correct manner. Keep your work area clean; wash your hands to prevent contamination.

**References**

<http://www.unimelb.edu.au/ehsm/5.html#5.1.1>. (Australian Standards in

Laboratories) AS 2243 Safety in Laboratories, Parts 1 – 10.

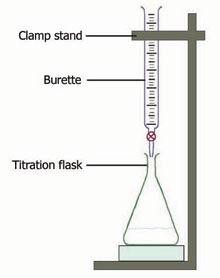
**General Introduction**

In this course, the practical processes will be focused on the qualitative analysis (identification by a chemical test) and quantitative analysis (determination of % through titration or UV- spectrophotometry). These two types of assays could be briefly explained as:

**A) Titration assay**

It based on one of four different types of titration according to the type and structure of the drug (analyte) and the reaction type with the titrant molecules using suitable indicator that produce characteristic colour change at end point. These four types are:

i- Direct Acid-base Titration (e.g. Ethylene diamine in aminophylline vs. H2SO4).



ii- Back Acid-base Titration (e.g. Aspirin vs. NaOH). iii- Redox Titration (e.g. Ascorbic acid vs. Ce4+).

iv- Complexometric Titration (e.g. Ca. lactate vs. EDTA-disodium salt).

**B) UV-spectrophotometric assay**

This type of assay based on the Beer-Lambert's law (A = A1% X C), which could be represented in one of the two following forms

**Titrant**

**Analyte soln.**

**+ Indicator**

**!Error**

A = A1% X C …………………..(1), if the C measured in % (g/100 ml), L = 1 cm.

A = ξ X C …………… ……..(2), if the C measured in molar, L = 1 cm. Where:



A = the absorbance of UV at certain **λ**

A1% = A physical constant for each drug defined as the absorbance o[f 1 %](http://en.wikipedia.org/wiki/File%3ASpektrofotometri.jpg) soln. from this

Drug at its **λ**max (C = 1 % in eq. 1).

ξ (Molar absorbance or molar extinction coefficient) = A physical constant for each drug defined as the absorbance of 1 M soln. from this drug at its **λ**max (C = 1 M in eq. 2).

**Definitions**

***Chromophore***: A covalent unsaturated group (contains π-electrons), responsible for UV-absorption e.g. C=O, C=C, - NO2, C6H6.

***Auxochrome***: A saturated group with hetero atom bearing non-bonded electrons which become conjugated with π-eles. in chromophores to alter **λ**max and intensity of the absorption (e.g. – OH, - NH2, Cl).

**O NH**

**CH3**

**Auxochromes Chromophores**

**OH**

**UV-Cuvette** or **cell**: is made from quartz (doesn't absorb UV-light in this range). The travelling pathway for light = 1 cm.

**UV-Calibration curve:** A graph of Absorption (A) vs. Concentration (C); it could be used

for: a) Determination of unknown concentration. **A**

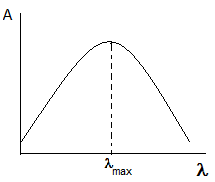
b) Determination of A1% (= slope if the C in %). **A1**

c) Determination of ξ (= slope if the C in molar).

**A2**

Slope = A1% or ξ = (A1 – A2) / (C1 – C2)

**UV-spectrum**: A graph of Absorption (A) vs. Wave length (**λ**);

it could be used for determination of unknown **λ**max

and hence identification of a drug.

**Important applications for Beer – Lambert's Law:**

**slope = A1%**

**or =** 

**C2 C1 C**

First: A1% = (A1 / C1 ) = (A2 / C2) = ….. etc (could be used for calculation of unknown C

or A for assumed solution of certain drug.

Second: (C)practical = (A / A1% ) (could be used for calculation of practical C of certain drug sample, then for determination of % of the active drug in this sample from the eq. :

% of active drug = (Cpractical / (Ctheoritical) X 100

**Lab. 2**

***Analysis of Paracetamol tablets***

**Paracetamol** is a widely used [over-the-counter](http://en.wikipedia.org/wiki/Over-the-counter_drug) [analgesic](http://en.wikipedia.org/wiki/Analgesic) (pain reliever) and [antipyretic](http://en.wikipedia.org/wiki/Antipyretic) (fever reducer). It is commonly used for the relief of [headaches](http://en.wikipedia.org/wiki/Headache) and other minor aches and pains and is a major ingredient in numerous [c](http://en.wikipedia.org/wiki/Common_cold)old and flu remedies.

**Chemical Structure**

**O**

- **λmax:** 257 nm

- **Chemical name:**

**NH C**

**OH**

**CH3**

4-Acetaminophenol

4-Hydroxyacetanilide

N-Acetyl-4-hydroxyaniline

N-(4-hydroxyphenyl)-acetamide

1. **Materials:** Distilled H2O – Paracetamol tablets – 0.1 N NaOH – 1% alcoholic FeCl3 soln.

2. **Glasswares:** 1 Beaker (50 ml) – 1 Vol. flask. (100 ml) - Vol. flask. (50 ml) – 4 Vol. flask. (25 ml – 1, 2, 3, and 4 ml pipettes for each group).

3. **Identification:** Add few drops of 1% alcoholic FeCl3 soln. to 1 – 2 ml paracetamol (5%

methanolic soln.).

Result: Formation of pale green colour due to presence of free phenolic –OH group.

4. **Determination of A1%** (Mathematically and graphically)

**Basis: UV-spectrophotometry**

**Procedure**

- **Soln. A**: Dissolve 50 mg paracetamol powder in 10ml 0.1 N NaOH by strong shaking in 100 ml volumetric flask, then complete it to 100 ml dist. H2O.

- **Soln. B**: Dilute (5 ml from soln.-A + 5 ml of 0.1 N NaOH) with dist. H2O in 50 ml volumetric flask.

- **Standard serial dil. Solns.**: Dilute individually 1, 2, 3 and 4 ml from soln. B with dist. H2O

in four 25 ml vol. flasks to form solns., 1 – 4.

- Measure A257 for each standard at λmax: 257 nm using UV-spectrophotometer and report the results according to the following report.

**Report**

- **Calculation:**

**Soln. A**: Its conc. is 50mg/100 i.e. 0.05g/100ml or = 0.05 %.

**Soln. B**: CA X VA = CB X VB

CB = (CA X VA)/VB = (0.05 X 5) / 50 = 0.005 %

**Soln. 1**: CB X VB = C1 X V1

C1 = (CB X VB)/V1 = (0.005 X 1) / 25 = (0.005 / 25) = (1/5) X 10 3 = 2 X 10 4 %

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **C X 10 4 (%)** | **A257** | **A1% (mathematically)** |
| **1** |  |  |  |
| **2** |  |  |  |
| **3** |  |  |  |
| **4** |  |  |  |
| **Average** |  |  |  |

Draw the calibration curve (A257 vs. C) and determine **A1%** = slope according to Beer- Lambert's law A = A1% X C ………. i. e. A1% = A / C ……………………………… (see page 9)

**From Graph:**

A1% = (A1 – A2) / (C1 – C2) = ---------------------------- = **(graphically)**

**Lab. 3**

***Analysis of aspirin tablets***

**Aspirin** is used as an [analgesic](http://en.wikipedia.org/wiki/Analgesic) to relieve minor aches and pains, as an [antipyretic](http://en.wikipedia.org/wiki/Antipyretic) to reduce fever, and as an [anti-inflammatory](http://en.wikipedia.org/wiki/Anti-inflammatory) medication. It also has an [antiplatelet](http://en.wikipedia.org/wiki/Antiplatelet_drug) effect by inhibiting the production of [thromboxane,](http://en.wikipedia.org/wiki/Thromboxane) which under normal circumstances binds [platelet](http://en.wikipedia.org/wiki/Platelet) molecules together to create a patch over damaged walls of blood vessels. Because the platelet patch can become too large and also block blood flow, locally and downstream, aspirin is also used long-term, at low doses, to help prevent [heart attacks](http://en.wikipedia.org/wiki/Myocardial_infarction), [strokes,](http://en.wikipedia.org/wiki/Stroke) and [blood clot](http://en.wikipedia.org/wiki/Thrombus) formation in people at high risk of developing blood clots.

**Chemical Structure**

**COOH**

**CH3**

- **MF**: C9H8O4

- **MWt**.: 180 Da

**O**

- **Chemical name:**

**O**

Acetylsalicylic acid

2-acetyloxybenzoic acid

1. **Materials:** Aspirin tablets – 0.5 M NaOH – 5 M NaOH – 0.5 M HCl - Phenol red – pure salicylic acid (0.01 %) - 1% aq. FeCl3 soln. – Fe[NH4(SO4)2] – EtOH (96 %).

2. **Glasswares:** 1 Beaker (50 ml) – 5 cm funnel - 2 Con. flask. (250 ml) – 1 burette (50 ml) –

2 Nessler cylinders – 1 Vol. flask (100 ml), 1 pipette (30 ml).

3. **Identification (Alkali hydrolysis or saponification)**:

**COOH**

**O**

**CH3**

**O**

**+ 2 NaOH**

**(5 ml, 5M)**

**1 - 3 min, heating**

**COONa**

**OH**

**NaO**

**+**

**CH3**

**O**

**(0.3 g)**

**1) Cool**

**CH3**

**HO**

**COOH**

**OH**

**Sod. salicylate Sod. acetate**

**1) Filtration**

**2) Salicylic acid soln.**

**Violet colour**

**2) dropwise H2SO4**

**nutralisation**

**+ 3) + FeCl3 soln.**

**O**

**Acetic acid (viniger odour) Salicylic acid (white ppt,)**

**4. Limit test: (determination of free salicylic acid in the aspirin sample)**

**Tube-1:** 50 ml from



(0.45 % sample soln. in 4 ml EtOH + 96 ml H2O)

+ 1 ml of 1 % Fe [NH4(SO4)2].

**Tube-2:** 50 ml from

(3 ml of 0.01 % standard Salicylic acid + 47 ml H2O) + 1 ml of 1 % Fe [NH4(SO4)2]

Compare the intensity of violet colour that will be developed in both tubes. The sample will be valid if the intensity in tube **1** less than that in **2**

5. **Assay** (% acetylsalicylic acid in the aspirin tablets)

**Basis:**  back acid-base titration using excess 0.5 M NaOH and phenol red as indicator with

0.5 M HCl for neutralization of the remained NaOH.

**Procedure**

- Weigh 0.56 g powder (≡ 0.5 g aspirin) in 250 ml conical flask.

- Using a pipette, add excess NaOH (30 ml, 0.5 M).

- Boil the mixture for 10 min. on hot plate.

- Cool, then add few drops phenol red and titrate with 0.5 M HCl up to the end point (violet

→ pink – Red). The Vol. of HCl = V1 excess (unreacted 0.5N NaOH).

- Titrate blank 30 ml 0.5 M NaOH with 0.5 M HCl using phenol red. Record the end point

Vol. as V2.

- Accordingly, the vol. of NaOH reacted with aspirin = (V2 – V1).

- Record your results in the following report.

**Report**

- **Calculation:**

Where the following eq. describes the complete reaction of NaOH with aspirin

**C9H8O4 + 2 NaOH → HO.C6H4.COONa + CH3COO.Na + H2O**

**So, …………………** 1 mol aspirin ≡ 2 mol of NaOH

 180 g ≡ 2000 ml . 1 M

 0.180 g ≡ 2 ml . 1 M

 0.090 g ≡ 1 ml . 1 M

 0.045 g ≡ 1 ml . 0.5 M

and where (X)g ≡ (V2 – V1) ml

 (X) g = 0,045 X (V2 – V1)

% Purity = (X / 0.5) X 100 = ……. % and % aspirin in tablet = (X / 0.56) X 100 =

……. %

**Note**: The theoretical E. P. Vol. that will be expected to react with 0.5 g of aspirin:  0.045 g ≡ 1 ml . 0.5 M,  0.5 g ≡ (V) ml . 0.5 M

Then, exp. Equivalent vol = (0.5 X 1) / 0.04 = 11.1 ml.

So, the 0.5 N HCl volume at the E. P. = 30 – 11.1 ml

**Quiz-1:** Define limit test ? Why limit test **? Quiz-2:** What is the source of free acid in aspirin **? Quiz-3:** Define Back titration **? Quiz-4:** Why back titration **?**

**Lab. 4**

***Analysis of Aminophylline tablets***

**Aminophylline** is a [bronchodilator](http://en.wikipedia.org/wiki/Bronchodilator). It is a compound of the bronchodilator [theophyll](http://en.wikipedia.org/wiki/Theophylline)ine with [ethylenediamine](http://en.wikipedia.org/wiki/Ethylenediamine) in 2:1 ratio. The ethylenediamine improves solubility, and the aminophylline is usually found a[s a dihydrate.](http://en.wikipedia.org/wiki/Hydrate)

**H3C**

**Chemical Structure**

**O**

- **A1%**: 650

- **λmax**: 275 nm

**N N**

**O N N**

**CH2 NH2**

**CH2 NH2**

**2 H2O**

- **Two constituents:**

Theophylline: 76 - 86 %, C7H8N4O2

**CH3 H 2**

**MWt**.: 60 Da (Ethylene diamine)

Ethylene diamine: ≥ 10.9 %, C2H8N2

- **Chemical name**

1,3-dimethyl-7H-purine-2,6-dione ethane-

1,2-diamine

**1. Materials:** Aminophylline tablets – 1 % CuSO4 – 0.1 M NaOH – 0.01 M NaOH - 0.05 M H2SO4

– Bromocresol green (Indicator).

**2. Glasswares:** 1 Beaker (50 ml) – 5 cm funnel - 1 Con. flask. (250 ml) – 1 burette (50 ml) – 2

Test tubes – 100 ml Vol. flask - 50 ml Vol. flask – 10 ml measuring cylinder – 1 ml pipette.

**3. Identification (**with 1% CuSO4**)** in a test tube:

0.5 g powder + 5 ml dist. H2O (filtration) → → → then 2 ml (filtrate) + 2 ml CuSO4 (pale green)

→ → → purple-blue colour (compare this colour with blank colour of CuSO4).

**4. Assay**-1 (% theophylline in the aminophylline tablets, 76 - 86 %)

- **Basis:** UV – Spectrophotometry at **λmax**: 275 nm and **A1%**: 650.

**Procedure-1** (Total wt. of I Tablet = 0.2 g ≡ 0.1 g aminophylline)

 0.2 g ≡ 0.1 g aminophylline,  0.08 g ≡ 0.04 g aminophylline

- **Soln.-1**: 0.08 g powder + 10 ml 0.1 M NaOH + 30 ml dist. H2O (shake well for 10 mins., then complete) → → → with dist. H2O to 100 ml in vol. flask.

- **Soln.-2**: Filter soln. 1 and then complete 1 ml from the filtrate with 0.01 M NaOH to 50 ml in Vol. Flask.

- Measure A275 of soln. – 2, then calculate % theophylline taking A1% = 650.

**5. Assay-2** (% ethylene diamine in the aminophylline tablets, ≥ 10.9 %)

**Basis:** direct acid-base titration using 0.05 M H2SO4 and Bromocresol green as indicator.

**Procedure-2**

- Weigh 0.6 g powder (≡ 0.3 g aminophylline) in 250 ml conical flask.

- Add 20 ml H2O, then boil the mixture for 10 min. on hot plate.

- Cool, then add few drops bromocresol green and titrate with 0.05 M H2SO4 up to the end point

(blue-alkaline → green – acid).

**Report-1**

 soln.-1 = 0.08 g powder ≡ 0.04 g /100 ml, C1 = ………………….. %.

and  C1 X V1 = C2 X V2, (C2)Theoritical = (……. X …….) / ……. =

………………….. %.

and  A275 = A1% X (C2)practical, (C2)practical = ………….. / ………... =

………………….. %.

 % theophylline in tablet = (………….... / …….……...) X 100 = ………….. %.

**Report-2**

- **Calculation:**

Where every 1 mol Aminophylline contains 1 mol ethylene diamine

and  1 mol ethylene diamine ≡ 1 mol of H2SO4

 60 g ≡ 1000 ml . 1 M

 0.060 g ≡ 1 ml . 1 M

 0.03 g ≡ 1 ml . 0.5 M

 0.003 g ≡ 1 ml . 0.05 M

and where (X)g ≡ (V) ml at the end point

 (X) g = 0.003 X (V)E.P. = 0.003 X ………….. = ……… g ------------ (1)

**Note**: The theoretical E. P. Vol. that will be expected to react with 0.3 g of aminophylline:  Theoretical content Ethylene diamine **in 0.6 g** ≈ 0.6 X (11 / 100) = ………… g and  0.003 g ≡ 1 ml . 0.05 M, ……….. g ≡ theoretical (V)E.P.

Then, theoretical expected (V)E.P. = …………. / 0.003 = …………. ml

 From eq (1)  (X) g = ……………………….

and % ethylene diamine in tablet = (…….. / ……..) X 100 = ………. %

**Quiz-1:** 1 M H2SO4 = …… N ? **Quiz-2:** 1 M NaOH = …… N?

**Lab. 5**

***Analysis of Pyridostigmine tablets or ampoules***

**Pyridostigmine** is a [parasympathomimetic](http://en.wikipedia.org/wiki/Parasympathomimetic) and a reversible [cholinesterase inhibitor](http://en.wikipedia.org/wiki/Cholinesterase_inhibitor). Since it is a quaternary amine, it is poorly absorbed in the gut and does not cross the [blood–brain barrie](http://en.wikipedia.org/wiki/Blood%E2%80%93brain_barrier)r, except possibly in stressful conditions

**Chemical Structure**

**CH3**

**A1%**: 186

**λmax: 270 nm**

**N**

**Br CH3**

**N**

**O CH3**

**O**

**Chemical name:**

(dimethylcarbamoyl)oxy]-1-methylpyridinium bromide

**Purity** 92-107%; **% in Tablet** 17.14%

**1. Materials:** Distilled H2O – Pyridostigmine tablets or ampoules.

**2. Glasswares:** 1 Beaker (50 ml) – 1 Vol. flask. (100 ml) – 4 vol. flask (50 ml) – 1, 2, 3 and 4 ml (pipettes for each group).

**3. Assay and determination of A1%** (Mathematically and graphically)

**Basis: UV-spectrophotometry**

**Procedure**

- **Soln. A**:

**Case of tablets**: Total wt. of 1 tablet ≡ 0.350 g ≡ 0.06 g pyridostigmine bromide. Dissolve 0.350 g powder in 100 ml volumetric flask with 100 ml dist. H2O. Mix well and filter immediately.

**Case of ampoules**: Every 1 ampoule (2 ml) ≡ 10 mg ≡ 0.01 g pyridostigmine bromide. Mix content of 6 ampoules (0.06 g) with dist. H2O, completing it to 100 ml in volumetric flask.

- **Standard serial dil. Solns.**: Dilute individually 1, 2, 3 and 4 ml from soln. A with dist.

H2O in four 50 ml vol. flasks to form solns., 1 – 4.

- Measure A270 for each solution at λmax: 270 nm using UV-spectrophotometer and report the results according to the following report.

**Report**

- **Calculation:**

**Soln. A**: Its conc. is 60 mg/100 i.e. 0.06g/100ml or = 0.06 %.

**(CA)T= 0.35 %**

**Soln. 1**: CA X VA = C1 X V1

C1 = (CA X VA)/V1 = (0.06 X 1) / 50 = ……… X 10 4 % Similarly, (C1)T= 0.35 / 50 = 0.007 % = …70. X 10 4 %

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **S. No.** | **C X 10 4 (%)** | **CT X 10 4 (%)** | **A270** | **A1%** |
| **1** |  |  |  |  |
| **2** |  |  |  |  |
| **3** |  |  |  |  |
| **4** |  |  |  |  |
| **Average** |  |  |  |  |

Draw the calibration curve (A270 vs. C) and determine **A1%** = slope according to Beer- Lambert's law A = A1% X C ………. i. e. A1% = A / C …………… (Mathematically, use

averages)

 A1% = ………… / ………… = ………….

**From Graph:**

A1% = (A1 – A2) / (C1 – C2) = ---------------------------- = ………….. (Graphically)

**From Table:**

 average (C)practical = average (A) / A1% = **…………** / 186 = ………….. %

 % purity = [average (C)practical / average (C)theoretical] X 100 = ………….. %

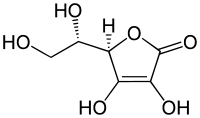
**and ** % in Tablet = [average (C)practical / average (C)T] X 100 = ………….. %

**Lab. 6**

***Analysis of Vit-C (Ascorbic acid) tablets***

**Ascorbic acid** is a naturally occurring [organic compound](http://en.wikipedia.org/wiki/Organic_compound) [with antioxidant](http://en.wikipedia.org/wiki/Antioxidant) properties.

**Chemical Structure MF**: C6H8O6

**MWt**.: 176 Da

**Chemical name:** (5*R*)-[(1*S*)-1,2- dihydroxyethyl]-3,4-dihydroxyfuran-2(5*H*)-one

**Purity:** 95 – 107 %; **in tablet**: 33.3 %

**or** 1 tablet = 4.5g ≡ 1g → 22.7 %

**1. Materials:**Vit-C tablets – 0.1 M Ce[(NH4)4(SO4)4] – 1 M H2SO4 – 2,6- dichlorophenolindophenol (DCPIP, 0.1%)–1% aq. AgNO3 soln. and ferroin sulfate indicator

**2. Glasswares:** 1 Beaker (25 ml) – 5 cm funnel - 2 Con. flask. (250 ml) – 1 burette (50 ml) –

2 test tubes.

**3. Identification (**Oxid-Red Reactions**)**:

- Dissolve 0.25 g of Vit-C powder in 5 ml dist. H2O through strong shaking, then filter and divide it into two eq. volumes in two test tubes.

- Add to the first tube 2,6-DCPIP dropwise:



(**Its deep blue colour will be decolourized**).

- Add to the second tube AgNO3 soln.

(**Black ppt. from Ag-metal will be formed**).

1 ml AgNO3 + 2 ml filtrate → Ago ↓ **black**

**4. Assay** (Purity and % ascorbic acid in tablets)

**1) DCPIP 2) AgNO3**

**Basis:** Redox titration *vs*. 0.1 M Ce[(NH4)4(SO4)4] and ferroin sulfate as indicator.

**Procedure**

- Weigh 0.45 g powder (≡ 0.15 g Vit-C) in 250 ml conical flask. (Wt. 1 Tablet = 1.5 ≡ 0.5 g

Vit-C) and dissolve it in 30 ml dist. H2O and 20 ml 1 M H2SO4 by shaking well.

- Add few drops ferroin sulfate and titrate *vs*. 0.1 M Ce[(NH4)4(SO4)4] up to the end point

(green colour → colourless).

- Determine the E. P. Volume (V)E.P and calculate % Ascorbic acid in the Tablet.

- **Calculation:**

**Report**

Where the following eq. describes the complete Redox reaction of Vit-C with CeIV+ ion

**2 Ce4+ + 2e → 2 Ce3+**

**C6H8O6 + 2 Ce4+ → C6H6O6 + 2 Ce3+ + 2 H+**

**So, …………………** 1 mol Ascorbic acid ≡ 2 mol of **Ce4+**

 176 g ≡ 2000 ml . 1 M

 0.176 g ≡ 2 ml . 1 M

 0.088 g ≡ 1 ml . 1 M

 0.0088 g ≡ 1 ml . 0.1 M

and where (X)g ≡ (V)E.P ml

 (X) g = 0,0088 X (V)E.P

% Purity = (X / 0.15) X 100 = ……. % and % Vit-C in tablet = (X / 0.45) X 100 =

……. %

**Note - 1**: The theoretical E. P. Vol. that will be expected to react with 0.15 g of Vit-C:  0.0088 g ≡ 1 ml . 0.1 M,  0.15 g ≡ (V) ml . 0.1 M

Then, exp. Equivalent vol = (0.15 X 1) / 0.0088 = 17.03 ml.

**Note - 2**: The Vit-C Tablet should be kept free from contact with light, metal and moisture.

**Quiz-1:** Define the oxidation and reduction reactions on the basis of electrons transfer, oxidation number, hydrogen transfer and oxygen transfer **?**

**Quiz-2:** 1 M H2SO4 is necessary Why **? Quiz-3:** What is the basis of identification reactions **? Quiz-4:** Comment on decolorization of DCPIP and precipitation of Ag-metal **?**

**O**

**N**

**FeSO4 . 8H2O N**

**Cl Cl**

**N**

**3**

**1,10-phenanthroline ferrous sulfate octahydrate**

**OH**

**2,6-DCPIP**

Lab. 7 (7th week)

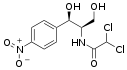
First practical exam............................................. (lOpts)

**Lab. 8**

***Analysis of Chloramphenicol capsules***

**Chloramphenicol** is a [bacteriostatic](http://en.wikipedia.org/wiki/Bacteriostatic) [antimicrobial](http://en.wikipedia.org/wiki/Antimicrobial) and also known as chlornitromycin, is effective against a wide variety of [Gram-positive](http://en.wikipedia.org/wiki/Gram-positive_bacteria) and [Gram-negative](http://en.wikipedia.org/wiki/Gram-negative_bacteria) [bacteria,](http://en.wikipedia.org/wiki/Bacteria) including most [anaerobic organisms.](http://en.wikipedia.org/wiki/Anaerobic_organism) Due to resistance and safety concerns, it is no longer a [first-line agent](http://en.wikipedia.org/wiki/First-line_agent) for any infection in developed nations, although it is sometimes used topically for [eye infections](http://en.wikipedia.org/wiki/Conjunctivitis). Nevertheless, the global problem of advancing bacterial resistance to newer drugs has led to renewed interest in its use.

**Chemical Structure**



**NO2**

**HO H**

**A1%**: 298 **λmax:** 278 nm **Chemical name:**

**Cl** 2,2-dichloro-N-[1,3-dihydroxy-1-(4-

**H NH2 C CH**

***1R,2R***-isomer

**Cl**

**CH2OH O**

nitrophenyl)propan-2-yl]acetamide

**MF:** C11H12Cl2N2O5; **MWt**: 323 Da

**1. Materials:** Distilled H2O – Chloramphenicol capsules – 2-naphthol – Zn-powder – 1M H2SO4 – 10 N NaOH – Urea.

**2. Glasswares:** 1 Beaker (50 ml) – 1 Vol. flask. (100 ml) – 1 vol. flask (50 ml) - 5 vol. flask

(25 ml) – 1, 2, 3, 4, 5 and 10 ml (pipettes for each group).

**3. Identification** (**principle:** Azo-dye formation with 2–naphthol)

**NO2**

**1) Reduction**

**Zn / H2SO4**

**o**

**2) Diazotisation**

**NH2 NaNO / H SO N N.HSO4**

**2 2 4**

**Chloramphenicol 1 ry-aromatic amine**

**(HNO2, 0 oC)**

**Diazonium salt**

**HO**

**Red Azo-dye**

**or yellow in case of phenol N N**

**instead of 2-naphthol**

**3) Diazocoupling**

**2-Naphthol / NaOH**

**Procedure**

- Dissolve 10 mg chloramphenicol in 2 ml (50% EtOH).

- Add 4.5 ml (1M H2SO4) and Zn-powder (50mg), then shake well and allow to stand 10 min.

- Decant or filter, then cool in ice bath and add 0.5 ml NaNO2 (1% in H2O)**.**

- After 2 min. add 1g urea, then 1 ml 2-naphthol and 2 ml NaOH (10 M).

- A red dye will be formed soonly.

**Note:**

- If this reaction was repeated without Zn powder, no dye will be produced because the formation of the primary aromatic amine is necessary.

- Also, cooling of reaction-2 at 0 oC is necessary because HNO2 which will be formed from the reaction of NaNO2 with H2SO4 is very unstable above 5 oC.

**4. Determination of A1%** and/or ξ **(**Mathematically and graphically**) Basis: UV-spectrophotometry**

**Procedure**

- **Soln. A**:

**Chloramphenicol powder**: Dissolve 0.050 g powder (or equivalent amount in case of capsules) with 70 ml warm dist. H2O by strong shaking in 100 ml volumetric flask, then complete into 100 ml with H2O.

- **Soln. B**:

Dilute 10 ml from soln. A with dist. H2O in a 50 ml vol. flask.

- **Standard serial dil. Solns.**: Dilute individually 1, 2, 3, 4 and 5 ml from soln. B with dist.

H2O in five 25 ml vol. flasks to form solns., 1 – 5.

- Measure A278 for each standard at λmax: 278 nm using UV-spectrophotometer and report the results according to the following report.

**Report**

- **Calculation:**

**Soln. A**: Its conc. is 0.05 g/100 ml or = 0.05 %.

**Soln. B**: Its conc.; according to the eq. CA X VA = CB X VB

**Where** CA = 0.05 %; VA = 10 ml; VB = 50 ml**;** then CB = 0.01 %.

**Soln. 1**: CB X VB = C1 X V1

C1 = (CB X VB)/V1 = (0.01 X 1) / 25 = ……… X 10 4 %

**Complete the following table:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **S. No.** | **C X 10 4**  **(%)** | **A278** | **A1%** | **C X 10 5**  **(M)** | ξ **X 105** |
| **1** |  |  |  |  |  |
| **2** |  |  |  |  |  |
| **3** |  |  |  |  |  |
| **4** |  |  |  |  |  |
| **5** |  |  |  |  |  |
| **Average** |  |  |  |  |  |

**Draw the calibration curve** (A278 vs. C) and determine **A1%** = slope according to Beer- Lambert's law A = A1% X C ………. i. e. A1% = A / C …………… (Mathematically, use

averages)

 A1% = ………… / ………… = ………….

**From Graph:**

A1% = (A1 – A2) / (C1 – C2) = ---------------------------- = ………….. (Graphically)

**From the previous table:**

 average (C)practical = average (A) / A1% = **…………** / 298 = ………….. %

 % purity = [average (C)practical / average (C)theoretical] X 100 = ………….. %

**Quz:** Graphically, determine ξ from the calibration curve (A278 vs. C, in M) ?

**Lab. 9**

***Analysis of Calcium lactate tablets***

**Calcium lactate** is used in foods (a[s a baking powde](http://en.wikipedia.org/wiki/Baking_powder)r) and given medicinally as an [antacid](http://en.wikipedia.org/wiki/Antacid) and also to treat [calcium deficiencies.](http://en.wikipedia.org/wiki/Hypocalcaemia)

**Chemical Structure**

**MF**: C6H10CaO6**.** 5H2O

**COO HO H**

**CH3**

**O**

**2+**

**Ca . 5 H2O**

**2 O**

**OH**

**O 2+**

Ca

**O**

**OH**

**MWt**.: 308 Da

**Chemical name:** Calcium 2-hydroxypropanoate pentahydrate

**Purity:** 95 – 105 %

**1. Materials:** Ca-lactate tablets – 0.05 M disodium salt of EDTA – 1 M H2SO4 – KMnO4 (1%)

– Conc. HCl – Pt-wire - Hydroxynaphthol blue as indicator.

**2. Glasswares:** 1 Beaker (25 ml) – 1 Con. flask. (25 ml) - 5 cm funnel - 1 Con. flask. (250 ml)

– 1 burette (50 ml).

**3. Identification (Oxid-Red Reaction)**:

**A)** Warm 0.25 g of Ca-lactate powder in 5 ml dist. H2O for 10 mins., acidify, then filter and add few drops KMnO4.

- **Detection**: odour of acetaldehyde then vinegar and decolourization of KMnO4 soln. due to oxidation cleavage of Lactate with permanganate as oxidizing agent.

**B)** Moist 0.1 g of Ca-lactate powder with HCl and introduce it (on a Pt-wire) into Bunsen burner flame.

- **Detection**: Reduction into Brick-red colour.

**4. Assay** (Purity or % Ca-lactate in the sample)

**Basis:**  Complexometric titration *vs*. 0.05 M EDTA-disodium salt and hydroxynaphthol blue as indicator.

**Procedure**

- Dissolve an amount (≡ 0.35 g Ca-lactate powder) with 150 ml dist. H2O and 2 ml HCl (3 M)

in a 250 ml conical flask by stirring well (3 – 5 mins).

- Add 15 ml EDTADS salt from pre-adjusted burette (50 ml), then 15 ml of NaOH soln. (1

M).

- Mix well and add few crystals of indicator (pink colour), then continue the titration *vs*. 0.05

M EDTADS up to the end point (pink colour → blue).

- Determine the E. P. Volume (V)E.P and calculate % active Ca-lactate according to the following report:

- **Calculation:**

**Report**

Where the following eq. describes the complexation reaction with EDTADS

**Ca2+ + [H2X]2 → [CaX]2 + 2H+**

**So, …………………** 1 mol Ca-lactate ≡ 1 mol of **EDTADS**



|  |  |  |  |
| --- | --- | --- | --- |
| ……….. | g | ≡ | 1000 ml . 1 M |
| ……….. | g | ≡ | 1 ml . 1 M |
| ……….. | g | ≡ | 1 ml . 0.5 M |





 0.0154 g ≡ 1 ml . 0.05 M

and where (X)g ≡ (V)E.P ml

 (X) g = 0.0154 X (V)E.P = …………….. g

% Purity = (X / 0.35) X 100 = (……. / 0.35) X 100 = ………. %

**Note :** The theoretical E. P. Vol. that will be expected to react with 0.35 g of Ca-lactate

 0.0154 g ≡ 1 ml . 0.05 M,  0.35 g ≡ Expected (V) ml . 0.05 M Then, Expected (V) ml equivalent = (0.35 X 1) / 0.0154 = ……….. ml.

**Quiz-1:** Define complexometric titration **? Quiz-2:** What is the adequate identifying agent of Ca- lactate **? Quiz-3:** Comment on decolorization of KMnO4 soln. when added to Ca-lactate soln. **?**

**Quiz-4:** How many chiral C-atoms and optical isomers expected in Ca-lactate ?

**CH2**

**CH2COO. Na**

**N**

**CH2COOH**

**Ca2+**

**CH2 N**

**CH2COO. Na**

**CH2COO CH2COO**

**Ca2+**

**+ 2H+**

**CH2 N**

**CH2COOH**

**CH2COO. Na**

**CH2 N**

**CH2COO. Na**

**EDTADS Complex formation**

**Lab. 10**

***Analysis of Inderal (propranolol hydrochloride) tablets***

**Propranolol** is a [sympatholytic](http://en.wikipedia.org/wiki/Sympatholytic) non-selective [beta blocker.](http://en.wikipedia.org/wiki/Beta_blocker) Sympatholytics are used to treat hypertension, anxiety and panic. It was the first successful beta blocker developed. Propranolol is available in generic form as propranolol hydrochloride, as well as under the brand names Inderal, , Avlocardyl, Deralin, Dociton, Inderalici, InnoPran XL, Sumial, Anaprilinum, Bedranol SR.

**Chemical Structure**

**O NH**

**OH H Cl**

**C16H21NO2.HCl**

- **A1%**: 210

- **λmax:** 290 nm

- **Chemical name:**

(±)-1-[(1-methylethyl)amino]-3-(1- naphthalenyloxy)-2-Propanol hydrochloride

- **Purity:** 92.5 – 107.5 %

- **Content** = 19.5 % Inderal

**1. Materials:** MeOH – Inderal tablets or powder.

**2. Glasswares:** 1 Beaker (50 ml) – 2 Vol. flask. (50 ml) –1 pipette (5 ml).

**3. Assay of inderal** (purity and % in tablet).

**Basis: UV-spectrophotometry**

**Procedure**

- **Soln. A**:

Dissolve 51.25 mg powder ( ≡ 10 mg inderal) by strong shaking for 10 mins. With 30 ml

MeOH, then complete into 50 ml with MeOH in volumetric flask (50 ml).

- **Soln. B**:

Transfer 5 ml from soln. A to a vol. flask (50 ml), then completing it with MeOH.

- Measure A290 for soln. B at λmax: 290 nm using UV-spectrophotometer.

- Calculate purity and % of inderal in tablet using Beer-Lambert's law according to the following report.

**Report**

- **Calculation:**

 Total wt. of 1 Tablet = 205 mg ≡ 40 mg inderal. →→→ (content = 19.5 % Inderal)  Total powder of 51.25 mg ≡ 10 mg inderal.

**Soln. A**: is 10 mg /50 ml i.e. 20 mg/100 ml or ………… g /100 ml, then its conc. CA = ………………. %

**Soln. B**:  CA X VA = CB X VB …………. X ………… = CB X …………

CB = (CA X VA)/VB = (………… X ………) / 50 = ……… X 10 3 % → (Theoretical).

**From Beer-Lambert's law:**

 A = A1% X C (CB)practical = A290 / A1% = **…………** / 210 = ………….. %

 **% purity** = **[**(CB)practical / (CB)theoretical] X 100 = ………….. %

**For calculation of % inderal in Tablet:**

 51.25 mg powder dissolved in 50 ml (soln. A) i.e. 102.5 mg /100 ml

 The total (CA)theoretical = 0.102 g / 100 ml = ………….. %

 The total (CB)theoretical = (CA)theoretical / 10 = ………….. %

 **% of inderal in tablet** = **[**(CB)practical / total (CB)theoretical] X 100 = ………….. %

**Quiz-1:** Is inderal optical active drug ? How many optical isomers expected in inderal ?

**Lab. 11**

***Analysis of Penicillamine capsules***

Penicillamine is used as a form of [immunosuppression](http://en.wikipedia.org/wiki/Immunosuppression) to treat [rheumatoid arthritis](http://en.wikipedia.org/wiki/Rheumatoid_arthritis).

**Chemical Structure**

**HS O**

**4 1**

**3 2 OH**

**H2N**

**MF**: C5H11NO2S

**MWt**.: 149.212 Da

**Chemical name:** (2*S*)-2-amino-3-methyl-3- sulfanyl-butanoic acid

**Purity:** 95 – 100.5 %

**Content:** 1 capsule contains 150 mg. (total wt.)

***R*- or *l*-isomer (toxic) & *S*- or *d*-isomer**

**(active)**

**1. Materials:** Penicillamine capsules – Phosphotungestic acid – 0.02 M Hg(NO3)2 – 1M NaOH – 1% ethanolic Dithiazone as indicator.

**2. Glasswares:** 1 Beaker (25 ml) – 1 Con. flask. (25 ml) - 5 cm funnel - 1 Con. flask. (250 ml)

– 1 burette (50 ml).

**3. Identification**:

Dissolve 20 mg of penicillamine powder in 5 ml dist. H2O by strong shaking, then filter and add 2 ml phosphotungestic acid and allow it to stand for 2 min.

- **Detection**: formation of  ***deep blue colour***.

**4. Assay** (Purity or % penicillamine in the sample)

**Basis:** Complexometric titration *vs*. 0.02 M Hg(NO3)2 and Dithiazone as indicator.

**Procedure**

- Dissolve an amount (≡ 0.1 g penicillamine powder) with 50 ml dist. H2O and 20 ml of

NaOH (1 M) in a 250 ml conical flask by strong shaking or stirring for about 10 mins.

- Add 0.2 ml or few drops of dithiazone indicator to produce clear yellowish-orange colour.

- Mix well, then titrate *vs*. 0.02 M Hg(NO3)2 up to the end point (Violet colour).

- Determine the E. P. Volume (V)E.P and calculate % active penicillamine in the sample according the following report:

- **Calculation:**

**Report**

Where the following eq. describes the complexation reaction with Hg(NO3)2

**SH**

**O**

**HS**

**2+**

**H**

**g**

**2 NH2**

**O**

**OH**

**O H2N**

**H2N O**

**O**

**Hg2+**

**+ 2H+**

**Penicillamine**

**SH Complex formation**

**So, …………………** 2 mol penicillamine ≡ 1 mol of Hg(NO3)2



|  |  |  |
| --- | --- | --- |
| 2 X ……….. g | ≡ | 1000 ml . 1 M |
| ……….…… g | ≡ | 1000 ml . 0.5 M |
| ……….……..g | ≡ | 1 ml . 0.5 M |
| ……….……..g | ≡ | 1 ml . 0.1 M |
| 0.00596 g | ≡ | 1 ml . 0.02 M |









and where (X)g ≡ (V)E.P ml

 (X) g = 0.00596 X (V)E.P = …………….. g

% Purity = (X / 0.1) X 100 = (……. / 0.1) X 100 = ………. %

**Note - 1**: The theoretical E. P. Vol. that will be expected to react with 0.1 g of penicillamine  0.00596 g ≡ 1 ml . 0.02 M titrant,  0.1 g ≡ Expected (V) ml . 0.02 M Then, Expected (V) ml equivalent = (0.1 X 1) / 0.00596 = ……….. ml.

**Quiz-1:** Is the active ampicilline S or R-isomer **?**

**Quiz-2:** What is the adequate identifying agent of penicillamine **?**

**Quiz-3:** How many chiral C-atoms and optical isomers expected in penicillamine ?

**Lab. 12**

***Analysis of Warfarin (as Sodium salt) tablets***

**Warfarin** (also known under the brand names **Coumadin**, **Jantoven**, **Marevan**, **Lawarin**, **Waran**, and **Warfant**) is an [anticoagulant](http://en.wikipedia.org/wiki/Anticoagulant) normally used in the prevention of [thrombosis](http://en.wikipedia.org/wiki/Thrombosis) and [thromboembolism](http://en.wikipedia.org/wiki/Thromboembolism), the formation of blood clots in the blood vessels and their migration elsewhere

in the body respectively.

**Chemical Structure**

**O**

- **A1%**: 405

- **λmax:** 308 nm

- **Chemical name:**

**OH**

(±)- or (*RS*)-4-hydroxy-3-(3-oxo-1-phenylbutyl)-

2*H*-chromen-2-one or coumarin

**O O** - **Purity:** 92.5 – 107.5 %

- **Content** = 0.455 % warfarin sod

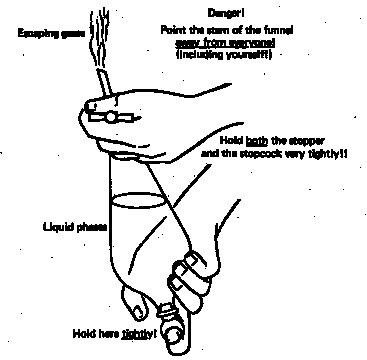
**MF:** C19H16O4; **MWt**: 308.33 Da or g/mol

**1. Materials:** Distilled H2O – warfarin tablets – 0.01 M NaOH – conc. HCl – CHCl3.

**2. Glasswares:** 1 Beaker (50 ml) – 1 Vol. flask. (25 ml) – 1 Vol. flask (100 ml) – 5 cm funnel

– 1 measuring cylinder (20-25 cm) - 1 pipette (20 ml) - 1 separatory funnel (50 ml).

**3. Assay of warfarin** (purity and % in tablet).

**Basis:** UV-spectrophotometry and **liquid-liquid** extraction technique for purification.



**Procedure**

**Soln. A**

- Dissolve 440 mg powder ( ≡ 2 mg warfarin) by strong shaking for 15 mins. with 25 ml 0.01

M NaOH in a volumetric flask (25 ml). → → → → → → → → (A).

**Soln. B**

- Filter soln. A and transfer 20 ml from the filtrate into the separatory funnel using pipette.

- Add 0.15 ml conc. HCl to obtain free warfarin, then extract with 3 x 15 ml CHCl3 portions.

- Extract the collected 45 ml three CHCl3 layers (contain free warfarin) with 3 x 20 ml 0.01 M NaOH using the separatory funnel once again to obtain warfarin sodium salt.

- Collect the aqueous layers (60 ml) in a vol. flask (100 ml) and complete it into 100 ml with

0.01 M NaOH. → → → → → → → → (B).

- Measure A308 for soln. B at λmax: 308 nm using UV-spectrophotometer.

- Calculate purity and % of warfarin in tablet using Beer-Lambert's law according to the following report.

**Report**

- **Calculation:**

 Total wt. of 1 Tablet = 220 mg ≡ 1 mg warfarin. →→→ (content = 0.455 %

warfarin)

 Total powder of 440 mg ≡ 2 mg warfarin.

**Soln. A**: is 2 mg (total 440 mg) /25 ml *i.e.* 8 mg (total 1760 mg) /100 ml, Then its theoretical concentration is:

 (CA)warfarin = ………………. % warfarin & (CA)total = ………………. %

**Soln. B**: 20 ml from A diluted into 100 ml (the concentration was reduced by factor = 5)

so the equation CA X VA = CB X VB can be used or:

(CB)warf = (CA)warf / 5 = ……………… X 10 4 % & (CB)total = (CA)total / 5 =

…………… X 10 4 %

**From Beer-Lambert's law:**

 A = A1% X C (CB)practical = A308 / A1% = **…………** / 405 = ………….... X 10 4 %

 **% purity** = **[**(CB)practical / (CB)warf] X 100 = ………….. %

**For calculation of % warfarin in Tablet:**

 **% of warfarin in tablet** = **[**(CB)practical / (CB)total] X 100

= **(………………..** / ……………….) X 100 = ………….. %

**Note:**

The extraction process aimed at isolation and purification of warfarin from any polar or non-polar additive substances present in the tablet for good formulation.

**Quiz-1:** Is warfarin optical active drug ? How many optical isomers are expected ?

**Quiz-2:** Write down the different methods to get red off emulsion if produced during extraction ?

**Lab. 13**

***Analysis of Kemistine (2% chloramphenicol) or Riachol (1%) ointment***

**Chemical Structure**

See Lab. 8

- **A1%**: 298

- **λmax:** 278 nm

- **Chemical name:** See Lab. 8

**1. Materials:** Distilled H2O – Riachol ointment – Benzene.

**2. Glasswares:** 1 Dry beaker (100 ml) – 1 Vol. flask. (200 ml) – 1 Vol. flask (50 ml) – 5 cm funnel – 1 measuring cylinder (20-25 cm) - 1 pipette (10 ml) - 1 separatory funnel (250 ml).

**3. Assay of chloramphenicol** (purity and % in ointment).

**Basis:** UV-spectrophotometry and **liquid-liquid** extraction technique for purification.

**Procedure**

**Soln. A**:

- Dissolve 1000 mg Riachol ( ≡ 10 mg chloramphenicol) by strong stirring with 40 ml benzene in a dry beaker (100 ml).

- Transfer that soln. to the separatory funnel (150-250 ml).

- Wash the beaker with 10 ml benzene portion and add it to the separatory funnel.

- Extract with 3 x 50 and then for once with 30 ml portions warm water to collect 180 ml aqueous layer containing chloroamphenicol.

- Transfer the collected portions to a 200 ml vol. flask and complete with water up to 200 ml to form soln. A.

**Soln. B**:

- Filter soln. A and discard first 20 ml filtrate, then transfer 10 ml from the filtrate to a vol. flask (50 ml).

- Complete into 50 ml with water to produce soln. B.

- Measure A270 for soln. B at λmax: 270 nm using UV-spectrophotometer.

- Calculate purity and % of chloramphenicol in ointment using Beer-Lambert's law according to the following report.

**Report**

- **Calculation:**

 Total wt. of ointment = 1000 mg ≡ 10 mg chloramphenicol. →→→ (content = 1 %

chloramphenicol).

So, theoretical concentration can be calculated as following:

**Soln. A**: is 10 mg (total 1000 mg) /200 ml *i.e.* ……….. mg (total …………. mg) /100 ml, then

 (CA)chloramphenicol = ………………. % & (CA)total = ………………. %

**Soln. B**: 10 ml from A diluted into 50 ml (the concentration was reduced by factor = 5)

so the equation CA X VA = CB X VB can be used or:

(CB)chloramphenicol = (CA)chl / 5 = ……………… % & (CB)total = (CA)total / 5 =

…………… %

**From Beer-Lambert's law:**

 A = A1% X C (CB)practical = A270 / A1% = **…………** / 298 = …………....

%

 **% purity** = **[**(CB)practical / (CB)chloramphenicol] X 100

= (…………….. / …………….) X 100 = ………….….. %

**For calculation of % chloramphenicol in Riachol ointment:**

 **% of chloramphenicol in ointment** = **[**(CB)practical / (CB)total] X 100

= **(………………..** / ……………….) X 100 = ………….. %

**Note:**

In case of Kemistine ointment the same procedure and calculation will be used starting with 0.5 g instead of 1 g used in case of Riachol.

**Lab. 14**

***Analysis of Methyldopa tablets***

**Methyldopa** is an [alpha-adrenergic agonist](http://en.wikipedia.org/wiki/Alpha-adrenergic_agonist) (selective for α2-adrenergic [rece](http://en.wikipedia.org/wiki/Receptor_(biochemistry))ptors) [psychoactive drug](http://en.wikipedia.org/wiki/Psychoactive_drug) used as a [sympatholytic](http://en.wikipedia.org/wiki/Sympatholytic) or [antihypertensive.](http://en.wikipedia.org/wiki/Antihypertensive) Its use is now mostly deprecated following the introduction of alternative safer classes of agents. However, it continues to have a role in otherwise difficult to treat [hypertension](http://en.wikipedia.org/wiki/Hypertension) and [gestational hypertension](http://en.wikipedia.org/wiki/Gestational_hypertension) (also known as pregnancy-induced

hypertension**.**

**Chemical Structure**



**MF:** C10H13NO4; **MWt**: 211.215 Da or g/mol

- **A1%**: 89

- **λmax:** 550 nm

- **Chemical name:**

(*S*)-2-amino-3-(3,4-dihydroxyphenyl)-2-methyl- propanoic acid

- **Purity:** 95 – 105 %

**1. Materials:** Distilled H2O – methyldopa tablets – 5 M NaOH - 0.01 M HCl – 0.05 M H2SO4

– 1% FeCl3 – 5 M Ammonia soln. – glycine buffer – Ferrous sulphate citrate - .

**2. Glasswares:** 1 Beaker (50 ml) – 2 test tubes - 2 Vol. flask. (100 ml) – 1 measuring cylinder

(20-25 cm) - 1 pipette (20 ml) – 1 funnel (5 cm).

**3. Identification**:

- Add 5 ml (0.4 % MDOPA in 0.1 M HCl) to 0.1 ml FeCl3, divide it into two eq. volumes in two test tubes.

- Add to the first tube excess amount



5 M ammonia

(**Purple colour will be produced**).

- Add to the second tube excess amount

5 M NaOH.

(**Red colour will be produced**).

**4. Assay of MDOPA** (purity and % in tablet).

**Basis:** UV-spectrophotometry.

**1) Ammonia 2) NaOH**

**Procedure**

**Soln. A**:

- Dissolve 100 mg powder (≡ 100 mg MDOPA) by shaking for 15 mins. with 70 ml 0.05 M H2SO4 in a volumetric flask (100 ml), then complete to 100 ml and filter → → → → → (A).

**Soln. B**:

- Add 2 ml ferrous sulphate citrate and 8 ml glycine buffer to 5 ml from the soln. A, then

complete to 100 ml in vol. flask with dist. water → → → → → (B).

- Measure A for soln. B at λmax: 550 nm using UV-spectrophotometer.

- Calculate purity and % of MDOPA in tablet using Beer-Lambert's law according to the following report.

**Report**

- **Calculation:**

**Soln. A**: is 100 mg MDOPA /100 ml,

Then its theoretical concentration is:

 (CA)MDOPA = ………………. %

**Soln. B**: 5 ml from A diluted into 100 ml (the concentration was reduced by 20 factor)

so the equation CA X VA = CB X VB can be used or: (CB)MDOPA = (CA)MDOPA / 20 = ……………… %

**From Beer-Lambert's law:**

 A = A1% X C (CB)practical = A550 / A1% = **………..…** / 89 = ………….... %

 **% purity** = **[**(CB)practical / (CB)MDOPA] X 100

= (………………… / …………….) X 100 = ………….. %

**Quiz-1:** Is MDOPA optical active drug ? How many optical isomers are expected ?

**Further useful readings**

**A- Use of a separatory funnel or "How to do an upscale extraction"**

A separatory funnel is a standard piece of equipment in synthetic chemistry.



It generally consists of a conical or pear-shaped glass body with a stopcock and a stopper on top. The shape allows for the clean separation of the two layer because of a small interface on the low end. Although it looks very easy to use, there are some important points that have to be considered.

**1. Stopper**

The stopper on top can be made from glass or Teflon. It is imperative that it fits tightly, so that the

solution does not leak out when the separatory funnel is inverted. If a ground glass joint does not fit perfectly, a *minute* amount of grease is applied to the upper part of the joint to get a better seal.

The stopper has to be removed when draining the lower layer. If the stopper were not removed, a vacuum will build up above the liquid part and prevent the solution from draining. After some time, the vacuum will suck air in (from the stem) and the phases will mix again.

**2. Stopcock plug**

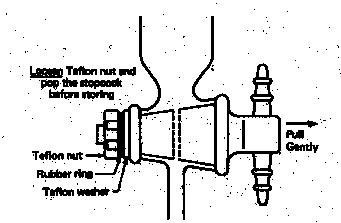
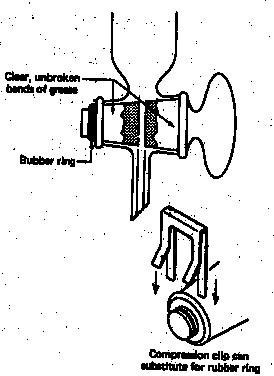
The stopcock plugs can be made from glass (left side) or Teflon (right side). It is important to have

a good seal here as well. Again, a very *small* amount of grease can be applied to the glass plugs (shown on the left) to improve the seal and allow for better movement of the plug. **The grease should be used sparingly, because it will clog up the hole in the plug!**

The glass plug has to be held in place with a metal clip. Teflon plugs usually possess a thread (some glass manufacturers offer those version for glass plugs as well), which allows to place a nut on it to hold it in place. Teflon plugs should not be lubricated!

In order to find out which plug is needed, the glass joint has to be examined. Glass stopcocks

require a ground glass joint (looks milky if clean and is rough on the inside). Teflon stopcocks use a polished joint (clear). **Do not attempt to fit a glass stopcock into a teflon joint!** The slopes on the plugs are slightly different and the joint will break!



The hole of the plug has to be open and match up with the holes in the stem and separatory funnel.

**3. Before you start**

Perform the following tests before you start. Suspend the separatory funnel in an iron ring (make

sure it does not fall through!).While the stopcock is closed, pour ~20 mL of acetone into the separatory funnel (with a short stem funnel). Check if the solvent leaks out at the stopcock. If this does not occur, place the stopper on top and invert the funnel. Does the acetone leak out now? If

not, place the funnel back in the ring stand and remove the stopper. Open the stopcock and drain the solvent. If the solvent does not drain, you probably used too much grease to lubricate the stopcock. Pour the solvent out and remove the stopcock plug and check the hole. If it is clogged, use a

wooden applicator or piece of wire to open it up. Put it back into the stopcock, secure it and start over with the tests. It is better to solve problems in the beginning and not with your product solution in the funnel later on.

**4. Performing an extraction**

a. Place the separatory funnel in an iron ring. Remove the stopper and make sure that the stopcock

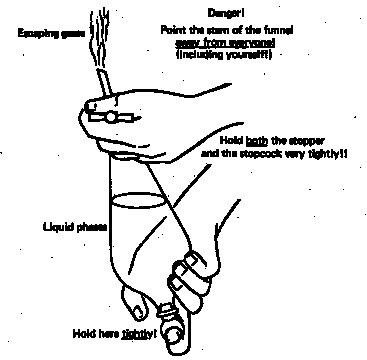
is closed.

b. Add the solution to be extracted. Do not fill the funnel more than half at this point. Add the washing/extraction solution and place the stopper on top. There should still be some room afterwards.

c. Take the separatory funnel out of the ring and hold it tightly at the stopper and the stopcock. Invert it slowly and vent (open the stopcock) towards the back of the hood. You will hear a kind of whistle when the pressure is released.

d. Close the stopcock and shake the funnel gently, watching out for emulsions. Vent it again. Repeat this step until no more gas escapes.

e. Place the separatory funnel back in the iron ring. Allow the layers to separate. Then remove the stopper and drain the bottom layer into a clean container. At this point, you need to know which layer contains your desired product.



**5. Warnings**

If you extract or wash acidic solutions with sodium carbonate or sodium bicarbonate solutions,

carbon dioxide will form due to an acid-base reaction. A significant pressure will build up in the funnel. Hence, you need to be more careful in the beginning and vent more often.

If you use low boiling solvents like diethyl ether, pentane, dichloromethane, chloroform, etc. for extraction, you will observe a significant build up of pressure as well.

Due to the pressure build up, you will need to hold on to the stopper and stopcock very tightly. It might be a good idea to wrap a paper towel around the stopper and joint.

When you vent the funnel, point the stem of the funnel away from everybody, so that the solvent and gases released are not blown into somebody's face. The best protocol is to vent it in the back of a hood.

**Never throw any layer away, until you are absolutely sure that you isolated your final product.** It is easier to isolate it from a small amount of solution than from the waste container.

If you are not sure which layer is organic and which one is aqueous, take a small sample of both layers and add some water, Which layer did increase in volume? Most [common organic solvents](http://www.chem.ucla.edu/%7Ebacher/General/30BL/tips/solvent.html) possess a lower density than water. However, halogenated solvents like dichloromethane, chloroform or carbon tetrachloride are significantly heavier than water (or most diluted aqueous solutions).

**B- Types of titration**

**Titration**, also known as **titrimetry**, is a common laboratory method of [quantitative](http://en.wikipedia.org/wiki/Quantitative_research) [chemical analysis](http://en.wikipedia.org/wiki/Analytical_chemistry) that is used to determine the unknown [concentration](http://en.wikipedia.org/wiki/Concentration) of an identified [analyte.](http://en.wikipedia.org/wiki/Analyte) Because [volume](http://en.wikipedia.org/wiki/Volume) measurements play a key role in titration, it is also known as **volumetric analysis**. A [reagent,](http://en.wikipedia.org/wiki/Reagent) called the *titrant* or *titrator* is prepared as a [standard solution.](http://en.wikipedia.org/wiki/Standard_solution) A known concentration and volume of titrant reacts with a solution of analyte or *titrand* to determine concentration.

There are many types of titrations with different procedures and goals. The most common types of qualitative titration are [acid-base titrations](http://en.wikipedia.org/wiki/Acid-base_titration) and [redox titrations](http://en.wikipedia.org/wiki/Redox_titration).

**Acid-base titration**

Acid-base titrations depend on the [neutralization](http://en.wikipedia.org/wiki/Neutralization_(chemistry)) between an acid and a base when mixed in solution. In

addition to the sample, an appropriate [indicator](http://en.wikipedia.org/wiki/PH_indicator) is added to the titration chamber, reflecting the pH range of the equivalence point. The acid-base indicator indicates the endpoint of the titration by changing color. The endpoint and the equivalence point are not exactly the same because the equivalence point is determined by the stoichiometry of the reaction while the endpoint is just the color change from the indicator.

**Redox titration**

Redox titrations are based on a [reduction-oxidation reaction](http://en.wikipedia.org/wiki/Redox) between an oxidizing agent and a

reducing agent. A [potentiometer](http://en.wikipedia.org/wiki/Potentiometer) or a [redox indicator](http://en.wikipedia.org/wiki/Redox_indicator) is usually used to determine the endpoint of the titration, as when one of the constituents is the oxidizing agent**.**

**Complexometric titration**

Complexometric titrations rely on the formation of a [complex](http://en.wikipedia.org/wiki/Complex_(chemistry)) between the analyte and the titrant. In general,

they require specialized [indicators](http://en.wikipedia.org/wiki/Complexometric_indicator) that form weak complexes with the analyte. Common examples are [Eriochrome Black T](http://en.wikipedia.org/wiki/Eriochrome_Black_T) for the titration of [calcium](http://en.wikipedia.org/wiki/Calcium) and [magnesium](http://en.wikipedia.org/wiki/Magnesium) ions, and the [chelating agent](http://en.wikipedia.org/wiki/Chelating_agent) [EDTA](http://en.wikipedia.org/wiki/EDTA) used to titrate metal ions in solution.

**Endpoint and equivalence point**

Though equivalence point and endpoint are used interchangeably, they are different terms. *Equivalence point*

is the theoretical completion of the reaction: the volume of added titrant at which the number of [moles](http://en.wikipedia.org/wiki/Mole_(unit)) of titrant is equal to the number of moles of analyte, or some multiple thereof (as in [polyprotic](http://en.wikipedia.org/wiki/Polyprotic) acids). *Endpoint* is what is actually measured, a physical change in the solution as determined by an [indicator](http://en.wikipedia.org/wiki/PH_indicator) or an

instrument mentioned above.

There is a slight difference between the endpoint and the equivalence point of the titration. This error is referred to as an indicator error, and it is indeterminate.

**Back titration**

Back titration is a titration done in reverse; instead of titrating the original sample, a known excess of

standard reagent is added to the solution, and the excess is titrated. A back titration is useful if the endpoint of the reverse titration is easier to identify than the endpoint of the normal titration, as with [precipitation](http://en.wikipedia.org/wiki/Precipitation_(chemistry)) reactions. Back titrations are also useful if the reaction between the analyte and the titrant is very slow, or when the analyte is in a non-[soluble](http://en.wikipedia.org/wiki/Solubility) solid.

***Student Name*:** ……………………………………… ***Univ. ID*:** *…………………………………………………* ***Group No.***: …………………………………………… ***Time***: *……………………………………………………..*