AV-8915

Zoology, B. Sc –Fifth Semester
LZC-504 (Biotechniques)

SECTION- A

Q1. Answer

i.) a ii) a iii) a iv) b v) b vi) b vii) b viii) c ix) c x) b

SECTION-B

Q.2. Answer:

Chromatography is a method for separating the components of a mixture by differential migration between a stationary phase and a mobile (moving) phase. In all chromatography there is a mobile phase and a stationary phase. The stationary phase is the phase that doesn't move and the mobile phase is the phase that does move. The mobile phase moves through the stationary phase picking up the compounds to be tested. As the mobile phase continues to travel through the stationary phase it takes the compounds with it. At different points in the stationary phase the different components of the compound are going to be absorbed and are going to stop moving with the mobile phase. This is how the results of any chromatography are gotten, from the point at which the different components of the compound stop moving and separate from the other components.

In paper and thin-layer chromatography the mobile phase is the solvent. The stationary phase in paper chromatography is the strip or piece of paper that is placed in the solvent. In thin-layer chromatography the stationary phase is the thin-layer cell. Both these kinds of chromatography use capillary action to move the solvent through the stationary phase.

The retention factor, Rf, is a quantitative indication of how far a particular compound travels in a particular solvent. The Rf value is a good indicator of whether an unknown compound and a known compound are similar, if not identical. If the Rf value for the unknown compound is close or the same as the Rf value for the known compound then the two compounds are most likely similar or identical.

The retention factor, Rf, is defined as

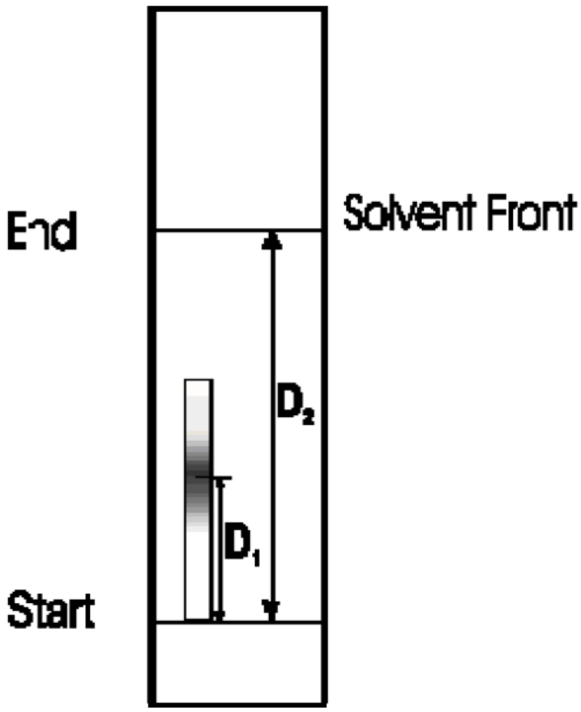
Rf = distance the solute (D1) moves divided by the distance traveled by the solvent front (D2)

$R_f = D_1 / D_2$

where

D1 = distance that color traveled, measured from center of the band of color to the point where the food color was applied

D2 = total distance that solvent traveled



Q3. Answer

Paper Chromatography is one of the most common types of chromatography. It uses a strip of paper as the stationary phase. Capillary action is used to pull the solvents up through the paper and separate the solutes.

Thin-layer Chromatography uses an absorbent material on flat glass or plastic plates. This is a simple and rapid method to check the purity of an organic compound. It is used to detect pesticide or insecticide residuesin food. Thin-layer chromatography is also used in forensics to analyze the dye composition of fibers.

Gas Chromatography is used in airports to detect bombs and is used is forensics in many different ways. It is used to analyze fibers on a persons body and also analyze blood found at a crime scene. In gas chromatography helium is used to move a gaseous mixture through a column of absorbent material.

Q.4 Answer

Cryopreservation is the technique of freezing cells and tissues at very low temperatures (-80^{o}C-196 ^{o}C) at which the biological material remains genetically stable and metabolically inert, while minimizing ice crystal formation. In general, when a tissue is subjected to low temperatures, ice crystals will eventually form. These crystals may disrupt the cell membrane leading to the death of the cell. The goal of cryopreservation is to replace some of the water with other compounds that will not form large crystals when frozen. The most commonly used replacements are DMSO (dimethyl sulfoxide) and glycerol. These are mixed into a solution with media or serum in which cells are suspended and placed in a liquid nitrogen freezer. As the media begins to freeze, the salt concentration outside the cells will become greater than that in the cells and water will leave the cells to be replaced by the cryopreservative.

Cryopreservation media generally consists of a base medium, protein source, and a cryopreservative. The cryopreservative both protects the cells from mechanical and physical stress and reduces the water content within the cells, thus minimizing the formation of cell-lysing ice crystals. The protein source, often fetal bovine serum (FBS), also protects the cells

from the stress associated with the freeze-thaw process. Cells are frozen slowly, at 1C/minute, using programmable coolers or by techniques outlined below.

Cell viability is a determination of living or dead cells, based on a total cell sample. Viability measurements may be used to evaluate the death or life of cancerous cells and the rejection of implanted organs. In other applications, these tests might calculate the effectiveness of a pesticide or insecticide, or evaluate environmental damage due to toxins.

Since everything living is made up of cells, cell viability counts have a tremendous number of applications. Testing for it usually involves looking at a sample cell population and staining the cells or applying chemicals to show which are living and which are dead. There are numerous tests and methods for measuring this.

When a sample is stained with various dyes or treated with chemicals, it is then subject to microscopic examination to evaluate cell viability. These measurements can be used to evaluate the effectiveness or lack thereof of certain treatments to cells.

The dyes or testing measurements used for determining cell viability are frequently called reagents. These are substances designed to provoke chemical reactions. When reagents are applied to cells, they may perform several actions, which allow scientists to examine cells in many different ways. Sometimes reagents are tested merely to show how they may affect the cells themselves, thus giving scientists information on which reagents should be avoided in order to not corrupt testing.

Cell sorting by flow cytometer

Answer 5. Flow cytometry sorters allow physical separation of sub-populations of cells of interest from a heterogeneous population, with a high degree of purity, for further analysis. Two kinds of sorting mechanisms are found:

1.- mechanical sorting : within a flow cell

Some flow cytometers, such as the FACSCalibur, use a mechanical device called the « catcher tube » to sort the cells of interest. The catcher tube is located in the upper portion of the flow cell and moves into the stream to collect the cells (fig.1). When cells pass through the laser beam the system determines if each cell belongs to the selected population defined by boundaries in the cytogram. If the cell is identified as a cell of interest, it is captured by the catcher tube and collected into a tube or into a concentration module, otherwise it is dispatched to the waste tank. The operator can choose the purity level of the sort among three levels (three sort modes).

The FACS Calibur can sort a maximum of 300 cells/second.

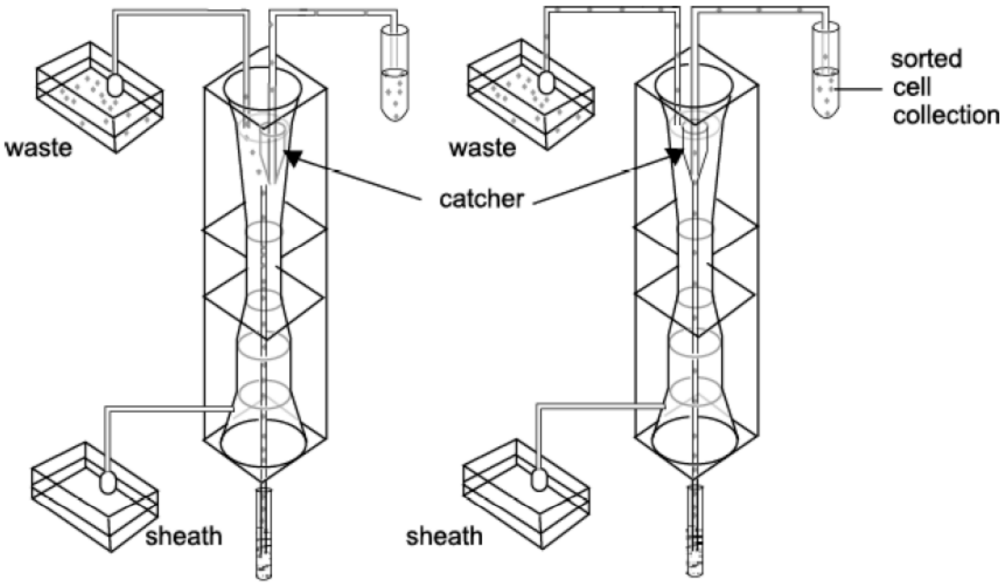


Figure 1: (Left) catcher tube in sheath stream; (right) catcher tube in sample stream (BD Biosciences)

2.- electrostatic sorting : « stream in air »

Most of the high speed cell sorters use the electrostatic deflection of droplets method. In this case there is no flow cell but the stream is focused in a vibrating nozzle and exits in a jet which is broken into regularly spaced droplets (fig. 2). The droplets containing a cell of interest are charged electrically (positively or negatively). When a charge droplet passes through a high voltage electrostatic field, between the deflection plates, it is deflected and collected into the corresponding collection tube. The deflection of the droplet is towards the oppositely charged plate, so that this droplet is separated from uncharged and oppositely charged droplets. In this case it is possible to sort two different populations of the same sample. Generally, the sorted cells are collected in a tube but they can also be collected on slides or in multi-well plates. For precise sorting it is very important to adjust several parameters, including :

- the nozzle vibration conditioned by the ddf (drop drive frequency which is the number of drops formed per second) and its amplitude level, the particle rate, i.e. the speed which influences the distance between each cell,
- the dead time : time taken by the instrument to measure a particle's signal and reset to measure the next particle (i.e. time necessary to analyze one particle),

- the drop delay : distance between the laser beam interception of the cell and the break-off point, the point where the stream beaks into droplets…

The operator can choose the purity level of the sort among four levels (four sort modes).

The FACSVantage SE can sort up to 10 000 cells/second when it is equipped with a turbo sort option and the appropriate nozzle.

Note : The orifice diameter of the nozzle tip used for sorting should be seven to ten times the diameter of the cells to be sorted.

The main drawbacks of the mechanical sorting is the possibility to sort only one population of cells at a slow speed of sorting. Nevertheless there is no aerosol, so that it is safe to sort samples which have been treated with toxic substances such as radioactive compounds.

The advantage of the electrostatic sorting is the possibility to sort two sub-populations of cells at a high speed. However, it generates aerosols so it is not appropriate to sort samples which have been treated with toxic substances. The high pressure generated in electrostatic sorting can also damage the sorted cells.

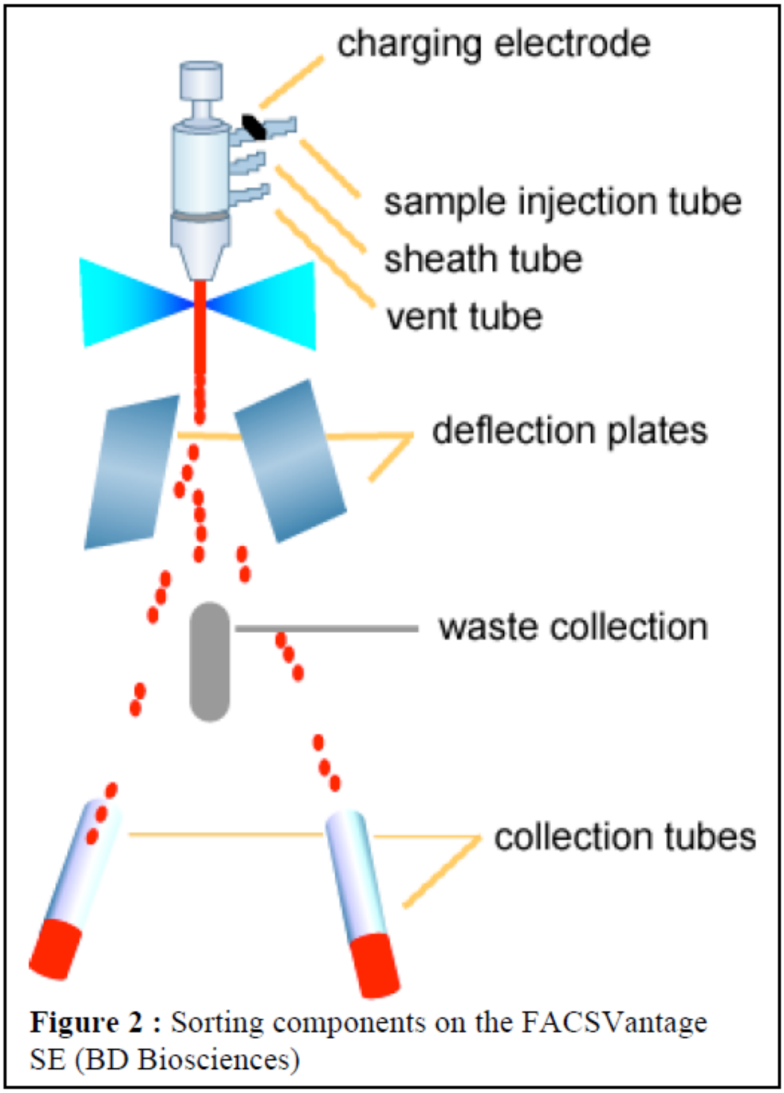


Figure 2 : Sorting components on the FACSVantage SE (BD Biosciences)

Answer 6. Phase-contrast microscope makes highly transparent objects more visible. Different parts of an object can be distinguished because they affect light differently from

one another. One basis for such differences is the refractive index. Cell organelles are made up of different proportions of various molecules: DNA, RNA, protein, lipid, carbohydrate, salts, and water. Regions of different composition are likely to have different refractive indexes. Normally such differences cannot be detected by our eyes. However, the phase-contrast microscope converts differences in refractive index into differences in intensity (relative brightness and darkness), which are visible to the eye. Phase-contrast microscopes accomplish this result by

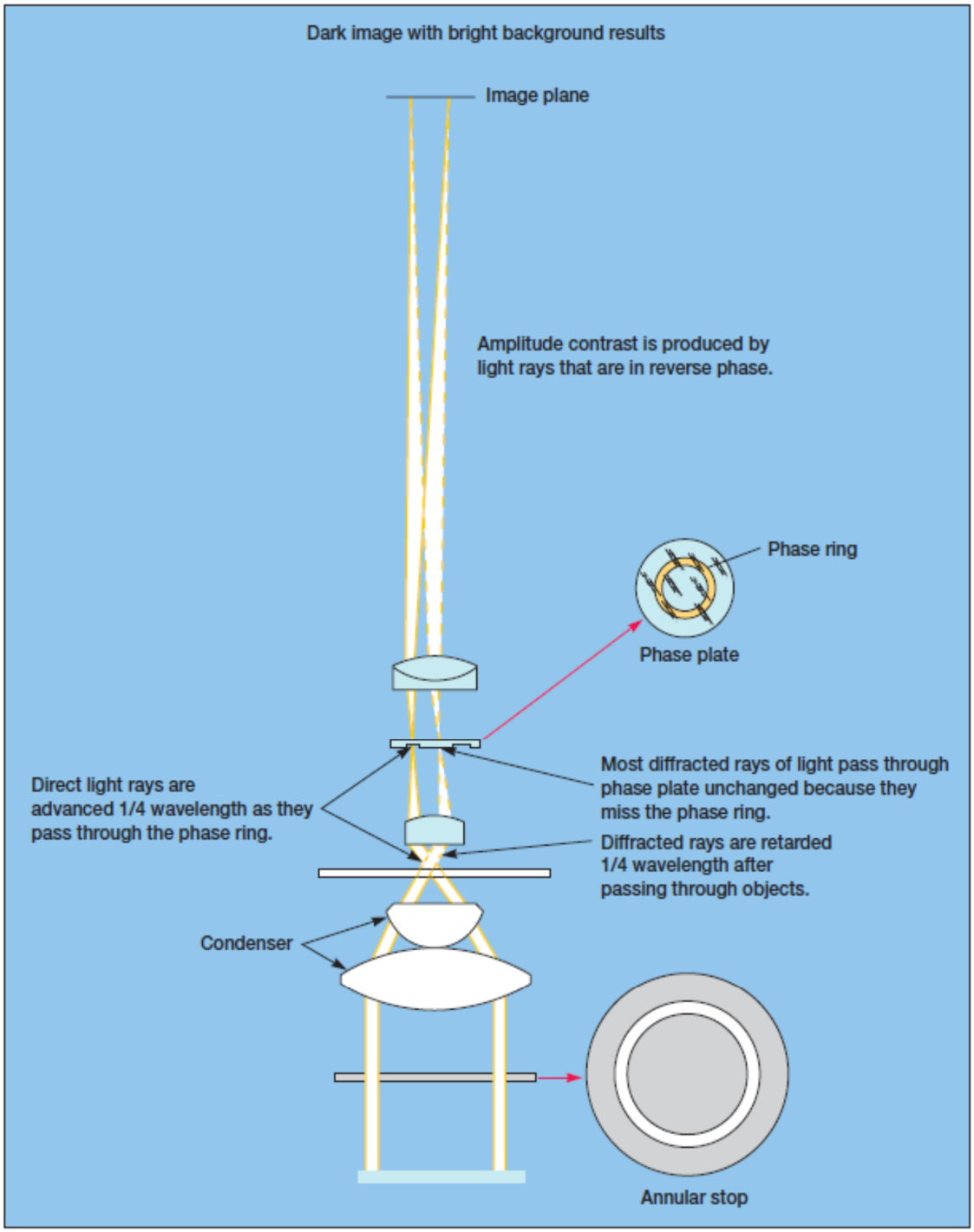
(1) Separating the direct light that enters the objective lens from the diffracted light emanating from the specimen and

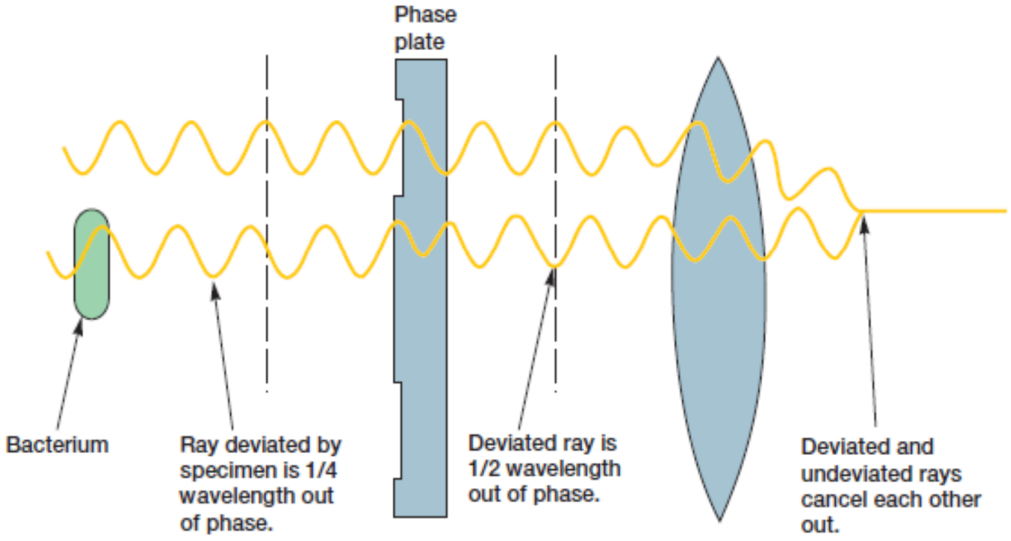
(2) Causing light rays from these two sources to *interfere* with one another.

The relative brightness or darkness of each part of the image reflects the way in which the light from that part of the specimen interferes with the direct light. Unpigmented living cells are not clearly visible in the brightfield microscope because there is little difference in contrast between the cells and water. Thus, microorganisms often must be fixed and stained before observation to increase contrast and create variations in color between cell structures. A **phase-contrast microscope** converts slight differences in refractive index and cell density into easily detected variations in light intensity and is an excellent way to observe living cells. The condenser of a phase-contrast microscope has an annular stop, an opaque disk with a thin transparent ring, which produces a hollow cone of light. As this cone passes through a cell, some light rays are bent due to variations in density and refractive index within the specimen and are retarded by about 1/4 wavelength. The deviated light is focused to form an image of the object. Undeviated light rays strike a phase ring in the

phase plate, a special optical disk located in the objective, while the deviated rays miss the ring and pass through the rest of the plate. If the phase ring is constructed in such a way that the undeviated light passing through it is advanced by 1/4 wavelength, the deviated and undeviated waves will be about 1/2 wavelength out of phase and will cancel each other when they come together to form an image. The background, formed by undeviated light, is bright, while the unstained object appears dark and well-defined. This type of microscopy is called **dark-phase-contrast microscopy.** Color filters often are used to improve the image. Phase-contrast microscopy is especially useful for studying microbial motility, determining the shape of living cells, and detecting bacterial components such as endospores and inclusion bodies that contain poly- -hydroxybutyrate, polymetaphosphate, sulfur, or other

substances. These are clearly visible because they have refractive indexes markedly different from that of water.





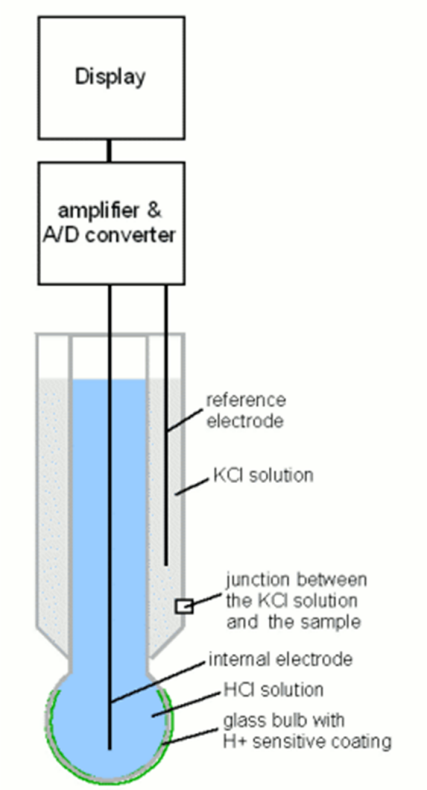
Answer 7. (i) pH meter: pH electrode is an example of ion selective electrode that responds to one specific ion in solution. They sely on the technique of potentiometry, which involves the measurement of a potential of An electrode without a current flowing.

pH electrode consists of a thin glass, porous membrane sealedee at the end of a hard glass tube containing x0.1M HCl into which is immersed a silver wire coated with AgCl. This Ag/AgCl electrode acts as an internal reference that generarte a constant potential. This outer and inner side of porous membrane contain exchanged binding sites for H^+ or Na^+ ions. On the inside of membrane the exchange sites are predominantly occupied by H^+ ions from HCl, inhibits on the outside the exchange sites are occupied by sodium ion/hydrogen ions. Hydrogen ions in the test tube solution can diffuse in the opposite direction replacing bound odium ions in the porous called ion exchange equilibrium. Any other types of cations present in the test solution ate unable to bind to the exchange sites. Thus, ensuring the high specificity of electrode.

The principle of operation of pH electrodes is based upon the fact that, if there is a gradient of proton activity across the membrane , this will generate a potential. In this case the potential is determined solely by the proton gradient across the membrane. Since proton activity on the inside of membrane is constant (owing to the rise of 0.1M HCl) the observed potential is directly dependent upon the proton activity of the test solution.

Operation & calibration:

The glass membrane of pH electrode is fragile and easily damaged. It is important that its surface remains hydrated and so it should be kept immersed in water when not in use.



Calibration should be normally carried out using two standard buffer solutions that span the pH range for which the electrode is to be used. The clean and blotted dry electrode should be immersed in the buffer solution allowed to equilibrate and the pH meter adjusted to the known pH valueusing the calibration on the instrument. The process is the repeated with second buffer solution and any adjustment made using the slope and temperature.

(ii)Answer

Culture media is a liquid or gel designed to support the growth of cells. The culture medium is the most important and complex factor to control in making cells "happy". Besides meeting the basic nutritional requirement of the cells, the culture medium should also have any necessary growth factors, regulate the pH and osmolality, and provide essential gases (O_2 and CO_2).

There are following types of cell culture media.

i) Natural Media-It contains biological fluid which is very useful but still it has not known the exact composition of these natural media

ii) Artificial Media

- Serum containing media
- Serum-free media (defined culture media)
- Chemically defined media

- Protein-free media

Basic Components of Culture Media

Culture media (as a powder or as a liquid) contains:

- amino acids
- Glucose
- Salts
- Vitamins
- Other nutrients
- Natural buffering system
- HEPES
- Phenol red as a pH indicator (yellow or purple)
- Inorganic salt
- Amino Acids (L-glutamine)
- Carbohydrates
- Proteins and Peptides (important in serum-free media. Serum is a rich source of proteins and includes albumin, transferrin, aprotinin, fetuin, and fibronectin
- Fatty Acids and Lipids
- Vitamins
- Trace Elements

Media Supplements

- Serum in Media
  - Basic nutrients
  - Growth factors and hormones
  - Binding proteins
  - Promote attachment of cells to the substrate
  - Protease inhibitors
  - Provides minerals, like Na+, K+, Zn2+, Fe2+, etc
  - Protects cells from mechanical damages during agitation of suspension cultures
  - Acts a buffer
- Antibiotics

	Media Type	Examples
Natural media	Biological Fluids	plasma, serum, lymph, human placental cord serum, amniotic fluid
	Tissue Extracts	Extract of liver, spleen, tumors, leucocytes and bone marrow, extract of bovine embryo and chick embryo
	Clots	coagulants or plasma clots
Artificial media	Balanced salt solutions	PBS, DPBS, HBSS, EBSS
	Basal media	MEM DMEM
	Complex media	RPMI-1640, IMDM

Answer 8. Specdtrophotometer and colorimeter work on the principle of Lamber-Beer's Law which correlates absorption of a specific wavelength of light to the concentration of absorbing species and path length of the monochromatic light passed through the solution. A wide range of biomolecules absorb light at characteristic wavelengths, just as tryptophan absorbs light at 280 nm. Measurement of light absorption by a spectrophotometer is used to detect and identify molecules and to measure their concentration in solution.

The fraction of the incident light absorbed by a solution at a given wavelength is related to the thickness of the absorbing layer (path length) and the concentration of the absorbing species. These two relationships are combined into the Lambert-Beer law,

$$\log \frac{I_0}{I} = \varepsilon c l$$

where

I_0 is the intensity of the incident light,

I is the intensity of the transmitted light,

ratio I/I_0 (the inverse of the ratio in the equation) - the transmittance,

ε - the molar extinction coefficient (in units of liters per molecentimeter),

c is the concentration of the absorbing species (in moles per liter),

l - the path length of the light-absorbing sample (in centimeters).

The Lambert- Beer law assumes that the incident light is parallel and monochromatic (of a single wavelength) and that the solvent and solute molecules are randomly oriented. The expression

$\log(I_0/I)$ is called the absorbance, designated *A*.

It is important to note that each successive millimeter of path length of absorbing solution in a 1.0 cm cell

absorbs not a constant amount but a constant fraction of the light that is incident upon it. However, with an absorbing layer of fixed path length, *the absorbance,* A, *is directly proportional to the concentration of the absorbing solute.*

The molar extinction coefficient varies with the nature of the absorbing compound, the solvent, and the wavelength, and also with pH if the light-absorbing species is in equilibrium with an ionization state that has different absorbance properties.