Semester – I

I7MBP104 **BIOINSTRUMENTATION** **4H – 4C**

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

This course will explore both theoretical and practical concepts of bioinstrumentation and its application in a variety of scientific disciplines. Analytical experiments will be performed in the laboratory portion of the class.

OBJECTIVES

This course highlights the basic laboratory skills that are essential for beginning-level employment in clinical, pharmaceutical, microbiology, biochemistry and biotechnology laboratories. Upon successful completion of this course, students are expected to be able to explain bioinstrumentation techniques, design and application.

UNIT – I

Spectroscopy – properties of electromagnetic radiations. Instrumentation and applications of – UV-Visible spectrophotometer, spectrofluorimeter, atomic spectroscopy, FTIR, NMR spectroscopy, MALDI-TOF and flow cytometer.

UNIT – II

Centrifugation: principle and types of centrifuges. Principles and applications of analytical and preparative centrifuges. Relative molecular mass determination and sedimentation coefficient. Sub-cellular Fractionation of cellular components. Density gradient and ultra centrifugation.

UNIT – III

Chromatography – principle, instrumentation and applications of ion exchange, affinity, gel filtration, Low pressure liquid chromatography (LPLC) and high performance liquid chromatography (HPLC) and fast protein liquid chromatography (FPLC), gas liquid chromatography-mass spectroscopy (GC-MS).

UNIT – IV

Electrophoresis - principle, instrumentation and applications of agarose gel electrophoresis, native PAGE, sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing, immuno electrophoresis, pulse field gel electrophoresis, capillary electrophoresis, gel documentation – applications.

UNIT – V

Radioisotopic techniques – introduction, nature of radio activity, types and rate of radioactive decay, units of radio activity, detection and measurement of radio activity. Principle, instrumentation and applications of Geiger-Muller counter, solid and liquid scintillation counter and autoradiography. Biosafety methods in radioactive laboratory.

SUGGESTED READINGS

TEXT BOOKS

1. John Enderle., (2006). *Bioinstrumentation*. (2006). Morgan and Claypool Publishers, NJ.
2. Richard Normann. (1988). *Principles of bioinstrumentation*. Wiley Publishers,US.
3. Keith Wilson and John Walker. (2010). *Principle and Techniques of Biochemistry and molecular biology.* (7th ed.). Cambridge university press, NY.

REFERENCES

1. Boyer, R. (2000). *Modern Experimental Biochemistry*. (3rd ed.). Addison Wesley Longman, New Delhi.
2. Chatwal, G.R., and Anand, S.K., (2003). *Instrumental Methods of Chemical Analysis*. (5th ed.). Himalaya Publishing House, Mumbai
3. Friedfelder, D. (2001). *Physical Biochemistry: Applications to biochemistry and molecular biology.* Oxford Publishers, New York.
4. Sharma, B.K. (2007). *Instrumental Methods of Chemical Analysis*, Krishna Prakashan Media (P) Ltd, India.
5. Wilson, K., and Walker, J., (2010). *Principles and Techniques of Biochemistry and Molecular Biology*, (7th Low Price ed.). Cambridge University Press, India.

DEPARTMENT OF MICROBIOLOGY
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I M. Sc. MICROBIOLOGY 2017 – 2019

BIOINSTRUMENTATION (17MBP104)

SEMESTER – I **4H – 4C**

UNIT I

S. No	Duration	Topic	Reference
1	1	Spectroscopy – Introduction	T1: 287
2	1	Properties of electromagnetic radiation	T1: 287
3	1	Instrumentation and application of UV-visible spectrophotometer	T1: 301
4	1	Spectrofluorimeter	T1: 326
5	1	Atomic spectroscopy	T1: 358
6	1	Fourier transform infra red spectroscopy	T1: 357
7	1	NMR-spectroscopy	T1: 354
8	1	MALDI-TOF and flow cytometer	W1,W2
9	1	Unit revision	
Total Hrs: 9			

T1: Bioinstrumentation – L. Veerakumari

W1: https://en.wikipedia.org/wiki/MALDI_imaging

W2: https://en.wikipedia.org/wiki/Flow_cytometry

2017

Bioinstrumentation

Unit-I Notes

Prepared by Dr S. Ramalakshmi, Asst Prof, Dept of Microbiology, KAHE, CBE-21

Unit – 1

Spectroscopy

Spectroscopic techniques employ light to interact with matter and thus probe certain features of a sample to learn about its consistency or structure. Light is electromagnetic radiation, a phenomenon exhibiting different energies, and dependent on that energy, different molecular features can be probed.

Properties of electromagnetic radiation

The interaction of electromagnetic radiation with matter is a quantum phenomenon and dependent upon both the properties of the radiation and the appropriate structural parts of the samples involved. This is not surprising, since the origin of electromagnetic radiation is due to energy changes within matter itself. The transitions which occur within matter are quantum phenomena and the spectra which arise from such transitions are principally predictable. Electromagnetic radiation is composed of an electric and a perpendicular magnetic vector, each one oscillating in plane at right angles to the direction of propagation. The wavelength λ is the spatial distance between two consecutive peaks (one cycle) in the sinusoidal waveform and is measured in submultiples of metre, usually in nanometres (nm). The maximum length of the vector is called the amplitude. The frequency ν of the electromagnetic radiation is the number of oscillations made by the wave within the timeframe of 1 s. It therefore has the units of 1 s^{-1} = 1 Hz. The frequency is related to the wavelength via the speed of light c (c=2.998x108 m s^{-1} in vacuo) by ν = ¼c λ^{-1}. A historical parameter in this context is the wave number which describes the number of completed wave cycles per distance and is typically measured in 1 cm^{-1}.

UV-visible spectrophotometer

Instrumentation

UV/Vis spectrophotometers are usually dual-beam spectrometers where the first channel contains the sample and the second channel holds the control (buffer) for correction. Alternatively, one can record the control spectrum first and use this as internal reference for the sample spectrum. The latter approach has become very popular as many spectrometers in the laboratories are computer-controlled, and baseline correction can be carried out using the software by simply subtracting the control from the sample spectrum. The light source is a tungsten filament bulb for the visible part of the spectrum, and a deuterium bulb for the UV region. Since the emitted light consists of many different wavelengths, a monochromator, consisting of either a prism or a rotating metal grid of high precision called grating, is placed between the light source and the sample. Wavelength selection can also be achieved by using colored filters as monochromators that absorb all but a certain limited range of wavelengths. This

limited range is called the bandwidth of the filter. Filter-based wavelength selection is used in colorimetry, a method with moderate accuracy, but best suited for specific colorimetric assays where only certain wavelengths are of interest. If wavelengths are selected by prisms or gratings, the technique is called spectrophotometry.

Applications

The application of UV/Vis spectroscopy to further analytical purposes is rather limited, but possible for systems where appropriate features and parameters are known. Most commonly, this type of spectroscopy is used for quantification of biological samples either directly or via colorimetric assays. In many cases, proteins can be quantified directly using their intrinsic chromophores, tyrosine and tryptophan. Protein spectra are acquired by scanning from 500 to 210 nm. The characteristic features in a protein spectrum are a band at 278/280 nm and another at 190nm. The region from 500 to 300nm provides valuable information about the presence of any prosthetic groups or coenzymes. Protein quantification by single wavelength measurements at 280 and 260nm only should be avoided, as the presence of larger aggregates (contaminations or protein aggregates) gives rise to considerable Rayleigh scatter that needs to be corrected.

Spectrofluorimeter

Instrumentation

Fluorescence spectroscopy works most accurately at very low concentrations of emitting fluorophores. UV/Vis spectroscopy, in contrast, is least accurate at such low concentrations. One major factor adding to the high sensitivity of fluorescence applications is the spectral selectivity. Due to the Stokes shift, the wavelength of the emitted light is different from that of the exciting light. Another feature makes use of the fact that fluorescence is emitted in all directions. By placing the detector perpendicular to the excitation pathway, the background of the incident beam is reduced.

The schematic of a typical spectrofluorimeter has two monochromators, one for tuning the wavelength of the exciting beam and a second one for analysis of the fluorescence emission. Due to the emitted light always having a lower energy than the exciting light, the wavelength of the excitation monochromator is set at a lower wavelength than the emission monochromator. The better fluorescence spectrometers in laboratories have a photon-counting detector yielding very high sensitivity. Temperature control is required for accurate work as the emission intensity of a fluorophore is dependent on the temperature of the solution. Two geometries are possible for the measurement, with the 90° arrangement most commonly used. Pre- and post-filter effects can arise owing to absorption of light prior to reaching the fluorophore and the reduction of emitted radiation. These phenomena are also called inner filter effects and are more evident in solutions

with high concentrations. As a rough guide, the absorption of a solution to be used for fluorescence experiments should be less than 0.05. The use of microcuvettes containing less material can also be useful. Alternatively, the front-face illumination geometry can be used which obviates the inner filter effect. Also, while the 90° geometry requires cuvettes with two neighbouring faces being clear (usually, fluorescence cuvettes have four clear faces), the front-face illumination technique requires only one clear face, as excitation and emission occur at the same face. However, front-face illumination is less sensitive than the 90° illumination.

Applications

There are many and highly varied applications for fluorescence despite the fact that relatively few compounds exhibit the phenomenon. The effects of pH, solvent composition and the polarisation of fluorescence may all contribute to structural elucidation. Measurement of fluorescence lifetimes can be used to assess rotation correlation coefficients and thus particle sizes. Non-fluorescent compounds are often labeled with fluorescent probes to enable monitoring of molecular events. This is termed extrinsic fluorescence as distinct from intrinsic fluorescence where the native compound exhibits the property. Some fluorescent dyes are sensitive to the presence of metal ions and can thus be used to track changes of these ions in invitro samples, as well as whole cells. Since fluorescence spectrometers have two monochromators, one for tuning the excitation wavelength and one for analyzing the emission wavelength of the fluorophore, one can measure two types of spectra: excitation and emission spectra. For fluorescence excitation spectrum measurement, one sets the emission monochromator at a fixed wavelength (λ_{em}) and scans a range of excitation wavelengths which are then recorded as ordinate (x-coordinate) of the excitation spectrum; the fluorescence emission at λ_{em} is plotted as abscissa. Measurement of emission spectra is achieved by setting a fixed excitation wavelength (λ_{exc}) and scanning a wavelength range with the emission monochromator. To yield a spectrum, the emission wavelength lem is recorded as ordinate and the emission intensity at λ_{em} is plotted as abscissa.

ATOMIC SPECTROSCOPY

Instrumentation

In general, atomic spectroscopy is not carried out in solution. In order for atoms to emit or absorb monochromatic radiation, they need to be volatilized by exposing them to high thermal energy. Usually, nebulizers are used to spray the sample solution into a flame or an oven. Alternatively, the gaseous form can be generated by using inductively coupled plasma (ICP). The variations in temperature and composition of a flame make standard conditions difficult to achieve. Most modern instruments thus use an ICP. Atomic emission spectroscopy (AES) and atomic absorption spectroscopy (AAS) are generally used to identify specific elements present in

the sample and to determine their concentrations. The energy absorbed or emitted is proportional to the number of atoms in the optical path. Strictly speaking, in the case of emission, it is the number of excited atoms that is proportional to the emitted energy. Concentration determination with AES or AAS is carried out by comparison with calibration standards. Sodium gives high backgrounds and is usually measured first. Then, a similar amount of sodium is added to all other standards. Excess hydrochloric acid is commonly added, because chloride compounds are often the most volatile salts. Calcium and magnesium emission can be enhanced by the addition of alkali metals and suppressed by addition of phosphate, silicate and aluminate, as these form nondissociable salts. The suppression effect can be relieved by the addition of lanthanum and strontium salts. Lithium is frequently used as an internal standard. For storage of samples and standards, polyethylene bottles are used, since glass can absorb and release metal ions, and thus impact the accuracy of this sensitive technique. Cyclic analysis may be performed that involves the estimation of each interfering substance in a mixture. Subsequently, the standards for each component in the mixture are doped with each interfering substance. This process is repeated two or three times with refined estimates of interfering substance, until self-consistent values are obtained for each component. Flame instability requires experimental protocols where determination of an unknown sample is bracketed by measurements of the appropriate standard, in order to achieve the highest possible accuracy. Biological samples are usually converted to ash prior to determination of metals. Wet ashing in solution is often used, employing an oxidative digestion similar to the Kjeldahl method

Applications

Atomic emission and atomic absorption spectrophotometry Sodium and potassium are assayed at concentrations of a few p.p.m. using simple filter photometers. The modern emission spectrophotometers allow determination of about 20 elements in biological samples, the most common being calcium, magnesium and manganese. Absorption spectrophotometers are usually more sensitive than emission instruments and can detect less than 1 p.p.m. of each of the common elements with the exception of alkali metals. The relative precision is about 1% in a working range of 20–200 times the detection limit of an element. AES and AAS have been widely used in analytical chemistry, such as environmental and clinical laboratories. Nowadays, the technique has been superseded largely by the use of ion-selective electrodes.

Fourier transform infrared spectroscopy (FTIR)

Is a technique which is used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high spectral resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time.

The term Fourier transform infrared spectroscopy originates from the fact that a Fourier transform (a mathematical process) is required to convert the raw data into the actual spectrum. Fourier transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many frequencies of light at once, and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterwards, a computer takes all these data and works backwards to infer what the absorption is at each wavelength.

The beam described above is generated by starting with a broadband light source one containing the full spectrum of wavelengths to be measured. The light shines into a Michelson interferometer a certain configuration of mirrors, one of which is moved by a motor. As this mirror moves, each wavelength of light in the beam is periodically blocked, transmitted, blocked, transmitted, by the interferometer, due to wave interference. Different wavelengths are modulated at different rates, so that at each moment, the beam coming out of the interferometer has a different spectrum.

As mentioned, computer processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength). The processing required turns out to be a common algorithm called the Fourier transform (hence the name, "Fourier transform spectroscopy"). The raw data is sometimes called an "interferogram".

There are three principal advantages for an FT spectrometer compared to a scanning (dispersive) spectrometer.

1. The multiplex or Fellgett's advantage. This arises from the fact that information from all wavelengths is collected simultaneously. It results in a higher Signal-to-noise ratio for a given scan-time. For a spectrum with m resolution elements, this increase is equal to the square root of m. alternatively; it allows a shorter scan-time for a given resolution. In practice multiple scans are often averaged, increasing the signal-to-noise ratio by the square root of the number of scans.
2. The throughput or Jacquinot's advantage. This results from the fact that in a dispersive instrument, the monochromator has entrance and exit slits which restrict the amount of light that passes through it. The interferometer throughput is determined only by the diameter of the collimated beam coming from the source. Although no slits are needed, FTIR spectrometers do require an aperture to restrict the convergence of the collimated beam in the interferometer. This is because convergent rays are modulated at different frequencies as the path difference is varied. Such an aperture is called a Jacquinot stop.[1] For a given resolution and wavelength this circular aperture allows more light through than a slit, resulting in a higher signal-to-noise ratio.

3. The wavelength accuracy or Connes advantage. The wavelength scale is calibrated by a laser beam of known wavelength that passes through the interferometer. This is much more stable and accurate than in dispersive instruments where the scale depends on the mechanical movement of diffraction gratings. In practice, the accuracy is limited by the divergence of the beam in the interferometer which depends on the resolution.

Another minor advantage is less sensitivity to stray light that is radiation of one wavelength appearing at another wavelength in the spectrum. In dispersive instruments, this is the result of imperfections in the diffraction gratings and accidental reflections. In FT instruments there is no direct equivalent as the apparent wavelength is determined by the modulation frequency in the interferometer

Nuclear magnetic resonance (**NMR**)

NMR is a physical phenomenon in which nuclei in a magnetic field absorb and re-emit electromagnetic radiation. This energy is at a specific resonance frequency which depends on the strength of the magnetic field and the magnetic properties of the isotope of the atoms; in practical applications, the frequency is similar to VHF and UHF television broadcasts (60–1000 MHz). NMR allows the observation of specific quantum mechanical magnetic properties of the atomic nucleus. Many scientific techniques exploit NMR phenomena to study molecular physics, crystals, and non-crystalline materials through nuclear magnetic resonance spectroscopy. NMR is also routinely used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI).

All isotopes that contain an odd number of protons and/or neutrons (see Isotope) have an intrinsic magnetic moment and angular momentum, in other words a nonzero spin, while all nuclides with even numbers of both have a total spin of zero. The most commonly studied nuclei are 1H and 13C, although nuclei from isotopes of many other elements (e.g. 2H, 6Li, 10B, 11B, 14N, 15N, 17O, 19F, 23Na, 29Si, 31P, 35Cl, 113Cd, 129Xe, 195Pt) have been studied by high-field NMR spectroscopy as well.

Instrumentation

Schematically, an analytical NMR instrument is very similar to an EPR instrument, except that instead of a klystron generating microwaves two sets of coils are used to generate and detect radio frequencies. Samples in solution are contained in sealed tubes which are rotated rapidly in the cavity to eliminate irregularities and imperfections in sample distribution. In this way, an average and uniform signal is reflected to the receiver to be processed and recorded. In solid

samples, the number of spin–spin interactions is greatly enhanced due to intermolecular interactions that are absent in dissolved samples due to translation and rotation movements. As a result, the resonance signals broaden significantly. However, high-resolution spectra can be obtained by spinning the solid sample at an angle of 54.7° (magic angle spinning).
The sophisticated pulse sequences necessary for multidimensional NMR require a certain geometric layout of the radio frequency coils and sophisticated electronics. Advanced computer facilities are needed for operation of NMR instruments, as well as analysis of the acquired spectra.

Applications

Medicine
The application of nuclear magnetic resonance best known to the general public is magnetic resonance imaging for medical diagnosis and magnetic resonance microscopy in research settings, however, it is also widely used in chemical studies, notably in NMR spectroscopy such as proton NMR, carbon-13 NMR, deuterium NMR and phosphorus-31 NMR. Biochemical information can also be obtained from living tissue (e.g. human brain tumors) with the technique known as in vivo magnetic resonance spectroscopy or chemical shift NMR Microscopy.
Chemistry
By studying the peaks of nuclear magnetic resonance spectra, chemists can determine the structure of many compounds. It can be a very selective technique, distinguishing among many atoms within a molecule or collection of molecules of the same type but which differ only in terms of their local chemical environment. NMR spectroscopy is used to unambiguously identify known and novel compounds, and as such, is usually required by scientific journals for identity confirmation of synthesized new compounds.

Purity determination
NMR is primarily used for structural determination, however it can also be used for purity determination, providing that the structure and molecular weight of the compound is known. This technique requires the use of an internal standard of a known purity. Typically this standard will have a high molecular weight to facilitate accurate weighing, but relatively few protons so as to give a clear peak for later integration e.g. 1,2,3,4-tetrachloro-5-nitrobenzene. Accurately weighed portions of both the standard and sample are combined and analyzed by NMR.

MALDI, TOF mass spectrometry, MALDI-TOF

Matrix-assisted laser desorption ionisation (MALDI) produces gas phase protonated ions by excitation of the sample molecules from the energy of a laser transferred via a UV light-absorbing matrix. The matrix is a conjugated organic compound (normally a weak organic acid

such as a derivative of cinnamic acid and dihydroxybenzoic acid) that is intimately mixed with the sample. These are designed to maximally absorb light at the wavelength of the laser, typically a nitrogen laser of 337 nm or a neodymium/yttrium-aluminium-garnet (Nd-YAG) at 355 nm. The sample (1–10 pmol mm^{-3}) is mixed with an excess of the matrix and dried on to the target plate, where they co-crystallise on drying. Pulses of laser light of a few nanoseconds duration cause rapid excitation and vaporisation of the crystalline matrix and the subsequent ejection of matrix and analyte ions into the gas phase. This generates a plume of matrix and analyte ions that are analysed in a TOF mass analyser. The particular advantage of MALDI is the ability to produce large mass ions, with high sensitivity. MALDI is a very soft ionisation method that does not produce abundant amounts of fragmentation compared with some other ionisation methods. Since the molecular ions are produced with little fragmentation, it is a valuable technique for examining mixtures TOF is the best type of mass analyzer to couple to MALDI, as this technique has a virtually unlimited mass range. Proteins and other macromolecules of M_r greater than 400 000 have been accurately measured.

Sample concentration for MALDI

Maximum sensitivity is achieved in MALDI–TOF if samples are diluted to a particular concentration range. If the sample concentration is unknown a dilution series may be needed to produce a satisfactory sample/matrix spot of suitable concentration on the MALDI plate. Peptides and proteins seem to give best spectra at around 0.1 to 10 pmolmm^{-3}. Some proteins, particularly glycoproteins, may yield better results at concentrations up to 10 pmol mm^{-3}. Oligonucleotides give better spectra at around 10 to 100 pmol mm^{-3} while polymers require a concentration around 100 pmol mm^{3}.

Applications

MALDI-IMS involves the visualization of the spatial distribution of proteins, peptides, lipids, and other small molecules within thin slices of tissue, such as animal or plant. The application of this technique to biological studies has increased significantly since its introduction. MALDI-IMS is providing major contributions to the understanding of diseases, improving diagnostics, and drug delivery. Significant studies are of the eye, cancer research, drug distribution and neuroscience.

MALDI-IMS has been able to differentiate between drugs and metabolites and provide histological information in cancer research, which makes it a promising tool for finding new protein biomarkers. However, this can be challenging because of ion suppression, poor ionization, and low molecular weight matrix fragmentation effects. To combat this, chemical derivatization is used to improve detection.

Flow cytometer

Modern flow cytometers are able to analyze several thousand particles every second, in "real time," and can actively separate and isolate particles having specified properties. A flow cytometer is similar to a microscope, except that, instead of producing an image of the cell, flow cytometry offers "high-throughput" (for a large number of cells) automated quantification of set parameters. To analyze solid tissues, a single-cell suspension must first be prepared.

A flow cytometer has five main components:

- a flow cell - liquid stream (sheath fluid), which carries and aligns the cells so that they pass single file through the light beam for sensing
- a measuring system - commonly used are measurement of impedance (or conductivity) and optical systems - lamps (mercury, xenon); high-power water-cooled lasers (argon, krypton, dye laser); low-power air-cooled lasers (argon (488 nm), red-HeNe (633 nm), green-HeNe, HeCd (UV)); diode lasers (blue, green, red, violet) resulting in light signals
- a detector and Analogue-to-Digital Conversion (ADC) system - which converts analogue measurements of forward-scattered light (FSC) and side-scattered light (SSC) as well as dye-specific fluorescence signals into digital signals that can be processed by a binary computer
- an amplification system - linear or logarithmic
- a computer for analysis of the signals.

The process of collecting data from samples using the flow cytometer is termed 'acquisition'. Acquisition is mediated by a computer physically connected to the flow cytometer, and the software which handles the digital interface with the cytometer. The software is capable of adjusting parameters (e.g., voltage, compensation) for the sample being tested, and also assists in displaying initial sample information while acquiring sample data to ensure that parameters are set correctly. Early flow cytometers were, in general, experimental devices, but technological advances have enabled widespread applications for use in a variety of both clinical and research purposes. Due to these developments, a considerable market for instrumentation, analysis software, as well as the reagents used in acquisition such as fluorescently labeled antibodies has developed.

Modern instruments usually have multiple lasers and fluorescence detectors. The current record for a commercial instrument is ten lasers and 18 fluorescence detectors. Increasing the number of lasers and detectors allows for multiple antibody labeling, and can more precisely identify a target population by their phenotypic markers. Certain instruments can even take digital images of individual cells, allowing for the analysis of fluorescent signal location within or on the surface of cells.

Applications

The technology has applications in a number of fields, including molecular biology, pathology, immunology, plant biology and marine biology. It has broad application inmedicine (especially in transplantation, hematology, tumor immunology and chemotherapy, prenatal diagnosis, genetics and sperm sorting for sex preselection). Also, it is extensively used in research for the detection of DNA damage, caspase cleavage and apoptosis. In marine biology, the autofluorescent properties of photosynthetic planktoncan be exploited by flow cytometry in order to characterise abundance and community structure. In protein engineering, flow cytometry is used in conjunction with yeast displayand bacterial display to identify cell surface-displayed protein variants with desired properties.

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BIOINSTRUMENTATION (17MBP104)

SEMESTER – I **4H – 4C**

UNIT II

S. No	Duration	Topic	Reference
1	1	Centrifugation - Introduction	T1: 113
2	1	Principles and types of centrifuges	T1: 113 – 122
3	1	Principle and application of analytical centrifuges	T1: 128-130
4	1	Principle and application of preparative centrifuges	T1: 130 -136
5	1	Relative molecular mass determination and sedimentation coefficient	T1: 114 -115
6	1	Sub cellular fractionation of cellular components	T1: 113-114
7	1	Density gradient centrifugation	T1: 133 – 135
8	1	Ultra centrifugation	T1: 119 - 122
9	1	Unit revision	
Total Hrs: 9			

T1: Bioinstrumentation – L. Veerakumari

2017

BIOINSTRUMENTATION

Unit-II Notes

Prepared by Dr S. Ramalakshmi, Asst Prof, Dept of Microbiology, KAHE, CBE-21

UNIT – 2

Centrifugation is a process which involves the application of the centripetal force for the sedimentation of heterogeneous mixtures with a centrifuge, and is used in industrial and laboratory settings. This process is used to separate two miscible substances, but also to analyze the hydrodynamic properties of macromolecules. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate (pellet) to gather on the bottom of the tube. The remaining solution (supernatant) may be discarded with a pipette.

Centrifugation is a technique used for the separation of particles using a centrifugal field. The particles are suspended in liquid medium and placed in a centrifuge tube. The tube is then placed in a rotor and spun at a definitive speed. Rotation of the rotor about a central axis generates a centrifugal force upon the particles in the suspension.
Two forces counteract the centrifugal force acting on the suspended particles:

- Buoyant force: This is the force with which the particles must displace the liquid media into which they sediment.
- Frictional force: This is the force generated by the particles as they migrate through the solution.

Particles move away from the axis of rotation in a centrifugal field only when the centrifugal force exceeds the counteracting buoyant and frictional forces resulting in sedimentation of the particles at a constant rate.

Particles which differ in density, size or shape sediment at different rates. The rate of sedimentation depends upon:
1. The applied centrifugal field
2. Density and radius of the particle.
3. Density and viscosity of the suspending medium.

Angular velocity = w radians / second;
since one revolution = 360^o = 2p radians,

Types of Centrifuges and their Uses:

There are four major types of centrifuges. They are:

1. Small Bench Centrifuges:

They are used to collect small amount of material that rapidly sediment like yeast cells, erythrocytes etc. They have maximum relative centrifugal field of 3000-7000 g.

2. Large Capacity Refrigerated Centrifuges:

They have refrigerated rotor chamber and have capacity to change rotor chambers for varying size. They can go up to maximum of 6500 g and use to sediment or collect the substances that sediment rapidly like erythrocytes, yeast cell, nuclei and chloroplast.

3. High Speed Refrigerated Centrifuges:

They can generate speed of about 60000g and are used to collect micro-organism, cellular debris, larger cellular organelles and proteins precipitated by ammonium sulphate.

Design and Types of Rotors:

1. Swinging Bucket Rotors:

The swinging bucket rotor has buckets that start off in a vertical position but during acceleration of the rotor swing out to a horizontal position so that during centrifugation the tube and hence the solution in the tube, is aligned perpendicular to the axis of rotation and parallel to the applied centrifugal field, the tube returning to its original position during deceleration of the rotor.

2. Fixed Angle Rotors:

In fixed angles the tubes are located in holes in the rotor body set at a fixed angle between 14° and 40° to the vertical. Under the influence of centrifugal field, particles move radially outward and have only a short distance to travel before colliding with, and precipitating on, the outer wall of the centrifuge tube. A region of high concentration is formed that has a density greater than surrounding medium, with the result that the precipitate sinks and collects as a small compact pellet at the outermost point of the tube.

3. Vertical Tube Rotors:

They are considered as zero angle fixed angle rotors in which the tubes are aligned vertically in the body of the rotors at all times.

4. Zonal Rotors:

The zonal rotors may be of the batch or continuous flow type. The former being more extensively used than the latter, and are designed to minimize the wall effect that is encountered in swinging- bucket and fixed angle rotors, and to increase sample size.

5. Elutriator Rotors:

The elutriator is a kind of continuous flow rotor that contains recesses to hold a single conical shaped separation chamber, the apex of which points away from the axis of rotation, and a bypass chamber on the opposite side of the rotor that serves as a counter balance and to provide the fluid outlet.

4. Ultra Centrifuges

(a) Analytical ultracentrifuge:

It is capable of operating at 500000 g. Three kinds of optical systems are available in analytical ultracentrifuges: a light absorption system, and the alternative Schlieren system and Rayleigh interferometric system, both of which detect changes in the refractive index of the solution.

In an analytical ultracentrifuge, a sample being spun can be monitored in real time through an optical detection system, using ultraviolet light absorption and/or interference optical refractive index sensitive system. This allows the operator to observe the evolution of the sample concentration versus the axis of rotation profile as a result of the applied centrifugal field. With modern instrumentation, these observations are electronically digitized and stored for further mathematical analysis. Two kinds of experiments are commonly performed on these instruments: sedimentation velocity experiments and sedimentation equilibrium experiments.

Sedimentation velocity experiments aim to interpret the entire time-course of sedimentation, and report on the shape and molar mass of the dissolved macromolecules, as well as their size-distribution. The size resolution of this method scales approximately with the square of the particle radii, and by adjusting the rotor speed of the experiment size-ranges from 100 Da to 10 GDa can be covered. Sedimentation velocity experiments can also be used to study reversible chemical equilibria between macromolecular species, by either monitoring the number and molar mass of macromolecular complexes, by gaining information about the complex composition from multi-signal analysis exploiting differences in each components spectroscopic signal, or by following the composition dependence of the sedimentation rates of the macromolecular system, as described in Gilbert-Jenkins theory.

Sedimentation equilibrium experiments are concerned only with the final steady-state of the experiment, where sedimentation is balanced by diffusion opposing the concentration gradients, resulting in a time-independent concentration profile. Sedimentation equilibrium distributions in the centrifugal field are characterized by Boltzmann distributions. This experiment is insensitive to the shape of the macromolecule, and directly reports on the molar mass of the macromolecules and, for chemically reacting mixtures, on chemical equilibrium constants.

The kinds of information that can be obtained from an analytical ultracentrifuge include the gross shape of macromolecules, the conformational changes in macromolecules, and size distributions of macromolecular samples. For macromolecules, such as proteins, that exist in chemical equilibrium with different non-covalent complexes, the number and subunit stoichiometry of the complexes and equilibrium constant constants can be studied.

(b) Preparative ultracentrifuge:

It can produce relative centrifugal force of about 600000g and its chamber is refrigerated, sealed and evacuated. It is employed for separation of macromolecules/ligands binding kinetic studies, separation of various lipoprotein fractions from plasma and deprotonisation of physiological fluids for amino acid analysis. Preparative ultracentrifuges are available with a wide variety of rotors suitable for a great range of experiments. Most rotors are designed to hold tubes that contain the samples. Swinging bucket rotors allow the tubes to hang on hinges so the tubes

reorient to the horizontal as the rotor initially accelerates. Fixed angle rotors are made of a single block of material and hold the tubes in cavities bored at a predetermined angle. Zonal rotors are designed to contain a large volume of sample in a single central cavity rather than in tubes. Some zonal rotors are capable of dynamic loading and unloading of samples while the rotor is spinning at high speed.

Preparative rotors are used in biology for pelleting of fine particulate fractions, such as cellular organelles (mitochondria, microsomes, ribosomes) and viruses. They can also be used for gradient separations, in which the tubes are filled from top to bottom with an increasing concentration of a dense substance in solution. Sucrose gradients are typically used for separation of cellular organelles. Gradients of caesium salts are used for separation of nucleic acids. After the sample has spun at high speed for sufficient time to produce the separation, the rotor is allowed to come to a smooth stop and the gradient is gently pumped out of each tube to isolate the separated components.

Differential centrifugation

Differential centrifugation is a common procedure in microbiology and cytology used to separate certain organelles from whole cells for further analysis of specific parts of cells. In the process, a tissue sample is first lysed to break the cell membranes and mix up the cell contents. The lysate is then subjected to repeated centrifugations, each time removing the pellet and increasing the centrifugal force. Finally, purification may be done through equilibrium sedimentation, and the desired layer is extracted for further analysis.

Separation is based on size and density, with larger and denser particles pelleting at lower centrifugal forces. As an example, unbroken whole cells will pellet at low speeds and short intervals such as 1,000g for 5 minutes. Smaller cell fragments and organelles remain in the supernatant and require more force and greater times to pellet. In general, one can enrich for the following cell components, in the separating order in actual application:

- Whole cells and nuclei;
- Mitochondria, chloroplasts, lysosomes, and peroxisomes;
- Microsomes (vesicles of disrupted endoplasmic reticulum); and
- Ribosomes and cytosol.

Sample preparation

Before differential centrifugation can be carried out to separate different portions of a cell from one another, the tissue sample must first be lysed. In this process, a blender, usually a piece of porous porcelain of the same shape and dimension as the container is used. The container is, in most cases, a glass boiling tube.

The tissue sample is first crushed and a buffer solution is added to it, forming a liquid suspension of crushed tissue sample. The buffer solution is a dense, inert, aqueous solution which is designed to suspend the sample in a liquid medium without damaging it through chemical reactions or osmosis. In most cases, the solution used is sucrose solution; in certain cases brine will be used. Then, the blender, connected to a high-speed rotor, is inserted into the container holding the sample, pressing the crushed sample against the wall of the container.

With the rotator turned on, the tissue sample is ground by the porcelain pores and the container wall into tiny fragments. This grinding process will break the cell membranes of the sample's cells, leaving individual organelles suspended in the solution. This process is called cell lysis. A portion of cells will remain intact after grinding and some organelles will be damaged, and these will be catered for in the later stages of centrifugation.

Ultracentrifugation

The lysed sample is now ready for centrifugation in an ultracentrifuge. An ultracentrifuge consists of a refrigerated, low-pressure chamber containing a rotor which is driven by an electrical motor capable of high speed rotation. Samples are placed in tubes within or attached to the rotor. Rotational speed may reach up to 100,000 rpm for floor model, 150,000 rpm for bench-top model (Beckman Optima Max-XP or Sorvall MTX150), and creating centrifugal speed forces of 800,000g to 1,000,000g. This force causes sedimentation of macromolecules, and can even cause non-uniform distributions of small molecules.

Since different fragments of a cell have different sizes and densities, each fragment will settle into a pellet with different minimum centrifugal forces. Thus, separation of the sample into different layers can be done by first centrifuging the original lysate under weak forces, removing the pellet, then exposing the subsequent supernatants to sequentially greater centrifugal fields. Each time a portion of different density is sediment to the bottom of the container and extracted and repeated application produces a rank of layers which includes different parts of the original sample. Additional steps can be taken to further refine each of the obtained pellets.

Sedimentation depends on mass, shape, and partial specific volume of a macromolecule, as well as solvent density, rotor size and rate of rotation. The sedimentation velocity can be monitored during the experiment to calculate molecular weight. Values of sedimentation coefficient (S) can be calculated. Large values of S (faster sedimentation rate) correspond to larger molecular weight. Dense particle sediments more rapidly. Elongated proteins have larger frictional coefficients and sediment more slowly to ensure accuracy.

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I M. Sc. MICROBIOLOGY 2017 – 2019

BIOINSTRUMENTATION (17MBP104)

SEMESTER – I **4H – 4C**

UNIT III

S. No	Duration	Topic	Reference
1	1	Chromatography – Introduction	T2: 60-61
2	1	Ion exchange chromatography	T1: 203-206
3	1	Affinity chromatography	T1: 207-210
4	1	Gel filtration chromatography	T1: 211-215
5	1	Low pressure liquid chromatography (LPLC)	T2: 74-78
6	1	High performance liquid chromatography (HPLC)	W3
7	1	Fast protein liquid chromatography (FPLC)	W4
8	1	Gas liquid chromatography –Mass spectroscopy (GC-MS)	W5
9	1	Unit revision	
Total Hrs: 9			

T1: Bioinstrumentation – L. Veerakumari

T2: Analytical biochemistry (Biochemical technique) Dr. P. Asokan

W3: https://en.wikipedia.org/.wiki/highperformance liquid chromatography

W4: https://en.wikipedia.org/wiki/Fast_protein_liquid_chromatography

W5: https://en.wikipedia.org/wiki/Gas_chromatography%E2%80%93mass_spectrometry

2017

Bioinstrumentation

Unit III Notes

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UNIT – 3

Chromatography

Chromatography is a versatile method of separating many different kinds of chemical mixtures. The process or technique of separating molecules or components in mixture solutions (gas or liquid) according to the differential absorption and elution. Invented in 1906 by Russian botanist Mikhail Tsvet. Chromatography is the physical separation of a mixture into its individual components. Used in qualitative and quantitative analysis of biological and chemical substances.

Basics of Chromatography

In any chromatographic technique, a **stationary phase** usually a solid, thick liquid or bonded coating that stays fixed in one place, and a **mobile phase** or **eluent** (usually a liquid or gas) moves through it or across it. A sample to be separated, when placed on the stationary phase, will gradually move along in the same direction as the mobile phase. If a sample compound (or **analyte**) has no interaction with the stationary phase, it will run right through and come out of the system (**elute**) at the same rate as the mobile phase. On the other hand, if an analyte has no interaction with the mobile phase, it will stick directly to the stationary phase and never elute. Neither of these are good outcomes.

Types of Chromatography

- ✓ Adsorption Chromatography
- ✓ Partition Chromatography
- ✓ Ion Exchange Chromatography
- ✓ Molecular Exclusion Chromatography
- ✓ Affinity Chromatography

Ion exchange chromatography:

Ion exchange chromatography is a process that allows separation of ions and polar molecules change. Ion exchange chromatography is coined by Smail, Stevens and Raumann. It can be used for almost any kind of charged molecules including large proteins, small nucleotides and amino acids. Ion exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge.

This type of chromatography is further subdivided into two. They are cation exchange chromatography and anion exchange chromatography.

The ionic compound consisting of the cationic species M^+ and the anionic species B can be retained by the stationary phase. Cationic exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group. Anion

exchange chromatography retains anion using positively charged functional group. Ion exchange is classified into resins, gels and inorganic exchanger.

Resins:

Resins are amorphous particles of organic materials. Ion exchange resins are used for the separation of small molecules.

Classification of ion exchange resins:

Strongly acidic cation exchanger – Sulphonic acid groups attached to styrene and divinyl benzene copolymer.

Weakly acidic cation exchanger – Carboxylic groups attached to acrylic and divinyl benzene copolymer.

Strongly basic anion exchanger – Quaternary ammonium groups attached to styrene and divinyl benzene copolymer.

Weakly basic anion exchanger – poly alkyl amine groups attached to styrene and divinyl benzene copolymer.

Ion exchange gels:

Cellulose and dextran ion exchangers, which are polymers of the sugar glucose, posses larger pore size and lower charge densities. Because they are much softer than polystyrene resins, dextran and its relatives are called gels. Ion exchange gels consist of,

Column: Glass, stainless steel or polymers, Length: Diameter, Ratio: 20:100, Packing column: Wet packing method, Application of the sample: After packing, sample is added to the top of the column, use syringe or pipette.

Mobile phase: Acids, alkalis, buffers.

Elution: Components of mixture separate and move down the column at different rates depending upon the affinity of the ion for ion exchanger.

The elutes are collected at different stages,

Analysis of elute: Spectrophotometric, flame

Method:

A sample is introduced either manually or with an auto sampler, into a sample loop of known volume. The mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material. Stationary phase material is used as a resin or gel matrix consisting of agarose or cellulose beads with covalently bonded charged functional groups. The target analytes are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary phase. The analyte of interest must then be detected by some means, typically by conductivity or UV/Visible light absorbance. A chromatography data system (CDS) is usually needed to control an IC.

Advantages:

- It is a non-denaturing technique. It can be used at all stages and scales of purification

- An IEX separation can be controlled by changing pH, salt concentration and/or the ion exchange media
- It can serve as a concentrating step. A large volume of dilute sample can be applied to a media, and the adsorbed protein subsequently eluted in a smaller volume
- It offers high selectivity; it can resolve molecules with small differences in charge.

Disadvantages:

- Costly equipment and more expensive chemicals
- Turbidity should be below 10 ppm.

Affinity chromatography:

Affinity chromatography is essential sample purification technique used primarily for biological molecules such as proteins. It is a method of separating a mixture of proteins or nucleic acids (molecules) by specific interaction of those molecules with a component known as a ligand, which is immobilized on a support. If a solution of a mixture of proteins is passed over the column, one of the proteins binds to the ligand on the basis of specificity and high affinity. The other proteins in the solution wash through the column because they were not able to bind to the ligand.

Principle:

Affinity chromatography is one of the most diverse and powerful methods for purification of a specific molecule or a group of molecules from complex mixtures. It is based on highly specific biological interactions between two molecules such as interactions between enzyme and substrate, receptor and ligand or antibody and antigen. These interactions which are typically reversible are used for purification by placing one if the interacting molecules referred to as affinity lignand onto a solid matrix to create a stationary pase while a target molecule are in the mobile phase. Many of the commonly used ligands coupled to affinity matrices are now commercially available and are ready to use.

Chromatographic media:

A matrix in its use here is a substance, usually in bead form to which a specific ligand is covalently bound. In order to form the matrix to be effective it must have certain characters such as,

- It must be insoluble in solvents and buffers employed in the process.
- It must be chemically and mechanically stable.
- It must be easily coupled to a ligand or spacer arm onto which the ligand can be attached.
- It must exhibit good flow properties and have a relatively large surface area for attachment.

Immobilized ligand:

- The ligand can be selected only after the nature of the macromolecule to be isolated is known.
- When a hormone receptor protein is to be purified by affinity chromatography, the hormone itself is an ideal candidate for the ligand.
- For antibody isolation, an antigen or hapten may be used as ligand.
- If an enzyme is to be purified, a substrate analog, inhibitor, cofactor or effector may be used as the immobilized ligand.

Attachment of ligand to matrix:

Several procedures have been developed for the covalent attachment of the ligand to the stationary phase. All procedures for gel modification proceed in two separate chemical steps:

- Activation of the ligand to the functional group on the matrix.
- Joining of the ligand to the functional group on the matrix.

Steps:

1. Loading affinity column
2. Proteins sieve through matrix of affinity beads.
3. Proteins interact with affinity ligand with some binding loosely and others tightly.
4. Wash off proteins that do not bind.
5. Wash off proteins that bind loosely.
6. Elute proteins that bind tightly to ligand and collect purified protein of interest.

Applications:

1. It is used for isolation and purification of all biological macromolecule.
2. It is used to purify nucleic acid, antibodies, enzymes etc.
3. To notice which biological compounds bind to a particular substance.
4. To reduce an amount of substance in a mixture.

High-performance liquid chromatography

High-performance liquid chromatography (**HPLC**; formerly referred to as **high-pressure liquid chromatography**), is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture. It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

Instrumentation: The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector. The sampler brings the sample mixture into the mobile phase stream which carries it into the column. The pumps deliver the desired flow and composition of the mobile phase through the column. The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components. A digital microprocessor and user software control the HPLC instrument and provide data analysis.

Operation

The sample mixture to be separated and analyzed is introduced in a discrete small volume (typically microliters), into the stream of mobile phase percolating through the column. The components of the sample move through the column at different velocities, which are function of specific physical interactions with the sorbent (also called stationary phase). The velocity of each component depends on its chemical nature, on the nature of the stationary phase (column) and on the composition of the mobile phase. The time at which a specific analyte elutes (emerges from the column) is called its retention time. The retention time measured under particular conditions is considered an identifying characteristic of a given analyte.

Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol). Some HPLC techniques use water-free mobile phases. The aqueous component of the mobile phase may contain acids (such as formic, phosphoric or trifluoroacetic acid) or salts to assist in the separation of the sample components. The composition of the mobile phase may be kept constant ("isocratic elution mode") or varied ("gradient elution mode") during the chromatographic analysis. Isocratic elution is typically effective in the separation of sample components that are not very different in their affinity for the stationary phase. In gradient elution the composition of the mobile phase is varied typically from low to high eluting strength. The eluting strength of the mobile phase is reflected by analyte retention times with high eluting strength producing fast elution (=short retention times). A typical gradient profile in reversed phase chromatography might start at 5% acetonitrile (in water or aqueous buffer) and progress linearly to 95% acetonitrile over 5–25 minutes. Periods of constant mobile phase composition may be part of any gradient profile. For example, the mobile phase composition may be kept constant at 5% acetonitrile for 1–3 min, followed by a linear change up to 95% acetonitrile.

The chosen composition of the mobile phase (also called eluent) depends on the intensity of interactions between various sample components ("analytes") and stationary phase (e.g. hydrophobic interactions in reversed-phase HPLC). Depending on their affinity for the stationary and mobile phases analytes partition between the two during the separation process taking place in the column. This partitioning process is similar to that which occurs during a liquid–liquid extraction but is continuous, not step-wise. In this example, using a water/acetonitrile gradient,

more hydrophobic components will elute (come off the column) late, once the mobile phase gets more concentrated in acetonitrile (i.e. in a mobile phase of higher eluting strength).

The choice of mobile phase components, additives (such as salts or acids) and gradient conditions depends on the nature of the column and sample components. Often a series of trial runs is performed with the sample in order to find the HPLC method which gives adequate separation.

Fast protein liquid chromatography (FPLC), is a form of liquid chromatography that is often used to analyze or purify mixtures of proteins. As in other forms of chromatography, separation is possible because the different components of a mixture have different affinities for two materials, a moving fluid (the "mobile phase") and a porous solid (the stationary phase). In FPLC the mobile phase is an aqueous solution, or "buffer". The buffer flow rate is controlled by a positive-displacement pump and is normally kept constant, while the composition of the buffer can be varied by drawing fluids in different proportions from two or more external reservoirs. The stationary phase is a resin composed of beads, usually of cross-linked agarose, packed into a cylindrical glass or plastic column. FPLC resins are available in a wide range of bead sizes and surface ligands depending on the application.

In the most common FPLC strategy, ion exchange, a resin is chosen that the protein of interest will bind to the resin by a charge interaction while in buffer A (the running buffer) but become dissociated and return to solution in buffer B (the elution buffer). A mixture containing one or more proteins of interest is dissolved in 100% buffer A and pumped into the column. The proteins of interest bind to the resin while other components are carried out in the buffer. The total flow rate of the buffer is kept constant; however, the proportion of Buffer B (the "elution" buffer) is gradually increased from 0% to 100% according to a programmed change in concentration (the "gradient"). At some point during this process each of the bound proteins dissociates and appears in the effluent. The effluent passes through two detectors which measure salt concentration (by conductivity) and protein concentration (by absorption of ultraviolet light at a wavelength of 280nm). As each protein is eluted it appears in the effluent as a "peak" in protein concentration and can be collected for further use.

FPLC was developed and marketed in Sweden by Pharmacia in 1982 and was originally called **fast performance liquid chromatography** to contrast it with HPLC or high-performance liquid chromatography. FPLC is generally applied only to proteins; however, because of the wide choice of resins and buffers it has broad applications. In contrast to HPLC the buffer pressure used is relatively low, typically less than 5 bar, but the flow rate is relatively high, typically 1-5 ml/min. FPLC can be readily scaled from analysis of milligrams of mixtures in columns with a total volume of 5ml or less to industrial production of kilograms of purified protein in columns with volumes of many liters. When used for analysis of mixtures the effluent is usually collected in fractions of 1-5 ml which can be further analyzed, e.g. by MALDI mass

spectrometry. When used for protein purification there may be only two collection containers, one for the purified product and one for waste.

A typical laboratory FPLC consists of one or two high-precision pumps, a control unit, a column, a detection system and a fraction collector. Although it is possible to operate the system manually, the components are normally linked to a personal computer or, in older units, a microcontroller.

1. Pumps: GE/Pharmacia systems utilize two two-cylinder piston pumps, one for each buffer, combining the output of both in a mixing chamber. Waters systems use a single peristaltic pump which draws both buffers from separate reservoirs through a proportioning valve and mixing chamber. In either case the system allows the fraction of each buffer entering the column to be continuously varied. The flow rate can go from a few milliliters per minute in bench-top systems to liters per minute for industrial scale purifications. The wide flow range makes it suitable both for analytical and preparative chromatography.

2. Injection loop: A segment of tubing of known volume which is filled with the sample solution before it is injected into the column. Loop volume can range from a few microliters to 50ml or more.

3. Injection valve: A motorized valve which links the mixer and sample loop to the column. Typically the valve has three positions for loading the sample loop, for injecting the sample from the loop into the column, and for connecting the pumps directly to the waste line to wash them or change buffer solutions. The injection valve has a sample loading port through which the sample can be loaded into the injection loop, usually from a hypodermic syringe using a Luer-lock connection.

4. Column: The column is a glass or plastic cylinder packed with beads of resin and filled with buffer solution. It is normally mounted vertically with the buffer flowing downward from top to bottom. A glass frit at the bottom of the column retains the resin beads in the column while allowing the buffer and dissolved proteins to exit.

5. Flow Cells: The effluent from the column passes through one or more flow cells to measure the concentration of protein in the effluent (by UV light absorption at 280nm). The conductivity cell measures the buffer conductivity, usually in millisiemens/cm, which indicates the concentration of salt in the buffer. A flow cell which measures pH of the buffer is also commonly included. Usually each flow cell is connected to a separate electronics module which provides power and amplifies the signal.

6. Monitor/Recorder: The flow cells are connected to a display and/or recorder. On older systems this was a simple chart recorder, on modern systems a computer with hardware interface and display is used. This permits the experimenter to identify when peaks in protein concentration occur, indicating that specific components of the mixture are being eluted.

7. Fraction collector: The fraction collector is typically a rotating rack that can be filled with test tubes or similar containers. It allows samples to be collected in fixed volumes, or can be controlled to direct specific fractions detected as peaks of protein concentration, into separate containers.

Many systems include various optional components. A filter may be added between the mixer and column to minimize clogging. In large FPLC columns the sample may be loaded into the column directly using a small peristaltic pump rather than an injection loop. When the buffer contains dissolved gas, bubbles may form as pressure drops where the buffer exits the column; these bubbles create artifacts if they pass through the flow cells. This may be prevented by degassing the buffers, or by adding a flow restrictor downstream of the flow cells to maintain a pressure of 1-5 bar in the effluent line.

The columns used in FPLC are large [mm id] tubes that contain small [µ] particles or gel beads that are known as stationary phase. The chromatographic bed is composed by the gel beads inside the column and the sample is introduced into the injector and carried into the column by the flowing solvent. As a result of different components adhering to or diffusing through the gel, the sample mixture gets separated.

Columns used with an FPLC can separate macromolecules based on size, charge distribution (ion exchange), hydrophobicity, reverse-phase or biorecognition (as with affinity chromatography). For easy use, a wide range of pre-packed columns for techniques such as ion exchange, gel filtration (size exclusion), hydrophobic interaction, and affinity chromatography are available.[6] FPLC differs from HPLC in that the columns used for FPLC can only be used up to maximum pressure of 3-4 MPa (435-580 psi). Thus, if the pressure of HPLC can be limited, each FPLC column may also be used in an HPLC machine.

Optimizing protein purification

Using a combination of chromatographic methods, purification of the target molecule is achieved. The purpose of purifying proteins with FPLC is to deliver quantities of the target at sufficient purity in a biologically active state to suit its further use. The quality of the end product varies depending the type and amount of starting material, efficiency of separation, and selectivity of the purification resin. The ultimate goal of a given purification protocol is to deliver the required yield and purity of the target molecule in the quickest, cheapest, and safest way for acceptable results. The range of purity required can be from that required for basic analysis (SDS-PAGE or ELISA, for example), with only bulk impurities removed, to pure enough for structural analysis (NMR or X-ray crystallography), approaching >99% target molecule. Purity required can also mean pure enough that the biological activity of the target is retained. These demands can be used to determine the amount of starting material required to reach the experimental goal. If the starting material is limited and full optimization of purification protocol cannot be performed, then a safe standard protocol that requires a minimum adjustment and optimization steps are expected. This may not be optimal with respect to

experimental time, yield, and economy but it will achieve the experimental goal. On the other hand, if the starting material is enough to develop more complete protocol, the amount of work to reach the separation goal depends on the available sample information and target molecule properties. Limits to development of purification protocols many times depends on the source of the substance to be purified, whether from natural sources (harvested tissues or organisms, for example), recombinant sources (such as using prokaryotic or eukaryotic vectors in their respective expression systems), or totally synthetic sources.

No chromatographic techniques provide 100% yield of active material and overall yields depend on the number of steps in the purification protocol. By optimizing each step for the intended purpose and arranging them that minimizes inter step treatments, the number of steps will be minimized.

A typical multistep purification protocol starts with a preliminary capture step which many times utilize ion exchange chromatography (IEC). The media (stationary phase) employed range from large bead resins (good for fast flow rates and little to no sample clarification at the expense of resolution) to small bead resins (for best possible resolution with all other factors being equal). Short and wide column geometries are amenable to high flow rates also at the expense of resolution, typically because of lateral diffusion of sample on the column. For techniques such as size exclusion chromatography to be useful, very long, thin columns and minimal sample volumes (maximum 5% of column volume) are required. Hydrophobic interaction chromatography (HIC) can also be used for first and/ or intermediate steps. Selectivity in HIC is independent of running pH and descending salt gradients are used. For HIC, conditioning involves adding ammonium sulphate to the sample to match the buffer A concentration. If HIC is used before IEC, the ionic strength would have to be lowered to match that of buffer A for IEC step by dilution, dialysis or buffer exchange by gel filtration. This is why IEC is usually performed prior to HIC as the high salt elution conditions for IEC are ideal for binding to HIC resins in the next purification step. Polishing is used to achieve the final level of purification required and is commonly performed on a gel filtration column. An extra intermediate purification step can be added or optimization of the different steps is performed for improving purity. This extra step usually involves another round of IEC under completely different conditions.

Although this is an example of a common purification protocol for proteins, the buffer conditions, flow rates, and resins used to achieve final goals can be chosen to cover a broad range of target proteins. This flexibility is imperative for a functional purification system as all proteins behave differently and often deviate from predictions.

Gas chromatography–mass spectrometry (**GC-MS**) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental

analysis, explosives investigation, and identification of unknown samples, including that of material samples obtained from planet Mars during probe missions as early as the 1970s. GC-MS can also be used in airport security to detect substances in luggage or on human beings. Additionally, it can identify trace elements in materials that were previously thought to have disintegrated beyond identification.

Instrumentation

The GC-MS is composed of two major building blocks: the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the column's dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% phenyl polysiloxane). The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the column will promote separation of the molecules as the sample travels the length of the column. The molecules are retained by the column and then elute (come off) from the column at different times (called the retention time), and this allows the mass spectrometer downstream to capture, ionize, accelerate, deflect, and detect the ionized molecules separately. The mass spectrometer does this by breaking each molecule into ionized fragments and detecting these fragments using their mass-to-charge ratio.

These two components, used together, allow a much finer degree of substance identification than either unit used separately. It is not possible to make an accurate identification of a particular molecule by gas chromatography or mass spectrometry alone. The mass spectrometry process normally requires a very pure sample while gas chromatography using a traditional detector (e.g. Flame ionization detector) cannot differentiate between multiple molecules that happen to take the same amount of time to travel through the column (*i.e.* have the same retention time), which results in two or more molecules that co-elute. Sometimes two different molecules can also have a similar pattern of ionized fragments in a mass spectrometer (mass spectrum). Combining the two processes reduces the possibility of error, as it is extremely unlikely that two different molecules will behave in the same way in both a gas chromatograph and a mass spectrometer. Therefore, when an identifying mass spectrum appears at a characteristic retention time in a GC-MS analysis, it typically increases certainty that the analyte of interest is in the sample.

Types of mass spectrometer detectors

The most common type of mass spectrometer (MS) associated with a gas chromatograph (GC) is the quadrupole mass spectrometer, sometimes referred to by the Hewlett-Packard (now Agilent) trade name "Mass Selective Detector" (MSD). Another relatively common detector is the ion trap mass spectrometer. Additionally one may find a magnetic sector mass spectrometer, however these particular instruments are expensive and bulky and not typically found in high-throughput service laboratories. Other detectors may be encountered such as time of flight

(TOF), tandem quadrupoles (MS-MS) (see below), or in the case of an ion trap MS^n where n indicates the number mass spectrometry stages.

GC-tandem MS

When a second phase of mass fragmentation is added, for example using a second quadrupole in a quadrupole instrument, it is called tandem MS (MS/MS). MS/MS can sometimes be used to quantitate low levels of target compounds in the presence of a high sample matrix background.

The first quadrupole (Q1) is connected with a collision cell (q2) and another quadrupole (Q3). Both quadrupoles can be used in scanning or static mode, depending on the type of MS/MS analysis being performed. Types of analysis include product ion scan, precursor ion scan, selected reaction monitoring (SRM) (sometimes referred to as multiple reaction monitoring (MRM)) and neutral loss scan. For example: When Q1 is in static mode (looking at one mass only as in SIM), and Q3 is in scanning mode, one obtains a so-called product ion spectrum (also called "daughter spectrum"). From this spectrum, one can select a prominent product ion which can be the product ion for the chosen precursor ion. The pair is called a "transition" and forms the basis for SRM. SRM is highly specific and virtually eliminates matrix background.

Ionization

After the molecules travel the length of the column, pass through the transfer line and enter into the mass spectrometer they are ionized by various methods with typically only one method being used at any given time. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected.

The ionization technique chosen is independent of using full scan or SIM.

Electron ionization

By far the most common and perhaps standard form of ionization is electron ionization (EI). The molecules enter into the MS (the source is a quadrupole or the ion trap itself in an ion trap MS) where they are bombarded with free electrons emitted from a filament, not unlike the filament one would find in a standard light bulb. The electrons bombard the molecules, causing the molecule to fragment in a characteristic and reproducible way. This "hard ionization" technique results in the creation of more fragments of low mass to charge ratio (m/z) and few, if any, molecules approaching the molecular mass unit. Hard ionization is considered by mass spectrometrists as the employ of molecular electron bombardment, whereas "soft ionization" is charge by molecular collision with an introduced gas. The molecular fragmentation pattern is dependent upon the electron energy applied to the system, typically 70 eV (electron Volts). The use of 70 eV facilitates comparison of generated spectra with library spectra using manufacturer-supplied software or software developed by the National Institute of Standards (NIST-USA).

Spectral library searches employ matching algorithms such as Probability Based Matching and dot-product matching that are used with methods of analysis written by many method standardization agencies. Sources of libraries include NIST, Wiley, the AAFS, and instrument manufacturers.

Cold electron ionization

The "hard ionization" process of electron ionization can be softened by the cooling of the molecules before their ionization, resulting in mass spectra that are richer in information. In this method named cold electron ionization (Cold-EI) the molecules exit the GC column, mixed with added helium make up gas and expand into vacuum through a specially designed supersonic nozzle, forming a supersonic molecular beam (SMB). Collisions with the makeup gas at the expanding supersonic jet reduce the internal vibrational (and rotational) energy of the analyte molecules, hence reducing the degree of fragmentation caused by the electrons during the ionization process. Cold-EI mass spectra are characterized by an abundant molecular ion while the usual fragmentation pattern is retained, thus making Cold-EI mass spectra compatible with library search identification techniques. The enhanced molecular ions increase the identification probabilities of both known and unknown compounds, amplify isomer mass spectral effects and enable the use of isotope abundance analysis for the elucidation of elemental formulae.

Chemical ionization

In chemical ionization a reagent gas, typically methane or ammonia is introduced into the mass spectrometer. Depending on the technique (positive CI or negative CI) chosen, this reagent gas will interact with the electrons and analyte and cause a 'soft' ionization of the molecule of interest. A softer ionization fragments the molecule to a lower degree than the hard ionization of EI. One of the main benefits of using chemical ionization is that a mass fragment closely corresponding to the molecular weight of the analyte of interest is produced.

In positive chemical ionization (PCI) the reagent gas interacts with the target molecule, most often with a proton exchange. This produces the species in relatively high amounts.

In negative chemical ionization (NCI) the reagent gas decreases the impact of the free electrons on the target analyte. This decreased energy typically leaves the fragment in great supply.

Analysis

A mass spectrometer is typically utilized in one of two ways: full scan or selected ion monitoring (SIM). The typical GC-MS instrument is capable of performing both functions either individually or concomitantly, depending on the setup of the particular instrument.

The primary goal of instrument analysis is to quantify an amount of substance. This is done by comparing the relative concentrations among the atomic masses in the generated spectrum. Two

kinds of analysis are possible, comparative and original. Comparative analysis essentially compares the given spectrum to a spectrum library to see if its characteristics are present for some sample in the library. This is best performed by a computer because there are a myriad of visual distortions that can take place due to variations in scale. Computers can also simultaneously correlate more data (such as the retention times identified by GC), to more accurately relate certain data.

Another method of analysis measures the peaks in relation to one another. In this method, the tallest peak is assigned 100% of the value, and the other peaks being assigned proportionate values. All values above 3% are assigned. The total mass of the unknown compound is normally indicated by the parent peak. The value of this parent peak can be used to fit with a chemical formula containing the various elements which are believed to be in the compound. The isotope pattern in the spectrum, which is unique for elements that have many isotopes, can also be used to identify the various elements present. Once a chemical formula has been matched to the spectrum, the molecular structure and bonding can be identified, and must be consistent with the characteristics recorded by GC-MS. Typically, this identification done automatically by programs which come with the instrument, given a list of the elements which could be present in the sample.

A "full spectrum" analysis considers all the "peaks" within a spectrum. Conversely, selective ion monitoring (SIM) only monitors selected ions associated with a specific substance. This is done on the assumption that at a given retention time, a set of ions is characteristic of a certain compound. This is a fast and efficient analysis, especially if the analyst has previous information about a sample or is only looking for a few specific substances. When the amount of information collected about the ions in a given gas chromatographic peak decreases, the sensitivity of the analysis increases. So, SIM analysis allows for a smaller quantity of a compound to be detected and measured, but the degree of certainty about the identity of that compound is reduced.

Full scan MS

When collecting data in the full scan mode, a target range of mass fragments is determined and put into the instrument's method. An example of a typical broad range of mass fragments to monitor would be *m/z* 50 to *m/z* 400. The determination of what range to use is largely dictated by what one anticipates being in the sample while being cognizant of the solvent and other possible interferences. A MS should not be set to look for mass fragments too low or else one may detect air (found as *m/z* 28 due to nitrogen), carbon dioxide (*m/z* 44) or other possible interferences. Additionally if one is to use a large scan range then sensitivity of the instrument is decreased due to performing fewer scans per second since each scan will have to detect a wide range of mass fragments.

Full scan is useful in determining unknown compounds in a sample. It provides more information than SIM when it comes to confirming or resolving compounds in a sample. During instrument method development it may be common to first analyze test solutions in full scan mode to determine the retention time and the mass fragment fingerprint before moving to a SIM instrument method.

Selected ion monitoring

In selected ion monitoring (SIM) certain ion fragments are entered into the instrument method and only those mass fragments are detected by the mass spectrometer. The advantages of SIM are that the detection limit is lower since the instrument is only looking at a small number of fragments (e.g. three fragments) during each scan. More scans can take place each second. Since only a few mass fragments of interest are being monitored, matrix interferences are typically lower. To additionally confirm the likelihood of a potentially positive result, it is relatively important to be sure that the ion ratios of the various mass fragments are comparable to a known reference standard.

Applications

Environmental monitoring and cleanup

GC-MS is becoming the tool of choice for tracking organic pollutants in the environment. The cost of GC-MS equipment has decreased significantly, and the reliability has increased at the same time, which has contributed to its increased adoption in environmental studies.

Criminal forensics

GC-MS can analyze the particles from a human body in order to help link a criminal to a crime. The analysis of fire debris using GC-MS is well established, and there is even an established American Society for Testing and Materials (ASTM) standard for fire debris analysis. GCMS/MS is especially useful here as samples often contain very complex matrices and results, used in court, need to be highly accurate.

Law enforcement

GC-MS is increasingly used for detection of illegal narcotics, and may eventually supplant drug-sniffing dogs. It is also commonly used in forensic toxicology to find drugs and/or poisons in biological specimens of suspects, victims, or the deceased.

Sports anti-doping analysis

GC-MS is the main tool used in sports anti-doping laboratories to test athletes' urine samples for prohibited performance-enhancing drugs, for example anabolic steroids.

Security

A post–September 11 development, explosive detection systems have become a part of all US airports. These systems run on a host of technologies, many of them based on GC-MS. There are only three manufacturers certified by the FAA to provide these systems, one of which is Thermo Detection (formerly Thermedics), which produces the EGIS, a GC-MS-based line of explosives detectors. The other two manufacturers are Barringer Technologies, now owned by Smith's Detection Systems, and Ion Track Instruments, part of General Electric Infrastructure Security Systems.

Chemical warfare agent detection

As part of the post-September 11 drive towards increased capability in homeland security and public health preparedness, traditional GC-MS units with transmission quadrupole mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field portability and near real-time detection of chemical warfare agents (CWA) such as sarin, soman, and VX. These complex and large GC-MS systems have been modified and configured with resistively heated low thermal mass (LTM) gas chromatographs that reduce analysis time to less than ten percent of the time required in traditional laboratory systems. Additionally, the systems are smaller, and more mobile, including units that are mounted in mobile analytical laboratories (MAL), such as those used by the United States Marine Corps Chemical and Biological Incident Response Force MAL and other similar laboratories, and systems that are hand-carried by two-person teams or individuals, much ado to the smaller mass detectors. Depending on the system, the analytes can be introduced via liquid injection, desorbed from sorbent tubes through a thermal desorption process, or with solid-phase micro extraction (SPME).

Chemical Engineering

GC-MS is used for the analysis of unknown organic compound mixtures. One critical use of this technology is the use of GC-MS to determine the composition of bio-oils processed from raw biomass.

Food, beverage and perfume analysis

Foods and beverages contain numerous aromatic compounds, some naturally present in the raw materials and some forming during processing. GC-MS is extensively used for the analysis of these compounds which include esters, fatty acids, alcohols, aldehydes, terpenes etc. It is also used to detect and measure contaminants from spoilage or adulteration which may be harmful and which are often controlled by governmental agencies, for example pesticides.

Astrochemistry

Several GC-MS have left earth. Two were brought to Mars by the Viking program. Venera 11 and 12 and Pioneer Venus analysed the atmosphere of Venus with GC-MS. The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon, Titan. The material in the comet 67P/Churyumov-Gerasimenko will be analysed by the Rosetta mission with a chiral GC-MS in 2014.

Medicine

Dozens of congenital metabolic diseases also known as Inborn error of metabolism are now detectable by newborn screening tests, especially the testing using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering with metabolic disorders. This is increasingly becoming a common way to diagnose IEM for earlier diagnosis and institution of treatment eventually leading to a better outcome. It is now possible to test a newborn for over 100 genetic metabolic disorders by a urine test at birth based on GC-MS.

In combination with isotopic labeling of metabolic compounds, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C as the labeling and the measurement of ^{13}C-^{12}C ratios with an **isotope ratio mass spectrometer** (**IRMS**); an MS with a detector designed to measure a few select ions and return values as ratios.

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I M. Sc. MICROBIOLOGY 2017 – 2019

BIOINSTRUMENTATION (17MBP104)

SEMESTER – I **4H – 4C**

UNIT IV

S. No	Duration	Topic	Reference
1	1	Electrophoresis – Introduction	T1: 239
2	1	Agarose gel electrophoresis native PAGE	T1: 247-249
3	1	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis	T1: 254-257
4	1	Isoelectric focusing	T1: 257-259
5	1	Immuno electrophoresis	T1: 263-266
6	1	Pulse field gel electrophoresis	T1: 251-253
7	1	Capillary electrophoresis	T1: 260-263
8	1	Gel documentation – application	T1:271-273
9	1	Unit revision	
Total Hrs: 9			

T1: Bioinstrumentation – L. Veerakumari

2017

Bioinstrumentation

Unit-IV Notes

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UNIT – 4

Electrophoresis

Agarose gel electrophoresis is a method of gel electrophoresis used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of DNA or proteins in a matrix of agarose. The proteins may be separated by charge and/or size (isoelectric focusing agarose electrophoresis is essentially size independent), and the DNA and RNA fragments by length. Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix, and the biomolecules are separated by size in the agarose gel matrix.

Agarose gels are easy to cast and are particularly suitable for separating DNA of size range most often encountered in laboratories, which accounts for the popularity of its use. The separated DNA may be viewed with stain, most commonly under UV light, and the DNA fragments can be extracted from the gel with relative ease. Most agarose gels used are between 0.7 - 2% dissolved in a suitable electrophoresis buffer.

Principle

Principles of Gel Electrophoresis Electrophoresis are a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. As such, it is one of the most widely-used techniques in biochemistry and molecular biology.

Casting of gel

The gel is prepared by dissolving the agarose powder in an appropriate buffer, such as TAE or TBE, to be used in electrophoresis. The agarose is dispersed in the buffer before heating it to near-boiling point, but avoid boiling. The melted agarose is allowed to cool sufficiently before pouring the solution into a cast as the cast may warp or crack if the agarose solution is too hot. A comb is placed in the cast to create wells for loading sample, and the gel should be completely set before use.

The concentration of gel affects the resolution of DNA separation. For a standard agarose gel electrophoresis, a 0.8% gives good separation or resolution of large 5–10kb DNA fragments, while 2% gel gives good resolution for small 0.2–1kb fragments. 1% gels is often used for a standard electrophoresis. The concentration is measured in weight of agarose over volume of buffer used (g/ml). High percentage gels are often brittle and may not set evenly, while low percentage gels (0.1-0.2%) are fragile and not easy to handle. Low-melting-point (LMP) agarose gels are also more fragile than normal agarose gel. Low-melting point agarose may be used on its own or simultaneously with standard agarose for the separation and isolation of DNA. PFGE and FIGE are often done with high percentage agarose gels.

Loading of samples

Once the gel has set, the comb is removed, leaving wells where DNA samples can be loaded. Loading buffer is mixed with the DNA sample before the mixture is loaded into the wells. The loading buffer contains a dense compound, which may be glycerol, sucrose, or Ficoll, that raises the density of the sample so that the DNA sample may sink to the bottom of the well. If the DNA sample contains residual ethanol after its preparation, it may float out of the well. The loading buffer also include colored dyes such as xylene cyanol and bromophenol blue used to monitor the progress of the electrophoresis. The DNA samples are loaded using a pipette.

Electrophoresis

Agarose gel electrophoresis is most commonly done horizontally in a submarine mode whereby the slab gel is completely submerged in buffer during electrophoresis. It is also possible, but less common, to perform the electrophoresis vertically, as well as horizontally with the gel rose on agarose legs using an appropriate apparatus. The buffer used in the gel is the same as the running buffer in the electrophoresis tank, which is why electrophoresis in the submarine mode is possible with agarose gel.

For optimal resolution of DNA greater than 2 kb in size in standard gel electrophoresis, 5 to 8 V/cm is recommended (the distance in cm refers to the distance between electrodes, therefore this recommended voltage would be 5 to 8 multiplied by the distance between the electrodes in cm). Voltage may also be limited by the fact that it heats the gel and may cause the gel to melt if it is run at high voltage for a prolonged period, especially if the gel used is LMP agarose gel. Too high a voltage may also reduce resolution, as well as causing band streaking for large DNA molecules. Too low a voltage may lead to broadening of band for small DNA fragments due to dispersion and diffusion.

Since DNA is not visible in natural light, the progress of the electrophoresis is monitored using colored dyes. Xylene cyanol (light blue color) comigrates large DNA fragments, while Bromophenol blue (dark blue) comigrates with the smaller fragments. Less commonly used dyes include Cresol Red and Orange G which migrate ahead of bromophenol blue. A DNA marker is also run together for the estimation of the molecular weight of the DNA fragments. Note however that the size of a circular DNA like plasmids cannot be accurately gauged using standard markers unless it has been linearized by restriction digest, alternatively a supercoiled DNA marker may be used.

Staining and visualization

DNA as well as RNA is normally visualized by staining with ethidium bromide, which intercalates into the major grooves of the DNA and fluoresces under UV light. The ethidium bromide may be added to the agarose solution before it gels, or the DNA gel may be stained later after electrophoresis. Destaining of the gel is not necessary but may produce better images. Other

methods of staining are available; examples are SYBR Green, Gel Red, methylene blue, brilliant cresyl blue, Nile blue sulphate, and crystal violet. SYBR Green, Gel Red and other similar commercial products are sold as safer alternatives to ethidium bromide as it has been shown to be mutagenic in Ames test, although the carcinogenicity of ethidium bromide has not actually been established. SYBR Green requires the use of a blue-light transilluminator. DNA stained with crystal violet can be viewed under natural light without the use of a UV transilluminator which is an advantage; however it may not produce a strong band.

When stained with ethidium bromide, the gel is viewed with an ultraviolet (UV) transilluminator. Standard transilluminators use wavelengths of 302/312-nm (UV-B), however exposure of DNA to UV radiation for as little as 45 seconds can produce damage to DNA and affect subsequent procedures, for example reducing the efficiency of transformation, in vitro transcription, and PCR. Exposure of the DNA to UV radiation therefore should be limited. Using a higher wavelength of 365 nm (UV-A range) causes less damage to the DNA but also produces much weaker fluorescence with ethidium bromide. Where multiple wavelengths can be selected in the transillumintor, the shorter wavelength would be used to capture images, while the longer wavelength should be used if it is necessary to work on the gel for any extended period of time.

The transilluminator apparatus may also contain image capture devices, such as a digital or polaroid camera, that allow an image of the gel to be taken or printed.

Application

- Estimation of the size of DNA molecules following restriction enzyme digestion, e.g. in restriction mapping of cloned DNA.
- Analysis of PCR products, e.g. in molecular genetic diagnosis or genetic fingerprinting
- Separation of DNA fragments for extraction and purification.
- Separation of restricted genomic DNA prior to Southern transfer, or of RNA prior to Northern transfer.

Agarose gels are easily cast and handled compared to other matrices and nucleic acids are not chemically altered during electrophoresis. Samples are also easily recovered. After the experiment is finished, the resulting gel can be stored in a plastic bag in a refrigerator.

Electrophoresis is performed in buffer solutions to reduce pH changes due to the electric field, which is important because the charge of DNA and RNA depends on pH, but running for too long can exhaust the buffering capacity of the solution. Further, different preparations of genetic material may not migrate consistently with each other, for morphological or other reasons.

Polyacrylamide gel electrophoresis

Polyacrylamide gel electrophoresis (PAGE), describes a technique widely used in biochemistry, forensics, genetics, molecular biologyand biotechnology to separate

biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. Mobility is a function of the length, conformation and charge of the molecule.

As with all forms of gel electrophoresis, molecules may be run in their native state, preserving the molecules' higher-order structure, or a chemical denaturant may be added to remove this structure and turn the molecule into an unstructured linear chain whose mobility depends only on its length and mass-to-charge ratio. For nucleic acids, urea is the most commonly used denaturant. For proteins,sodium dodecyl sulfate (SDS) is an anionic detergent applied to protein samples to linearize proteins and to impart a negative charge to linearized proteins. This procedure is called **SDS-PAGE**. In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis. Proteins that have a greater hydrophobic content, for instance many membrane proteins, and those that interact with surfactants in their native environment, are intrinsically harder to treat accurately using this method, due to the greater variability in the ratio of bound SDS.

Sample preparation

Samples may be any material containing proteins or nucleic acids. These may be biologically derived, for example from prokaryotic or eukaryotic cells, tissues, viruses, environmental samples, or purified proteins. In the case of solid tissues or cells, these are often first broken down mechanically using a blender (for larger sample volumes), using a homogenizer (smaller volumes), by sonicator or by using cycling of high pressure, and a combination of biochemical and mechanical techniques – including various types of filtration and centrifugation – may be used to separate different cell compartments and organelles prior to electrophoresis. Synthetic biomolecules such as oligonucleotides may also be used as analytes.

The sample to analyze is optionally mixed with a chemical denaturant if so desired, usually SDS for proteins or urea for nucleic acids. SDS is an anionic detergent that denatures secondary and non–disulfide–linked tertiary structures, and additionally applies a negative charge to each protein in proportion to its mass. Urea breaks the hydrogen bonds between the base pairs of the nucleic acid, causing the constituent strands to separate. Heating the samples to at least 60 °C further promotes denaturation.

In addition to SDS, proteins may optionally be briefly heated to near boiling in the presence of a reducing agent, such as dithiothreitol (DTT) or 2-mercaptoethanol (beta-mercaptoethanol/BME), which further denatures the proteins by reducing disulfide linkages, thus overcoming some forms of tertiary protein folding, and breaking up quaternary protein structure (oligomeric subunits). This is known as reducing SDS-PAGE.

A tracking dye may be added to the solution. This typically has a higher electrophoretic mobility than the analytes to allow the experimenter to track the progress of the solution through the gel during the electrophoretic run.

Preparing acrylamide gels

The gels typically consist of acrylamide, bisacrylamide, the optional denaturant (SDS or urea), and a buffer with an adjusted pH. The solution may be degassed under a vacuum to prevent the formation of air bubbles during polymerization. Alternatively, butanol may be added to the resolving gel (for proteins) after it is poured, as butanol removes bubbles and makes the surface smooth. A source of free radicals and a stabilizer, such as ammonium persulfate and TEMED are added to initiate polymerization. The polymerization reaction creates a gel because of the added bisacrylamide, which can form cross-links between two acrylamide molecules. The ratio of bisacrylamide to acrylamide can be varied for special purposes, but is generally about 1 part in 35. The acrylamide concentration of the gel can also be varied, generally in the range from 5% to 25%. Lower percentage gels are better for resolving very high molecular weight molecules, while much higher percentages are needed to resolve smaller proteins.

Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the sample wells. After the gel is polymerized the comb can be removed and the gel is ready for electrophoresis.

Electrophoresis

Various buffer systems are used in PAGE depending on the nature of the sample and the experimental objective. The buffers used at the anode and cathode may be the same or different.

An electric field is applied across the gel, causing the negatively charged proteins or nucleic acids to migrate across the gel away from the negative electrode (which is the cathode being that this is an electrolytic rather than galvanic cell) and towards the positive electrode (the anode). Depending on their size, each biomolecule moves differently through the gel matrix: small molecules more easily fit through the pores in the gel, while larger ones have more difficulty. The gel is run usually for a few hours, though this depends on the voltage applied across the gel; migration occurs more quickly at higher voltages, but these results are typically less accurate than at those at lower voltages. After the set amount of time, the biomolecules have migrated different distances based on their size. Smaller biomolecules travel farther down the gel, while larger ones remain closer to the point of origin. Biomolecules may therefore be separated roughly according to size, which depends mainly on molecular weight under denaturing conditions, but also depends on higher-order conformation under native conditions. However, certain glycoproteins behave anomalously on SDS gels.

Further processing

Following electrophoresis, the gel may be stained (for proteins, most commonly with Coomassie Brilliant Blue R-250; for nucleic acids,ethidium bromide; or for either, silver stain), allowing visualization of the separated proteins, or processed further (e.g. Western blot). After staining, different species biomolecules appear as distinct bands within the gel. It is common to

run molecular weight size markers of known molecular weight in a separate lane in the gel to calibrate the gel and determine the approximate molecular mass of unknown biomolecules by comparing the distance traveled relative to the marker.

For proteins, SDS-PAGE is usually the first choice as an assay of purity due to its reliability and ease. The presence of SDS and the denaturing step make proteins separate, approximately based on size, but aberrant migration of some proteins may occur. Different proteins may also stain differently, which interferes with quantification by staining. PAGE may also be used as a preparative technique for the purification of proteins. For example, **q**uantitative **p**reparative **n**ative **c**ontinuous **p**oly**a**crylamide **g**el **e**lectrophoresis (QPNC-PAGE) is a method for separating native metalloproteins in complex biological matrices.

Polyacrylamide gel (*PAG)* had been known as a potential embedding medium for sectioning tissues as early as 1964, and two independent groups employed PAG in electrophoresis in 1959. It possesses several electrophoretically desirable features that make it a versatile medium. It is a synthetic, thermo-stable, transparent, strong, chemically relatively inert gel, and can be prepared with a wide range of average pore sizes. The pore size of a gel is determined by two factors, the total amount of acrylamide present (%T) (T = Total concentration of acrylamide and bisacrylamide monomer) and the amount of cross-linker (%C) (C = bisacrylamide concentration). Pore size decreases with increasing %T; with cross-linking, 5%C gives the smallest pore size. Any increase or decrease in %C from 5% increases the pore size, as pore size with respect to %C is a parabolic function with vertex as 5%C. This appears to be because of non-homogeneous bundling of polymer strands within the gel. This gel material can also withstand highvoltage gradients, is amenable to various staining and destaining procedures, and can be digested to extract separated fractions or dried for autoradiography and permanent recording.

Components

- **Chemical buffer** Stabilizes the pH value to the desired value within the gel itself and in the electrophoresis buffer. The choice of buffer also affects the electrophoretic mobility of the buffer counterions and thereby the resolution of the gel. The buffer should also be unreactive and not modify or react with most proteins. Different buffers may be used as cathode and anode buffers, respectively, depending on the application. Multiple pH values may be used within a single gel, for example in DISC electrophoresis. Common buffers in PAGE include Tris, Bis-Tris, or imidazole.
- **Counterion** balance the intrinsic charge of the buffer ion and also affect the electric field strength during electrophoresis. Highly charged and mobile ions are often avoided in SDS-PAGE cathode buffers, but may be included in the gel itself, where it migrates ahead of the protein. In applications such as DISC SDS-PAGE the pH values within the gel may vary to change the average charge of the counterions during the run to improve resolution. Popular counterions are glycine and tricine. Glycine has been used as the source of trailing ion or

slow ion because its pKa is 9.69 and mobility of glycinate are such that the effective mobility can be set at a value below that of the slowest known proteins of net negative charge in the pH range. The minimum pH of this range is approximately 8.0.

- **Acrylamide (C_3H_5NO; mW: 71.08).** When dissolved in water, slow, spontaneous autopolymerization of acrylamide takes place, joining molecules together by head on tail fashion to form long single-chain polymers. The presence of a free radical-generating system greatly accelerates polymerization. This kind of reaction is known as Vinyladdition polymerisation. A solution of these polymer chains becomes viscous but does not form a gel, because the chains simply slide over one another. Gel formation requires linking various chains together. Acrylamide is a neurotoxin. It is also essential to store acrylamide in a cool dark and dry place to reduce autopolymerisation andhydrolysis.
- **Bisacrylamide (*N*,*N*′-Methylenebisacrylamide) ($C_7H_{10}N_2O_2$; mW: 154.17).** Bisacrylamide is the most frequently used cross linking agent for polyacrylamide gels. Chemically it can be thought of as two acrylamide molecules coupled head to head at their non-reactive ends. Bisacrylamide can crosslink two polyacrylamide chains to one another, thereby resulting in a gel.
- **Sodium Dodecyl Sulfate (SDS) ($C_{12}H_{25}NaO_4S$; mW: 288.38).** (only used in denaturing protein gels) SDS is a strong detergent agent used to denature native proteins to unfolded, individual polypeptides. When a protein mixture is heated to 100 °C in presence of SDS, the detergent wraps around the polypeptide backbone. It binds to polypeptides in a constant weight ratio of 1.4 g SDS/g of polypeptide. In this process, the intrinsic charges of polypeptides become negligible when compared to the negative charges contributed by SDS. Thus polypeptides after treatment become rod-like structures possessing a uniform charge density that is same net negative charge per unit weight. The electrophoretic mobilities of these proteins are a linear function of the logarithms of their molecular weights.

  Without SDS, different proteins with similar molecular weights would migrate differently due to differences in mass-charge ratio, as each protein has an isoelectric point and molecular weight particular to its primary structure. This is known as Native PAGE. Adding SDS solves this problem, as it binds to and unfolds the protein, giving a near uniform negative charge along the length of the polypeptide.

- **Urea ($CO(NH_2)_2$; mW: 60.06).** Urea is a chaotropic agent that increases the entropy of the system by interfering with intramolecular interactions mediated by non-covalentforces such as hydrogen bonds and Vander Waals forces. Macromolecular structure is dependent on the net effect of these forces, therefore it follows that an increase in chaotropic solutes denatures macromolecules,
- **Ammonium persulfate (APS) ($N_2H_8S_2O_8$; mW: 228.2)**. APS is a source of free radicals and is often used as an initiator for gel formation. An alternative source of free radicals is riboflavin, which generated free radicals in a photochemical reaction.

- **TEMED (*N*, N, N′, N′-tetramethylethylenediamine)** ($C_6H_{16}N_2$; mW: 116.21). TEMED stabilizes free radicals and improves polymerization. The rate of polymerization and the properties of the resulting gel depend on the concentrations of free radicals. Increasing the amount of free radicals results in a decrease in the average polymer chain length, an increase in gel turbidity and a decrease in gel elasticity. Decreasing the amount shows the reverse effect. The lowest catalytic concentrations that allow polymerization in a reasonable period of time should be used. APS and TEMED are typically used at approximately equimolar concentrations in the range of 1 to 10 mM.

Chemicals for processing and visualization

The following chemicals and procedures are used for processing of the gel and the protein samples visualized in it:

- **Tracking dye**. As proteins and nucleic acids are mostly colorless, their progress through the gel during electrophoresis cannot be easily followed. Anionic dyes of a known electrophoretic mobility are therefore usually included in the PAGE sample buffer. A very common tracking dye is Bromophenol blue (BPB, 3', 3", 5', 5" tetrabromophenolsulfonphthalein). This dye is coloured at alkali and neutral pH and is a small negatively charged molecule that moves towards the anode. Being a highly mobile molecule it moves ahead of most proteins. As it reaches the anodic end of the electrophoresis medium electrophoresis is stopped. It can weakly bind to some proteins and impart a blue colour. Other common tracking dyes are xylene cyanol, which has lower mobility, and Orange G, which has a higher mobility.
- **Loading aids**. Most PAGE systems are loaded from the top into wells within the gel. To ensure that the sample sinks to the bottom of the gel, sample buffer is supplemented with additives that increase the density of the sample. These additives should be non-ionic and non-reactive towards proteins to avoid interfering with electrophoresis. Common additives are glycerol and sucrose.
- **Coomassie Brilliant Blue R-250 (CBB) ($C_{45}H_{44}N_3NaO_7S_2$; mW: 825.97)**. CBB is the most popular protein stain. It is an anionic dye, which non-specifically binds to proteins. The structure of CBB is predominantly non-polar, and it is usually used in methanolic solution acidified with acetic acid. Proteins in the gel are fixed by acetic acid and simultaneously stained. The excess dye incorporated into the gel can be removed by destaining with the same solution without the dye. The proteins are detected as blue bands on a clear background. As SDS is also anionic, it may interfere with staining process. Therefore, large volume of staining solution is recommended, at least ten times the volume of the gel.
- **Ethidium bromide (EtBr)** is the traditionally most popular nucleic acid stain.

- **Silver staining**. Silver staining is used when more sensitive method for detection is needed, as classical Coomassie Brilliant Blue staining can usually detect a 50 ng protein band, Silver staining increases the sensitivity typically 50 times. The exact chemical mechanism by which this happens is still largely unknown. Silver staining was introduced by Kerenyi and Gallyas as a sensitive procedure to detect trace amounts of proteins in gels. The technique has been extended to the study of other biological macromolecules that have been separated in a variety of supports. Many variables can influence the colour intensity and every protein has its own staining characteristics; clean glassware, pure reagents and water of highest purity are the key points to successful staining. Silver staining was developed in the 14th century for colouring the surface of glass. It has been used extensively for this purpose since the 16th century. The colour produced by the early silver stains ranged between light yellow and an orange-red. Camillo Golgi perfected the silver staining for the study of the nervous system. Golgi's method stains a limited number of cells at random in their entirety.
- **Western Blotting** is a process by which proteins separated in the acrylamide gel are electrophoretically transferred to a stable, manipulable membrane such as anitrocellulose, nylon, or PVDF membrane. It is then possible to apply immunochemical techniques to visualise the transferred proteins, as well as accurately identify relative increases or decreases of the protein of interest. For more, see Western Blot.

Isoelectric focusing takes place in a pH gradient. The charged molecules move towards the anode or the cathode until they reach a position in the pH gradient where their net charges are zero. This pH value is the "**isoelectric** point" (pI) of the substance.

Principle of Isoelectric Focusing

The use of isoelectric focusing is limited to molecules which can be either positively or negatively charged. Proteins, enzymes and peptides are such amphoteric molecules. The net charge of a protein is the sum of all negative and positive charges of the amino acid side chains, but the three-dimensional configuration of the protein also plays a role.

Isoelectric focusing takes place in a pH gradient. The charged molecules move towards the anode or the cathode until they reach a position in the pH gradient where their net charges are zero. This pH value is the isoelectric point of the substance. Since it is no longer charged, the electric field does not have any influence on it.

The method of separating proteins according to their isoelectric points in a gradient is called isoelectric focusing. This technique was discovered by H. Svensson and Sweden. This method has a high resolution power because ordinary paper electrophoresis resolves plasma proteins into six bands whereas isoelectric focusing resolves them into 40 bands.

In conventional electrophoresis, the pH between anode and cathode is constant and the positively charged ions migrate towards the cathode and negatively charged ions migrate towards the anode. But in isoelectric focusing, a stable pH gradient is arranged. The pH gradually increases from anode to cathode. When a protein is introduced at a pH which is lower than its isoionic point, it will possess a net positive charge and will migrate in the direction of the cathode. Due to the presence of pH gradient, the net charge of the molecule changes due to ionization as it moves is zero, it will stop migrating. This is the isoelectric point of protein. Each protein present in the mixture will migrate to its isoelectric point and stop its migration at that point. Thus, once a final, stable focusing is reached, the resolution will be retained for a long time.

Uses

Isoelectric focusing is widely used for the separation and identification of serum proteins. It is used in the food and agriculture industry, forensic and human genetic laboratories, for research in enzymology, immunology and membrane biochemistry, etc.

Immuno electrophoresis

Immunoelectrophoresis is a general name for a number of biochemical methods for separation and characterization of proteins based on electrophoresis and reaction with antibodies. All variants of immunoelectrophoresis require immunoglobulins, also known as antibodies, reacting with the proteins to be separated or characterized. The methods were developed and used extensively during the second half of the 20th century. In somewhat chronological order: Immunoelectrophoretic analysis (one-dimensional immunoelectrophoresis *ad modum*Grabar), crossed immunoelectrophoresis (two-dimensional quantitative immunoelectrophoresis *ad modum* Clarke and Freeman or *ad modum* Laurell), rocket-immunoelectrophoresis (one-dimensional quantitative immunoelectrophoresis *ad modum* Laurell), fused rocket immunoelectrophoresis *ad modum* Svendsen and Harboe, affinity immunoelectrophoresis *ad modum* Bøg-Hansen.

Agarose as 1% gel slabs of about 1 mm thickness buffered at high pH (around 8.6) is traditionally preferred for the electrophoresis as well as the reaction with antibodies. The agarose was chosen as the gel matrix because it has large pores allowing free passage and separation of proteins, but provides an anchor for the immunoprecipitates of protein and specific antibodies. The high pH was chosen because antibodies are practically immobile at high pH. An electrophoresis equipment with a horizontal cooling plate was normally recommended for the electrophoresis.

Immunoprecipitates may be seen in the wet agarose gel, but are stained with protein stains like Coomassie Brilliant Blue in the dried gel. In contrast to SDS-gel electrophoresis, the electrophoresis in agarose allows native conditions, preserving the native structure and activities of the proteins under investigation, therefore immunoelectrophoresis allows characterization of enzyme activities and ligand binding etc. in addition to electrophoretic separation.

Crossed immunoelectrophoresis is also called two-dimensional quantitative immunoelectrophoresis *ad modum* Clarke and Freeman or*ad modum* Laurell. In this method the proteins are first separated during the first dimension electrophoresis, then instead of the diffusion towards the antibodies, the proteins are electrophoresed into an antibody-containing gel in the second dimension. Immunoprecipitation will take place during the second dimension electrophorsis and the immunoprecipitates have a characteristic bell-shape, each precipitate representing one antigen, the position of the precipitate being dependent on the amount of protein as well as the amount of specific antibody in the gel, so relative quantification can be performed. The sensitivity and resolving power of crossed immunoelectrophoresis is than that of the classical immunoelectrophoretic analysis and there are multiple variations of the technique useful for various purposes. Crossed immunoelectrophoresis has been used for studies of proteins in biological fluids, particularly human serum, and biological extracts.

Rocket immunoelectrophoresis is one-dimensional quantitative immunoelectrophoresis. The method has been used for quantitation of human serum proteins before automated methods became available.

Fused rocket immunoelectrophoresis is a modification of one-dimensional quantitative immunoelectrophorsis used for detailed measurement of proteins in fractions from protein separation experiments.

Affinity immunoelectrophoresis is based on changes in the electrophoretic pattern of proteins through specific interaction or complex formation with other macromolecules or ligands. Affinity immunoelectrophoresis has been used for estimation of binding constants, as for instance with lectins or for characterization of proteins with specific features likeglycan content or ligand binding. Some variants of affinity immunoelectrophoresis are similar to affinity chromatography by use of immobilized ligands.

The open structure of the immunoprecipitate in the agarose gel will allow additional binding of radioactively labeled antibodies to reveal specific proteins. This variation has been used for identification of allergens through reaction with IgE.

Two factors determine that immunoelectrophoretic methods are not widely used. First they are rather work intensive and require some manual expertise. Second they require rather large amounts of polyclonal antibodies. Today gel electrophoresis followed by electroblotting is the preferred method for protein characterization because it's ease of operation, its high sensitivity, and its low requirement for specific antibodies. In addition proteins are separated by gel electrophoresis on the basis of their apparent molecular weight, which is not accomplished by immunoelectrophoresis, but nevertheless immunoelectrophoretic methods are still useful when non-reducing conditions are needed.

Pulsed field gel electrophoresis is a technique used for the separation of large deoxyribonucleic acid (DNA) molecules by applying to a gel matrix an electric field that periodically changes direction.

The procedure for this technique is relatively similar to performing a standard gel electrophoresis except that instead of constantly running the voltage in one direction, the voltage is periodically switched among three directions; one that runs through the central axis of the gel and two that run at an angle of 60 degrees either side. The pulse times are equal for each direction resulting in a net forward migration of the DNA. For extremely large molecules (up to around 2 Mb), switching-interval ramps can be used that increases the pulse time for each direction over the course of a number of hours—take, for instance, increasing the pulse linearly from 10 seconds at 0 hours to 60 seconds at 18 hours.

This procedure takes longer than normal gel electrophoresis due to the size of the fragments being resolved and the fact that the DNA does not move in a straight line through the gel.

Applications

PFGE may be used for genotyping or genetic fingerprinting. It is commonly considered a gold standard in epidemiological studies of pathogenic organisms. Subtyping has made it easier to discriminate among strains of *Listeria monocytogenes* and thus to link environmental or food isolates with clinical infections.

Capillary electrophoresis

Capillary electrophoresis (**CE**) is a family of electrokinetic separation methods performed in submillimeter diameter capillaries and in micro- and nanofluidic channels. Very often, CE refers to capillary zone electrophoresis (CZE), but other electrophoretic techniques including capillary gel electrophoresis (CGE), capillary isoelectric focusing (CIEF), capillary isotachophoresis and micellar electrokinetic chromatography (MEKC) belong also to this class of methods.[1] In CE methods, analytes migrate through electrolytesolutions under the influence of an electric field. Analytes can be separated according to ionic mobility and/or partitioning into an alternate phase via Non-covalent interactions. Additionally, analytes may be concentrated or "focused" by means of gradients inconductivity and pH.

The instrumentation needed to perform capillary electrophoresis is relatively simple. A basic schematic of a capillary electrophoresis system. The system's main components are a sample vial, source and destination vials, a capillary, electrodes, a high voltage power supply, a detector, and a data output and handling device. The source vial, destination vial and capillary are filled with an electrolyte such as an aqueous buffer solution. To introduce the sample, the capillary inlet is placed into a vial containing the sample. Sample is introduced into the capillary via capillary action, pressure, siphoning, or electrokinetically, and the capillary is then returned to the source vial. The migration of the analytes is initiated by an electric field that is applied between the source and destination vials and is supplied to the electrodes by the high-voltage power supply. In the most common mode of CE, all ions, positive or negative, are pulled through the capillary in the same direction by electroosmotic flow. The analytes separate as they migrate due to their electrophoretic mobility, and are detected near the outlet end of the capillary. The output of the detector is sent to a data output and handling device such as

an integrator or computer. The data is then displayed as an electropherogram, which reports detector response as a function of time. Separatedchemical compounds appear as peaks with different retention times in an electropherogram. Capillary electrophoresis was first combined with mass spectrometry by Richard D. Smith and coworkers, and provides extremely high sensitivity for the analysis of very small sample sizes. Despite the very small sample sizes (typically only a few nanoliters of liquid are introduced into the capillary), high sensitivity and sharp peaks are achieved in part due to injection strategies that result in concentration of analytes into a narrow zone near the inlet of the capillary. This is achieved in either pressure or electrokinetic injections simply by suspending the sample in a buffer of lower conductivity (*e.g.* lower salt concentration) than the running buffer. A process called field-amplified sample stacking (a form of isotachophoresis) results in concentration of analyte in a narrow zone at the boundary between the low-conductivity sample and the higher-conductivity running buffer.

To achieve greater sample throughput, instruments with arrays of capillaries are used to analyze many samples simultaneously. Such capillary array electrophoresis (CAE) instruments with 16 or 96 capillaries are used for medium- to high-throughput capillary DNA sequencing, and the inlet ends of the capillaries are arrayed spatially to accept samples directly from SBS-standard footprint 96-well plates.

Separation by capillary electrophoresis can be detected by several detection devices. The majority of commercial systems use UV or UV-Vis absorbance as their primary mode of detection. In these systems, a section of the capillary itself is used as the detection cell. The use of on-tube detection enables detection of separated analytes with no loss of resolution. In general, capillaries used in capillary electrophoresis are coated with a polymer (frequently polyimide or Teflon) for increased flexibility. The portion of the capillary used for UV detection, however, must be optically transparent. For polyimide-coated capillaries, a segment of the coating is typically burned or scraped off to provide a bare window several millimeters long. This bare section of capillary can break easily, and capillaries with transparent coatings are available to increase the stability of the cell window. The path length of the detection cell in capillary electrophoresis (~ 50 micrometers) is far less than that of a traditional UV cell (~ 1 cm). According to the Beer-Lambert law, the sensitivity of the detector is proportional to the path length of the cell. To improve the sensitivity, the path length can be increased, though this results in a loss of resolution. The capillary tube itself can be expanded at the detection point, creating a "bubble cell" with a longer path length or additional tubing can be added at the detection point. Both of these methods, however, will decrease the resolution of the separation. Post-column detection utilizing a sheath flow configuration has also been described.

Fluorescence detection can also be used in capillary electrophoresis for samples that naturally fluoresce or are chemically modified to contain fluorescent tags. This mode of detection offers high sensitivity and improved selectivity for these samples, but cannot be utilized for samples that do not fluoresce. Numerous labeling strategies are used to create fluorescent derivatives or conjugates of non-fluorescent molecules, including proteins and DNA. The set-up for

fluorescence detection in a capillary electrophoresis system can be complicated. The method requires that the light beam be focused on the capillary, which can be difficult for many light sources. Laser-induced fluorescence has been used in CE systems with detection limits as low as 10^{-18} to 10^{-21} mol. The sensitivity of the technique is attributed to the high intensity of the incident light and the ability to accurately focus the light on the capillary.[2] Multi-color fluorescence detection can be achieved by including multiple dichroic mirrors and bandpass filters to separate the fluorescence emission amongst multiple detectors (*e.g.*, photomultiplier tubes), or by using a prism or grating to project spectrally resolved fluorescence emission onto a position-sensitive detector such as a CCD array. CE systems with 4- and 5-color LIF detection systems are used routinely for capillary DNA sequencing and genotyping ("DNA fingerprinting") applications.

In order to obtain the identity of sample components, capillary electrophoresis can be directly coupled with mass spectrometers or Surface Enhanced Raman Spectroscopy (SERS). In most systems, the capillary outlet is introduced into an ion source that utilizes electrospray ionization (ESI). The resulting ions are then analyzed by the mass spectrometer. This set-up requires volatile buffer solutions, which will affect the range of separation modes that can be employed and the degree of resolution that can be achieved. The measurement and analysis are mostly done with a specialized gel analysis software.

For CE-SERS, capillary electrophoresis eluants can be deposited onto a SERS-active substrate. Analyte retention times can be translated into spatial distance by moving the SERS-active substrate at a constant rate during capillary electrophoresis. This allows the subsequent spectroscopic technique to be applied to specific eluants for identification with high sensitivity. SERS-active substrates can be chosen that do not interfere with the spectrum of the analytes.

Application

Capillary electrophoresis may be used for the simultaneous determination of the ions NH_4^+, Na^+, K^+, Mg^{2+} and Ca^{2+} in saliva

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I M. Sc. MICROBIOLOGY 2017 – 2019

BIOINSTRUMENTATION (17MBP104)

SEMESTER – I **4H – 4C**

UNIT V

S. No	Duration	Topic	Reference
1	1	Radio isotopic technique	W6
2	1	Nature of radioactivity and rate of radioactive decay	W6
3	1	Units of radioactivity and detection	W6
4	1	Measurement of radioactivity	W6
5	1	Principle, instrumentation and application of Geiger-Muller counter	W7
6	1	Solid and liquid scintillation counter	W8. W9
7	1	Autoradiography	W10
8	1	Biosafety methods in radioactive laboratory	T1: 398
9	1	Unit revision	
Total Hrs: 9			

T1: Bioinstrumentation – L. Veerakumari

W6: https://en.wikipedia.org/wiki/Radioactive_decay

W7: https://en.wikipedia.org/wiki/Geiger_counter

W8: https://en.wikipedia.org/wiki/Scintillation_counter

W9: https://en.wikipedia.org/wiki/Liquid_scintillation_counting

W10: https://en.wikipedia.org/wiki/Autoradiograph

2017

Bioinstrumentation

Unit V Notes

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Unit - 5

Radio isotopic

An atom consists of an extremely small; positively charged nucleus surrounded by a cloud of negatively charged electrons. Nuclei consist of positively charged protons and electrically neutral neutrons held together by nuclear force.

Units of radioactive decay

The International System of Units (SI) unit of radioactive activity is the becquerel (Bq), named in honour of the scientist Henri Becquerel. One Bq is defined as one transformation (or decay or disintegration) per second.

An older unit of radioactivity is the curie, Ci, which was originally defined as "the quantity or mass of radium emanation in equilibrium with one gram of radium (element)". Today, the curie is defined as 3.7×10^{10} disintegrations per second, so that 1 curie (Ci) = 3.7×10^{10} Bq. For radiological protection purposes, although the United States Nuclear Regulatory Commission permits the use of the unit curiealongside SI units, the European Union European units of measurement directives required that its use for "public health ... purposes" be phased out by 31 December 1985.

Radioactive decay (also known as **nuclear decay** or **radioactivity**) is the process by which the nucleus of an unstable atom loses energy by emitting radiation, including alpha particles, beta particles, gamma rays, and conversion electrons. A material that spontaneously emits such radiation is considered **radioactive**.

Radioactive decay is a stochastic (i.e. random) process at the level of single atoms, in that, according to quantum theory, it is impossible to predict when a particular atom will decay, regardless of how long the atom has existed. For a collection of atoms however, the collection's decay rate can be calculated from their measured decay constants or half-lives. This is the basis of radiometric dating. The half-lives of radioactive atoms have no known lower or upper limit, spanning a time range of over 55 orders of magnitude, from nearly instantaneous to far longer than the age of the universe. A radioactive source emits its decay products isotropically (all directions and without bias) in the absence of external influence.

Types of radioactive decay

Early researchers found that an electric or magnetic field could split radioactive emissions into three types of beams. The rays were given the names alpha, beta, and gamma, in order of their ability to penetrate matter. While alpha decay was observed only in heavier elements of atomic number 52 (tellurium) and greater, the other two types of decay were produced by all of the elements. Lead, atomic number 82, is the heaviest element to have any isotopes stable (to the

limit of measurement) to radioactive decay. Radioactive decay is seen in all isotopes of all elements of atomic number 83 (bismuth) or greater. Bismuth, however, is only very slightly radioactive, with a half-life greater than the age of the universe; radioisotopes with extremely long half-lives are considered effectively stable for practical purposes.

In analysing the nature of the decay products, it was obvious from the direction of the electromagnetic forces applied to the radiations by external magnetic and electric fields that alpha particles carried a positive charge, beta particles carried a negative charge, and gamma rays were neutral. From the magnitude of deflection, it was clear that alpha particles were much more massive than beta particles. Passing alpha particles through a very thin glass window and trapping them in a discharge tube allowed researchers to study the emission spectrum of the captured particles, and ultimately proved that alpha particles are helium nuclei. Other experiments showed beta radiation, resulting from decay and cathode rays, were high-speed electrons. Likewise, gamma radiation and X-rays were found to be high-energy electromagnetic radiation.

The relationship between the types of decays also began to be examined: For example, gamma decay was almost always found to be associated with other types of decay, and occurred at about the same time, or afterwards. Gamma decay as a separate phenomenon, with its own half-life (now termed isomeric transition), was found in natural radioactivity to be a result of the gamma decay of excited metastable nuclear isomers, which were in turn created from other types of decay.

Although alpha, beta, and gamma radiations were most commonly found, other types of emission were eventually discovered. Shortly after the discovery of the positron in cosmic ray products, it was realized that the same process that operates in classical beta decay can also produce positrons (positron emission), along with neutrinos (classical beta decay produces antineutrinos). In a more common analogous process, called electron capture, some proton-rich nuclides were found to capture their own atomic electrons instead of emitting positrons, and subsequently these nuclides emit only a neutrino and a gamma ray from the excited nucleus (and often also Auger electrons and characteristic X-rays, as a result of the re-ordering of electrons to fill the place of the missing captured electron). These types of decay involve the nuclear capture of electrons or emission of electrons or positrons, and thus act to move a nucleus toward the ratio of neutrons to protons that have the least energy for a given total number of nucleons. This consequently produces a more stable (lower energy) nucleus.

(A theoretical process of positron capture, analogous to electron capture, is possible in antimatter atoms, but has not been observed, as complex antimatter atoms beyond antihelium are not experimentally available. Such decay would require antimatter atoms at least as complex as beryllium-7, which is the lightest known isotope of normal matter to undergo decay by electron capture.)

Shortly after the discovery of the neutron in 1932, Enrico Fermi realized that certain rare beta-decay reactions immediately yield neutrons as a decay particle (neutron emission). Isolated proton emission was eventually observed in some elements. It was also found that some heavy elements may undergo spontaneous fission into products that vary in composition. In a phenomenon called cluster decay, specific combinations of neutrons and protons other than alpha particles (helium nuclei) were found to be spontaneously emitted from atoms.

Other types of radioactive decay were found to emit previously-seen particles, but via different mechanisms. An example is internal conversion, which results in an initial electron emission, and then often further characteristic X-rays and Auger electrons emissions, although the internal conversion process involves neither beta nor gamma decay. A neutrino is not emitted, and none of the electron(s) and photon(s) emitted originate in the nucleus, even though the energy to emit all of them does originate there. Internal conversion decay, like isomeric transition gamma decay and neutron emission, involves the release of energy by an excited nuclide, without the transmutation of one element into another.

Rare events that involve a combination of two beta-decay type events happening simultaneously are known (see below). Any decay process that does not violate the conservation of energy or momentum laws (and perhaps other particle conservation laws) is permitted to happen, although not all have been detected. An interesting example discussed in a final section, is bound state beta decay of rhenium-187. In this process, beta electron-decay of the parent nuclide is not accompanied by beta electron emission, because the beta particle has been captured into the K-shell of the emitting atom. An antineutrino is emitted, as in all negative beta decays.

Radionuclides can undergo a number of different reactions. These are summarized in the following table. A nucleus with mass number A and atomic number Z is represented as (A, Z). The column "Daughter nucleus" indicates the difference between the new nucleus and the original nucleus. Thus, ($A - 1$, Z) means that the mass number is one less than before, but the atomic number is the same as before.

If energy circumstances are favorable, a given radionuclide may undergo many competing types of decay, with some atoms decaying by one route, and others decaying by another. An example is copper-64, which has 29 protons, and 35 neutrons, which decays with a half-life of about 12.7 hours. This isotope has one unpaired proton and one unpaired neutron, so either the proton or the neutron can decay to the opposite particle. This particular nuclide (though not all nuclides in this situation) is almost equally likely to decay through positron emission (18%), or through electron capture (43%), as it does through electron emission (39%). The excited energy states resulting from these decays which fail to end in a ground energy state, also produce later internal conversion and gamma decay in almost 0.5% of the time.

More common in heavy nuclides is competition between alpha and beta decay. The daughter nuclides will then normally decay through beta or alpha, respectively, to end up in the same place.

The **Geiger counter** is an instrument used for measuring ionizing radiation used widely in such applications as radiation dosimetry, radiological protection, experimental physics and the nuclear industry.

It detects ionizing radiation such as alpha particles, beta particles and gamma rays using the ionization effect produced in a Geiger–Müller tube; which gives its name to the instrument. In wide and prominent use as a hand-held radiation survey instrument, it is perhaps one of the world's best-known radiation detection instruments.

The original detection principle was discovered in 1908 at the Cavendish laboratory, but it was not until the development of the Geiger-Müller tube in 1928 that the Geiger-Müller counter became a practical instrument. Since then it has been very popular due to its robust sensing element and relatively low cost. However, there are limitations in measuring high radiation rates and the energy of incident radiation.

A Geiger counter consists of a Geiger-Müller tube, the sensing element which detects the radiation, and the processing electronics, which displays the result.

The Geiger-Müller tube is filled with an inert gas such as helium, neon, or argon at low pressure, to which a high voltage is applied. The tube briefly conducts electrical charge when a particle or photon of incident radiation makes the gas conductive by ionization. The ionization is considerably amplified within the tube by the Townsend discharge effect to produce an easily measured detection pulse, which is fed to the processing and display electronics. This large pulse from the tube makes the G-M counter relatively cheap to manufacture, as the subsequent electronics is greatly simplified. The electronics also generates the high voltage, typically 400–600 volts that has to be applied to the Geiger-Müller tube to enable its operation.

Readout

There are two types of radiation readout; counts or radiation dose. The counts display is the simplest and is the number of ionizing events displayed either as a count rate, commonly "counts per second", or as a total over a set time period (an integrated total). The counts readout is normally used when alpha or beta particles are being detected. More complex to achieve is a display of radiation dose rate, displayed in a unit such as the sievert which is normally used for measuring gamma or X-ray dose rates. A G-M tube can detect the presence of radiation, but not its energy which influences the radiation's ionising effect. Consequently, instruments measuring dose rate require the use of an energy compensated G-M tube, so that the dose displayed relates to the counts detected. The electronics will apply known factors to make this conversion, which is specific to each instrument and is determined by design and calibration.

The readout can be analog or digital, and increasingly, modern instruments are offering serial communications with a host computer or network.

There is usually an option to produce audible clicks representing the number of ionization events detected. This is the distinctive sound normally associated with hand held or portable Geiger counters. The purpose of this is to allow the user to concentrate on manipulation of the instrument whilst retaining auditory feedback on the radiation rate.

Limitations

There are two main limitations of the Geiger counter. Because the output pulse from a Geiger-Müller tube is always the same magnitude regardless of the energy of the incident radiation, the tube cannot differentiate between radiation types. A further limitation is the inability to measure high radiation rates due to the "dead time" of the tube. This is an insensitive period after each ionization of the gas during which any further incident radiation will not result in a count, and the indicated rate is therefore lower than actual. Typically the dead time will reduce indicated count rates above about 10^4 to 10^5 counts per second depending on the characteristic of the tube being used.[2] Whilst some counters have circuitry which can compensate for this, for accurate measurements ion chamber instruments are preferred for high radiation rates.

The application and use of a Geiger counter is dictated entirely by the design of the tube, of which there are a great many, but they can be generally categorised as "end-window", or windowless "thin-walled" or "thick-walled", and sometimes hybrids of these types.

Types and application of Geiger Muller counter

Particle detection

The first historical uses of the Geiger principle were for the detection of alpha and beta particles, and the instrument is still used for this purpose today. For alpha particles and low energy beta particles the "end-window" type of G-M tube has to be used as these particles have a limited range even in free air, and are easily stopped by a solid material. Therefore, the tube requires a window which is thin enough to allow as many as possible of these particles through to the fill gas. The window is usually made of mica with a density of about 1.5 - 2.0 mg/cm^2.

Alpha particles have the shortest range, and to detect these windows should ideally be within 10mm of the radiation source due to alpha particle attenuation in free air. However, the G-M tube produces a pulse output which is the same magnitude for all detected radiation, so a Geiger counter with an end window tube cannot distinguish between alpha and beta particles.[2] A skilled operator can use distance to differentiate alpha and high energy beta, but with the detector in close contact with the radiation source the types are indistinguishable. The "pancake" Geiger-Muller detector is a variant of the end window probe, but designed with a larger detection area to make checking quicker. However the pressure of the atmosphere against the low pressure of the fill gas limits the window size due to the limited strength of the window membrane.

High energy beta particles can also be detected by a thin-walled "windowless" G-M tube, which has no end window. Although the tube walls have a greater stopping power than a thin end window, they still allow these more energetic particles to reach the fill gas.

End-window G-M detectors are still used as a general purpose portable radioactive contamination measurement and detection instrument, owing to their relatively low cost, robustness and their relatively high detection efficiency; particularly with high energy beta particles. However, for discrimination between alpha and beta particles or provision of particle energy information, scintillation counters or proportional counters should be used. Those instrument types are manufactured with much larger detector a area, which means that checking for surface contamination is quicker than with a G-M instrument.

Gamma and X-ray detection

Geiger counters are widely used to detect gamma radiation, and for this the windowless tube is used. However, efficiency is generally low due to the poor interaction of gamma rays compared with alpha and beta particles. For instance, a chrome steel G-M tube is only about 1% efficient over a wide range of energies.

The article on the Geiger-Muller tube carries a more detailed account of the techniques used to detect photon radiation. For high energy gamma it largely relies on interaction of the photon radiation with the tube wall material, usually 1–2 mm of chrome steel on a "thick-walled" tube, to produce electrons within the wall which can enter and ionize the fill gas. This is necessary as the low pressure gas in the tube has little interaction with high energy gamma photons. However, for low energy photons there is greater gas interaction and the direct gas ionisation effect increases. With decreasing energy the wall effect gives way to a combination of wall effect and direct ionisation, until direct gas ionisation dominates. Due to the variance in response to different photon energies, windowless tubes employ what is known as "energy compensation" which attempts to compensate for these variations over a large energy range.

Low energy photon radiation such as low energy X rays or gamma rays interacts better with the fill gas. Consequently, a typical design for low energy photon detection for these is a long tube with a thin wall or with an end window. The tube has a larger gas volume than a steel walled tube to give an increased chance of particle interaction.

Neutron detection

A variation of the Geiger tube is used to measure neutrons, where the gas used is boron trifluoride or helium-3 and a plastic moderator is used to slow the neutrons. This creates an alpha particle inside the detector and thus neutrons can be counted.

Gamma measurement—personnel protection and process control

The term "Geiger counter" is commonly used to mean a hand-held survey type meter; however the Geiger principle is in wide use in installed "area gamma" alarms for personnel protection, and in process measurement and interlock applications. A Geiger tube is still the sensing device, but the processing electronics will have a higher degree of sophistication and reliability than that used in a hand held survey meter.

Physical design

For hand-held units there are two fundamental physical configurations: the "integral" unit with both detector and electronics in the same unit, and the "two-piece" design which has a separate detector probe and an electronics module connected by a short cable.

In the 1930's a mica window was added to the cylindrical design allowing low-penetration radiation to pass through with ease.

The integral unit allows single-handed operation, so the operator can use the other hand for personal security in challenging monitoring positions, but the two piece design allows easier manipulation of the detector, and is commonly used for alpha and beta surface contamination monitoring where careful manipulation of the probe is required or the weight of the electronics module would make operation unwieldy. A number of different sized detectors are available to suit particular situations, such as placing the probe in small apertures or confined spaces.

Gamma and X-Ray detectors generally use an "integral" design so the Geiger–Müller tube is conveniently within the electronics enclosure. This can easily be achieved because the casing usually has little attentuation, and is employed in ambient gamma measurements where distance from the source of radiation is not a significant factor. However, to facilitate more localised measurements such as "surface dose", the position of the tube in the enclosure is sometimes indicated by targets on the enclosure so an accurate measurement can be made with the tube at the correct orientation and a known distance from the surface.

There is a particular type of gamma instrument known as a "hot spot" detector which has the detector tube on the end of a long pole or flexible conduit. These are used to measure high radiation gamma locations whilst protecting the operator by means of distance shielding.

Particle detection of alpha and beta can used in both integral and two-piece designs. A pancake probe (for alpha/beta) is generally used to increase the area of detection in two-piece instruments whilst being relatively light weight. In integral instruments using an end window tube there is a window in the body of the casing to prevent shielding of particles. There are also hybrid instruments which have a separate probe for particle detection and a gamma detection tube within the electronics module. The detectors are switchable by the operator, depending the radiation type that is being measured.

Guidance on application use

In the United Kingdom the HSE has issued a user guidance note on selecting the best portable instrument type for the radiation measurement application concerned. This covers all radiation protection instrument technologies and is a useful comparative guide to the use of G-M detectors. The guide does not recommend the G-M detector for mixed alpha and beta

contamination detection, and they are only recommended as "satisfactory" for beta-only contamination. However, for gamma and low-voltage X-rays they are recommended as the best instrument type.

In 1908 Hans Geiger, under the supervision of Ernest Rutherford at the Victoria University of Manchester (now the University of Manchester), developed an experimental technique for detecting alpha particles that would later be used in the Geiger-Müller tube. This early counter was only capable of detecting alpha particles and was part of a larger experimental apparatus. The fundamental ionization mechanism used was discovered by John Sealy Townsend by his work between 1897 and 1901, and is known as the Townsend discharge, which is the ionization of molecules by ion impact.

It was not until 1928 that Geiger and Walther Müller (a PhD student of Geiger) developed the sealed Geiger-Müller tube which developed the basic ionization principles previously used experimentally. This was relatively small and rugged, and could not only detect alpha and beta radiation such as prior models but also gamma radiation. Now a practical radiation instrument could be produced relatively cheaply, and so the Geiger-Muller counter was born. As the tube output required little electronic processing, a distinct advantage in the thermionic valve era due to minimal valve count and low power consumption, the instrument achieved great popularity as a portable radiation detector.

Modern versions of the Geiger counter use the halogen tube invented in 1947 by Sidney H. Liebson. It superseded the earlier Geiger tube because of its much longer life and lower operating voltage, typically 400-600 volts.

Scintillation counter

A scintillation counter is an instrument for detecting and measuring ionizing radiation by using the excitation effect of incident radiation on a scintillator material, and detecting the resultant light pulses.

Scintillation counters are used to measure radiation in a variety of applications including hand held radiation survey meters, personnel and environmental monitoring for radioactive contamination, medical imaging, radiometric assay, nuclear security and nuclear plant safety.

Several products have been introduced in the market utilizing scintillation counters for detection of potentially dangerous gamma-emitting materials during transport. These include scintillation counters designed for freight terminals, border security, and ports, weigh bridge applications, scrap metal yards and contamination monitoring of nuclear waste. There are variants of scintillation counters mounted on pick-up trucks and helicopters for rapid response in case of a security situation due to dirty bombs or radioactive waste. Hand-held units are also commonly used.

Solid scintillation counter

Scintillation counter consists of a scintillation chamber connected by a light tube to a photomultiplier tube. In solid scintillation counter the sample is placed close to the flour crystal which in turn is placed adjacent to a photomultiplier. The photomultiplier is connected to a high voltage supply and a scaler. Radiations entering the chamber activate the scintillator to emit visible light photons, which pass through the light tube to fall on the photoelectric cathode surface of the photomultiplier. The photomultiplier converts the optical signal to an electrical one and provides a large degree of amplification. This consists of an evacuated coated at one end with a photocathode made of alkali metals, which is maintained at a large negative potential. Photons liberate electrons from the cathode which are accelerated towards a dynode and here knock out a number of secondary electrons. This process continues down the dynode chain until eventually a large signal is collected. The vast number of electrons finally impinges on a collector plate to generate a current pulse, which can be visually observed and electronically recorded. Solid scintillation counting is useful for measurement of gamma emitting isotopes.

Liquid scintillation counter

Liquid scintillation counting is the measurement of activity of a sample of radioactive material which uses the technique of mixing the active material with a liquid scintillator, and counting the resultant photon emissions. The purpose is to allow more efficient counting due to the intimate contact of the activity with the scintillator. It is generally used for alpha and beta particle detection.

Samples are dissolved or suspended in a "cocktail" containing a solvent (historically aromatic organics such as benzene or toluene, but more recently less hazardous solvents are used), typically some form of a surfactant, and small amounts of other additives known as "fluors" or scintillators. Scintillators can be divided into primary and secondary phosphors, differing in their luminescence properties.

Beta particles emitted from the isotopic sample transfer energy to the solvent molecules: the π cloud of the aromatic ring absorbs the energy of the emitted particle. The energized solvent molecules typically transfer the captured energy back and forth with other solvent molecules until the energy is finally transferred to a primary scintillator. The primary phosphor will emit photons following absorption of the transferred energy. Because that light emission may be at a wavelength that does not allow efficient detection, many cocktails contain secondary phosphors that absorb the fluorescence energy of the primary phosphor and re-emit at a longer wavelength.

The radioactive samples and cocktail are placed in small transparent or translucent (often glass or plastic) vials that are loaded into an instrument known as a liquid scintillation counter. Newer machines may use 96-well plates with individual filters in each well. Many counters have two photo multiplier tubes connected in a coincidence

circuit. The coincidence circuit assures that genuine light pulses, which reach both photo multiplier tubes, are counted, while spurious pulses (due to line noise, for example), which would only affect one of the tubes, are ignored.

Counting efficiencies under ideal conditions range from about 30% for tritium (a low-energy beta emitter) to nearly 100% for phosphorus-32, a high-energy beta emitter. Some chemical compounds (notably chlorine compounds) and highly colored samples can interfere with the counting process. This interference, known as "quenching", can be overcome through data correction or through careful sample preparation.

High-energy beta emitters, such as phosphorus-32, can also be counted in a scintillation counter without the cocktail, instead using an aqueous solution. This technique, known as **Cherenkov counting**, relies on the Cherenkov radiation being detected directly by the photomultiplier tubes. Cherenkov counting in this experimental context is normally used for quick, rough measurements, since the geometry of the sample can create variations in the output.

Autoradiography

An **autoradiograph** is an image on an x-ray film or nuclear emulsion produced by the pattern of decay emissions (e.g., beta particles or gamma rays) from a distribution of a radioactive substance. Alternatively, the autoradiograph is also available as a digital image (digital autoradiography), due to the recent development of scintillation gas detectors or rare earth phosphor imaging systems. The film or emulsion is opposed to the labeled tissue section to obtain the autoradiograph (also called an autoradiogram). The *auto-* prefix indicates that the radioactive substance is within the sample, as distinguished from the case of historadiography or micro radiography, in which the sample is X-rayed using an external source. Some autoradiograph can be examined microscopically for localization of silver grains (such as on the interiors or exteriors of cells or organelles) in which the process is termed micro-autoradiography. For example, micro-autoradiography was used to examine whether atrazine was being metabolized by the hornwort plant or by epiphytic microorganisms in the biofilm layer surrounding the plant.

Applications

In biology, this technique may be used to determine the tissue (or cell) localization of a radioactive substance, either introduced into a metabolic pathway, bound to a receptor or enzyme, or hybridized to a nucleic acid.

The use of radiolabeled ligands to determine the tissue distributions of receptors is termed either *in vivo* or *in vitro* **receptor autoradiography** if the ligand is administered into the circulation (with subsequent tissue removal and sectioning) or applied to the tissue sections, respectively. The ligands are generally labeled with ^{3}H (tritium) or ^{125}I(radioiodine). The distribution of RNA transcripts in tissue sections by the use of radiolabeled, complementary oligonucleotides or ribonucleic acids ("riboprobes") is called in situ hybridization

histochemistry. Radioactive precursors of DNA and RNA, [^{3}H]-thymidine and [^{3}H]-uridine respectively, may be introduced to living cells to determine the timing of several phases of the cell cycle. RNA or DNA viral sequences can also be located in this fashion. These probes are usually labeled with ^{32}P, ^{33}P, or ^{35}S. In the realm of behavioral endocrinology, autoradiography can be used to determine hormonal uptake and indicate receptor location; an animal can be injected with a radio labeled hormone, or the study can be conducted *in vitro*.

Biosafety methods in radioactive laboratory

All ionizing radiations can have effect on the human body. When radiations damages living cells, it can destroy or mutate the cells, possibly causing a cancerous growth. There is a great difference in the penetrating powers of alpha particles, beta particles and gamma particles. Of the three types of radiation, alpha particles are easiest to stop. A sheet of paper is all that is needed for their absorption. However, it may require a material with a greater thickness and density to stop beta particles. Gamma rays have the most penetrating power of the three radiation types. Hence the most important safety aspect when dealing with radioactive sources is that of shielding often simply keeping a reasonable distance from the source will be sufficient as the air act as a shield. When working with more intense sources, some form of shielding may be required. Sealed sources should be handled with tongs or a special source holder never with the fingers. One should not probe inside sealed sources or allow them to come into contact with any substance, which might attack or dissolve the source or its container. When not in use sealed sources should always be returned to their lead lined storage boxes. Radioactive substances should be handled with the same care and respect as concentrated acids. Washing of hands thoroughly after radioactive source is very essential.

Unit - I

Atomic absorption spectrophotometer is used to assay _____.
Electromagnetic waves has _____ energy
The region of visible light spectrum is _____ nm
Human vision detects electromagnetic wavelengths extending from _____nm.
DNA may be measured at _____ nm
Quartz is used_____ region
Light source for IR spectrophotometer is
_____ give better resolution in the chromatographic separation
Cosmic rays has_____energy
Shorter wavelength in visible region is _____
Nitrogen laser produce _____ nm
Which is used as absorbent in TLC is_____
Flame photometry is used to measure _____
Photons are known as _____
In a spectrophotometer, the band width of the wavelength is determined by _____
Modern spectrophotometer has_____ facility
Units for absorbance is _____
Autoclave is an example of _____ technology
Transmission (T)is expressed as _____
FID is _____ dector
Arrangement of the electromagnetic waves is
The typical temperature for an autoclave at 15 psi is _____ degree Celsius
The solid sample can be prepared with _____
Color filter in spectrophotometer produces bandwidths of _____ nm
_____ monochromator is used in spctrofluorimeter
_____ is heart of the spectrophotometer
Frequency of the electromagnetic radiation is
AAS is used to determine the concentration of _____
Lambda maximum is _____
Absorption of light energy is directly proportional to
Spectrophotometer is used for
Frequency of the electromagnetic radiation is
Best monochromator is
The region of Ultra violet is _____ nm
Nitrogen laser produce _____ nm
The radio waves has_____ wavelength
Wavelength expressed as
pH range is between _____

52 The energy source in the atomic spectrophotometer is _____
53 Complementary color for green is _____
54 Incubator maintains the temperature between _____ degree Celsius
55 Spectrophotometer is used for
56 Oven maintains the temperature between _____ degree Celsius
57 100 % transmittance is
58 Sodium levels in biological fluids can be determined by _____
59 Ground state of an elelement is _____- energy
60 Sodium levels in biological fluids can be determined by _____
61 Longer wavelength in visible region is _____
62 Absorbance (A) is expressed as_____
63 Proteins may be measured at _____ nm
64 Maximum light absorption of the sample is_____-
65 Fluorescence emission occurs in _____ direction
66 The exit slit in spectrophotometer is useful in selection of
67 Arrangement of the visible light energy is
68 In a clorimeter, the band width of the wavelength is determined by _____
69 Hydrogen lamp emits _____ nm
70 Atomic absorption is_____ ionization process
71 Transmittance is indirectly proportional to _____
Structure of the drug can be detected by _____
72 IR region is _____ um
73 Units used for transmittance is _____
74 Prism in spectrophotometer produces bandwidths of _____ nm
75 Melting point of the pure water is_____degree Celsius
76 Nucleic acids may be measured at _____ nm
77 Complementary color for red is _____
78 pH of the lemon juice is _____
79 Radiataion source for AAS is _____lamp
80 The radiation used in the fluorimeter is _____
81 _____ is used as detector in spectrophotometer
82 Exited state of an atom is _____ energy
83 AAS is used to characterize _____
84 **Unit - II**
85 Trade name for agarose is _____
86 The column may be made up of
87 The size of the microporous supports used in the HPLC system is _____ nm diameter
88 The maximum _____ number of reservoirs can be used in HPLC system
89 LS chromatography is_____
90 Amino acid separtion can be done in_____
91 Cellulose is a_____
92 LPLC_____
93 Ion exchange property may be altered by _____
94 The chromatographic technique discovered in _____
95 GL chromatography is_____
96 The metal column may be used in_____
97 Chromatographic system consists of
98 Sample concentration for chromatographic techniques should be between _____ percentage
99 Cellulose commonly used in _____ chromatography
100 Increase the density of the sample by the addition of _____
101 Mobile phase is called_____
102 The Chromatography techniques was discovered by

The laboratory column chromatography has _____ cm diameter
Sulphopropyl Polystyrene is a _____ exchanger
The silica matrix used in chromatography stable between_____ pH
Conventional coloumns used in for HPLC generally made of _____
Solvent movement in ascenting type of paper charomatography is_____
_____ is used to protect the stationary phase in column chromatography
Trade name for Polyacrylamide is _____

BPC stands for _____

The stationary phase may be _____
GS chromatography is_____
TLC_____ layer charomatography
_____ give better resolution in the chromatographic separation
The size of the conventional column used in the HPLC is _____ cm
Commercial name for dextran is _____
The mobile phase may be _____
The laboratory column length is _____ cm
The matrix needs to have _____
An 80-100 mesh is equivalent to _____ nm
_____mesh is most common for routine chromatographic use
Paper chromatography is used for identification of_____
Solvent movement in decenting type of paper charomatography is_____
LL chromatography is_____
Staionar phase is called _____

GLC is the abbreviation of _____

Agarose is a_____
Trade name for dextran cross linked is _____

The internal size of the conventional column used in the HPLC is _____ mm diameter

The gel filtration chromatography is used for_____ determination
The glass may be used in _____
Aminoethyl agarose _____ exchanger
The HPLC pump can produce the pressure upto _____ Pa
_____ of mobile phase is essential for HPLC analysis
_____ pump is used for pumping the mobile phase
The agarose matrix used in chromatography stable between_____ pH
Trade name for dextran is _____
Carboxy methyl cellulose is a _____ exchanger
Dextran is a _____
Commercial name for Agarose is _____

HPLC refers to _____

Unit - III

Differential centrifugation is useful for separation of _____
Analytical centrifuge is useful for _____
Ultracentrifuge rotor made up of _____
Gradient centrifugation is useful for separation of different_____ molecules
Low spped cnetrifuge rotor made up of _____
Rotor must be protected from _____
Large volume can be separated by_____ rotors
Glass centrifuge tubes are usually used for _____centrifugation
Cell debris may separated at _____ rpm
Molecular weight of the macromolecules can be determined by using _____
Rotors are made up of _____material

153 In fixed – angle rotors the tubes are located in holes in the rotor body set at a fixed angle of between _____ to the vertical
154 Caesium chloride used in the preparation of _____
155 Mitochondria may separated at _____ rpm
156 Laboratory centrifuges are _____
157 Ultra centrifuge must be with _____
158 At 100000 g _____ separated
159 _____ used in Lipoprotein fractionations
160 RCF is an abbreviation of _____
161 Cesium salt gradients are used in the separation of _____
162 Sodium iodide is used for separating of _____
163 Analytical cells are made up of _____
164 The best rotor used in centrifuge is _____
165 At 1000 g _____ separated
166 Zonal rotors are used in _____
167 Sedimentation pattern can be obtained in ultracentrifugation is _____
168 Centrifuge consists of _____
169 Sucrose concentration upto _____ % can be used in centrifugation
170 Small volume can be separated by_____
171 Sedimentation is based on _____
172 The rate of sedimentation is dependent upon the centrifugal field (G) is determined by _____
173 High weight rotor will affect the _____
174 _____ lamp is used in ultracentrifuge
175 Swinging rotor is most useful for _____
176 Sedimentation coefficient may be calculated by using _____
177 Relative molecular mass determination can be done by using _____
178 Fixed angle rotor is useful for _____
179 Analytical centrifuge developed by _____
180 Vertical rotor is useful for _____
181 Centrifuge mainly used for _____
182 rmax is more in _____
183 Sodium bromide is used for separating of _____
184 High speed centrifuge may be _____
185 Rotor must be _____
186 _____ % of sucrose solution is used density gradients
187 Ultra centrifuge must be enclosed with _____
188 High speed centrifuge must be enclosed with _____
189 Sub cellular fraction can be done with _____
190 RCF value is _____
191 Differential centrifugation is useful for _____
High sedimentation rate may be achieved by _____
193 Analytical centrifuge is used for _____
194 Rotor may be washed with_____
195 Isopynic solution is_____
196 Motor and rotor is connected by a_____
197 Cystol may be separated at _____ rpm
198 Caesium chloride is used for separation of _____
199 Rotor must be kept in a _____ condition
200 Gravitational field is _____
201 _____ will determine the sedimentation rate
202 Fixed angle rotors are ideal tool for_____
203 Analytical centrifuge is useful for _____
204 Analytical centrifuge is useful for _____

Unit IV

Electrophoresis is the migration of charged particle in a solution under the influence of an_____

Low voltage electrophoresis runs at_____

Silver staining is used for_____

Agarose gel is used for separation of _____

_____will affect the electrophoresis separation process

High voltage electrophoresis can be operated under _____condition

_____will affect the electrophoresis separation process

_____ % w/v is used for polymerization

_____will affect the electrophoresis separation process

Usually_____ % of gels are used for separating of proteins

SDS is used in electrophoresis for_____

% of gradient gel used for separation is_____

_____ will affect the electrophoresis separation process

Coomassive Brilliant Blue is used for_____

DNA can be detected by_____

Low voltage electrophoresis runs at_____

In capillary blotting the gel is placed on a_____

TEMED used in polyacrylamide gel electrophoresis_____

Ampholytes are_____

Capillary electrophoresis _____amount of samples are separated

Acryl amide is a_____

_____ is used in electrophoresis unit

High voltage electrophoresis_____

Power pack provides_____

Photo polymerization can be done by using_____

Bromophenol is used as a_____

SDS is a _____

_____ % of stacking gels used for separating proteins

Agarose is a product extracted from_____

Pulse field gel electrophoresis is used for separating_____

_____will affect the electrophoresis separation process

Unit - V

Windowless detector is useful for_____

1 Curie is_____

_____ is used as secondary fluor in scintillation counter

Proton is_____ charged

_____flour is used to detect gamma emission

Mass number (A) _____

_____ flour is used to detect alpha emission

Neutron is converted into proton is _____ emission

Neutron mass is approximately_____ to that of proton

Alpha rays has energetic value of _____ MeV

In an atom _____ and _____ is equal

Gamma rays has energetic value of _____ MeV

The proton is bigger than_____

The d.p.s is _____

The stable isotopes are equal number of _____

Solubilising agents such as _____ decreases point quenching

Half life of ^{35}S is_____ days

PPO has an emission maximum of _____nm

257 Half life of ^{3}H is _____ years
258 POPOP has an emission maximum of_____nm
259 Half life of 32 P is_____ days
260 Half life of 14 C is_____ years
261 Scintillation cocktail is a mixture of_____
262 Half life of 125 I is_____ days
263 POPOP is_____
264 The d.p.m is _____
265 PPO is_____
266 The unstable isotopes are unequal number of_____
267 The old system of radioactivity is measured in terms of_____
268 The proton is_____ bigger than
269 t ½ is_____ / lambda
270 Usually atom is _____ charged
271 Beta rays has energetic value of _____ MeV
272 Number of neutron in an atom is always _____
273 Proton is converted into neutron is _____emission
274 _____ flour is used to detect bets emission
275 Radioisotope is _____
276 _____ is used as primary fluor in scintillation counter
277 Nucleus of the atom consists of_____
278 End window ionization counter is _____
279 PMT is_____
280 Emulsifier is used in_____
281 The basic International unit of radioactivity is_____
282 Autoclave is an example of _____technology.
283 The pressure used in an autoclave is measured in _____.
284 The material used in instrumentation of incubator is _____.
285 The space between the two adjacent walls of hot air oven is called _____.
286 The optimum temperature used in the hot air oven for sterilization is_____.
287 The electrode used to check the pH of the sample is called_____.
288 The size of pores in HEPA filter is_____micron.
289 The colorimeter works on the principle of _____.
290 The wavelength of UV rays is in the _____spectrum.
291 Frequency of electromagnetic radiation is measured in _____ units.
292 _____ focuses the parallel beam of light in to fine beams in UV-Vis Spectrophotometry.
293 _____ is used in the photon multiplier tubes to amplify the light rays.
294 _____ filter paper is commonly used in paper chromatography.
295 Silica used in TLC method serves as _____.
296 Commercial name of Polyacrylamide gel used in column chromatography is_____.
297 The process of extracting the separated compound from the column matrix is called_____.
298 _____ pump is cost effective for use in HPLC.
299 Ratio between the distance travelled by solute and solvent in a stationary phase is called_____.
300 Chromatography was discovered by _____.
301 The carrier gas used as mobile phase in chromatography is _____.
302 The material used to make preparative column chromatography is _____.
303 _____used to make heating element of the column in gas chromatography.
304 Propulsion of atom in Geiger Muller counter is made possible by _____
305 The central part of the centrifuge attached to the main motor is called _____.
306 The usual refrigeration temperature of ultra centrifuges is _____degree.
307 What is the role of indicator light in hot air oven?
308 Which of the following is a subatomic particle?
309 The atomic reaction in which the dissociation of nucleus occurs is _____.

310 The no of electron in the last orbital of an atom is called its _____.

311 EEO refers to _____.

312 Low EEO agarose is suitable for _____molecular weight seperation.

313 High EEO agarose is suitable for _____molecular weight seperation.

314 Adsoption of liquid sample to a semisolid stationary phase is used in _____chromatograph

315 Adsoption of solid sample to a solid stationary phase with ionic surface is used in _____chromatography.

KARPAGAM UNIVERSITY (KARPAGAM ACADEMY OF HIGHER EDUCATION)
DEPARTMENT OF MICROBIOLOGY
BIOINSTRUMENTATION 17MBP104

Option 1	Option 2	Option 3
Enzyme	Potassium	Sugars
Power of	Quantum of	Electricity
500-900	100-400	200-900
500-900	100-400	400-700
200	400	260
IR	Visible	NMR
Tungsten lamp	Hydrogen lamp	Sodium lamp
Gradient solvent	None of the above	Both a and b
high	medium	moderate
Violet	Yellow	Blue
200	600	337
gum	iron	copper
Fluorescence	reflection	Scattering
opaque	transparent	quanta
Tungsten lamp	UV region	Monochromator
Double beam	All the above	Printer
OD	No units	Kg
Moist heat	Dry heat	Moist and dry heat
T = I	T= a/b	T = A & C
free ionization	fuel ionization	file ionization
Cosmic rays / UV / Visible / IR / Microwaves	UV / Visible / IR / Microwaves / Cosmic rays	UV / Cosmic rays / Visible / IR / Microwaves
160	250	200
Hydrogen	Nujol	Carbon
15-30	550-600	200-500
Three	Four	Two
Light source	Read out	Monochromater
gram	Hertz	decibel
molecules	medium	substrates
Minimum absorption	Poor absorption	Medium absorption
Concentration of the reagents	None of the above	Concentration of light intensity
Qualitative analysis	Quantitative analysis	Both a and b
gram	Hertz	decibel
Filter	grating	Prism
100-300	300-550	500-600
200	600	355
high	medium	moderate
micrometer	centimeter	nanometer
1-14	7-14	1-7

Tungsten filament	X –rays	Laser rays
Red	Yellow	Blue
20-60	100-150	75-100
Qualitative analysis	Quantitative analysis	Both a and b
100-300	100-125	75-100
opaque	transparent	turbidity
Electrophoresis	Colorimetry	Chromatography
high	medium	low
Electrophoresis	Colorimetry	Chromatography
Red	Yellow	Blue
T = I	T= a/b	T = A & C
320	600	550
lambda mimimum	lambda poor	lambda average
40	90	60
Laser	Light intensity	Path length
VIBYGOR	VIOGBYR	VIBORGY
detector	light	sample
500-600	200-400	300-550
Hot	poor	Medium
Concentration of the reagents	Concentration of the solution	Concentration of light intensity
UV-Vis spectroscopy	Cosmic rays	X- ray
100-200	200-250	150-250
Mg	cm	Kg
More than one	More than ten	More than five
10	100	50
200	400	260
Red	Yellow	Blue
acidic	None of the above	neutral
hydrogen	tungston	UV
UV lamp	Deuterium lamp	Hydrogen lamp
Light tubes	Electrical signal	Monitor
high	medium	low
Nitrogen content	metal ions	Protein content
Separose	Sepahdex	Sephacryl
Plastic	Rubber	Metal
5-10.5	50-100	10-50.5
10	20	15
large-solid	liquid-space	large-space
APLC	GPLC	fermentor
Polystyrene	polymer	Nylon
Low performance liquid chromatography	Low performance large chromatography	Low pressure large chromatography
Temperature	Mesh size	Pressure
1925	1800	1903
gas-lab	gas-lemon	gas-large
HPLC	Both b and c	GLC
mobile phase	none of the above	both a and b
50-60	80-90	60-70
Bio-afffinity	Dye-ligand	Ion-exchange
Agar	Yeast extract	Sucrose
eluent	solid	solvent
Louis	Johnson	Harris

0.5-5	20-30	0.2-0.8
Strong basic cation	Weak basic cation	Weak acidic cation
2-14	2-12	3-8
Plastic	Copper	Stainless steel
downward	all directions	side ways
Stone	Metal	Glass
Separose	Sepahdex	Sephacryl
Bold phase chromatography	Buffer phase chromatography	Base phase chromatography
Solid	Liquid	Gas and liquid
gas-solid	gas-space	gas-sample
thin	tear	tuff
Gradient solvent	Soluble solvent	Both a and b
1-3.5	100-200	50-100
Biogel-P	Sepharose	Sephadex
liquid	Both b and c	Gas
100-200	250-300	150-250
Good flow rates	Good chemical stability	Good absorption
0.6-1.0	3.0-4.0	1.0-2.0
200-300	350-400	300-350
proteins	hormones	enzymes
downward	all directions	side ways
liquid-arge	liquid-lab	large-liquid
eluent	Gas	solvent
Gas liquid chromatography	Gas large chromatography	Glass large chromatography
Polystyrene	polymer	Nylon
Separose	Sepahdex	Sephacryl
4	20	10
pH	Ion exchange property	Temperature
HPLC	All the above	GC
Strong basic anion	Weak basic anion	Weak acidic anion
$5.5x10^{7}$	$5.5x10^{12}$	$5.5x10^{10}$
Degassing	Boiling	Acidification
Peristaltic	Centrifugal	Centripetal
3-14	8-12	3-8
Separose	Sepahdex	Sephacryl
Strong basic cation	Weak basic cation	Weak acidic cation
Polystyrene	polymer	Nylon
Biogel-P	DEAE Cellulose	Sephadex
High performance liquid chromatography	High performance large chromatography	High pressure large chromatography
protein	Nucleic acids	chloroplast
Detection of conformational changes	collecting of molecules	Separation of molecules
titanium alloy	steel	gold
density	plasmids	structure
aluminium	steel	copper
moisture	rain	temperature
vertical	Fixed angle	swinging bucket
Low speed	All the above	High speed
1000	600	320
Low speed centrifuge	Medium speed centrifuge	Ultra centrifuge
brass and steel	copper	steel

15^{0} and 50^{0}	14^{0} and 40^{0} 4	16^{0} and 90^{0}
Enzyme solution	gradient solution	vitamin solution
10000	1500	2000
refrigerated	chiller	non-refrigerated
temperature control	vacuum control	speed control
cystol	DNA	Mitochondria
Sodium bromide	alcohol	Methanol
relative centrifugal field	relative computer field	relative centrifuge field
Nucleic acid	cell debris	Mitochondria
DNA and RNA	plasmids	protein
quartz	steel	glass
steel	aluminum	titanium alloy
Cell debris	DNA	Mitochondria
industry	Clinical labs	Laboratory
UV system	Louis system	Schlieren system
rotor, motor	rotor and soft	rotor, motor and soft
100	60	90
Effendorf tube	Fixed angle	swinging bucket
speed	medium	centrifugal field
$G - Wr^2$	Gwr^3	$G\ w^2r$
Separation process	high speed centrifugation	speed
UV	mercury	xenon
gradient separation	Pelleting of molecules	isopynic separation
Ultra centrifuge	Low volume centrifuge	low speed centrifuge
Ultra centrifuge	Low volume centrifuge	Low speed centrifuge
separation of molecules	pelting of molecules	pelting of molecules
Johnson	Marry	Louis
isopynic separation	none of the above	gradient separation
pelleting and sedimentation		sedimentation
vertical rotor	zonal rotor	swinging bucket rotor
DNA	Nucleic acids	lipoprotein
refrigerated	none of the above	room temperature
corrosive resistant	glass	high weight
10-20.0	50-100	20-30
steel	glass	heavy armour plating
steel	glass	heavy armour plating
Analytical centrifuge	Medium speed centrifuge	Preparative centrifuge
$1.12 \times 10^5 rpm^2r$	$4.12 \times 10^5 rpm^2r$	$2.12 \times 10^5 rpm^2r$
biochemical research	physical and chemical	chemical research
motor	flexible soft	rotor
Ligand-binding studies	Pelleting of molecules	Separation of molecules
Distilled water	Salt water	Hot water
Same concentration	None of the above	Different concentration
Flexible rod	needle	wire
10000	4000	20000
DNA	enzymes	Protein
dry	liquid	hot
$9.81 cm/s^2$	$981 cm/s^2$	$98.1 cm/s^2$
speed	soft	motor
pelleting	sedimentation	quantification
Separation of molecules	sedimentation	Determination of purity
Determination of relative mass	sedimentation	Separation of molecules

density	Electromagnetic	Magnetic field
0-500 V	0-200 V	0-100 V
detecting carbohydrates	detecting RNA	detecting DNA
all the above	DNA	protein
none of the above	ionic strength of the buffer	pressure
high pressure	cold	vacuum
none of the above	size of the sample	pressure
0.1-1.2	0.1-0.6	0.1-0.3
none of the above	electric field	pressure
10-25.0	0-5	1-15.0
none of the above	naturing of proteins	denaturing of proteins
20-50	0-10	5-25.0
size of the gel	pressure	pH
detecting carbohydrates	detecting RNA	detecting DNA
sodium lamp exposure	tungsten lamp exposure	UV exposure
TEMED	bisacrylamide	acrylamide
Water	Urea and formamide	APS
pH modifier	Inhibitor	Catalysts
gel	buffer	pH gradients
kilograms	Milligrams	Nanograms
verotoxic	cytotoxic	Nurotoxic
aluminum	gold	platinum wire
500-2000 V	500-1000 V	500-10000 V
none of the above	alternative current	direct current
Ammonium phosphate	Ammonium sulphate and TEMED	Rifoflavin
gradient	electricity	Tracking dye
cationic detergent	anionic detergent	anionic dye
16	8	4
water	Wood	Plant
All the above	RNA	DNA
vacuum	pressure	temperature

Strong emission	None of the above	Moderate emission
3.70×10^{7} Bq	3.70×10^{10} Bq	3.70 x 10 Bq
POPOP	ZOPOP	BOPOP
positively	none of the above	both positive and negative
sodium iodide	acetic acid	antracene
Proton and neutron	Electrons	Electron and neutron
sodium iodide	acetic acid	antracene
Poistron	gamma	Alpha
Equal	Small	Big
4-8.0	30-35	25-30
Electron and neutron	Electrons	Proton and neutron
4-8.0	3-8.0	2-10.0
electron	neutrons	Electron and neutron
Disintegration per sample	Disintegration per surface	Disintegration per squire
Proton and electron	Protons and neutrons	Electron and neutron
Primary Flour	Scalar	Coincidence
87.2	3	2
220	380	330

12.26	3	2
220	380	420
14.2	36	25
5760	3	2
Solvent and flour	Solute and solvents	Two solutes
60	3	2
1, 5 – bis – 2 – (5- Phenyloxazolyl) benzene	2, 5 – Diphenyloxazole	Oxole
Disintegration per mass	Disintegration per mat	Disintegration per million
2, 5 – Polypenylazole	2, 5 – Diphenyloxazole	2, 3 – Polyphenylazole
Proton and electron	None of the above	Electron and neutron
Millicurie	Becquerel	Curie
1850	300	200
1.23	3.8	2
Positively	both positive and negative	Neutrally
4-8.0	3-8.0	2-10.0
Equal	None of the above	Big
Poistron	gamma	Alpha
sodium iodide	acetic acid	antracene
Unstable form	Stable form	Both stable and unstable form
PPO	ZPO	BPO
Proton and neutron	Electrons	Electron and neutron
scintillation	X ray	Audoradigraphy
Photomagnifier tube	Phtomultipication tube	Photographic tube
scintillation	X ray	Audoradigraphy
Millicurie	Becquerel	Curie
dry heat	moist heat	moist and dry heat
Pa	psi	lbs
Aluminium	Mild Steel	Copper alloy
Thermal cover	jacket	inner compartment
110	90	160
Reference electrode	Calomel electrode	Mercury electrode
10	0.03	0.3
Electromagnetism	Thermodynamics	Beer-Lamberts law
100-400	420-500	510-600
Decibel	Kilogram	Hertz
Monochromator	Grating lens	Slit
Cathode	Tungsten	Anodydes
Cellulose	Whatmann No.3	Membrane filter
Mobile phase	Solvent phase	Stationary phase
Sephadex	Sephacyl	Bio-gel A
elution	evaporation	precipitation
displacement pump	isocratic pump	pneumatic pump
Rf	RT	K
Louis	Johnson	Michael
oxygen	nitrogen	helium
glass	plastic	polyurethane
iron	copper	aluminium
electron diffusion	electron impulsion	electromagnetism
median axis	central axis	central median axis
1	4	2
power supply	voltage indicator	voltage cut off
electron	proton	neutron
nuclear fission	nuclear fusion	nucler shift

mass number	atomic number	atomic mass
Electro endo osmosis	Electro ethno osmosis	Electric endo osmosis
high	medium	low
high	medium	low
HPLC	TLC	Paper
HPLC	TLC	Paper

Option 4

Protein
Discrete packet of
400-800
100-700
100
UV
Nernst glower
Isogradient solvent
lower
Green
500
siliga gel
Absorption
dark

Solution

Scan
Percentage
heat
T = I/Io
flame ionization
Cosmic rays / Visible / UV /
Microwaves
121
Nitrogen
100-400
One
Detector
meter
element
Maximum absorption

Concentration of the sample

Absorption
meter
Lense
200-400
500
lower
meter
0-14

UV rays
Green
0-10
Absorption
0-10
dark
Spectrofluorimetry
zero
Spectrofluorimetry
Green
1/T
280
lamda maximum
30
Wavelength
VIBGYOR
filter
100-300
Soft

Concentration of the sample

NMR
0.8-25
Percentage
Less than one
0
100
Green
basic
hallow cathode
Mercury vapor lamp
Photo tubes
zero
Carbon content

Bio-gel P
Both a and c

0-2

6
liquid-soild
TLC
Polysaccharide
Low pressure liquid chromatography
Strong pH
1950
gas-liquid
LPLC
stationary phase

0-10

Affinity
Maltose
adsorbent
Mikkhail Tvwett

0.001-0.002
Strong acidic cation
1-7
Glass
upward
Nylon disc
Bio-gel P
Bonded phase chromatography
Both a and b
Gas-substate
thick
Isogradient solvent
1-2.5
Biogel-A
Solid
10-100
Both a and b
0.18-0.15
100-120
pigments
upward
ligiud-liquid
adsorbent
Glass liquid chromatography
Polysaccharide
Bio-gel P

1

Molecular weight
LPLC
Strong acidic anion
$5.5x10^{9}$
Gassing
2 HP
1-7
Bio-gel P
Strong acidic cation
Polysaccharide
Biogel-A
High pressure laboratory chromatography

Both a and b
Pelleting of molecules
metal
mitochondria
gold
heat
Zonal
Ultra centrifuge
550
High speed centrifuge
silver

16^{0} and 60^{0}

protein solution
20000
ultra centrifuge
refrigerated
plasmids
mercury
relative common field
plasmids
carbohydrate
plastic
brass
plasmids
Medical field
Lamberts system
motor and soft
80
Zonal
moderate

Gwr

low density
tungsten
normal sedimentation
high speed centrifuge
High speed centrifuge
all the above
Svedberg
normal separation
separation of macromolecules
fixed angle rotor
RNA
above room temperature
plastic
10-60.0
plastic
plastic
High speed centrifuge
$3.12 \times 10^{5} rpm^{2} r$
physical research
centrifugal field
Attachment of molecules
Tap water
Gradient
Flexible soft
100000
mitochondria
Moisture
$0.981 cm/s^{2}$
rotor
qualitative
Pelleting of molecules
Pelleting of molecules

Electric field

0-300 V
detecting protein
RNA
vacuum
hot
vacuum
0.1-0.9
vacuum
0-10
both A and B
5-100
vacuum
detecting protein
mercury lamp exposure
ammonium per sulphate
Formalin
Crosslinking agent
density
Centigrams
leucotoxic
copper
500-1500 V
both A and B

Both A and B

electrode
cationic dye
12
Sea weed
Chromosome
all the above

Weak emission
3.70 x 10^{4} Bq
AOPOP
negatively
zinc sulphate
Proton and electron
zinc sulphate
Negatron
Unequal
20-25
Electron and proton
2-3.0
neutron
Disintegration per second
None of the above
Quenching
1
110

1
110
5
1
Two solvents
1

Benzene

Disintegration per minutes
2, 5 – Diphenyloxazole
Proton and neutron
Microcurie
100
0.693
Negatively
2-3.0
Unequal
Negatron
zinc sulphate
None of the above
APO
Proton and electron
Geiger Muller
Photomultiplier tube
Geiger Muller
Microcurie
none of the above
Hg/mm3
Iron
clear space
221
none of the above
0.001
Optical illusion
90-110
Nanometer
Filter
Electrocell
none of the above
Baoth a and b
Poly A gel-P

filtration

filter

Rt

Mikhail Tswett
carbondioxie
steel
boxite
electron shift
gauge scale
10
temperature indication
Higs-Bosson particle
nuclear drift

valency
Electron endo osmosis
very low
medium and high
LPLC

LPLC

Answer key

Potassium
Discrete packet of
400-800
400-700
260
UV
Nernst glower
Gradient solvent
high
Violet
337
siliga gel
Fluorescence
quanta

Monochromator

All the above
No units
Moist heat
T = I/Io
flame ionization
Cosmic rays / UV / Visible / IR / Microwaves
121
Nujol
15-30
Two
Monochromater
Hertz
element
Maximum absorption

Concentration of the sample

Both a and b
Hertz
grating
200-400
355
high
nanometer
0-14

Laser rays
Red
20-60
Both a and b
100-300
transparent
Spectrofluorimetry
zero
Spectrofluorimetry
Red
1/T
280
lamda maximum
90
Wavelength
VIBGYOR
filter
200-400
Hot

Concentration of the sample

NMR
0.8-25
Percentage
Less than one
0
260
Green
acidic
hallow cathode
Mercury vapor lamp
Photo tubes
high
metal ions

Separose
Both a and c

5-10.5

6
liquid-soild
TLC
Polysaccharide

Low pressure liquid chromatography

Strong pH
1903
gas-liquid
Both b and c
both a and b

0-10

Ion-exchange
Sucrose
eluent
Mikkhail Tvwett

0.5-5
Strong acidic cation
3-8
Stainless steel
upward
Nylon disc
Bio-gel P

Bonded phase chromatography

Both a and b
gas-solid
thin
Gradient solvent
1-3.5
Sephadex
Both b and c
10-100
Both a and b
0.18-0.15
100-120
pigments
downward
ligiud-liquid
adsorbent

Gas liquid chromatography

Polysaccharide
Sephacryl

4

Molecular weight
LPLC
Strong basic anion
$5.5x10^{7}$
Degassing
Peristaltic
3-8
Sepahdex
Weak acidic cation
Polysaccharide
Biogel-A

High performance liquid chromatography

Both a and b
Detection of conformational changes
titanium alloy
density
aluminium
moisture
Zonal
Low speed
1000
Ultra centrifuge
brass and steel

14^{0} and 40^{0} 4

gradient solution
20000
refrigerated
vacuum control
cystol
Sodium bromide
relative centrifugal field
Nucleic acid
DNA and RNA
quartz
titanium alloy
Cell debris
industry
Schlieren system
rotor, motor and soft
60
Effendorf tube
centrifugal field

$G\ w^2r$

separation process
xenon
gradient separation
Ultra centrifuge
Ultra centrifuge
all the above
Svedberg
isopynic separation

all the above

swinging bucket rotor
lipoprotein
refrigerated
corrosive resistant
10-60.0
heavy armour plating
heavy armour plating
Preparative centrifuge
$1.12 \times 10^5 rpm^2r$
biochemical research
centrifugal field
Separation of molecules
Distilled water
Same concentration
Flexible soft
100000
DNA
dry
$981 cm/s^2$
speed
pelleting
Determination of purity
Determination of relative mass

Electric field

0-500 V
detecting protein
all the above
ionic strength of the buffer
cold
size of the sample
0.1-0.3
electric field
1-15.0
denaturing of proteins
5-25.0
pH
detecting protein
UV exposure
TEMED
Urea and formamide
cross linking agent
pH gradients
Nanograms
Nurotoxic
platinum wire
500-10000 V
direct current

Both A and B

Tracking dye
anionic detergent
4
Sea weed
All the above
temperature

Weak emission
3.70×10^{10} Bq
POPOP
positively
antracene
Proton and neutron
sodium iodide
Negatron
Equal
4-8.0
Electron and proton
2-3.0
electron
Disintegration per second
Proton and neutron
Primary Flour
87.2
380

12.26
420
14.2
5760
Solute and solvents
60

1, 5 – bis – 2 – (5- Phenyloxazolyl) benzene

Disintegration per minutes
2, 5 – Diphenyloxazole
Proton and neutron
Curie
1850
2
Neutrally
2-3.0
None of the above
Poistron
zinc sulphate
Unstable form
PPO
Proton and neutron
Geiger Muller
Photomultiplier tube
Audoradigraphy
Becquerel
moist heat
lbs
mild steel
jacket
160
Calomel electrode
0.3
Beer Lamberts law
100-400
Hertz
Slit
Anodydes
Whatmann No.3
Stationary phase
Poly A-gel P

elution

pneumatic pump

Rf

Mikhail Tswett
helium
glass
copper
electro magnetism
central median axis
4
power supply
Higs-Bosson particle
nuclear fission

valency
Electro endo osmosis
high
low
LPLC

HPLC

Reg. No._____

[17MBP104]

KARPAGAM UNIVERSITY

Karpagam Academy of Higher Education

Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

(For candidates admitted from 2009, onwards)

MICROBIOLOGY

M. Sc., DEGREE INTERNAL EXAMINATION, August- 2017

BIOINSTRUMENTATION

Time: 2 hours **Maximum:50 marks**

Date:

Part - A (20 X 1 = 20 Marks)

1. Exited state of an atom is _____ energy
   a) high b) medium
   c) low d) zero
2. Differential centrifugation is useful for separation of _____
   a) protein b) Nucleic acids
   c) chloroplast d) Both a and b
3. Analytical centrifuge is useful for _____
   a) Detection of conformational changes b) collecting of molecules
   c) Separation of molecules d) Pelleting of molecules
4. Laboratory centrifuges are _____
   a) refrigerated b) chiller
   c) non-refrigerated d) ultra centrifuge
5. _____ monochromator is used in spectrofluorimeter
   a) Three b) Four
   c) Two d) One
6. Frequency of the electromagnetic radiation is _____
   a) Gram b) Hertz
   c) decibel d) meter
7. Radiation source for AAS is _____lamp
   a) Hydrogen b) tungsten
   c) UV d) hallow cathode
8. Differential centrifugation is useful for _____
   a) biochemical research b) physical and chemical
   c) chemical research d) physical research
9. Caesium chloride is used for separation of _____
   a) DNA b) enzymes
   c) Protein d) mitochondria
10. _____ will determine the sedimentation rate
    a) speed b) soft
    c) motor d) rotor
11. Prism in spectrophotometer produces bandwidths of _____ nm
    a) More than one b) More than ten
    c) More than five d) Less than one
12. Transmittance is indirectly proportional to _____
    a) Concentration of the reagents b) Concentration of the solution

c) Concentration of light intensity d) Concentration of the sample

13. Proteins may be measured at _____ nm
    a) 320 b) 600
    c) 550 d) 280
14. Absorbance (A) is expressed as_____
    a) T = I b) T= a/b
    c) T = A & C d) 1/T
15. Sedimentation pattern can be obtained in ultracentrifugation is _____
    a) UV system b) Louis system
    c) Schlieren system d) Lamberts system
16. Vertical rotor is useful for _____
    a) isopynic separation b) isogradient
    c) gradient separation d) normal separation
17. Sucrose concentration upto _____ % can be used in centrifugation
    a) 100 b) 60
    c) 90 d) 80
18. Swinging rotor is most useful for _____
    a) gradient separation b) Pelleting of molecules
    c) isopynic separation d) normal sedimentation
19. Atomic absorption spectrophotometer is used to assay _____
    a) Enzyme b) Potassium
    c) Sugars d) Protein
20. In a spectrophotometer, the band width of the wavelength is determined by _____
    a) Tungsten lamp b) UV region
    c) Monochromator d) Solution

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. Define swedberg unit (S).
22. What is Nuclear Magnetic Resonance?
23. Write the full form of FTIR spectroscopy and give its principle.

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a) Given an account about properties of electromagnetic radiation.

    (or)

    b) Explain in detail about principle and instrumentation of UV-Visible light spectrophotometer
25. a) Give a detailed account on NMR spectroscopy and its applications.

    (or)

    b) Discuss about spectrofluorimeter and its applications.
26. a)) Define chromatography? Explain in detail about applications of chromatography

    (or)

    b) Explain different types of centrifuges.

Reg. No._____

[17MBP104]

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MICROBIOLOGY

M. Sc., DEGREE INTERNAL EXAMINATION, August- 2017

BIOINSTRUMENTATION

Time: 2 hours **Maximum:50 marks**

Date:

PART-A

1.a)high

2.b)both a and b

3.a)detection of conformational changes

4.a)refrigerated

5.c)two

6.b)hertz

7.a)hallow cathode

8.a)biochemical research

9.a)DNA

10.a)speed

11.d)less than one

12.d)concentration of the sample

13.d)280

14.d)1/T

15.c)schlieren system

16.a)isopynic separation

17.b)60

18.a)gradient separation

19.b)potassium

20.c)monochromator

PART-B

21.Define Swedberg unit(S):

Sedmentation rate-using centrifuge-measure the particle size-rate of travel-high g-force.One swedberg unit is 10 power -13.

22.What is Nuclear Magnetic Resonance:

Nuclei magnetic field-absorb,reemit-electromagnetic radiation-strength of the magnetic field-magnetic properties of the isotope-observe quantum mechanical magnetic properties-atomic nucleus-use in advanced medical imaging techniques.

23.write the full form of FTIR spectroscopy and give its principle:

Fourier transform infrared spectroscopy-computer processing-raw data (light absorption for each mirror position)-desired result (light absorption of wavelength)-beam of many frequencies of light-raw data-measure beam absorbed-second data point-different combination of frequencies-then infer the absorption of wavelength.

PART-C

24.a)Properties of electromagnetic radiation:

Interaction of electromagnetic radiation-quantum phenomenon-properties of radiation-structural parts-sample. Origin of electromagnetic radiation-energy changes within matter-transitions occur-spectra arise-principally predictable.

Composed of electric-perpendicular magnetic vector-one oscillating plane-direction of propagation.

Wavelenth-distance-two consecutive peaks-sinusoidal waveform-nanometers-maximum wavelength-amplitude-frequency-wavelength via speed of light-number of completed wave cycle-one cycle-1 cm-1.

b)Principle and instrumentation of UV-Visible light spectrophotometer:

dual beam-sample-control (buffer)-one record control spectrum-internal reference-sample spectrum. Latter approach-computer controlled-baseline correction-substrating control from sample-tungten filament bulb-visible part-spectrum-deuterium bulb-UV region. Monochromator-between source and sample-wavelength selection-colored filters-absorb limited range-brandwidth of filter. Filter-based wavelength selection-certain wavelength-specific colorimetric assay-prisms or gratings-technique-specctrophotometry.

UV/V spectrophotometer-quantification of biological samples-direct-colorimetric assays-protein-chromophores,tyrosine,tryptophan-protein spectra-presence of prosthetic enzymes-coenzymes-protein quantification-presence of larger aggregates-Rayleigh scatter.

25.a)NMR spectroscopy and its applications:

Low concentration-emits flurorophores-spectral selectivity-stokes shift-fluorscence-emitted-all directions-detector-perpendicular-exitation pathway-incident beam reduced-two monochromators-tuning wavelength-analysis-emission-photon counting detector-pre and post filter effects-use of microcurvettes-front face illumination.used for structural elucidation-assess-rotation correlation coefficient-invitro samples-excitation-emission spectra-abscissa.

b)Spectrofluorimeter and its applications:

Klystron generating microwaves-sealed tubes-solid samples-angle54.7-intermolecular interaction-resonance signals broaden-multidimensional NMR-radio frequency coils-sophisticated electronics.

Medicine-magnetic resonance microscopy-research setting-chemical studies-vivo magnetic resonance spectroscopy. Chemistry-unambiguosly identify novel compounds-scientific journals-purity determination-structural determination-high molecular weights-accurate weighing.

26.a)Chromatography and its application in detail:

Physical separation-mixture-different kinds-individual components-differential absorption and elution. Environmental monitoring and clean up-GC-MS-tracking organic pollutants-criminal forensics-analyse the particles-human body-link a criminal-fire debris analysis-law enforcement-illegal narcotics-drug-sniffing dogs-find-drug or poisons-biological specimens-sport antidoping analysis-anabolic steroids.

Security based on GC-MS-ion track instruments-smith detection system-chemical warfare agent detection-analytes-liquid injection-thermal desorption process-solid-phase micro extraction (SPMC)-chemical engineering-composition of bio oils processed-food and beverage and perfume analysis-spoilage or adulteration-astrochemistry-analysed the atmosphere-Rosetta mission-chiral GC-MS-medicine-inborn error-compound in urine-minor concentration-genetic metabolic disorder-metabolic activity-isotope ratio mass spectrometer (IRMS).

b)Different types of centrifuge:

Small bench centrifuge-yeast cells-erythocytes-large capacity refrigerated centrifuge-refrigerated rotor chamber-collect the substances-nuclei,chloroplast-high speed refrigerated centrifuge –microorganisms,cellular denris, proteins-design and types of rotors-swingling bucket rotors-fixed angle rotors-vertical tube rotors-zonal rotors-elutriator rotors-ultra centrifuge-analytical ultracentrifuge-sedimentation velocity experiment-sedimentation equilibrium experiment-shape and molar mass-dissolved macromolecules-diffusion-opposing concentration gradients.

Preparation ultracentrifuge-separation of macromolecule/ligands-lipoprotein-plasma and deprotonisation-amino acid analysis-biology for pelleting-microsomes,ribosomes,viruses-gradients-caesium salts-separation nuclei acids-

lysate weak forces-differential centrifuge-lysed cell membranes-lysate-remove pellet-purification.

Reg. No._____

[17MBP104]

KARPAGAM UNIVERSITY

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(For candidates admitted from 2009, onwards)

MICROBIOLOGY

M. Sc., DEGREE SECOND INTERNAL EXAMINATION, SEPTEMBER- 2017

BIOINSTRUMENTATION

Time: 2 hours **Maximum:50 marks**

Date:

Part - A (20 X 1 = 20 Marks)

1. Conventional columns used in for HPLC generally made of _____
   a. Plastic b. Copper
   c. Stainless steel d. Glass
2. GS chromatography is_____
   a. gas-solid b. gas-space
   c. gas-sample d. Gas-substate
3. The gel filtration chromatography is used for_____ determination
   a. pH b. Ion exchange property
   c. Temperature d. Molecular weight
4. Gradient centrifugation is useful for separation of different_____ molecules
   a. Density b. plasmids
   c. structure d. mitochondria
5. Ultra centrifuge must be with _____
   a. temperature control b. vacuum control
   c. speed control d. refrigerated
6. Sedimentation coefficient may be calculated by using _____
   a. Ultra centrifuge b. Low volume centrifuge
   c. low speed centrifuge d. high speed centrifuge
7. The HPLC pump can produce the pressure upto _____ Pa
   a. $5.5x10^{7}$ b. $5.5x10^{12}$
   c. $5.5x10^{10}$ d. $5.5x10^{9}$
8. Analytical centrifuge is used for _____
   a. Ligand-binding studies b. Separation of molecules
   c. Pelleting of molecules d. Attachment of molecules
9. RCF value is _____
   a. 1.12 x 105rpm2r b. 4.12 x 105rpm2r
   c. 2.12 x 105rpm2r d. 3.12 x 105rpm2r
10. Ultra centrifuge must be enclosed with _____
    a. Steel b. glass
    c. heavy armour plating d. plastic
11. Gamma rays has energetic value of _____ MeV
    a. 4-8.0 b. 3-8.0
    c. 2-10.0 d. 2-3.0
12. POPOP is_____
    a. Oxole b.1,5–bis–2–(5-Phenyloxazolyl) benzene

c. 2, 5 – Diphenyloxazole d. Benzene

13. Proton converted to neutron is _____emission
    a. Poistron b. gamma
    c. Alpha d. Negatron
14. SDS is used in electrophoresis for_____
    a. naturing of proteins b. denaturing of proteins
    c. both A and B d. denaturing of proteins
15. TEMED used in polyacrylamide gel electrophoresis_____
    a. pH modifier b. Inhibitor
    c. Catalysts d. Cross-linking agent
16. Pulse field gel electrophoresis is used for separating_____
    a. Chromosome b. DNA
    c. RNA d. Nucleus
17. Coomassive Brilliant Blue is used for_____
    a. detecting carbohydrates b. detecting RNA
    c. detecting DNA d. detecting protein
18. Agarose is a product extracted from_____
    a. Water b. Wood
    c. Plant d. Sea weed
19. The stable isotopes are equal number of _____
    a. Proton and electron b. Electron and neutron
    c. Proton and neutron d. Neutrons
20. Radioisotope is _____
    a. Unstable form b. Both stable and unstable form
    c. Stable form d. Soluble form

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. Define chromatogram.
22. What is Scintillation cocktail?
23. Define ligand with examples.

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a) Explain in detail about principle and applications of ion exchange chromatography

(or)

b) Brief on the principle and instrumentation of HPLC.

25. a) Write detail notes on SDS-PAGE and its uses.

(or)

b) Explain separation of DNA using agarose gel electrophoresis?

26. a) Discuss the importance of safe handling of radioisotopes.

(or)

b) Give a brief notes on liquid scintillation counter.

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MICROBIOLOGY
M. Sc., DEGREE SECOND INTERNAL EXAMINATION, SEPTEMBER- 2017
BIOINSTRUMENTATION
Answer key

PART A

1.c)stainless steel

2.a)gas solid

3.d)molecular weight

4.a)density

5.b)vacuum control

6.a)ultracentrifuge

7.a)5.5×10 power7

8.a)ligand binding studies

9.a)1.12×10 power 5 rpm2r

10.c)heavy armour plating

11.d)2-3.0

12.b)1,5-bis-2-(5-phenyloxazolyl)benzene

13.a)positron

14.d)denaturing of protein

15.d)cross linking agent

16.all the above

17.d)detecting protein

18.d)sea weed

19.c)proton and neutron

20.a)unstable form

Part B

21. Define chromatogram.

The graph showing the results of seperated components of a mixture by chromatography.

22. What is Scintillation cocktail?

In liquid scintillation method- Samples -dissolved or suspended in a "cocktail" containing a solvent (historically aromatic organics such as benzene or toluene, but more recently less hazardous solvents are used), typically some form of a surfactant, and small amounts of other additives known as "fluors" or scintillators. Scintillators can be divided into primary and secondary phosphors, differing in their luminescence properties.

23. Define ligand with examples.

In affinity chromatography, the ligands are used fixed onto stationary phase, reversibly binds desired biomolecule prsesent in mobile phase.

Eg- serum proteins, antibodies, lectins triazine dyes.

Part – C

24. a) Explain in detail about principle and applications of ion exchange chromatography

- Ion exchange chromatography process that allows separation of ions and polar molecules change.
- Ion exchange chromatography coined by Smail, Stevens and Raumann.
- Ion exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions.
- The stationary phase surface displays ionic functional groups that interact with analyte ions of opposite charge.
- TypeS -cation exchange chromatography and anion exchange chromatography.The ionic compound consisting of the cationic species M^+ and the anionic species B can be retained by the stationary phase.
- Cationic exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group. Anion exchange

chromatography retains anion using positively charged functional group. Ion exchange is classified into resins, gels and inorganic exchanger.

- Resins are amorphous particles of organic materials. Ion exchange resins are used for the separation of small molecules.

Classification of ion exchange resins:

Strongly acidic cation exchanger – Sulphonic acid groups attached to styrene and divinyl benzene copolymer.

Weakly acidic cation exchanger – Carboxylic groups attached to acrylic and divinyl benzene copolymer.

Strongly basic anion exchanger – Quaternary ammonium groups attached to styrene and divinyl benzene copolymer.

Weakly basic anion exchanger – poly alkyl amine groups attached to styrene and divinyl benzene copolymer.

Advantages:

- It is a non-denaturing technique. It can be used at all stages and scales of purification
- An IEX separation can be controlled by changing pH, salt concentration and/or the ion exchange media
- It can serve as a concentrating step. A large volume of dilute sample can be applied to a media, and the adsorbed protein subsequently eluted in a smaller volume
- It offers high selectivity; it can resolve molecules with small differences in charge.

Disadvantages:

- Costly equipment and more expensive chemicals
- Turbidity should be below 10 ppm.

b) Brief on the principle and instrumentation of HPLC.

- **High-performance liquid chromatography** (**HPLC**; formerly referred to as **high-pressure liquid chromatography**- analytical chemistry used to separate, identify, and quantify each component in a mixture.
- It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material.
- Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out the column.

Instrumentation: The schematic of an HPLC instrument typically includes a sampler, pumps, and a detector.

- The sampler brings the sample mixture into the mobile phase stream which carries it into the column.
- The pumps deliver the desired flow and composition of the mobile phase through the column.
- The detector generates a signal proportional to the amount of sample component emerging from the column, hence allowing for quantitative analysis of the sample components.

- A digital microprocessor and user software control the HPLC instrument and provide data analysis.
- The sample mixture to be separated and analyzed is introduced in a discrete small volume (typically microliters), into the stream of mobile phase percolating through the column.
- Common mobile phases used include any miscible combination of water with various organic solvents (the most common are acetonitrile and methanol).

25. a) Write detail notes on SDS-PAGE and its uses.

- **Polyacrylamide gel electrophoresis** (PAGE)-technique widely used in biochemistry, forensics, genetics, molecular biology and biotechnology
- **TO** separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility. Mobility is a function of the length, conformation and charge of the molecule.
- For proteins,sodium dodecyl sulfate (SDS) is an anionic detergent applied to protein samples to linearize proteins and to impart a negative charge to linearized proteins. This procedure is called **SDS-PAGE**.
- In most proteins, the binding of SDS to the polypeptide chain imparts an even distribution of charge per unit mass, thereby resulting in a fractionation by approximate size during electrophoresis.
- SDS is an anionic detergent that denatures secondary and non–disulfide–linked tertiary structures, and additionally applies a negative charge to each protein in proportion to its mass. Urea breaks the hydrogen bonds between the base pairs of the nucleic acid, causing the constituent strands to separate. Heating the samples to at least 60 °C further promotes denaturation.
- In addition to SDS, proteins may optionally be briefly heated to near boiling in the presence of a reducing agent, such as dithiothreitol (DTT) or 2-mercaptoethanol (beta-mercaptoethanol/BME), which further denatures the proteins by reducing disulfide linkages, thus overcoming some forms of tertiary protein folding, and breaking up quaternary protein structure (oligomeric subunits). This is known as reducing SDS-PAGE.
- A tracking dye may be added to the solution. This typically has a higher electrophoretic mobility than the analytes to allow the experimenter to track the progress of the solution through the gel during the electrophoretic run.

b) Explain separation of DNA using agarose gel electrophoresis?

Principle

- Electrophoresis are a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. As such, it is one of the most widely-used techniques in biochemistry and molecular biology.

- used in biochemistry, molecular biology, genetics, and clinical chemistry to separate a mixed population of DNA or proteins in a matrix of agarose. The proteins may be separated by charge and/or size (isoelectric focusing agarose electrophoresis is essentially size independent), and the DNA and RNA fragments by length. Biomolecules are separated by applying an electric field to move the charged molecules through an agarose matrix, and the biomolecules are separated by size in the agarose gel matrix.

Agarose gels are easy to cast and are particularly suitable for separating DNA of size range most often encountered in laboratories, which accounts for the popularity of its use. The separated DNA may be viewed with stain, most commonly under UV light, and the DNA fragments can be extracted from the gel with relative ease. Most agarose gels used are between 0.7 - 2% dissolved in a suitable electrophoresis buffer.

The concentration of gel affects the resolution of DNA separation. For a standard agarose gel electrophoresis, a 0.8% gives good separation or resolution of large 5–10kb DNA fragments, while 2% gel gives good resolution for small 0.2–1kb fragments. 1% gels is often used for a standard electrophoresis. The concentration is measured in weight of agarose over volume of buffer used (g/ml). High percentage gels are often brittle and may not set evenly, while low percentage gels (0.1-0.2%) are fragile and not easy to handle.

26. a) Discuss the importance of safe handling of radioisotopes.

- All ionizing radiations can have effect on the human body.
- damages living cells, it can destroy or mutate the cells, possibly causing a cancerous growth.
- great difference in the penetrating powers of alpha particles, beta particles and gamma particles.
- alpha particles are easiest to stop.
- greater thickness and density materials to stop beta particles.
- Gamma rays have the most penetrating power of the three radiation types.
- Hence the most important safety aspect when dealing with radioactive sources is that of shielding often simply **keeping a reasonable distance** from the source will be sufficient as the air act as a shield.
- When working with more intense sources, **some form of shielding** may be required. Sealed sources should be handled with tongs or a special source holder never with the fingers.
- sealed sources should always be returned to their **lead lined storage boxes**.
- Radioactive substances should be handled with the same care and respect as concentrated acids.
- **Washing of hands** thoroughly after radioactive source is very essential.

b) Give a brief notes on liquid scintillation counter.

- **Liquid scintillation counting** is the measurement of activity of a sample of radioactive material which uses the technique of mixing the active material with a liquid scintillator, and counting the resultant photon emissions.
- The purpose is to allow more efficient counting due to the intimate contact of the activity with the scintillator. It is generally used for alpha and beta particle detection.

- Samples are dissolved or suspended in a **"cocktail" containing a solvent** (historically aromatic organics such as benzene or toluene, but more recently less hazardous solvents are used), typically some form of a surfactant, and **small amounts of other additives known as "fluors"** or scintillators. Scintillators can be divided into primary and secondary phosphors, differing in their luminescence properties.
- The primary phosphor will emit photons following absorption of the transferred energy. Because that light emission may be at a wavelength that does not allow efficient detection, many cocktails contain secondary phosphors that absorb the fluorescence energy of the primary phosphor and re-emit at a longer wavelength.
- The radioactive samples and cocktail are placed in small transparent or translucent (often glass or plastic) vials that are loaded into an instrument known as a liquid scintillation counter.
- Counting efficiencies under ideal conditions range from about 30% for tritium (a low-energy beta emitter) to nearly 100% for phosphorus-32, a high-energy beta emitter. Some chemical compounds (notably chlorine compounds) and highly colored samples can interfere with the counting process. This interference, known **as "quenching",** can be overcome through data correction or through careful sample preparation.