M. Sc (RT) Ist Sem Exam 2013

"Instrumentation and Techniques" (AS-2276)

Section A

(1) (Multiple Choice Questions)

(i) Eye piece of a microscope is — ocular lens (a)

(ii) Living unstained biological material can be seen by — (b) Phase contrast microscope

(iii) Combination of Techniques of iso electric focussing with size of molecule is termed as — (b) 2-D gel electrophoresis

(iv) Full form of TAE is — (a) Tris acetate EDTA

(v) Rotors of centrifuge are type of — (c) both

(vi) Vertical tube and zonal are type of — Rotors (b)

(vii) Absorbance spectroscopy is — (c) both

(viii) Flame under which atoms of metal are vaporized is — (d) inner zone

(ix) Bouin's solution is used in — (b) fixation

(x) Rotary microtome is used to cut sections embedded in — (d) All

(2) Principle of fluorescence microscopy –

Fluorescence is defined as a phenomenon in which wavelength of UV-rays is converted into a wavelength of light in visible range due to characteristic property of substances. The substance which emits fluorescence is termed as fluorosphore.

When U.V. is absorbed by molecules of fluorosphore, they get excited, absorb energy and change from ground state to excited state. Under such conditions, energy of each molecule is higher than that of its ground state. The surplus energy either gets dissipated as heat or gets emitted as fluorescence. The excited electrons often lose some of their energy and drops back down to original ground state emitting another photon as it does so. The emitted photon is always of longer wavelength (or lesser energy) than original photon that was absorbed. Hence, the fluorescence emitted from the material having wavelength of visible range causes "principle of fluorescence microscopy".

(3) Staining procedure of gel electrophoresis :-

The most common method for identification of isolated molecule after electrophoresis is its staining to make it visible. A solution of different stains may be used for this purpose according to type of class of molecule to be identified. Some of them are Buffalo black NBR, Azocarmine B, Amido-black 10B, Bromocresol green etc.

Excess dye/stain is removed from the gel with destaining process to get clear background. This is carried out by two methods — (1) diffusion destaining and (2) Electrophoretic destaining. Wash solution is composed of acetic acid and distilled water or it may be distilled water alone.

After that, gel is dried under vaccum and can be kept in polyethylene bags for future reference

4 Caring of rotors of centrifuge -

① The protective anodised coating on aluminum rotors is very thin and does not provide a high degree of protection against corrosion, thus rotors should always be handled with care to prevent scratching.

② The use of acid solutions, strong alkaline detergents and salt solutions can also easily damage the protective coating, leading to corrosion and eventually failure of rotor. After use, rotors should be thoroughly washed, preferably with deionized water. Because moisture is a potential source of corrosion, left it to drain and dry upside down in a warm atmosphere.

③ Rotors then should be stored in a clean, dry environment.

④ Swinging-bucket rotors should never be completely immersed in water because the bucket hanging mechanism is difficult to dry and can rust.

⑤ Sample loads should be balanced with the limits — i.e. each opposite pair of sample containers being balanced individually

⑥ Swinging-bucket rotors should not be run with any buckets or caps removed or individual rotor buckets interchanged.

(5) Histochemical or Histological localization—

Identification of location of major class of biomolecules in histological preparation is known as histochemical or histological localization. Or we may say that identification of areas of presence of proteins, carbohydrates, lipids or nucleic acids are termed as histochemical localization. It includes following stages —

(1) Test for proteins – Location of proteins may be identified with the help of Dinitrofluorobenzene (DNFB). If positive, next stage may be followed for different nature of proteins.

(2) Test for acidic groups – Acidic groups such as nucleic acids may be identified in this stage. Methyl green pyronine mixture or Toluidine blue may be required to identify acidic groups in histological preparation.

(3) Test for carbohydrates – Controlled periodic acid-schiff (PA test can be used to identify location of carbohydrates. If positive, another specific testes can be applied for identification of specific carbohydrates.

(4) Test for lipids – Sudan black is good general indicator of lipids that can be used for identification of location of lipids in histological preparations. If positive, another stage is followed for identification of specific lipids.

(5) When above are tested, minor components of histological preparations may also be identified if required.

6- Principle of atomic absorption spectrophotometer –

The atomic absorption spectrophotometry involves the study of absorption of radiations (usually in U.V. and/or visible region) by neutral atoms in the gaseous state. Thus, in this technique, the sample is first converted into atomic vapor and then the absorption of atomic vapor is measured at a selected wavelength, which is characteristics of each individual elements. The measured absorbance is proportional to the concentration and analyses are made by comparing this absorbance with that given under same experimental conditions by reference samples of known compositions.

We may say that principle of atomic absorption spectrophotometer are similar basically to those considered for absorption of UV-visible radiation by solution, but the equipment, sample handling techniques and appearance of spectra are quite different.

(7) Principle of phase contrast microscope —

The phase contrast microscopy is used for examining living and unstained cells, which converts invisible small phase changes caused by cell components into visible intensity changes.

We already know that when light rays pass through ~~a~~ medium it undergoes some physical changes i.e. retardation, according to refractive indices of the mediums. When light rays pass through a living cell, it undergoes invisible phase changes due to different refractive indices and thickness of the cell organelles. When light rays pass through cell organelles they are transmitted at a velocity inversally proportional to refractive index of the organelle. Since all organelles are of different refractive indices, the light rays emerging would show variable phase change.

Generally unstained or living cell causes phase change by $\frac{1}{4}\lambda$. This value of phase change is imperceptible to human eye. The principle behind phase contrast microscope is to convert this imperceptible phase change into perceptible phase change with the help of two adaptors called anular ~~diaphragm~~ diaphragm and annular phase plate.

Transpasent living cell retards light rays by $1/4\lambda$ and phase plate also retards light by $1/4\lambda$. By addition of these two retardation, phase retardation is enhanced to $\frac{1}{2}\lambda$ which is perceptible to human eye.

(a) Different types of centrifuges - Centrifuges may be classified into four major groups — (1) Small bench centrifuge, (2) large capacity refrigerated centrifuge (3) High speed refrigerated centrifuge and (4) ultracentrifuges of two types < ① Preparative ② Analytical

(1) Small bench centrifuges — These are simplest and least expensive centrifuges and exist in many types of design. They are often used to collect small amounts of material that rapidly sediment and generally have a maximum speed of 4000 – 6000 rpm, with maximum RCF of 3000 to 4000g. Most operate at ambient temperature, the flow of air around the rotor controls rotor temperature.

(2) Large capacity refrigerated centrifuges —

These have refrigerated rotors chambers and vary only in their maximum carrying capacity, all being capable of utilizing a variety of interchangeable swinging bucket and fixed angle rotors enabling separation to be achieved in 10, 50 and 100 cc. tubes. These have a maximum speed of 6000rpm and produce a maximum relative centrifugal field approaching 6500g. These instruments are most often used to compact or collect substances that sediment rapidly, for instance, Erythrocytes, yeast cells, nuclei and chloroplasts.

(3) High speed refrigerated centrifuges - These instruments generally have a total capacity of up to 1.5 dm^3 and a range of interchangeable fixed angle and swinging-bucket rotors. These are available with maximum rotor speeds in the region of 25000 rpm generating a RCF of about 60,000 g. These instruments are most often used to collect microorganism, cellular debris, larger cellular organelles and protein precipitations. They cannot generate sufficient centrifugal force to effectively sediment viruses or smaller organelles such as ribosomes.

(4) Ultracentrifuges - These instruments are of two types -

(i) Preparative ultracentrifuges - Preparative ultracentrifuges are capable of spinning rotors to a maximum speed of 80,000 rpm and can produce a relative centrifugal field of upto 600000 g. The rotor chamber is refrigerated, sealed and evacuated to minimise any excessive roto temperature being generated by frictional resistance between air and spinning rotor. Temperature monitoring system is more sophisticated than in simpler instrument

For safety reasons, rotor chambers of high speed and ultracentrifuges are always enclosed in heavy armour plating.

(ii) Analytical ultracentrifuges — These instruments are capable of operating at speed of about 70,000rpm and can produce a rcf of upto 500000 g. It consists of a motor, a rotor contained in a protective armoured chamber that is refrigerated and evacuated and an optical system to enable the sedimenting material to be observed throughout the duration of centrifugation to determine concentration distribution in the sample at any time during centrifugation. Three types of optical system are available in the analytical ultracentrifuge ;- a light absorption system, and an alternative Schlieren system and Rayleigh interferometric system, both of which detect changes in the refractive index of the solution.

9 Principle of atomic absorption spectrophotometer.

AAS is meant for the study of absorption of UV- or visible radiations by neutral atoms in the gaseous state. The sample is first converted into atomic vapors and then the absorption of atomic vapor is measured at a selected wavelength, which is characteristics of each individual element. The measured absorbance is proportional to the concentration and ~~any~~ analyses is made by comparing this absorbance with that given under same experimental conditions by reference sample of known composition.

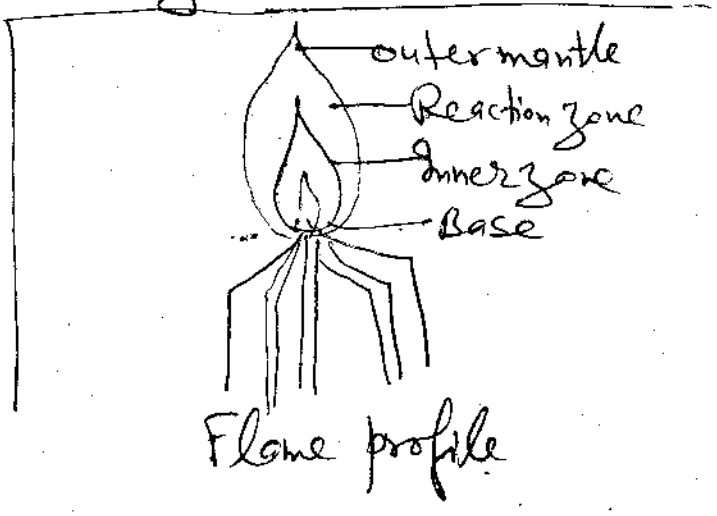
Instruments — The basic component of an instrument for atomic absorption measurements are similar to those of a spectrophotometer for the absorbance ~~of~~ by solutions. Detailed structure of instrument is as follows —

① Source of radiation — These are required to emit spectra of elements to be determined and these spectra should have sharp lines and high stability. The hollow cathod tube is the most common source of radiation, which has widely been used in AAS.

(2) Flame — Air–coal gas, Air–propane, air–acetylene and oxyacetylene flames have been used but the most widely used flame is air–acetylene.

Flame profile – In a flame atomizer, all or a part of solution of the sample is sprayed as a fine mist into a flame that is located in the path of radiation from the source. Important regions from bottom to top of the flame are— ① Base, ② Inner zone ③ Reaction zone ④ Outer mantle.

The sample in the form of small minute droplets enters the base of flame. Within this region, water evaporates from a substantial fraction of the droplets and some of the sample enters the inner zone in the form of solid particles. Here, vaporization as well as decomposition to the atomic state takes place. Moreover, excitation and absorption processes starts. The atoms are converted to oxides upon entering reaction zone. The oxides then passed into outer mantle and subsequently ejected from the flame.

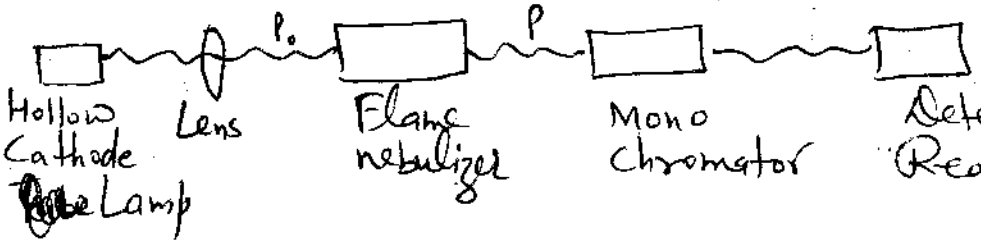


Flame profile

③ Monochromators or Filters – The function of monochromator is to select a given line in the emmission spectrum of the light source and isolate it from all other lines. For some of the alkali metals, which have only a few widely spaced resonance lines in the visible region, a glass filter is quite satisfactory.

④ Optical system, Detectors – These components are fundamentally as same as for the typical UV-Vis spectrophotometer. The radiant energy signals can be converted into electrical ones by making use of a multiplier tube. Photomultiplier tubes are used for the conversion of radiant energy signal to an electrical one.

⑤ Readout device – Now a days readout device is computerized part of the machine, however, basically it is a chart recorder, which is a potentiometer.



Block diagram of an AAS

(10) Rotary microtome - This is an ideal microtome for cutting the tissue embedded in paraffin and is most widely used. Rotary microtome are also available in many models. Rotary microtomes are also called because of the feed mechanism is actuated by rotating a circular drive wheel at one side of the machine. In all models, knife is fixed, edge uppermost and the object moves against the knife according to thickness selected, raising and falling vertically. One rotation of the rotating wheel produce a complete cycle of downward cutting stroke, upward return stroke and advancement of ~~machine~~ mechanism. The downward travel of block holder is not spring assisted and is under greater control of operator. The mechanism is usually encased to prevent access of the dirt and dust. The feed mechanism of rotary microtome lends itself to power drive attachment and apparatus has been described where the fly wheel is connected to a electric motor. This aid may be advantageous for certain types of work, particularly preparation of multiple sections from a single block but often with a wide variety of tissue, "the feel" of the block obtained by manual operation gives a valuable indication as to the best cutting technique for the material.

A device for setting the microtome to give sections of desired thickness is provided. A circular grooved disc is present at the rear end which moves anticlockwise by the exact distance as the desired thickness of section for which microtome has been set. This is called advance wheel. A central axle moves the front portion of microtome forward by exactly the same distance simultaneously as the front part moves up and down. To this front part a block holder carrying the paraffin block is fixed.

The razor is mounted on a pair of handss with a groove and adjusting screws. This is the razor holding device which is movable forward and backwards along two grooves on the heavy base of the microtome, so that the razor may also be brought nearer to the block if required.

Rotary microtome is good general purpose instrument. They are used mainly for paraffin wax embedded material and are well suited for the production of serial sections. A special knife holder, allowing the knife to be set at an oblique angle to the specimen may be used on some model for cutting cellulose nitrate embedded tissue.

(11) Construction and Function of fluorescence microscope —

(i) Source of illumination — The U.V. rays near visible range is used as a source of illumination. It is provided at a wavelength corresponding to the excitation maximum of the fluorospho The fluorescence microscope consist of (1) lamp (2) optical system and (3) a system of observation.

(1) Lamps - There are three types of lamps which are generally used -

(a) Tungsten - halogen lamp - which were popular earlier but they are now ~~to~~ no longer used.

(b) Mercury arc lamps — are most commonly used.

(c) Xenon arc lamps - provide a substantially continuous spectrum. These are most expensive but in the long run, the cost / hrs lamp time is cheaper

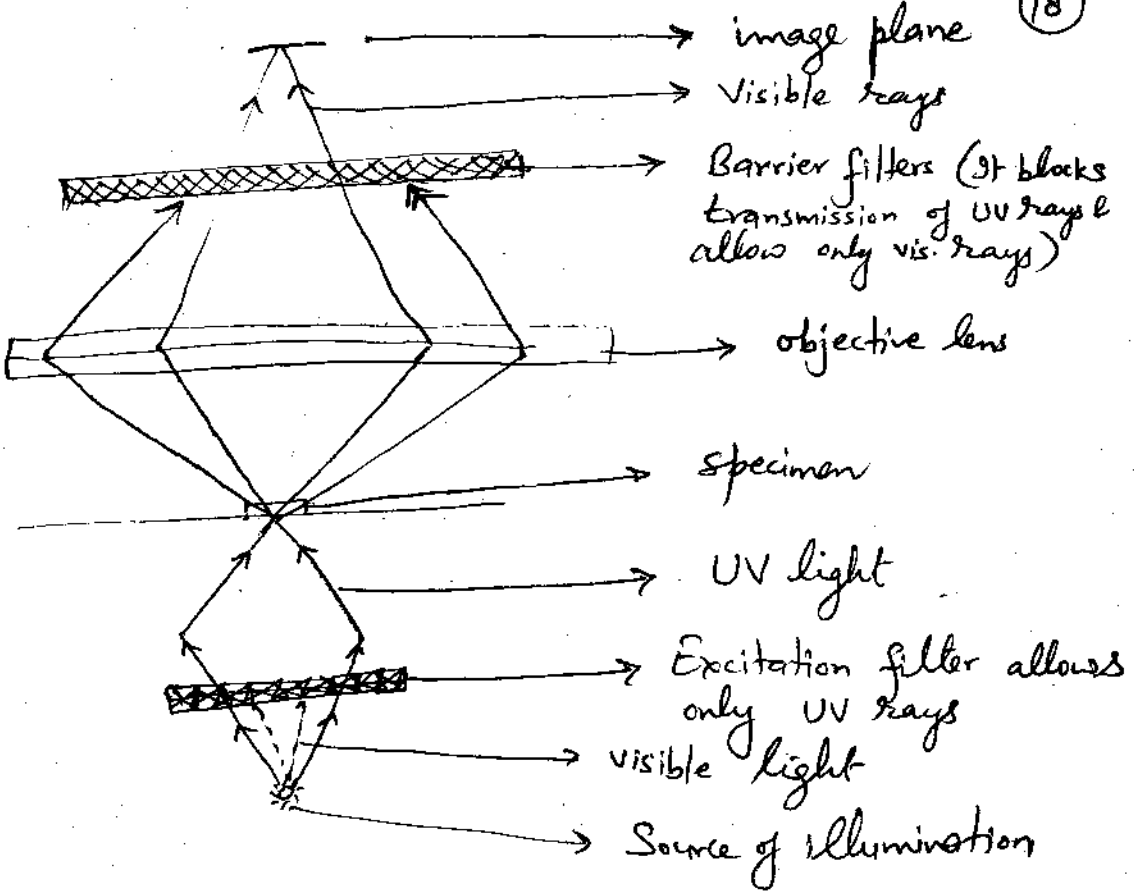
(ii) Optical system - The U.V. near the visible region excites the fluorosphore and emits light of longer wavelength in the visible range. The optical system of fluorescent microscope caters to this requirement. This requirement is met by using following filters.

(a) Primary or excitation filter - It is placed between UV source and specimen. It absorbs light of longer wavelength emitted from UV. sources. It gives normally monochromatic illumination depending upon the requirement of fluorosphore under study.

(b) Secondary or Barrier filter —

It is fitted in the body tube of microscope i.e. between the objective and eye. It comes in the observation pathway in image formation and prevents light of excitation wavelength from reaching the observer's eye. But its transmission should be maximum for the spectral region of emission from the fluorosphore.

(iii) Observation system — The objective and oculars are used for the formation of image of fluorescence. The lense system consist of quartz or fluorite (CaF_2) or lithium fluorite. All these materials are transparent to the electromagnetic rays which may be as low as 2000A°. The wavelength of UV used generally near the visible range i.e. approximately between 3500 – 3800A°. This range of UV can be easily converted into wavelength belonging to the visible range. Since UV is harmful to human eye, ocular lens is made of ordinary glass. This helps in preventing UV reaching the eye, but the fluorescent can be easily observed, which appears bright against the dark background.



OPTICS OF FLUORESCENCE MICROSCOPE

Applications of fluorescence microscope -

① Detection of material → Fluorescent microscope is used for studying biologicals like DNA, RNA, proteins etc. As it is a highly sensitive technique, it can be conveniently used for detection and demonstration of materials like nucleic acids, proteins, carbohydrates, chlorophyll etc. in extremely low concentration.

② Detection of cell components with labelled antibody —
Antibodies are covalently attached to certain organic molecules capable of fluorescence. Thus, fluorescent antibodies are used to detect cell components or organelles.