**Guru Ghasidas Vishwavidyalaya, Bilaspur**

# B. Pharm. Sem-II

# Pharmaceutical Analysis-II

# MODEL ANSWER

**NOTE: Section A is compulsory. Attempt any four questions from Section B.**

**Section A: Multiple Choice Questions: All questions are compulsory.** **12x2 = 24**

**Answer to Section A has been highlighted with yellow color and bold faced letters.**

1.1 The example of bulk property detector used in HPLC is
**(a) Refractive index detector** (b) UV detector (c) fluorescence detector (d) UV-visible detector

1.2 In-vitro hydrolysis studies of drugs & kinetic studies of reaction can be performed by
(a) Polarimetry (b) Refractometry (c) Potentiometry **(d) Conductometry**

1.3 The alkenes and aromatic compounds can be suitably detected using
(a) Refractive index detector (b) conductivity detector
**(c) Spectrophotometric detector** (d) Potentiometric detector

1.4 Formic acid is an example of
**(a) protogenic solvent** (b) protophillic solvent (c) amphiprotic solvent (d) Aprotic solvent

1.5 The relationship between concentration, temperature & potential of a solution is given by
(a) Ilkovic equation (b) Henderson equation **(c) Nernst equation** (d) Hassalbach equation

1.6 In refractometric analysis, if temperature is increased by 1 °C then refractive index decreases by
(a) 0.001 to 0.002 (b) 0.002 to 0.003 (c) 0.003 to 0.004 **(d) 0.004 to 0.005**

1.7 Sucrose can be determined after silylation using which chromatographic technique
(a) HPLC (b) Gel chromatography **(c) Gas liquid chromatography** (d) Paper chromatography

1.8 The composition of Silica gel G is
(a) silica gel without binder **(b) silica gel + $CaSO_4$** (c) Silica gel + alumina (d) silica gel + $MaSO_4$

1.9 The formula for resolution (R) between peaks in gas chromatography is (where d = distance between peak 1 and 2; $W_1$ and $W_2$ are width of peak 1 and 2, respectively)
**(a) $2d / (W_1+W_2)$** (b) $d / (W_1+W_2)$ (c) $2d / (W_1-W_2)$ (d) $d / (W_1-W_2)$

1.10 Which of the following can not be used as carrier gas in gas chromatography
(a) Hydrogen (b) Nitrogen (c) Helium **(d) oxygen**

1.11 Snells law is related to
**(a) Refractometry** (b)Potentiometry (c) Non-aqueous titrations (d) Chromatography

1.12 Relative flow ($R_f$) value ranges from
**(a) 0 to 1** (b) 0 to 2.0 (c) +2 to -2 (d) +1 to -1

**Section B: Long Questions: Attempt any four questions.** **04x14=56**

**Question 2. Write a complete note on instrumentation and applications of gas chromatography**

**Answer**

**INSTRUMENTATION OF GAS CHROMATOGRAPHY (Figure 1)**

**1. Carrier Gas**: The main purpose of the carrier gas is to transport sample components through the column. For selection of carrier gas, following factors must be considered:

1) It should be chemically inert and should not interact with sample or stationary phase.

2) It should be suitable for detector to be utilized and the type of sample analysis.

3) It should be readily available, cheap and high purity.

4) It should not cause the risk of fire or explosion hazard.

The carrier gases commonly used are hydrogen, helium, argon and nitrogen. For most application with thermal conductivity detector either hydrogen or helium is used. Between hydrogen and helium, the latter should be preferred for safety reasons. Hydrogen is used because it is cheap and when helium is not available. The commonly employed source of carrier gas is a compressed gas cylinder.

**2. Sample Injection System:** The injection port contains a gas tight self –sealing type rubber septum through which the sample is injected by means of syringe. Immediately after injection, the sample has to be vapourised instantaneously. For this purpose injection ports are heated and specially designed. The temperature of sample injection port is kept 20-50˚c above the column temperature. Liquid samples are generally injected in µl quantities (0.1-10 µl) with the help of hypodermic syringe. Gases can also be injected by similar syringe which have gas tight (Teflon tipped) plunger but are of large capacity (1-10ml).

**a) Gas Samples:** Gases are most conveniently introduced by typical hexport gas sampling valve. In these, at specific position, only carrier gas flows through the column. The sample gas can also be injected at the top of the column by means of a hypodermic syringe. The Hamilton Teflon coated gas syringe is particularly suitable.

**b) Liquid sample:** Liquids are most conveniently introduced by means of microsyringe which is of different sizes. Small samples can also be introduced by micropipette.

**c) Solid samples:** solid samples should be made to vapourise as quickly as possible by heating the injection port by means of small coil. Generally samples can be weighed into thin glass ampules, sealed and placed in the gas stream and then crushed in the ancillary tube which is heated by heating coils to vapourise the samples. An alternative method is to dissolve the solid sample in a volatile solvent and injected like liquid sample.

**3. Column technology:** Column is very important unit of GC, which is said to be heart of the system.

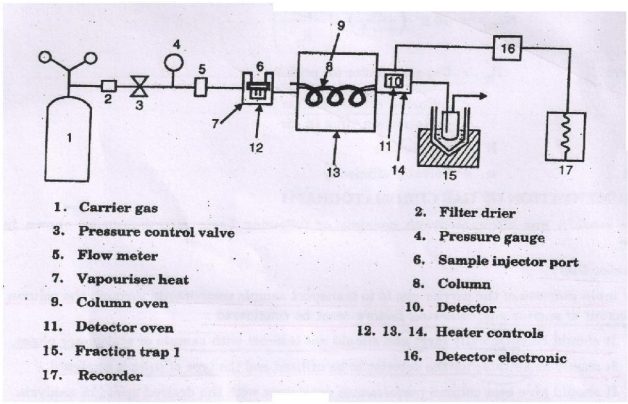


Figure1: Instrumentation of Gas chromatography

**a) The column:** coiled helical shape is most efficient shape but here the problem arises in uniform, even packing. U-tube columns are advantageous due to its short length and easy, even packing. The column may be made up of glass, aluminium, copper, steel. Tubes may be 2-10mm in diameter and from 204 meters in length.

**b) Support medium:** The purpose of solid support is to provide large and inert surface area for holding the liquid phase in thin and uniform film. It should also be chemically inert, heat stable and having

sufficient mechanical strength to prevent fractionating with normal handling. Two types are available namely firebrick and kieselguhr. glass beads, fluorinated resins are also used as support medium.

**c) Liquid phase:**

Liquids may be classified as:

1. Very polar: Glycols, glycerols, amino alcohols.
2. Polar: Alcohols, fatty acids, phenols, primary and secondary amines.
3. Intermediate: Ethers, Ketones, aldehydes, esters.
4. Low polarity: Chloroform, dichloromethane
5. Non Polar: Saturated hydrocarbon.

**d) Preparation of chromatographic columns:**

**1. Packed columns:** These are prepared by packing metal or glass tubing's with granular stationary phase. For gar solid chromatography the columns are packed by coating the liquid phase over an inert solid support.

**2. Open Tubular Columns:** The inside wall of capillary tubing is coated by liquid phase in the form of thin and uniform film. The carrier gas faces least resistance as there is no packing in the column. The sample capacity of this column is very low.

**3. Support coated open tubular columns:** It is made by depositing a micron size porous layer of support material on the inside wall of a capillary column and than coating with a thin film or liquid phase. These columns are of course having higher sample capacity and yield better resolution.

**e) Equilibration of the column:** Before introduction of the samples, complete equilibration or conditioning must be obtained. Conditioning is achieved by passing carrier gas for at least 6 hours or generally 24 hours. A properly conditioned column will show zero base line on the recorder.

**f) Control of column temperature:** A temperature programming is now used where the column is not kept at constant temperature but it is subjected to controlled rise which reduce the retention times of the less volatile samples to be analyzed more rapidly. For this, various methods have been used i.e. vapour jacket, electrically heated air baths and liquid bath or metal block etc.

**4. Detector:**

After the resolution of solutes, each vaporised component emerges in turn from column and is carried into the detector mixed with carrier gas.

a) **Thermal conductivity detectors:** The TCD is based on the fact that the rate of loss of heat from body depends upon thermal conductivity of the surrounding gas. Thus the crate of loss of heat is related to the composition of the surrounding gas. The filaments gets heated due to passage of a small constant current, are quite matching when only a carrier gas is passing in both the cells. As

the sample component enters the sensing cell, the temperature of filaments change due to widely different thermal conductivity of the sample component than that of the carrier gas. TCD filament are made of platinum, tungsten or alloys having large temperature coefficient of resistant and corrosion resistant.

b) **Flame ionization detector:** A tiny flame of hydrogen is maintained at a capillary jet made of quartz or platinum, air or oxygen is introduced through aide by side inlet for supporting the combustion. Column effluents are led into the flame wherein ionization of components may take place. An electrode system located close by picks up the ionization current which is than amplified and fed to the recorder. When only carrier gas passes through the flame, there is no or very small and constant ionization current recorded. When sample component elutes and passes through the flame, its molecules are ionized and the resulting ionization current after amplification is fed to the suitable recorder. A FID is sensitive to almost all the organic compounds but in sensitive to noble gases, oxygen, nitrogen, CO, $CO_2$ , water.

c) **Electron capture detector:** It responds to only those compounds whose molecules have an affinity for electrons e.g. chlorinated compounds, unsaturated compounds etc. On the other side it responds very little to compounds such as hydrocarbons. A tritium foil placed inside the cell, ionizes the carrier gas molecules to form electrons that move slowly toward the anode under a fixed voltage. Thus a standing current is produced which is amplified by an electrometer. When a component having affinity for electrons elutes out of the column and enters the detector, it absorbs some electrons causing drop in standing current.

**5. Recorder:** the signal from a gas chromatograph is continuously recorded as a function of time.

**6. Integrator:** An integrator is employed for simultaneous measurement of areas under chromatographic peaks by mechanical/ electronic means. Manual techniques for measurement of peak area are time consuming, tedious, and are less precise.

**GLC Applications:**

Industrial

1 Agriculture

2 Air pollution

3 Petroleum Industry

4 Fermentation

5 Food Industry

5 Insecticides

6 Cosmetics

7 Pharmaceutical Industry
(Various Categories of drugs)

Biochemical and Medical
1 Estimation of fatty acid ester
2 Steroids, amino acid
3 Estimation of normal metabolite
4 Drug metabolism Studies

Microbiological
1 Identification of Microorganism
2 Characterization of growth product
3 Analysis of body fluids for detection of microorganism

Question 3 Discuss and differentiate between thin layer and paper chromatography.

## Answer

Thin layer chromatography is a simple and rapid method carried out using thin layer of absorbents on plates. The various adsorbents used for the development of plate are Inorganic adsorbent i.e., silica gel G, Alumina, kieselguhr etc; Organic adsorbents i.e., charcoal, starch etc. The stationary phase can be applied by various methods i.e., pouring, dipping, spraying, spreading. The plates are placed in oven for activation followed by placing in chamber saturated with vapors of solvent system. The location of spots on TLC plate is performed by using spraying specific reagents. Various development techniques of TLC are ascending, descending, step-wise/multiple and two dimensional techniques.

TLC not only combines the advantages of paper and column chromatography but in certain aspects it is found to be superior to either method. The advantages are:

i) It requires little equipment.
ii) It requires very little time for separation (less than 1 hour) while in case of column and paper, it requires several hours or days.
iii) It is more sensitive i.e. separation effects are usually superior to those of other methods
iv) The lower detection limit of analytical sample in TLC is approximately one decimal lower than that in paper chromatography and very small quantities of sample is sufficient for analysis.

v) Spraying with corrosive agents for identification is also permitted which is not possible in paper chromatography as cellulose destroyed.

vi) The individual samples do not get diffused as compare to paper chromatography hence sensitivity of detection is more.

vii) The method is used for adsorption, partition, ion exchange chromatography as there is wide range of adsorbents available.

viii) The components which are separated can be recovered easily by scratching the powder coating of plate and quantitative separation of spots or zones are possible.

ix) It is possible to visualize the components for identification by UV light as the inorganic adsorbent background does not fluoresce.

x) This method can be applied to preparative separation with the aid of thicker layers of adsorbents.

**Comparison of TLC and Paper chromatography**

| **TLC chromatography** | **Paper chromatography** |
|---|---|
| It requires less amount of substances | It requires more amount of substances |
| Less time consuming | More time consuming |
| Sharpness of separation is good | Sharpness of separation is less than |
| Capacity of thin layers of adsorbent is higher | Less in paper chromatography |
| Strong acid can be safely identified | It is not possible in paper |
| Corrosive reagents may be coated on glass plates | Also not possible in paper |
| Physical strength is more | Physical strength is less |
| It can be heated in oven | Not possible |
| Sensitivity is more | Sensitivity is less |

Question 4 Explain in detail about principle of Gel chromatography.

## Answer

**Gel chromatography**: This method is mainly based on the differences in molecular dimensions and has different names like gel filtration. Molecular sieve filtration restricted diffusion chromatography, exclusion chromatography, molecular sieve chromatography etc.

Gel chromatography method separates different substances depending on their molecular size. This technique differs from other partition chromatographic techniques.

In this technique the particle which comes from stationary phase in the column are uncharged gels. The gel swills in the same solvent which percolates through the bed.

The stationary phase is a porous polymer matrix where the pores are completely filled with the solvent to be used as the mobile phase. The pore size is very important. The basis of the separation is the

molecules above a certain size are totally excluded from entering and occupying the pores and the interior of the pores is accessible partly or wholly to smaller molecules. The flow of the mobile phase will cause larger molecules to pass through the column unhindered without penetrating the gel matrix. whereas small molecules will be retarded because of their penetration in the gel.

Thus the components of the mixture emerge from the column in order of relative molecular mass, the largest first. Any components which are completely excluded from the gel will not be separated from each other, and similarly small molecules which completely penetrate the gel will not be separated from the gel. The molecules of intermediate size will be retarded to a degree dependent on their penetration of the matrix. If the substances are of similar chemical type, they are eluted in order of relative mass. Absorption effects on the surface of gel particles are ignored and thus gel chromatography may be looked upon as a kind of partition chromatography.

**MECHANISM**

There are three mechanisms which have been proposed to describe the separation process. It is the process in which solute molecules are distributed between two liquid phases.

**(a) Steric exclusion effect**:

In this it is presumed that different fractions of the total pore volume are accessible to different size molecules. This is because the gel particles contain a distribution of pore size. The large molecules get small number of pores into which they enter. Thus small molecules can enter large number of pores.

The steric exclusion effect is more prominent when major particles are larger than many pores of the gel.

**(b) Restricted diffusion mechanism** :

The process is diffusion controlled i. e. there is no diffusional equilibrium. Retention volume will now be affected by changes in flow rates. The absence of diffusional equilibrium is most pronounced at very high linear velocities.

**(c) Secondary exclusion effect** :

If the mixture of large and small molecules is placed on the gel, small molecules diffuse rapidly into the pores of gel and the diffusion of larger molecules in unoccupied pores is reduced.

Thus the larger molecules move further down till they unoccupied pores and results in enhancement of separation.

Question 5 Describe instrumentation and applications of polarimetry.

## Answer

**Instumentation of polarimeter (Figure 2)**

**Polarizer**: used to measure the angle of rotation of plane polarized light.

**Polarimeter:**

1) **Light source:-** The most common light sources are

i) Sodium vapour lamp- Emit light of wavelength 5890 Å and 5896 Å.

ii) Mercury vapour lamp- Emit light of wavelength 4368, 4916, 5461, 5770 Å and 5791 Å each of these lines can be isolated by proper use of filter.

2) **Polarizer & analyzer**: both are same & composed of calcite or quartz

- Different varieties of polarizing & analyzer are available but most common are glan-Thompson prism and nicol prism
- Polarizer is fixed while analyzer can be rotated about the axial of instrument.

3) **Sample tubes-** these are cylindrical tubes (usually 5-25cm in length)
- For temp control they are surrounded by jacket.

4) **Detectors**: eye piece serve as detector in polarimeter

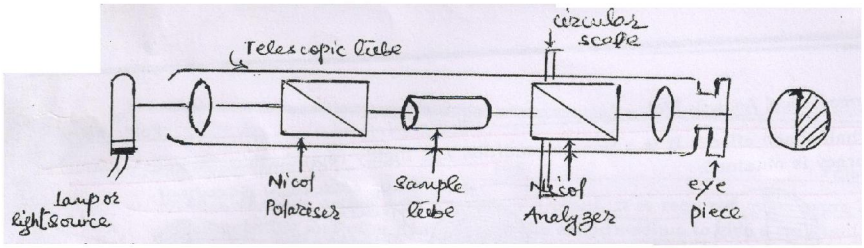


**Figure 2 : Instrumentation of Polarimeter**

**Working:** Analyzer can be used to set up zero position which is view in eye piece as half bright & half dark.

- Set zero & record reading on circular scale.
- Sample is placed in sample tube.
- On looking through the telescope of the polarimeter, the fields appear uniform bright since optically active substance rotated the plane of polarized light through a certain angle. Now analyzer is rotated to set zero condition and reading is noted from circular scale.
- The difference between second reading & first reading gives the angle through which plane of polarized light has been rotated by the optically active substance.

**Application of polarimetry :**

The application of polarimetry may broadly be classified as Qualitative applications and quantitative applications.

**Quantitative application:** Specific rotation is of importance in such application

1) If the specific rotation of a sample is known its concentration in the solution can be estimated.
2) If the concentration of the material in the sample is known, then its specific rotation can be determined.
3) The technique may be extended to the determination of optical substances in the presence of optically inactive species.
4) The optical rotation is additive reading can also be made in the presence of known amount of other active compound.
5) The observed specific rotation is a function of concentration. Therefore, quantitative analysis o optically active solutes may be carried out polarimetrically over a small range of concentration. The observed rotation is linear to a particular concentration. At higher concentration this linearity is lost, Quantitative analysis may be done by reading unknown concentration from the standard calibration curve of $a$ Vs concentration obtained from solution of known concentration.

   The formula used for all the calculation is---

$$(\alpha) = 100.\ \theta / Lc$$

$\alpha$ = specific rotation

$L$ = layer thickness in decimeters

$\theta$ = observed rotation

$c$ = concentration of solute in grams per 100ml

Temperature of measurement is included by superscript. The wavelength used is shown by subscript.

6) Quantitative poarimeteric analysis is of special importance in sugar industry and is called saccharimetry. In the absence of other optically active substance sucrose can be determined directly by measuring the angle of rotation, $(\alpha)^{20}_{D}$ being equal to +66.5. If other active substances are present, it is required to measure optical rotation before and after hydrolysis in the acidic medium to give a resultant D and L mixture of isomers. This has an optical rotation of -20.2°. the amount of sucrose present is calculated from the different in rotation before and after inversion

$$\underset{\text{Sucrose}}{C_{12}H_{22}O_{11}} \xrightarrow{\text{Inversion}} \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{Fructose}}{C_6H_{12}O_6}$$

(+66.5) (+52.7) (-92.4)

7) Simultaneous determination of penicillin and penicillinase enzyme can carried out.

**Qualitative application:** these are few in number and not of much importance.

1) ORD and CD measurement can be used for studying configuration and conformation in UV region.
2) Optical activity is a physical constant specific to particular substance and here an important criteria for the identification and determination of purity of substance.

Optical activity is the parameter available for distinguishing between D and L isomeric forms.

Question 6. Write assay of magnesium sulphate by complexometric analysis.

## Answer

**Procedure:** Weight accurately about 0.3 gm of the sample and dissolve in 50 ml water. Add10ml of strong ammonia-ammonium chloride solution and titrate with 0.05M disodium ethylenediaminetetraacetate using 0.1g of mordant black 11 mixture as indicator until the pink color is discharged and the solution becomes full blue.

Each ml of 0.05M disodium ethylenediaminetetraacetate is equivalent to 0.00602g of $MgSo_4$.

**Calculation:**

$$\text{Percentage purity} = \frac{V \times M \times 0.00602}{0.05 \times w} \times 100$$

V= volume of the titrant; M= molarity of the titrant; W= weight of sample

The buffer, strong ammonia-ammonium chloride solution, may be prepared by dissolving 67.5g of ammonium chloride in 740ml of strong ammonia solution and adding sufficient water to produce 1000 ml.

Question 7. Discuss in detail about instruments used for measurement of refraction.

## Answer

**Instrumentation (Figure 3)**

Refractometer determines the refractive index by measuring the position of critical ray. There are three types of refractometers; viz.; abbe's refractometer, immersion or dipping refractometer and the pulfrich refractometer. Out of these abbe's instrument is most convenient and widely used refractometer.

(a) **Abbe's refractometer**: it consists of a stationary telescope and two prisms held in contact with each other in a metal case. The prism system is attached with an atom which moves over the scale to read the refractive index. The abbe's refractometer has a facility of controlling the temperature of prisms by circulating water of constant temperature.

A beam of white light reflected from mirror enters into prism 'A' called illuminating prism whose upper rough surface acts as a source of infinite number of rays which passes through 0.1 mm thin layer of liquid in all directions. This ray then passes through the polished surface of prism 'B' and produces a critical boundary like a rainbow. This is due to the dispersion effect. To eliminate the color band, an auxiliary optical system called compensator is incorporated into the telescope which refracts the dispersed critical rays by exactly same value as for sodium D line critical ray. Thus, the optical compensator sharpens the boundary and produces a critical ray with white light which equivalent to that with sodium D line critical ray. The refractive index range is between 1.300 to 1.710.

(b) **Dipping/immersion refractometer:** it is quite similar to the regular abbe's refractometer except that the telescope is fixed to the refracting prism and there is no diffusion prism. It is very simple to operate but requires about 10-15 ml of sample. The single prism is mounted in the telescope containing compensator and eye piece. This is commonly use for analyzing solutions.

The scale is mounted below the eye piece inside the telescope tube. The lower surface of the prism is immersed in a sample contained in a small beaker. The mirrors present below the beaker refract the light through the liquid. The position of critical ray emerging from the prism is read by a fixed telescope with a graduated scale mounted inside. The range of immersion refractometer is less but precision is more than the abbe's refactometer i.e.± 0.000037 in $n_p$ while for abbe's refractometer it is ± 0.0002. The reading from the linear scale of dipping refractometer can be converted into refractive indices by using appropriate table.

(c) **Pulfrich refractometer**: in pulfrich refractometer the refracting prism is located under the sample. A beam of monochromatic radiation is allowed to pass along the surface of the prism at grazing incidence. Like abbe's refractometer the refracted angle ($r_C$) is observed by a telescope connected to a graduated arc.

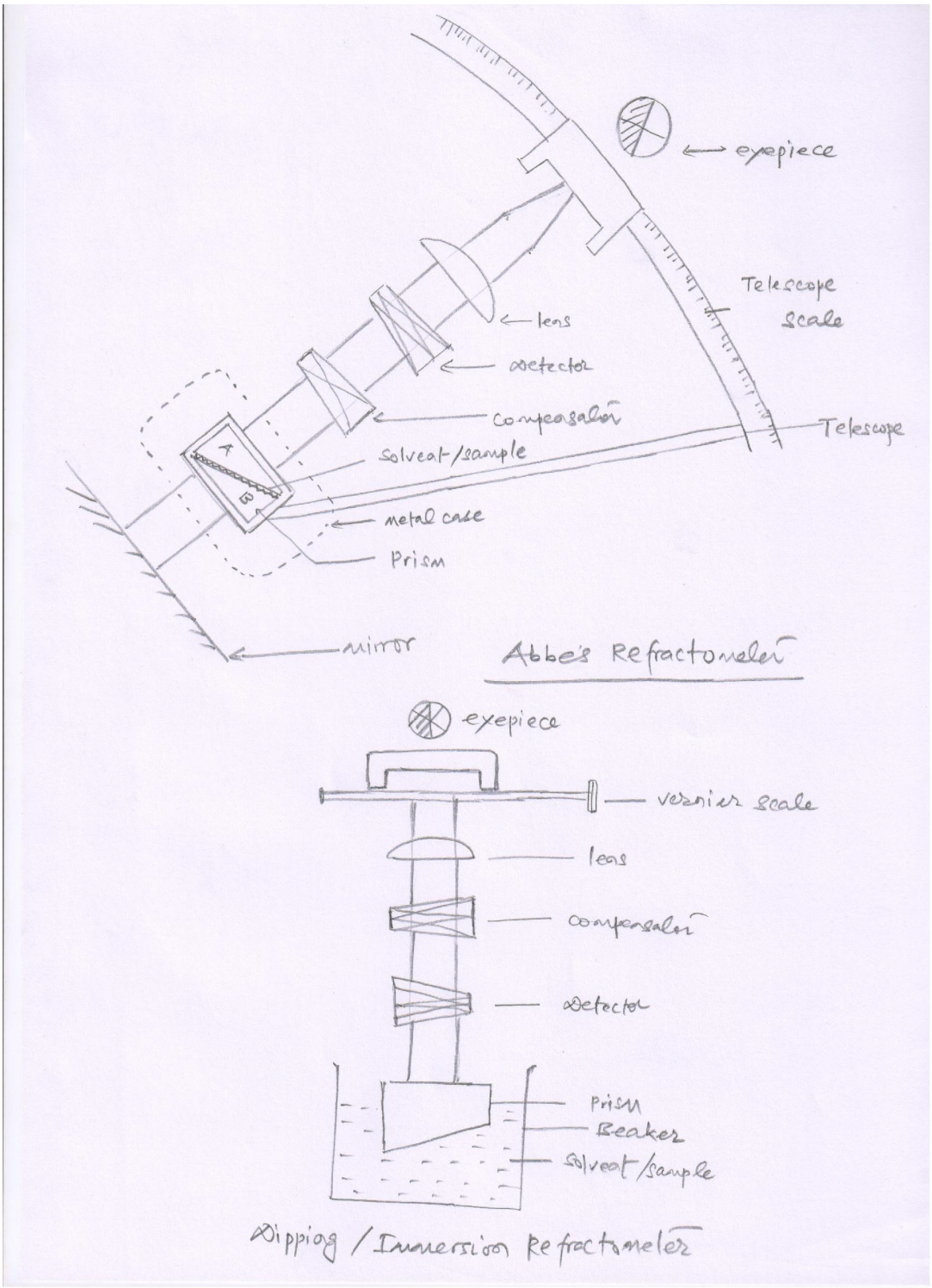


**Figure 3:** Intrumentation of Abbes and Immersion Refractometer.

The accuracy of this instrument is about 0.0001 units in n and the range of measurements is 1.33 to 1.60.

The other instrument used for measurement of refractive index includes the image refractometers and differential refractometers.