Q.P. Code: 00053290

S.Y.B.Sc. Biotechnology Semester III Examination

Model Answers

Biotechnology:- Biophysics

Q 1	Do as directed (Any fifteen)	15
1.	What is wavelength of Electromagnetic Radiation? 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).	
2.	Define the term 'Interference' Interference in light waves occurs whenever two or more waves overlap at a given point.	
3.	What is spectroscopy? The branch of science concerned with the investigation and measurement of spectra produced when matter interacts with or emits electromagnetic radiation.	
4.	Explain the term Stoke's shift. Fluorescence emission peak wavelength is red-shifted with respect to absorption peak wavelength. This shift may vary typically from 5 to more than 100 nm, depending on the electronic structure of the molecule.	
5.	State Beer's Law The Beer's law is the linear relationship between absorbance and concentration of an absorbing species.----1/2M Equation --- 1/2M	
6.	What is the role of pumping agent in a laser? Ans: To raise the level of energy of atoms of active medium from lower level to higher level.	
7.	List any two chemical compounds used in specimen preparation of electron microscopy. Any two – Examples, glutaraldehyde, osmium tetroxide, uranyl acetate, lead citrate etc.	

8.	Enlist any two modes of heat transfer. Ans: Any two from : Conduction / Convection / Radiation.	
9.	The unit for measuring frequency of sound is the _____. Ans: Hertz	
10.	The dip of the earth's magnetic field is measured with a _____. Ans: Dip circle.	
11.	The SI unit of viscosity is _____ Ans: N.s/m-2. (Newton.second per meter squared).	
12.	Explain the term : Terminal velocity. Ans: The maximum velocity with which a body falls through a viscous fluid under the influence of gravity.	
13.	What is surface energy? Ans: The extra energy that a fluid surface layer has is called surface energy.	
14.	_____ waves of the electromagnetic spectrum have the longest wavelength. Ans: Radio	
15.	Define Electrophoresis. Ans: Seperative- analytical technique involving migration and separation of charged particles in an applied electric feild.	
16.	Name any one tracking dye used in electrophoresis Ans: Bromophenol blue, Xylene cyanol or any other correct example.	
17.	What is zwitterion? Ans: An amino acid that is neutral due to presence of both positive (amino group) and negative (carboxyl group) charge.	
18.	State true or false: SDS PAGE is the second dimension in 2D PAGE. Ans: True	
19.	State true or false: Ethidium bromide is used to stain DNA in AGE. Ans: True	
20.	Give the full form of TEMED. Ans: Tetramethylethylenediamine N,N,N',N'-tetramethylethane-1,2-diamine.	

Q3

Q 2 A	Explain working principle and construction of SEM Working – 4 M Construction with diagram – 4M	**08**
Q 2 B	Give detailed account of Single beam spectrophotometer and list its limitations. Construction with working – 6M Any two limitations – 1M	**07**
	OR	
Q 2 C	Explain principle of florescent microscope with an application. Principle – 3 M Working with diagram – 3M Application with explanation - 2 M	**08**
Q 2 D	Explain dual beam spectrophotometer. Ans: Principle – 3M Working with diagram - 4M	**07**
Q 3 A	Enlist and explain the various uses of the Doppler effect. Points : Any four applications with 4 points per application or any eight applications with 2 points per application.	**08**
Q 3 B	Explain the principle, construction, working and use of an Ostwald viscometer. Points : Each point is 0.5 mark. 1 mark for principle, 2 marks each for construction, working and use. Diagram to be credited for a maximum of one mark in lieu of missing / insufficient points.	**07**
	OR	
Q 3 C	Explain the principle behind the construction and working of a platinum resistance electrode. Points : Made from platinum wires of specific purity, Generates resistance on heating due colliding electrons, How the coefficient of resistance is used to measure temperature, Calibration, Modification for different uses (2 marks each, diagram can be credited in lieu of missing points).	**08**
Q 3 D	Explain the different types of magnetism observed in nature. Points – Paramagnetism, diamagnetism and Ferromagnetism with	**07**

	diagrams (2, 2.5 and 2.5 marks respectively with 4, 5 and 5 relevant points if diagram absent; or else – 0.5 marks for diagram of each type of magnetism).	
Q 4 A	Describe principle of SDS PAGE and give any four applications. Principle of SDS PAGE:- polymerization – 2 Marks role of SDS -1 Mark, role of staining and destaining solutions- 1 mark Applications:- 4 Marks	**08**
Q 4 B	Discuss the various support matrices used in electrophoresis. Support matrices- 1 Mark List- paper, agarose, starch gels, polyacrylamide 2 Marks Explanation of each – 4 Marks	**07**
	OR	
Q 4 C	Discuss principle and applications of Agarose Gel 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 macromolecules such as DNA or proteins in a matrix of agarose, one of the two main components of agar. 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.[1] 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.[2] Agarose gel is easy to cast, has relatively fewer charged groups, and is 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. Agarose gel is a three-dimensional matrix formed of helical agarose molecules in supercoiled bundles that are aggregated into three-dimensional structures with channels and pores through which	**08**

	biomolecules can pass.[3] The 3-D structure is held together with hydrogen bonds and can therefore be disrupted by heating back to a liquid state. The melting temperature is different from the gelling temperature, depending on the sources, agarose gel has a gelling temperature of 35-42 °C and a melting temperature of 85-95 °C. Low-melting and low-gelling agaroses made through chemical modifications are also available. Agarose gel has large pore size and good gel strength, making it suitable as an anticonvection medium for the electrophoresis of DNA and large protein molecules. A number of factors can affect the migration of nucleic acids: the dimension of the gel pores (gel concentration), size of DNA being electrophoresed, the voltage used, the ionic strength of the buffer, and the concentration of intercalating dye such as ethidium bromide if used during electrophoresis.[11] Smaller molecules travel faster than larger molecules in gel, and double-stranded DNA moves at a rate that is inversely proportional to the log10 of the number of base pairs. Principle 4 marks and Applications- 4 Marks	
Q 4 D	Discuss Paper electrophoresis. Paper Electrophoresis is one of the zone electrophoresis. This is very important method in all laboratories. Principle: "The charge carried by a molecule depends on the pH of the medium. Electrophoresis at low voltage is not usually to separate low molecular weight compounds because of diffusion, but it is easier to illustrate the relationship between charge and pH with amino acids than with proteins (or) other macromolecules". Filter paper: Paper of good quality should contain at least 95% α-cellulose and should have only a very slight adsorption capacity.	**07**

	Apparatus: • The equipment required for electrophoresis consists basically of two items, a POWER PACK and an ELECTROPHORETIC CELL. • Power pack provides a stabilized direct current & has controls for both voltage & current out put, which have an out put of 0 to 500V and 0 to 150mA are available. • The Electrophoretic cell contains the electrodes, buffer reservoirs, a support for paper and a supporting transparent insulating cover. The electrodes are usually made of platinum. • The two arrangements of the filter strips are commonly used. The horizontal& vertical arrangements. Both the arrangements are equally viable & the choice usually depends upon personal preferences. Sample application: The sample may be applied as a spot (about 0.5cm in diameter) or as a narrow uniform streak. Special devices are available commercially for this purpose. The sample can be applied before the paper has been equilibrated with buffer (or) after it. Procedure: After the sample has been applied to the paper and the paper has been equilibrated with the buffer. The device providing stable voltage (or) current is available. Frequent observation is necessary to run electrophoretic apparatus. Overheating can be avoided by placing the entire equipment in the cold room. The process does not take longer than two hours. After 2 hours switched off the power and paper is removed. Once removed, the paper is dried in hot oven at 110C.	
Q 5	Write Short notes on **any three** of the following	**15**
a	Lasing action of a laser. Points : Stages of lasing - i.e. Energy transfer by pumping agent (xenon lamp), activation of active medium, population inversion, role of	

	resonator, resonance and emission of laser beam. (5 relevant stages – 1 mark per stage).	
b	Dispersion Definition – 1M Explanation with diagram – 3M Application – 1M	
c	Setup for calculation of η by falling sphere method. Points : Description of the setup (constant temperature water bath with thermometer, marked test tube with capillary to drop spheres, stop watch, test liquid) – 4 marks. Use – 1 mark.	
d	Angle of contact Points: Description of concept – 2 Marks (1 mark for diagram – expected). Cohesive and Adhesive forces and their relation to angle of contact (2 marks), Two examples (one each of angle of contact greater than and less than 90^{o}).	
e	Iso-electric focusing (IEF). Isoelectric focusing (IEF), also known as electrofocusing, is a technique for separating different molecules by differences in their isoelectric point (pI). It is a type of zone electrophoresis, usually performed on proteins in a gel, thattakes advantage of the fact that overall charge on the molecule of interest is a function of the pH of its surroundings. IEF involves adding an ampholyte solution into immobilized pH gradient (IPG) gels. IPGs are the acrylamide gel matrix co-polymerized with the pH gradient, which result in completely stable gradients except the most alkaline (>12) pH values. The immobilized pH gradient is obtained by the continuous change in the ratio of Immobilines. An Immobiline is a weak acid or base defined by its pK value. A protein that is in a pH region below its isoelectric point (pI) will be positively charged and so will migrate towards the cathode (negatively charged electrode). As it migrates through a gradient of increasing pH, however, the protein's overall charge will decrease until the protein reaches the pH region that corresponds to its pI. At this point it has no net charge and so migration ceases (as there is no electrical attraction	

towards either electrode). As a result, the proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its pI. The technique is capable of extremely high resolution with proteins differing by a single charge being fractionated into separate bands.

Molecules to be focused are distributed over a medium that has a pH gradient (usually created by aliphatic ampholytes). An electric current is passed through the medium, creating a "positive" anode and "negative" cathode end. Negatively charged molecules migrate through the pH gradient in the medium toward the "positive" end while positively charged molecules move toward the "negative" end. As a particle moves towards the pole opposite of its charge it moves through the changing pH gradient until it reaches a point in which the pH of that molecules isoelectric point is reached. At this point the molecule no longer has a net electric charge (due to the protonation or deprotonation of the associated functional groups) and as such will not proceed any further within the gel. The gradient is established before adding the particles of interest by first subjecting a solution of small molecules such as polyampholytes with varying pI values to electrophoresis.

The method is applied particularly often in the study of proteins, which separate based on their relative content of acidic and basic residues, whose value is represented by the pI. Proteins are introduced into an Immobilized pH gradient gel composed of polyacrylamide, starch, or agarose where a pH gradient has been established. Gels with large pores are usually used in this process to eliminate any "sieving" effects, or artifacts in the pI caused by differing migration rates for proteins of differing sizes. Isoelectric focusing can resolve proteins that differ in pI value by as little as 0.01. Isoelectric focusing is the first step in two-dimensional gel electrophoresis, in which proteins are first separated by their pI and then further separated by molecular weight through SDS-PAGE.